Taste masking of an active pharmaceutical ingredient for veterinary application

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Abstract

The aim of this thesis was to mask the bitter taste of praziquantel. The product into which the taste masked formulations were to be incorporated was an oral paste intended for the treatment of cats. Four different approaches to achieve taste masking were tested. First of all, microspheres with the active ingredient being embedded into a polymer matrix consisting of Eudragit E were produced. Moreover, taste masking as a result of complexation with β-cyclodextrin was tested as the formation of a true inclusion complex of praziquantel in β-cyclodextrin could be demonstrated. Another technique included incorporation into lipid microparticles. Finally, praziquantel being a racemic drug the isolated enantiomers were tested separately for their acceptance. The taste masked formulations were then incorporated into different pastes (bases: water, Miglyol or PEG) according to their properties and the acceptance by cats was tested. A poor result was scored for the Eudragit E microspheres in PEG which was mainly caused by the solubility of the active ingredient in this base. The drug-β-cyclodextrin complex and the lipid particles were both tested in Miglyol and water. In an oily paste rather low acceptance values were scored whereas in an aqueous paste satisfactory levels were reached. These could be increased slightly upon addition of a flavor. This result could be equaled with an in situ formed complex between praziquantel and β-cyclodextrin in an aqueous paste which was desired due to production and registration purposes. The separated praziquantel enantiomers were also tested in water and significantly different values were reached for them with $(-)$-praziquantel being preferred to its $(+)$-enantiomer. The acceptance level for $(-)$-praziquantel, however, was not significantly lower than that of the paste containing β-cyclodextrin and a flavor. Hence, an improvement of the results for $(-)$-praziquantel might be possible if taste masking agents were added. So in conclusion, taste masking of praziquantel was achieved in form of an aqueous paste containing β-cyclodextrin and a flavor as taste masking agents: the resulting paste is willingly taken by cats and also rated easily applicable by pet owners.

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Chapter 1

Introduction

To develop drug formulations for veterinary or for human use is quite similar in its first approach: The drug has to be administered to the patient. There are various application routes such as oral, parenteral, topical administration and application via mucous membranes. Many formulations in animal health like tablets or injections are similar to those for human use, but there are also a lot of formulations which are special for veterinary use such as medical collars, ruminal boluses, topical spot-ons and pour-ons. In addition, veterinary formulations are often more complex as special needs for both the patient and the animal holder have to be considered. For livestock, formulations have to be tailored for a mass treatment of herds which is important with respect to easy and fast (time-saving) application, low costs and season-long protection. Therefore, injections, feed additives, ruminal boluses and topical pour-ons are the formulation types most commonly used. For companion animals on the other hand, different factors such as pet and pet owner compliance, ease of use and dosing flexibility have to be considered. The first factor mentioned is especially important if the medication is used for treatment of chronic diseases. Therefore, the major formulation types used for companion animals are oral formulations, spot-ons, medicated collars, sprays, powders and shampoos (Ahmed & Kasraian, 2002).

It can be a rather challenging task for the pet owner to administer an oral product to cats or dogs as the animals often take their medicine very unwillingly, especially if it is badly tasting or smelling. One application method is the so called "poke down" method, where the medication is placed on the back of the animal's tongue and the throat is then massaged or the animal somehow distracted until the medication is swallowed. Especially with cats, this method is not easy and can be rather painful for the pet owner. Another method is to hide the medication in the animal's food. Unfortunately, there are also a few disadvantages with this method, because some drugs have to be administered in the fasted state and some drugs are too bitter to be masked successfully by the food. It is known that sometimes the animal will eat the food around the tablet which is left uneaten. Therefore, palatability is one of the main factors to be considered in development of oral formulations for companion animals, especially for cats as they have very sensitive taste buds and are much more independent than dogs. The term "palatability" refers to the voluntary (free choice) acceptance

or ingestion of a pharmaceutical composition by companion animals (Thombre, 2004). The simplest way to develop a palatable tablet is to add a well-accepted flavor. Commonly used flavors for dogs are beef or chicken, whereas cats prefer yeast, fish or milk flavor. However, the simple addition of a flavor may not be sufficient because this does not mask the taste of very bitter drugs and also does not cover odors of malodorous drugs, which is a big issue in veterinary formulations. Cats are especially sensitive to bad taste or odor and are not tricked as easy as dogs by simply adding a flavor. Other methods to achieve taste masking are coating, complexation, embedding in taste masking agents or inhibiting of taste buds by special excipients (Sohi et al., 2004). Coating may be the most efficient method in order to achieve taste masking because it encloses the drug and therefore also masks the odor. One disadvantage is that coating entire tablets is mostly not ideal because of the chewing habits of animals. They can bite the coating and once they taste the bitter active ingredient will refuse to swallow the tablet. A better result is achieved if granules or microparticles of the bitter drug are coated and then compressed to a tablet. In that case it has to be assured that the coating does not break up during tabletting. Other technologies include masking agents such as lipids, ion-exchange resins or complexation with cyclodextrins.

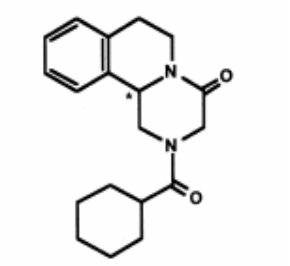

Figure 1.1: Praziquantel

Praziquantel (PZQ; Fig. 1.1; 2-(cyclohexylcarbonyl)-1,2,3,6,7, 11b-hexahydro-4H-pyrazino[2,1-a]isoquinoline-4-one) has a broad anthelmintic spectrum and is effective at a dose of 5mg/kg (Vetpharm, 2007). In animal health it is used against cestodes and trematodes (Geerts, 1994; Kruckenberg et al., 1981; Richards et al., 1989; Rommel et al., 1976). In human pharmaceuticals praziquantel is the drug of choice for the treatment of all forms of schistosomiasis (Andrews, 1981; Pearson & Guerrant, 1983; Liu et al., 1988; Mahmoud, 1987; Cioli & Pica-Mattoccia, 2003). The antiparasitic activity of the pyrazino isoquinoline ring system – the core structure of praziquantel – was observed in the early 1970s at the laboratories of Bayer, Germany (Andrews, 1981).

The aim of the present thesis is to develop a palatable oral formulation using different taste-masking technologies. Praziquantel was chosen as a model substance because of its very bitter taste and odor. Marketed animal health products of this drug only contain flavors as taste masking agents. However, this is not sufficient to mask the taste of praziquantel entirely as it is a very bitter active ingredient. In addition, the focus was placed on acceptability in cats because cats are much more challenging to treat. Various taste masking methods such as microencapsulation, inclusion complexes and embedding in masking agents are evaluated. The taste masking effect is first tested in vitro with dissolution studies and afterwards investigated in vivo by acceptance trials with cats.

1.1 Polymers

It is often stated that coating with different polymers is the simplest and most common technique to achieve taste masking (Sohi et al., 2004; Nanda et al., 2002; Douroumis, 2007). This does not only count for coating but also for methods where these polymers can also be used such as solid dispersions, melt extrusion or granulation (Douroumis, 2007). The coating acts as a physical barrier between the active ingredient and its surrounding, thus preventing drug dissolution in the oral cavity. Different substances can be used for these purposes: Carbohydrates such as different celluloses, shellac, gelatin, lipids or polymers.

Poly(meth)acrylates have proven particularly suitable as coating materials mainly because of their ability to be used for different targeted formulations and because they are pharmacologically inactive. Eudragit® polymers are copolymers derived from esters of acrylic and methacrylic acid. Their physicochemical properties are determined by their functional groups. A distinction is made between poly(meth)acrylates soluble in digestive fluids by salt formulation and those insoluble but permeable in digestive fluids. Eudragit L, S, FS and E belong to the first category which have either acidic (L,S,FS) or alkaline (E) groups to enable pH-dependent release of the active ingredient. They are used as protective coatings to increase the stability, for taste masking (E), as gastric resistance or controlled release coating in all sections of the intestine (L,S,FS). The poly(meth)acrylates insoluble and permeable in digestive fluids (Eudragit NE, RL, RS) have neutral functional groups (neutral esters and trimethylammonioethyl) and enable controlled release of the active ingredient by pH-independent swelling (Degussa Pharma Polymers, 2006).

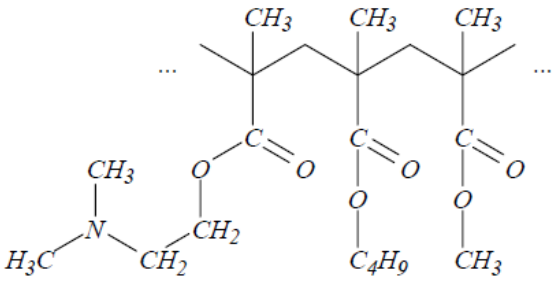

Figure 1.2: Eudragit E

As stated above the poly(meth)acrylate most often used for taste masking is Eudragit E (Ishikawa et al., 1999; Friend, 1992; Cerea et al., 2004). It is a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate (Fig. 1.2). It becomes water soluble via salt formation with acids thus providing gastro soluble coatings which are mainly used for taste masking or moisture protective coatings as they have a very low water vapor permeability. Moreover, good storage stability, protection of sensitive actives and improved passage of the dosage form are further advantages of Eudragit E coatings. No plasticizer is needed for Eudragit E as it is soft enough to build flexible coatings. However, separating agents such

as magnesium stearate or talcum often have to be added in order to avoid stickiness of the products (Degussa Pharma Polymers, 2006).

If Eudragit E is incompatible with the active ingredient, small amounts of Eudragit L or Eudragit RL can be used instead. A very thin film of these polymers (7-8 μm) reduces drug release only marginally so that no sustained release is obtained.

In addition to film coatings, Eudragit E can also be used as matrix in particles which are prepared for example by melt extrusion. The most important advantage of this process is the solvent free method in contrast to film coating where solubilization of the polymer is essential. These matrix particles can also be used for taste masking, furthermore for solubility enhancement and finally with neutral polymethacrylates sustained release pellets can be manufactured.

1.1.1 Coacervation

Microencapsulation in general can be described as a process in which very thin coatings of polymeric materials are deposited around particles which are either solid or droplets of liquids. In the pharmaceutical industry it is used to achieve prolonged or sustained release, taste masking of bitter drugs, reduced gastric irritation, separation of incompatible ingredients and protection of labile components (Voigt, 2006).

Coacervation, a very special microencapsulation technology, is also known as phase separation. The polymer is dissolved in a liquid phase and through various changes of the test conditions (change of temperature or pH or addition of a second substance such as a concentrated aqueous ionic salt solution or a non-solvent) its solubility is reduced which leads to a separation and building of a new phase. This new, polymer-rich phase becomes a clear homogeneous layer which is deposited around drug particles. Finally, this film solidifies to form the wall of the microcapsules (Voigt, 2006; Bauer et al., 1999; Dobetti & Pantaleo, 2002).

The process of coacervation is commonly divided in simple and complex coacervation. Phase separation in the case of simple coacervation requires a high polymer concentration and is induced either by a change of pH or temperature, for example ethyl cellulose. Another possible method is the addition of a non-solvent or another chemical compound which leads to precipitation of the polymer, for example electrolyts. In the case of a water soluble polymer as coating material such as cellulose acetate phthalate water can be used as a solvent and phase separation can then be achieved for example by adding a strong hydrophilic substance such as ethanol or sodium sulfate (Dobetti & Pantaleo, 2002; Thomasin et al., 1998). Complex coacervation involves the use of an ionic polymer as coating material with low concentrations of it in water. In this case coacervation is induced by the addition of an opposite charged polymer to the ionic polymer used as coating material which leads to a neutralization resulting in phase separation. An example for complex coacervation is the gelatine-gum arabic system where gelatine is positively charged below its isoelectric point but gum arabic is negatively charged (Voigt, 2006; Thomasin et al., 1998).

Taste masking of bitter drugs by coacervation has often been described in literature (Weiss et al., 1995; Al Omran et al., 2002; Palmieri et al., 2002; Weiss et al., 1993). It can be achieved by microencapsulation because the complete coating of the drug particle prevents contact with the taste sensors in the mouth. Moreover, it is important that the microcapsules are small enough (< 100 μm) to prevent mouth feel or even crushing by chewing which is crucial in animal health because especially cats tend to chew their meals very carefully. To achieve only a taste masking but no sustained release effect the coating applied should be nearly insoluble in the animal's mouth, but rapidly dissolve in gastric medium to release the active ingredient.

1.1.2 Solvent evaporation

Another method to achieve taste masking is the so-called solvent evaporation technique. In this process the active ingredient is not coated with a polymer film but is homogeneously dispersed in a polymer matrix in order to form microspheres. Although the drug is not completely covered by the polymer as it is with coacervation or film coating the dissolution of the active ingredient is also delayed. Drug dissolution from the matrix is mainly driven by diffusion and – in case of larger particles – by erosion. Hence, these kind of particles are most often used for sustained release. However, if a polymer with a pH-dependent solubility is used another dissolution profile can be achieved: in a polymer insoluble pH-range only a small amount of drug is dissolved but by change of pH the polymer dissolves and thus drug dissolution should occur rapidly and follow a first order kinetic. Thus, this technique can be used in order to achieve taste masking if the polymer used is insoluble at neutral pH (mouth of animals pH 6.8) so that the active drug remains encapsulated in the matrix during its stay in the oral cavity and only a few drug molecules from the surface area are dissolved. However, it is desired for the polymer to be soluble in acidic medium in order to release the drug without any sustained release effect as soon as the drug formulation is swallowed. If the polymer used is insoluble and/or only swellable in stomach and intestines drug dissolution is controlled by diffusion of the active ingredient through the matrix material leading to a zero order release kinetic and by this to a retarded effect. This is often not desired for veterinary drugs, e.g. praziquantel, so that Eudragit E (soluble in gastric fluid) is thought to be quite suitable for this case.

Microsphere preparation by solvent evaporation basically consists of four major steps: (1) dissolution or dispersion of the drug in an organic solvent containing the matrix forming material; (2) emulsification of this organic phase in a second continuous (frequently aqueous) phase immiscible with the first one; (3) evaporation of the solvent from the dispersed phase, thereby transforming the droplets into solid microspheres; (4) harvesting and drying of the microspheres (Fig. 1.3) (Freitas et al., 2005).In addition to evaporation of the solvent microspheres can also be created by solvent extraction. This is achieved if either more continuous phase or an additional extraction agent is given to the drug/polymer suspension.

Particle size of the microspheres prepared by solvent evaporation is dependent on several factors. Obviously, the impeller speed is the main parameter for controlling

the drug/polymer droplet size in the continuous phase. Increased mixing speed generally leads to a decrease in the mean size of the microspheres as it produces smaller emulsion droplets through stronger shear forces and increased turbulence. Another important aspect is the viscosity of the drug/matrix dispersion; the higher this viscosity is the larger are the produced microspheres. This is due to increased shear forces which are needed for droplet disruption. Besides, coalescence of drug/polymer dispersion droplets which would lead to a bigger particle size can be prevented by surface active stabilizer. Increased stabilizer concentration frequently results in reduced particle size (Freitas et al., 2005). For taste masking the particle size should not exceed <100 μm, meaning the particles are small enough to avoid a sandy and unpleasant feel in the animal's mouth and can thus not be chewed by the animal.

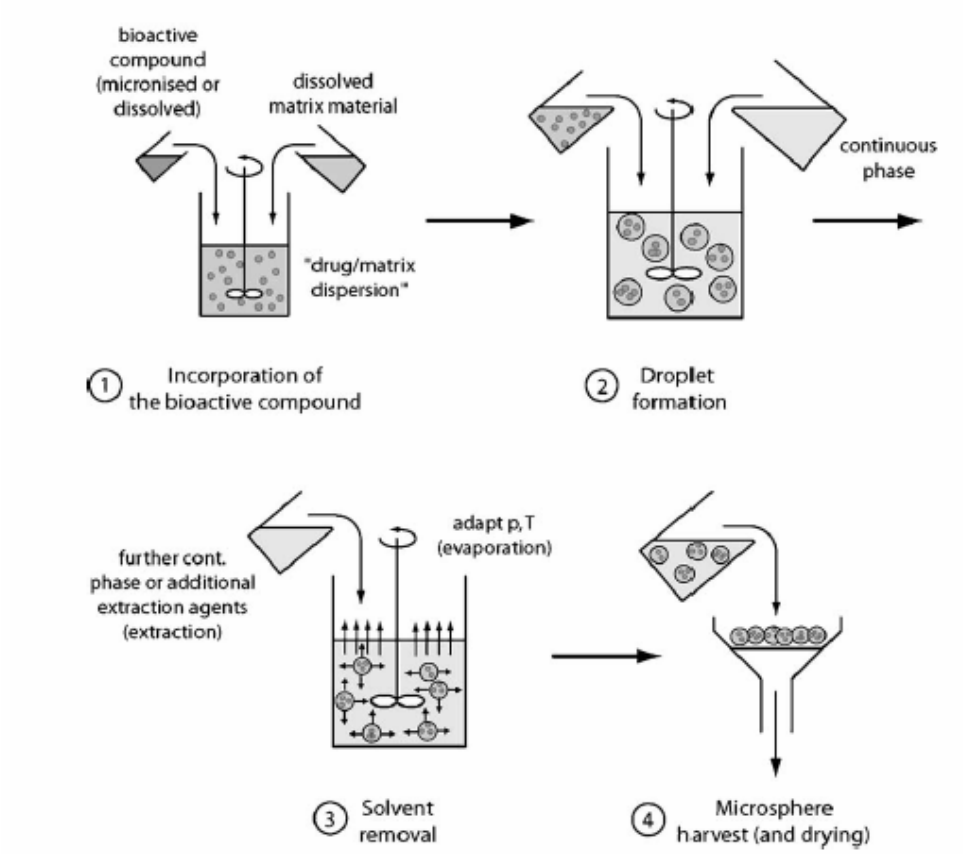

Figure 1.3: Schematic overview of the principle process steps in microsphere preparation (from Freitas et al., 2005).

However, when using organic polymer solutions in an aqueous phase the technique described above can lead to low encapsulation efficiencies for certain drug substances (Bodmeier et al., 1994). Moreover, this system can not be used for water soluble substances. To avoid this, the drug and polymer can be dissolved in an aqueous solution and then poured into an organic solution, thereby building a w/o emulsion.

1.2 Inclusion complexation with cyclodextrins

1.2.1 General

Cyclodextrins are cyclic, water-soluble oligosaccharides. Several different cyclodextrins consisting of either six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) glucopyranose units linked by α-(1,4) bonds occur naturally (Fig. 1.4) (Del Valle, 2004; Szejtli, 1990). Due to these bonds cyclodextrins form a cyclic structure where the hydrophilic hydroxyl groups are located on the wider edge of the ring (better: conical cylinder) and the hydrophobic hydrogen atoms and ether-like oxygen atoms face towards the inside of the cylinder. This results in a molecule with a hydrophilic outside and a hydrophobic cavity. Because of this apolar cavity, cyclodextrins are able to form inclusion complexes with a wide variety of hydrophobic guest molecules which are soluble in water due to their hydrophilic outside (Del Valle, 2004).

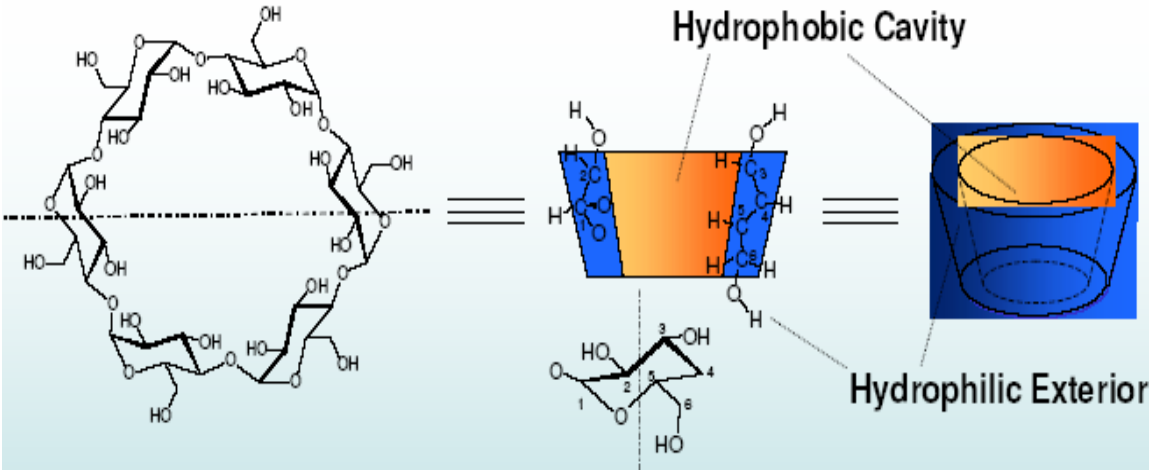

Figure 1.4: Chemical structure of α-cyclodextrin (from Wacker, 2002).

The cavity of the cyclodextrins is occupied by water molecules which are in direct contact with the apolar cavity. This polar-apolar interaction leads to an energetically unfavorable state so that the included water molecules can be easily substituted by guest molecules which are less polar than water and geometrically fit into the cyclodextrins' cavity (Szejtli & Szente, 2005). One or two guest molecules can be entrapped by one, two or three cyclodextrins whereby the most frequent host:guest ratio is 1:1. Inclusion in cyclodextrins exercises a significant effect on the physicochemical properties of guest molecules as they are temporarily locked within the host cavity. This can result in solubility enhancement, stabilization against UV light or heat, control of volatility and sublimation, physical isolation of incompatible compounds, taste and odor masking and controlled release (Del Valle, 2004).

The three naturally occurring cyclodextrins show different physical properties depending on their structure. The most important difference is the size of the cavity which is a result of the amount of glucopyranose units: α-cyclodextrin has the

smallest cavity with a medium volume of 0.174 nm^3 whereas β-cyclodextrin and γ-cyclodextrin exhibit greater cavities with 0.262 nm^3 and 0.427 nm^3 respectively (Szejtli, 1990; Wacker, 2002). Based on these volume dissimilarities inclusion complexes are formed with different guest molecules. In contrast to α-cyclodextrin which prefers simple aliphatic chain structures, β-cyclodextrin is most suitable for aromatic structures and simple ring systems whereas γ-cyclodextrin fits well with steroidal structures and larger ring systems (Wacker, 2002). Another property which differs between the cyclodextrins is their water solubility where β-cyclodextrin is the least soluble with only 1.8 g/100 ml at 25 °C. For α-cyclodextrin and γ-cyclodextrin the solubility lies at 14.5 g/100 ml and 23.2 g/100 ml, respectively. However, they all show increasing water solubility with rising temperature (Szejtli, 1990).

Based on these naturally occurring cyclodextrins, many cyclodextrin derivatives have been synthesized. These derivatives usually are produced by aminations, esterifications or etherifications of hydroxyl groups of the cyclodextrins. Depending on the substituent, the solubility of the cyclodextrin derivatives usually differs from that of their parent cyclodextrins. Nearly all derivatives have a changed hydrophobic cavity volume, improved solubility and stability against light or oxygen and can help to control the chemical activity of guest molecules (Del Valle, 2004).

Taste masking with cyclodextrins is achieved by inclusion complexes with bad tasting substances. This complexation mostly also leads to an increase in drug solubility which would normally also enhance the bad taste sensation because only dissolved substances elicit taste at all. However, due to the strongly hydrated outer surface of the complexes and the natural barrier of the cyclodextrin cylinder, the direct contact between the solubilized active ingredient and taste sensors is inhibited which results in eliminating the bad taste sensation.

1.2.2 Complex formation

Complex formation can occur either in solution or in solid state. In the latter case the active ingredient is simply added to dry cyclodextrin and mixed. Inclusion complex formation with this method can only take place if the cyclodextrin still contains its crystal water (i.e. has not been dehydrated by previous heating for hours over 100 °C in a vacuum (Szejtli & Szente, 2005)) so that the active ingredient can be complexed by substitution of the crystal water molecules. The main advantage of this method is that no water or other solvent is needed which have to be removed later on; disadvantages are insufficient mixing leading to incomplete complexation and the long duration of the complex formation. This process takes extremely long with very hydrophobic molecules with high melting points, but is quite rapid with liquids, oils and sublimable molecules. In contrast to the dry mixing method complexation can also occur in solution whereby different techniques are applied. Complex formation can be achieved either by co-precipitation or by kneading. Co-precipitation is the method most widely used in laboratories. Hereby cyclodextrin is dissolved in water and the guest molecule is added whilst stirring. Complexation conditions such as cyclodextrin concentration and temperature are chosen so that the solubility of

the complex is exceeded as the reaction proceeds and the complex can therefore be collected as a precipitate by decanting, centrifugation or filtration. The cyclodextrin does not have to be completely dissolved to form an inclusion complex. Therefore, another method needing less water can be applied for complexation, the so-called kneading method. Depending on the amount of water used this method can be divided in "slurry" or "paste" method, the latter requiring less water than the first. In both cases the basic principle is the same: The aqueous phase is saturated with cyclodextrin in solution and the guest compound can build a complex with the dissolved cyclodextrin molecules. As soon as the complex saturates the water phase it precipitates. Cyclodextrin crystals can then dissolve again and continue to saturate the water phase and build more inclusion complexes with the guest molecule. The amount of time needed to complete the complexation is variable and depends mostly on the guest molecule. Additives may be used to promote complexation such as ethanol or ammonia for enhanced complexation efficiency of basic drugs (Del Valle, 2004).

1.2.3 Phase-solubility techniques

To determine the stability of the complex and the amount of complexation two methods can be applied: phase-solubility techniques according to Higuchi with calculation of the stability constant and determination of the complexation efficiency according to Loftsson (Higuchi & Connors, 1965; Loftsson et al., 2005). The first techniques is based upon drug solubility analysis with interacting components. Molecular interactions between a substrate (S; drug) and a complexing agent (ligand L; in this case cyclodextrin) are studied by means of solubility. An equal weight of drug in a considerable excess of its solubility is added into several vials. A constant volume of solvent (mostly water) and a successively increasing amount of complexing agent are added and the liquid is stirred until the equilibrium is obtained. The total concentration of the dissolved drug is then measured. The results are plotted as a phase diagram of the molar concentration of the substrate (vertical axis) against the molar concentration of the ligand. Conclusions regarding complexation properties can be drawn from the slope: if drug and cyclodextrin form a soluble complex a steady increase of drug concentration can be seen (Fig. 1.5). Complexes with only one ligand result in a linear increase of drug solubility (S_mL) (A_L diagram). The reverse statement is usually adopted, although it does not necessarily follow. If more ligand molecules are involved in complex formation this leads to a slope of higher order (A_P diagram). Finally, the last diagram (A_N) in Fig. 1.5 is of uncertain origin. Possible explanations may be a change in the nature of the solvent in presence of large amounts of ligand leading to a change of the complex formation constant. Another reason might be a self association of the ligand which effects the apparent degree of complexation. Sometimes Type A diagrams show a plateau in the concentration of the drug. There are two different causes of this: either S is completely dissolved and thus further addition of L cannot lead to an increase of solubility or the ligand is not highly water soluble and thus the solution is saturated with L. This might be the case for some cyclodextrins, especially β cyclodextrin, which has a limited water solubility.

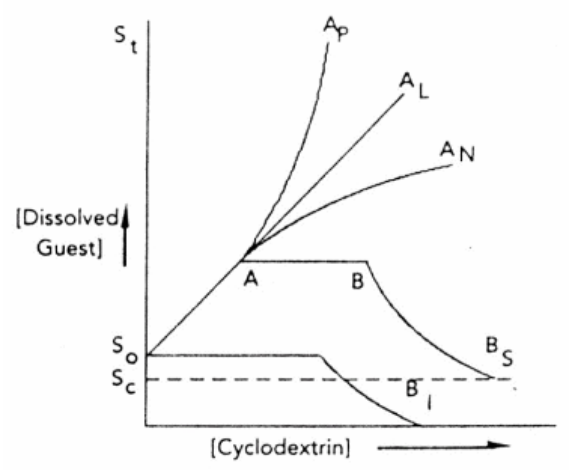

Figure 1.5: Phase-solubility diagram types

The second type of phase-solubility diagrams (B type) are obtained when insoluble complexes are formed (Fig. 1.5). The solubility limit of the complex is reached at a certain time point and upon further addition of ligand the complex precipitates. Dependant on the solubility of the complex this might be the case earlier or later (Type B_S and B_I, respectively). These diagrams are of less importance for cyclodextrin complexes.

Further conclusions on the complexation can be drawn from the diagrams in respect of stoichiometry and stability (equilibrium constants) of the complexes. For Type A_L diagrams it is rather difficult to determine the exact stoichiometry of the complexes (general formula S_mL_n). If the slope is greater than one at least one complex must be formed which contains more S molecules than L (m>1). In contrast, a slope less than one does not necessarily suggest a complex of the type 1:1 although this assumption is usually made in absence of additional information. In the special case of a plateau because of the precipitation of solid L it is possible to calculate a stoichiometric ratio based on the different turning points in the graph. For insoluble complexes with a Type B diagram the stoichiometric ratio can always be calculated although if more than one complex is formed it might lead to an "average" ratio. The stoichiometry ratio can – as previously described for the special Type A_L diagram – be calculated from the turning points in the curve which are marked by the formation of the soluble amount of the complex and precipitation of the complex after exceeding its solubility limit.

For the estimation of equilibrium constants the stoichiometry ratio is needed because otherwise no unambiguous interpretation of the complex formation constant is possible. As a result, the calculation of an apparent constant based on a reasonable stoichiometric ratio has to be sufficient if the ratio is not definitely known. For Type A diagrams with a supposed single complex, S_mL_n, being responsible for the increase in the solubility, the complex formation (or stability) constant can be calculated from the chemical equation

$$mS + nL \rightleftharpoons S_m L_n \tag{1.1}$$

with the equilibrium constant calculated as

$$K = \frac{S_m L_n}{S^m \cdot L^n} \tag{1.2}$$

in which the following quantities are applied:

$$S = S_0 \tag{1.3}$$
$$S_m L_n = (S_t - S_0)/m \tag{1.4}$$
$$L = L_t - n \cdot (S_m L_n) \tag{1.5}$$

with S_0 equilibrium solubility of S; S_t total concentration of dissolved S; L_t total added concentration of L.

Of special interest for the stability constant is the case n=1 and m=1, in particular for cyclodextrin complexes as 1:1 drug/cyclodextrin complexes are the most common type (Loftsson et al., 2005). In this special case, the constant can be calculated by

$$K_{1:1} = \frac{slope}{S_0 \cdot (1 - slope)} \tag{1.6}$$

It can also be calculated by combinations of Equ. 1.2 to 1.5:

$$K_{1:1} = \frac{S_t - S_0}{S_0 \cdot (L_t - S_t + S_0)} \tag{1.7}$$

The last two equations are equally able to be used for the calculation of the stability constants on the basis of a 1:1 stoichiometry. A 1:1 ratio is the most common one for cyclodextrins, so the calculations for the constants of the other diagram types are not explained here in detail but can be referred to in literature (Higuchi & Connors, 1965).

For cyclodextrin complexes the observed value of $K_{1:1}$ is mostly between 50 and 2000 M^{-1} (Connors, 1995). On average β-cyclodextrin shows the highest values of the natural cyclodextrins (490 M^{-1}) compared to α-cyclodextrin (129 M^{-1}) and γ-cyclodextrin (355 M^{-1}). The higher the constant the more stable is the complex. A high $K_{1:1}$ value is desired for complex stability in solution and thus also for taste masking. However, in order to achieve improved bioavailability the stability constant should not be too high so that the drug is still released from the cyclodextrin cavity in vivo and can hence be absorbed. As a result values ranging from 200 to 5000 M^{-1} are meaningful for the absorption process of complexed drugs because of the improvement of bioavailability of hydrophobic drugs (Blanco et al., 1991; Szejtli, 1988).

1.2.4 Complexation efficiency

Additionally to the stability constant the complexation efficiency (CE) of cyclodextrin complexes can be used for their characterization. This value can also be calculated

from phase-solubility diagrams but without the influence of the intrinsic solubility of the drug. For calculation of the constant $K_{1:1}$ the intrinsic solubility of the drug S_0 is needed (see Equ. 1.6 and 1.7). This solubility should be equal to the intercept (S_{int}) determined by linear regression of the phase-solubility data, but this is quite often not the case, especially for poorly soluble drugs (Loftsson et al., 2005). One reason for this might be the non-ideality of water as a solvent (Schmid, 2001; Xantheas, 2000). If S_0 is either greater or smaller than S_{int} it can lead to an over- or underestimation of $K_{1:1}$, respectively. Moreover, the addition of common pharmaceutical excipients, for example polymers, can influence the intrinsic solubility of the drug (by forming complexes with small molecules in aqueous solutions). So for the calculation of $K_{1:1}$ the question is raised which solubility should be used as intrinsic solubility: The true intrinsic solubility S_0, the intercept S_{int} or – if polymers are present – the solubility of the drug in the presence of the polymer. Depending on which solubility is used the values for can differ significantly (Duan et al., 2005).

These considerations show that the phase-solubility method is not really suitable for the exact determination of the stability constant particularly if additionally formation of multicomponent complexes and simultaneous formation of inclusion and non-inclusion complexes are considered. Moreover, the main purpose of cyclodextrins in pharmaceutical formulations is to enhance the solubility of the drug, thus it is more desired to gain knowledge about the ability of the particular cyclodextrin to form inclusion complexes with the drug. For this reason Loftsson introduced the complexation efficiency which is determined by either the slope of the phase-solubility (similar to the stability constant but without the intrinsic solubility) or the complex to free cyclodextrin concentration ratio:

$$CE = S_0 \times K_{1:1} = \frac{[D/CD]}{[CD]} = \frac{slope}{1 - slope} \tag{1.8}$$

where $[D/CD]$ concentration of dissolved complex; $[CD]$ concentration of free cyclodextrin; $slope$ slope of phase-solubility diagram

For example, a CE of 0.2 then means that only about one out of every six cyclodextrin molecules form a complex with the drug if a 1:1 drug/cyclodextrin complex is presumed. From these results the amount of cyclodextrin needed to achieve the wanted drug solubility can be determined.

As mentioned above polymers can influence the solubility of drugs and complexes and as a result also the complexation efficiency of cyclodextrin complexes (Duan et al., 2005; Loftsson et al., 1999; Loftsson & Masson, 2004; Loftsson & Fridriksdottir, 1998; Loftsson et al., 1994a; Ribeiro et al., 2003). Water soluble polymers such as polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC) and carboxymethylcellulose (CMC) can increase the CE for certain drug/cyclodextrin complexes. Added to aqueous drug/cyclodextrin solutions in a concentration between 0.1 and 0.25% w/v these polymers can lead to a significant increase in CE due to formation of non-inclusion (ternary) complexes. These complexes are formed between drug/cyclodextrin complexes and polymer molecules. They result in greater struc-

tural inhibition which are compensated by more negative free energy which, in the end, leads to an increase in the complexation efficiency (Loftsson & Masson, 2004; Loftsson et al., 1994b; Mura et al., 2001). In average, an increase of 70% could be accomplished after addition of a small amount of polymer. For β-cyclodextrin this value is even higher (about 130%). This is due to its limited solubility in water, so the increased solubility is based upon the enhanced complexation efficiency as well as on the improved solubility of both drug/β-CD complex and β-CD due to formation of ternary complexes. This leads to a decrease in the β-cyclodextrin amount needed for the solubilization of the drug which is desired in the pharmaceutical industry for various reasons such as toxicological considerations, production costs and higher bioavailability (Loftsson & Masson, 2004).

1.3 Lipid embedding

Nearly all of the above described processes require solvents, i.e. water, organic solvents or mixtures. The use of organic solvents may lead to environmental problems, solvent residues and excessive costs for recovery (Achanta et al., 1997; Barthelemy et al., 1999). Furthermore, instability of the active ingredient in these solvents can occur. Aqueous solvents on the other hand generally prolong the duration of the processes. Several thermal techniques employing lipophilic waxes and thereby avoiding the use of solvents are for example melt granulation, melt pelletisation, hot-melt extrusion, spray congealing and hot-melt coating (Achanta et al., 1997; Hamdani et al., 2002; Liu et al., 2001; Saraiya & Bolton, 1990). They all show promising results for taste masking, gastric resistance, sustained release or bioavailability enhancement, based upon type of coating wax (Barthelemy et al., 1999; Hamdani et al., 2002; Robson et al., 1999). Sometimes these processes cannot be used for some thermally instable active ingredients because of the high temperatures needed. In such cases, lipophilic waxes with a low melting range i.e. Precirol ATO 5 are recommended.

Lipids can successfully be applied for taste masking (Robson et al., 1999; Sugao et al., 1998). For this, the lipid of choice must exhibit a melting point high enough to function as an effective barrier around the active and yet be soft enough to release the drug in the intestine in order to avoid a sustained release effect (Gattefossé, 2005). Precirol ATO 5 consists of atomized glyceryl palmitostearate and shows physical and chemical properties ideal for taste masking. The melting point is between 50-60 °C which ensures its barrier function at room temperature but at the same time is not too high so it can be easily used in manufacturing compared to lipids with high melting points. Moreover, Precirol ATO 5 shows a low viscosity which should provide a quick release of the drug out of the matrix without sustained release effect (Sinchaipanid et al., 2004). Methods employed for lipids to achieve taste masking are all based upon their ability to melt at rather low temperatures. The techniques most commonly used are hot melt coating in a fluidized bed and melt granulation/embedding. For hot melt coating a melted excipient is atomized on to the fluidized active drug particles which are coated individually. Melt granulation, however, is based on embedding

drug particles in a lipid matrix. Both methods can be applied for taste masking (Gattefossé, 2005; Sinchaipanid et al., 2004).

Dissolution of the drug from lipid particles can either be controlled by diffusion, erosion/digestion or by a combination of both incidents (Jannin et al., 2006; Ozyazici et al., 2006). Erosion is mainly of influence if large particles (capsule or tablet size) are used, but for microparticles this effect is of minor interest. Drug dissolution from Precirol microparticles is hence mainly dependent on drug diffusion from the matrix particles into the dissolution medium (Marchaud et al., 2006). From this, it can be concluded that the dissolution rate is the faster, the smaller the particles and the larger the surface area. If diffusion is the determining step for dissolution, drug matrix particles normally show a delayed release with drug dissolution over several hours. For veterinary products, mainly for cats and dogs, this is not reasonable as the gastrointestinal transit time is less in companion animals than in humans. A faster release is therefore desired which can be achieved by a smaller particle size, larger surface area and incorporation of hydrophilic substances into the lipid matrix.

1.4 Enantioseparation

Racemic mixtures of active ingredients are commonly employed in drug therapy. It has long been known that the human body is a highly stereo-specific environment. Hence, different enantiomers may show very dissimilar biological activity due to discrepancy in protein binding and transport, mechanism of action, rates of metabolism, changes in activity due to metabolism, etc. (Lee & Williams, 1990). Consequently, as is often the case, only one form of the drug has the desired therapeutic effects whilst its mirror image may be less efficient or may even have an additional undesirable effect (Lim et al., 1995).

The commercially used praziquantel is a racemic compound (El Arini et al., 1998). It is known from the literature that most of the anthelmintic activity is due to the $(-)$-enantiomer whereas the $(+)$-enantiomer is responsible for most of the side effects such as emesis and diarrhea (Andrews, 1985; Blaschke & Walther, 1985). The efficacy of $(-)$-praziquantel against schistosomas has been shown in several studies (Liu et al., 1988; Andrews, 1985; Andrews et al., 1983; Shu-Hua & Catto, 1989; Xiao et al., 1998) as it is of great interest in human medicine. Unfortunately, less information is available for the efficacy of praziquantel enantiomers against cestodes and other parasites common in animal health. Andrews et al. (1983) stated that the efficacy of $(-)$-praziquantel against cestodes is higher than that of $(+)$-praziquantel, but did not specify the exact data. If one enantiomer were more effective against cestodes than the racemic mixture it would implicate that less amount of drug is needed which could on the one hand lead to lower costs but also – and even more important so – might be of interest for the taste sensation. Additionally, as mentioned above different enantiomers can show different effects in the human or animal body. It might thus be thoroughly possible that only one enantiomer of praziquantel has a very bitter taste and its mirror image does not. Of course, if the more effective enantiomer were not

the one responsible for the bad taste, this would be the most elegant solution for taste masking!

Chapter 2

Eudragit microparticles

2.1 Short introduction

Eudragit E as a cationic poly(meth)acrylate has been successfully employed as coating material for the protection from moisture or for taste masking. The polymer is soluble in acidic medium which prevents drug dissolution in neutral and basic medium, but leads to a rapid dissolution in the stomach. This effect is desired for taste masking formulations which especially in animal health should not lead to a sustained release of the active ingredient.

At first powdered Eudragit E (EPO) was used for coacervation with praziquantel. This should result in small microcapsules with the active ingredient as core coated by the polymer. The particles thus should not release any drug in the mouth but dissolve very quickly in the acidic medium of the stomach. Simple coacervation was tried with different phase separation methods: precipitation of the polymer was induced either by pH-shift or by addition of a non-solvent. Solvent evaporation was used as another method for taste masking with Eudragit EPO. In these trials praziquantel was incorporated into an Eudragit EPO matrix. This was possible by emulsifying an aqueous solution (acetone) of polymer and drug in an organic phase (paraffin). The obtained particles were characterized by scanning electron microscopy (SEM) and determination of the particle size distribution.

Dissolution studies are an important tool to characterize drug product performance in vitro. Hence, the manufactured products were examined for their taste masking properties by dissolution studies in a neutral medium to imitate the pH of the animal's mouth. Results can be used to estimate the taste masking effect of the used technique. Moreover, stability studies were performed to test if the formulations were stable over a certain time under special storage conditions.

2.2 Materials and methods

2.2.1 Materials

The drug substance praziquantel was provided by PCAS, Limay, France. Eudragit EPO and L were supplied by Röhm GmbH, Pharma Polymers, Darmstadt, Germany. Aluminium-monostearate, light liquid paraffin, phosphate buffer pH 6.8 and buffer pH 3 were purchased from Fluka AG, Buchs, Switzerland. Polysorbate 20 (Tween 20), sodium chloride and buffer pH 5 were obtained from Riedel-de Haën AG, Seelze, Germany. Solutol HS 15 was provided by BASF, Ludwigshafen, Germany. Magnesium stearate was supplied by Faci Metalest, S.L., Zaragoza, Spain.

The solvents ethanol absolute, n-hexane, cyclohexane, hydrochloric acid, sodium hydroxide solution and acetone were used from Merck Inc. Darmstadt, Germany. Demineralised water was used from the laboratory tap prepared in-house.

2.2.2 Coacervation

The ratio of drug to polymer for coacervation was set at 70:30 to ensure a thick enough coating around the drug particles for taste masking (Voigt, 2006). Due to the very poor wettability of praziquantel in water a detergent (Tween 20) was needed.

For coacervation by pH-shift 5 g Eudragit EPO were dissolved in 100 ml buffer pH 5 by sonication. 11.7 g unmicronized praziquantel and 0.1 g Tween 20 were added to the polymer solution and stirred with a paddle agitator at 500 rpm (Eurostar digital, Ika-Werke GmbH, Staufen, Germany) until the suspension was homogeneous. Afterwards 100 ml of 0.1 N sodium hydroxide solution were added with 1 drop/3 seconds. The product was filtered and dried at 60 °C and 300 mbar for one hour (Salvis Trockenschrank, Typ KVTS11, Reussbühl, Switzerland).

Coacervation was further tried using a solvent/non-solvent system. 5.8 g praziquantel were suspended in 100 ml water using a detergent (0.1 g Tween 20). 50 ml of a 5% ethanolic Eudragit EPO-solution were added drop by drop while continuously stirring with a paddle agitator at 500 rpm. The product was filtered and dried under an extractor hood. For another process according to Okor (1990), Eudragit EPO (0.3 g) and praziquantel (0.6 g) were dissolved in 10 ml ethanol. Excess non-solvent containing a flocculating agent, 80 ml of 0.1 M sodium chloride solution in water, was added gradually (1 drop/3 seconds) with continuous stirring (600 rpm). The sticky polymer-drug precipitate was collected by filtration, washed with water to remove the salt and dried under an extractor hood.

For determination of the drug content in the products the coacervates were ground in a mortar and an appropriate amount was dissolved in 50.0 ml water containing 5% Tween 20. After sonication and agitation with a magnetic stirrer (IKAMAG® RET S8, Ika-Werke GmbH, Staufen, Germany) until complete dissolution the solutions were filtered (0.45 μm filter, Millipore Millex-HV, Billerica, USA) and measured spectrophotometrically at 263 nm (Spectrophotometer Lambda 2, Perkin Elmer AG, Schwerzenbach, Switzerland).

2.2.3 Solvent evaporation

The drug/polymer ratio was set at 1:3 because the active ingredient is embedded in a polymer matrix and therefore more polymer is needed than for coating where only a thin polymer film is applied around the drug particles. Different formulations were used for the production of microspheres. The exact compositions are displayed in Tab. 2.1.

Component	Microspheres A	Microspheres B	Microspheres C	Microspheres D
Praziquantel	1.25 g/20.4%	1.25 g/20.4%	0.7 g/22.6%	0.7 g/22.2%
Al-stearate	1.13 g/18.4%	1.13 g/18.4%	-	-
Mg-stearate	-	-	0.3 g/9.7%	0.35 g/11.1%
Eudragit L	3.75 g/61.2%	-	-	-
Eudragit E	-	3.75 g/61.2%	2.1 g/67.7%	2.1 g/66.7%

Table 2.1: Compositions of Eudragit microspheres

Solvent evaporation was first tried with Eudragit L (microspheres A) because this polymer has a higher glass transition temperature than Eudragit EPO and is therefore easier to handle. Eudragit L (3.75 g) was dissolved in acetone (30.3 ml) with the addition of water (0.94 ml) whilst stirring with a magnetic stirrer and praziquantel (1.25 g) was added. Aluminum-monostearate (1.13 g) was dispersed in light liquid paraffin (125 ml). The solution of Eudragit L and praziquantel was then poured into the light liquid paraffin at 800 rpm and stirred at room temperature (25 °C) for 24 h. After sedimentation of the particles, they were washed three times with cyclohexane on a fluted filter and subsequently dried in a vacuum oven (200 mbar, 25 °C) for 24 h.

Solvent evaporation using Eudragit EPO and praziquantel was first performed as described above but without the addition of water which is not necessary to dissolve Eudragit EPO in acetone (microspheres B).

In order to improve this formulation the method was changed according to Bogataj et al. (1991). The main changes were the use of magnesium-stearate as dispersing agent, which is better suitable for the soft Eudragit E than for Eudragit L, and the temperature adjustment before and during the experiment. Briefly, Eudragit EPO (2.1 g) and praziquantel (0.7 g) were dissolved in 11 ml acetone and magnesium-stearate (0.3 g or 0.35 g; microspheres C and D, respectively) was added. After homogeneously mixing, this dispersion was poured into light liquid paraffin (80 ml) which had been previously cooled to 4 °C. During the experiment the paraffin was heated in a water bath to approximately 45 °C and stirred with a three-blade stirrer (500 rpm, 4 h). Afterwards the product was filtered, washed with n-hexane and dried (200 mbar, 3 h).

Content measurements of the microspheres were performed by HPLC in acetonitrile. Solutions were prepared with an approximate drug concentration of 100 ppm, filtered (0.45 μm filter, Millipore Millex-HV, Billerica, USA) and determined by

HPLC according to the method described in the following chapter.

2.2.4 HPLC method

An HPLC-method was used for quantitative analysis of the active ingredient. For this purpose, an Agilent LC 1100 apparatus (Agilent Technologies, Basel, Switzerland) was employed. The column (250 mm length and 4.6 mm internal diameter) was packed with Nucleosil 5 μm, C18 (Macherey-Nagel, Düren, Germany) and maintained at ambient temperature. The elution medium consisted of a mixture of 0.05% phosphoric acid and acetonitrile and was kept constant. The flow rate was 1.1 ml/min during the whole analyze-run. A volume of 10 μl of the sample solution was injected per run with an auto-sampler. The samples were detected and analyzed with UV-light at 215 nm.

2.2.5 Particle size distribution

Particle size was determined by the polydisperse method using the Mastersizer X (Malvern Instruments Ltd., Malvern, UK) with a dry powder feeder unit, a range lens of 1000 mm and a beam length of 10 mm. Measurements were run in triplicate and results are reported as volumetric mean diameter D(4, 3).

2.2.6 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to study the size, morphology and especially the surface of different Eudragit microspheres. Prior to examination, samples were gold sputter-coated to render them electrically conductive. The SEM photographs were recorded using a Philips XL 30 ESEM (Philips Electron Optics, Eindhoven, The Netherlands). Different magnifications were applied to gain overall and detailed impressions.

2.2.7 Dissolution studies

Drug dissolution was performed using the paddle method according to USP 30 specification. As dissolution apparatus a Sotax AT 7 (Sotax AG, Allschwil, Switzerland) was employed. Paddle speed was set at 100 rpm and temperature at 37 $\pm$ 0.5 °C. The medium for drug dissolution from the coacervates consisted of water containing 5%(w/w) Tween 20. The microspheres prepared by solvent evaporation were tested in two media: water with 1.5%(w/w) 1 N HCl containing 5%(w/w) Tween 20 and phosphate buffer pH 6.8 containing 5%(w/w) Tween 20 or Solutol HS 15. 900 ml medium was used and the particles were weighed to reach a maximum concentration of 100 ppm praziquantel. All tests were performed in triplicate. Samples were taken after 1, 3, 5, 10, 15, 30 and 60 min, filtered and the drug content was determined by UV spectroscopy or by HPLC according to the method described in Chapter 2.2.4.

2.2.8 Stability measurements

To detect any changes in the microspheres formulation during storage stability of the microspheres was tested at standard conditions: 25 °C/60% relative humidity (RH), 30 °C/65% RH and 40 °C/75% RH. Samples were kept for three and six months and were then characterized by dissolution studies in phosphate buffer pH 6.8 with 5% Solutol HS 15 as described in the previous chapter; the only difference was that only 600 ml medium were used because there was not enough substance to reach a concentration of 100 ppm in 900 ml medium.

2.3 Results and discussion

2.3.1 Coacervation

Several methods were tried for the production of praziquantel-Eudragit E microspheres by coacervation. In the first experiment, the pH-dependent solubility of Eudragit E was used to induce precipitation of the polymer. Eudragit E was dissolved in buffer pH 5 and the pH of this solution was slowly increased through drop by drop addition of a base (0.1 N sodium hydroxide solution). Due to this steady change of pH the polymer was expected to precipitate around the drug and build small microcapsules.

Light-optical microscopy observations showed agglomerations of crystalline drug particles. Unfortunately, no polymer film was visible around these particles to indicate the formation of microcapsules. The obtained product also did not really consist of small, perfectly shaped microcapsules but of a big white lump which broke during drying but still did not look like microcapsules at all. The content of praziquantel in the coacervate was determined to be 63.5% which was close to the theoretical drug content (70%). The small loss of active ingredient may be due to its solubility in the coacervation medium. Another possibility could be incomplete coating of the drug by the polymer so that drug particles appeared more in the powdery parts of the coacervate than in the agglomerated product.

In order to improve the results new attempts of coacervation by pH-shift were made. Magnesium stearate was used as a lubricant to decrease the stickiness of both drug and polymer and the different products were washed with n-hexane for the purpose of hardening of the polymer coating. Unfortunately, neither of the alterations had the desired effect as in no case separate small microcapsules were obtained but only rather bulky agglomerates of drug and polymer particles.

Another method for coacervation was tested with solvent/non-solvent systems. In one trial the active ingredient was suspended in water (non-solvent) and a polymer solution in ethanol (solvent) was added. In a second experiment this system was reversed so that the polymer and active ingredient were both dissolved in ethanol (solvent) and to this solution water containing sodium chloride as flocculating agent (non-solvent) was added (Okor, 1990). The product of the first process was a white, to some extent powdery, partially crumbly substance with a drug content of 79.7%. The

drug content was slightly higher than the theoretical amount (70%). One reason for this might be that not all of the polymer precipitated but some was maybe retained in the filtrate which in fact was slightly turbid. So as in the previous experimental setup, no real microcapsules were produced.

In the second attempt of coacervation by a solvent/non-solvent system the product was rather a sticky polymer-drug precipitate than microcapsules. Quantitative analysis showed a similar drug content as in the previous experiment (about 79%) in the more powdery parts of the coacervate. The reason for the higher drug content than expected may be that the polymer precipitated in the big hard mass which was obtained after drying whereas the active ingredient may have precipitated next to the polymer and therefore measured by the analysis of the more powdery parts of the product. This method was also not successful in producing praziquantel-Eudragit EPO microcapsules by coacervation.

Dissolution studies were carried out to test the produced coacervates regarding their drug release and hence their ability to mask the taste of praziquantel. Because the products were mostly hard, bulky agglomerates it was difficult to perform the dissolution studies as large particles would sustain the release due to their size alone; yet it was tried to use smaller particles. Additionally, the drug release from the coacervate prepared by pH-Shift was tested with ground coacervate so that any possible coating would be crushed and no delayed effect should be observed. The results of the dissolution studies carried out with the coacervate prepared by pH-shift and the one precipitated with ethanol are displayed in Fig. 2.1.

From the coacervate prepared by pH-shift powdery parts of the product were used because only rather big parts could be broken of the cluster coacervate. As only about 14% of the active ingredient had been released after 3 min it is likely that some form of coating was obtained. In comparison, tests with ground coacervate revealed a drug release of 46% after 3 min which is nearly as fast as dissolution of the pure active ingredient (59% after 5 min). Yet, the drug disintegration from the ground coacervate is a bit slower than pure praziquantel which also leads to the conclusion that the polymer had precipitated around the drug. Thus, these results show that praziquantel was to a certain extent coated with Eudragit E, however, no microcapsules could be separated. Hence it is to be assumed that no real coacervation was achieved which would lead to microcapsules but rather big clusters of drug and polymer were produced which also lead to a delayed dissolution of praziquantel.

Dissolution studies with the coacervate prepared by addition of ethanol showed a drug release of about 15% after 3 min which indicates that at least part of the active ingredient has somehow been incorporated by the polymer. Here, no studies were performed with ground product, but it can be assumed that drug disintegration would have been enhanced as shown for the previously tested formulation. In addition, it can be likewise presumed that the polymer did not precipitate around separate drug particles thus building real microcapsules but rather praziquantel and Eudragit E formed agglomerates.

None of the coacervation techniques tested showed good results for the building

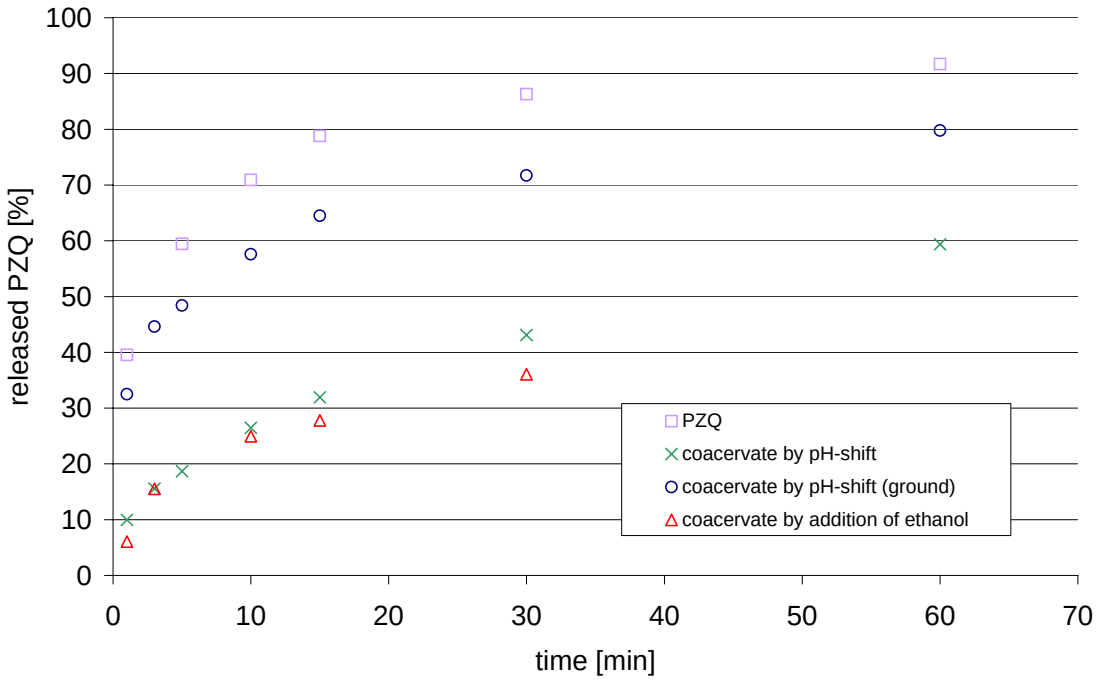

Figure 2.1: Dissolution of praziquantel from coacervates in water containing 5%(w/w) Tween 20; duplicate measurements.

of microcapsules of praziquantel and Eudragit E which could have been used for further testing. Therefore, this method was abandoned.

2.3.2 Development of microspheres by solvent evaporation

Different formulations were used for the production of praziquantel microspheres. First of all it was tested if praziquantel was generally suitable to be incorporated into microspheres. This was tested with Eudragit L and aluminum-monostearate as dispersing agent in an acetone/paraffin system according to a manufacturing specification from the Formulation Development Department of Novartis Animal Health (NAH, 2001) (microspheres A). The produced microspheres were very well shaped, free flowing and showed no signs of adherence. A drug content of 23.2% was determined which is slightly more than the theoretical content of 20.4%. This might be due to the fact that a small fraction of Al-stearate was removed during the washing with cyclohexane.

Afterwards the same experimental setup was used with Eudragit E (microspheres B) but without the addition of water which is not necessary to dissolve Eudragit E in acetone. The obtained product was not as nicely formed as the microspheres made with Eudragit L but more sticky and a white mass rather than separate roundly shaped microparticles. Measurement of the drug content showed not the desired amount; only 11% praziquantel were found in the (powdery) parts of the product. This leads to the assumption that the larger drug fraction was incorporated

into the polymer which precipitated as lump. However, the method which yielded very good results for Eudragit L did not work for Eudragit E. Therefore, another experimental setup was investigated and this formulation was not further studied.

In order to improve formulation B the method was changed according to Bogataj et al. (1991) (microspheres C). Instead of aluminum-monostearate magnesium-monostearate was used as lubricant, furthermore the paraffin was cooled before the experiment and heated to 45 °C during the experiment. After filtration the product was washed with n-hexane instead of cyclohexane. The produced microparticles were a little sticky but nicely formed and thus a much better result than in the case of Eudragit E and Al-monostearate (microspheres B). The drug content of the obtained microspheres revealed a praziquantel amount of 19.1% which is slightly less than the theoretical content (22.6%). It seems that the encapsulation efficiency for praziquantel and Eudragit E is not as good as with Eudragit L. Though, because taste masking was the desired aim microspheres with Eudragit E were further developed.

Formulation C of the microspheres generated quite good particles, although they did not show ideal properties yet as they were still a bit adhesive. Hence, an improvement of this formulation was tried by increasing the amount of Mg-monostearate to avoid stickiness of the microspheres. The same quantity of drug and polymer were used with a Mg-monostearate amount of 0.35 g (microspheres D). Due to this modification small, non-sticky microspheres with a praziquantel content between 17% and 19% for different batches were produced. Again the encapsulation efficiency is rather low with 81% in average, but no better results could be achieved with Eudragit E so the formulation used for upscaling was that of the microspheres D. Upscaling for acceptance tests and stability testing with 3.5 g praziquantel aroused some problems: with the former experimental setup for microspheres D, no satisfying results were obtained any longer. Instead of microspheres a sort of lump was produced. The polymer and drug precipitated somehow separately, so that very solid particles (polymer) and a greasy supernatant containing the drug appeared. This was rather unexpected as the experimental setup was unchanged. No reason could be found for this change. Unfortunately, one of these formulations had to be used for stability tests (see Chapter 2.3.6) because the time schedule was rather tight. The cluster was reduced to small pieces to be able to perform reasonably decent dissolution studies.

For palatability tests, of course, several changes in the experimental setup were tried so that the tests could be performed with real microspheres rather than with a crushed cluster. These modifications included a higher content of magnesium stearate, addition of water, decreased content of acetone and dispersion of praziquantel in paraffin instead of dissolution in acetone. In the end, the amount of acetone was reduced so that only the polymer was dissolved, but praziquantel was only partially dissolved in the polymer solution (microspheres E). This led to a precipitation of polymer with drug so that microspheres were obtained again where praziquantel was embedded in Eudragit E. With these microspheres dissolution studies were performed as well and the results in respect of taste masking (reduced release of praziquantel in the first minutes) were somewhat promising again and comparable to

the drug release from microspheres D but with a faster release towards the end (see Chapter 2.3.5).

2.3.3 Particle size distribution

For physical characterization of the manufactured microspheres measurements of the particle size were performed with microspheres A, placebo microspheres of formulation C and microspheres E (see previous chapter). The results are given in Tab. 2.2.

Microspheres A which were prepared using Eudragit L and aluminum-stearate had a mean diameter of 143 μm and a size distribution of 80% between 40 and 206 μm. A low amount (2%) of particles smaller than 10 μm could also be detected; this might be explained by aluminum-stearate which was not totally incorporated into the polymer matrix and also by abrasion during the size measurements.

Formulation	Mean diameter	D (v, 0.1)	D (v, 0.9)
Microspheres A	143 μm	40 μm	206 μm
Microspheres C (placebo)	412 μm	209 μm	631 μm
Microspheres E	204 μm	98 μm	301 μm

Table 2.2: Particle size distribution of Eudragit microspheres

In contrast to these microspheres, the placebo microspheres prepared as formulation C were more than twice as big with an average diameter of 412 μm and a size distribution (80%) between 209 and 631 μm. The main reason for this much higher particle size is most probably the lower speed of the stirrer: Microspheres A were prepared with 800 rpm whereas for these placebo microspheres a stirring rate of 450 rpm had to be used because of the lower amount of paraffin. Besides, Eudragit L seems to be better applicable for matrix formation mainly because it is harder due to its higher glass transition temperature.

Microspheres E were produced for acceptance tests and thus a larger batch was manufactured. Hence, the stirring rate could be increased and was set at 650 rpm. This and the higher magnesium stearate amount and reduced acetone volume most probably led to the smaller mean diameter (204 μm) than compared to the placebo microspheres. Altogether, the size distribution of the microspheres A and E was satisfactory because the particles were small enough to avoid a sandy feeling in the mouth and also are not broken by chewing which might lead to a unpleasant taste.

2.3.4 Scanning electron microscopy

SEM photographs of microspheres A, C (placebo), D and E are displayed in Fig. 2.2 to 2.5 with different magnifications. It can nicely be seen that microspheres A prepared with Eudragit L are more or less homogeneous and nicely spherically shaped. The larger particles on the surface of the microspheres might be agglomerations of other polymer droplets while the smaller powdery parts on the surface are most probably

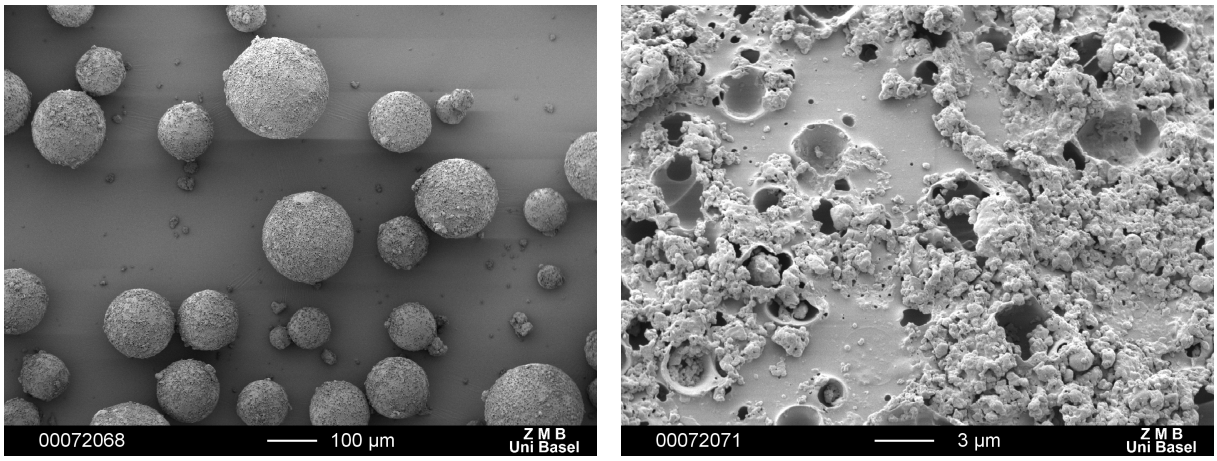

Figure 2.2: Micrographs of microspheres A (Eudragit L)

attributed to Al-stearate which was used as dispersing agent. In addition, on closer examination quite a lot of pores are detected on the surface. This is most probably explained by the solvent evaporating from the solidified polymer droplets in the paraffin thereby leaving holes in the polymer matrix. However, if the dissolution data are regarded (see Chapter 2.3.5) it might be assumed that these pores presumably only appear on the surface but not in the core of the microspheres as described by Esposito et al. (1999). Otherwise drug dissolution would be faster in a polymer insoluble medium due to water diffusion into the matrix through the pores and resulting release of the active ingredient. Finally, no drug crystals could be identified on the surface of the microspheres from which could be concluded that praziquantel was satisfactorily included into the polymer matrix.

In contrast to the homogeneous microspheres A the placebo microspheres of formulation C (Fig. 2.3) are not as nicely spherical shaped but have a more irregular appearance. The reason for this might be the lower glass transition temperature of Eudragit E in contrast to Eudragit L and its better solubility in acetone. This might result in a softening effect during the solidification phase so that the polymer structure collapses hence leading to irregular shaped particles. Although the size distribution was determined to be quite high (see previous chapter), the particles appear regular in this inspected sample. In a higher magnification an interesting surface structure becomes visible: the polymer does not seem to have precipitated as one big particle but the microspheres rather consist of many separate polymer plates sticking together. This might also be due to the lower glass transition temperature and thus the softened polymer so that the surface of the particles is disrupted into separate small sections by the evaporating solvent during the solidification process. This is not the case for Eudragit L where only pores are formed by evaporation of the solvent, but the polymer structure is unbroken. On the surface of the placebo microspheres of formulation C again the dispersing agent, here Mg-stearate, can be seen as powdery particles.

The micrographs of Eudragit E microspheres containing praziquantel are shown in Fig. 2.4 and 2.5. The appearance of the particles is slightly less regular as the

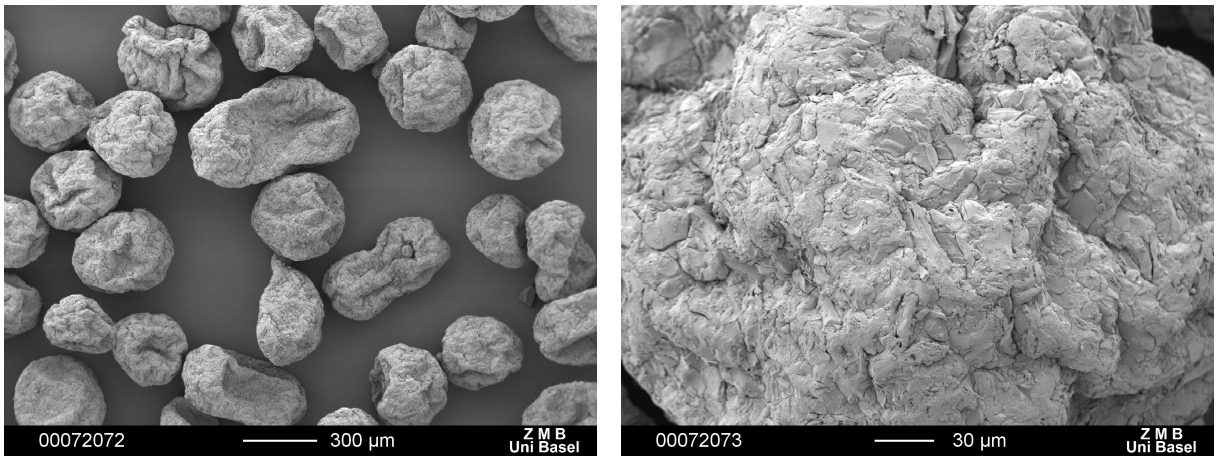

Figure 2.3: Micrographs of microspheres C (Eudragit E, placebo)

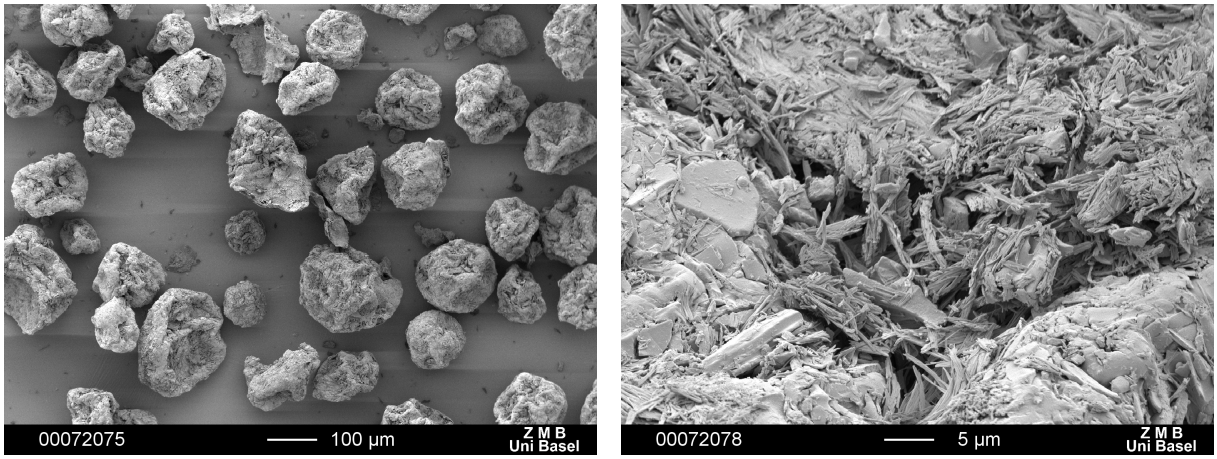

Figure 2.4: Micrographs of microspheres D (Eudragit E)

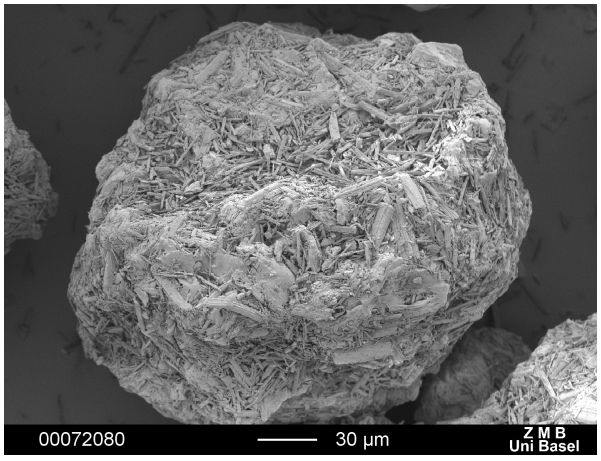

Figure 2.5: Micrograph of microspheres E (Eudragit E)

placebo microspheres with many smaller fractions and in the case of microspheres E also separate drug needles. Yet, they may have broken off during sample preparation. Furthermore, drug crystals clearly stand out of the surface of the microspheres which leads to the assumption that praziquantel was not completely incorporated into the Eudragit E matrix but also precipitated on the surface. Clear differences can be detected between the two formulations: while in the case of microspheres D the drug crystals are quite filigree and more of a polymer matrix can be seen this is not the case for microspheres E. Here, larger needles appear on the microspheres and individual drug crystals can also be seen separated from the polymer particles in the background. This can be based on the different manufacturing of the microspheres (see Chapter 2.3.2): microspheres D were prepared from a polymer-drug solution in acetone, while in the formulation E only the polymer was completely dissolved and the active ingredient was partly dissolved, but mainly suspended in acetone. This was known to be not optimal, but with the former experimental setup no satisfying results were obtained any longer although all parameters were unchanged. The only chance to achieve some form of microparticles again was the reduced amount of acetone so that only Eudragit E was dissolved and praziquantel suspended. As a result the drug crystals are larger because the drug was not dissolved completely and thus could not re-crystallize in smaller particles in the polymer framework but appear to be more or less unchanged and simply aggregated onto the polymer. Consequently, as the drug is not really incorporated into the polymer matrix but exists in discrete crystals separated and on the polymer particles, only a marginal taste masking effect might have been achieved with this formulation.

These pictures indicate that praziquantel was nicely incorporated into the Eudragit L matrix while this is not the case for Eudragit E. Here, drug crystals can clearly be seen which can dissolve quite rapidly once in contact with fluid in the mouth. Although it was known that some active ingredient must be on the surface of the microspheres due to the manufacturing procedure the extent of the drug amount was quite surprising but in the case of microspheres E explicable by the manufacturing procedure. Hence, taste masking effect of these particles on praziquantel might not be as pronounced as desired. Nonetheless, they were employed in acceptance tests to investigate their taste masking potential.

2.3.5 Drug dissolution studies

Feasibility studies for the use of the microspheres for taste masking were performed by dissolution studies. Samples were taken after 1, 3, 5, 10, 15, 30 and 60 min to observe any drug release especially in the initial phase which would then be responsible for bad taste in the animal's mouth.

The dissolution studies were conducted in two different media: acidic pH and pH 6.8 to imitate the conditions in the cat's mouth. These two media where chosen to investigate the microspheres' behavior in a medium in which the polymer is either soluble or at least allows drug dissolution (acidic pH for Eudragit E and neutral pH for Eudragit L) or insoluble (Eudragit L in acidic pH and neutral pH for Eudragit E).

The dissolution results in phosphate buffer pH 6.8 are displayed in Fig. 2.6 and dissolution in acidic medium is presented in Fig. 2.7. It can be seen that the pure active ingredient dissolves quite fast in both media with at least 40% disintegrated within the first minute. After 10 min more than two thirds of the total drug content have already been dissolved.

The effect of the different media can be seen nicely in the case of microspheres A with Eudragit L: if the polymer is insoluble nearly no drug diffuses from the matrix into the dissolution medium. After 5 min only 10% praziquantel have been released which is caused by the structure of the microspheres: in contrast to microcapsules where the drug core is completely covered by a polymer layer the microspheres consist of a drug-polymer matrix so that few drug particles exist on the surface of the particle and thus can dissolve from there into the dissolution medium. Even after 30 min this amount did not rise at all and after 60 min still only 14% drug had disintegrated from the microspheres.

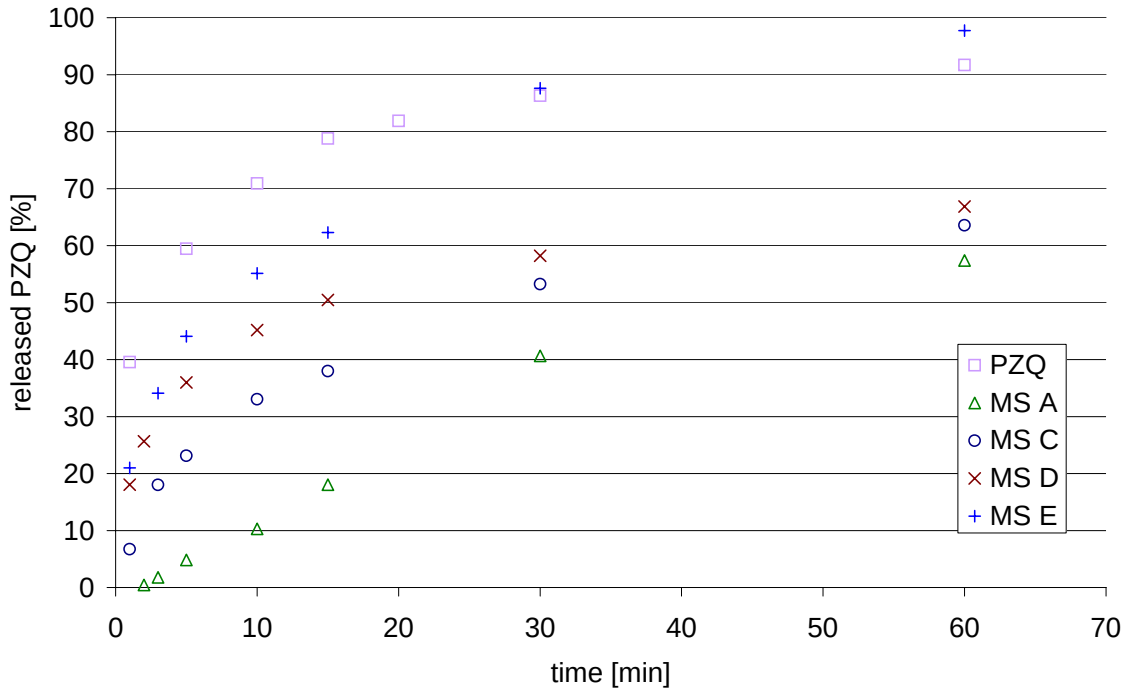

Figure 2.6: Dissolution of praziquantel from microspheres in phosphate buffer pH 6.8 containing 5%(w/w) detergent; duplicate (PZQ, MS A) or triplicate measurements.

Drug dissolution from Eudragit L microspheres in phosphate buffer pH 6.8 is not as fast as could have been expected if the polymer were soluble in the dissolution medium. The praziquantel release nearly follows a zero order kinetic which leads to the assumption that drug dissolution is mainly controlled by diffusion. This is most probably due to the pH which might not have been basic enough to enable a complete disintegration of the polymer. Even after 180 min still only 65% praziquantel have been released from the microsphere matrix (data not shown). Eudragit L allows

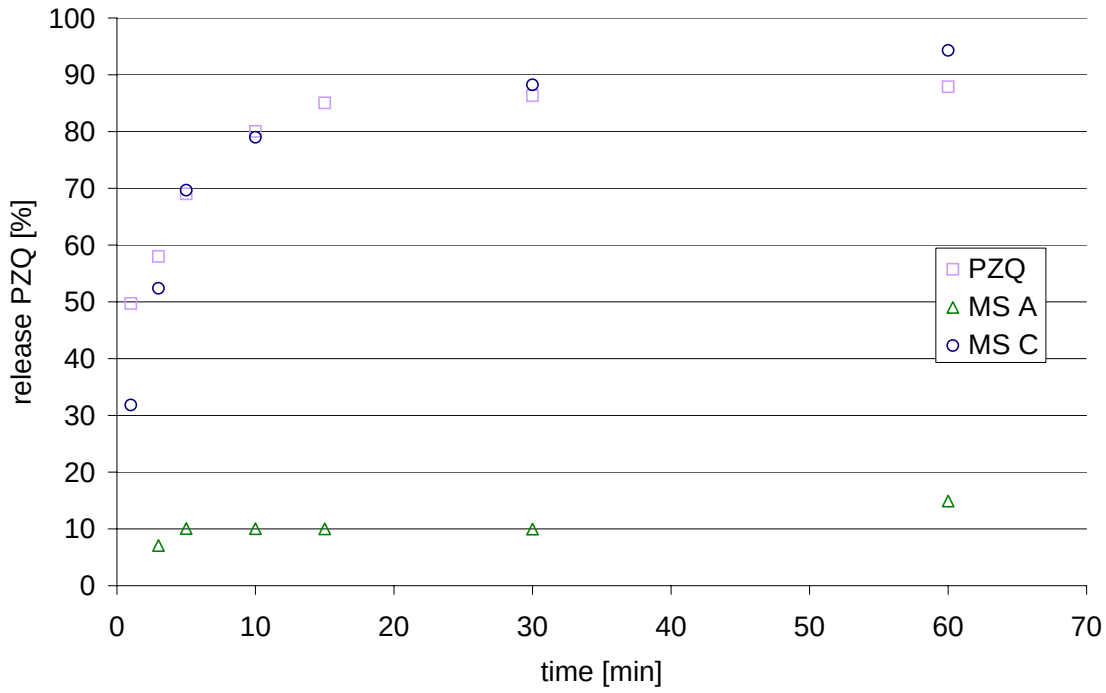

Figure 2.7: Dissolution of praziquantel from microspheres in acidic medium (pH 2) containing 5%(w/w) Tween 20; duplicate measurements.

drug dissolution above pH 6.0 but it does not seem to have become soluble in the tested phosphate buffer medium. The rather slow drug dissolution in pH 6.8 is thus explained that the polymer matrix does not dissolve completely in the given time but primarily enables the drug to diffuse out of the matrix. From these dissolution results it can be concluded that praziquantel is very well incorporated into the Eudragit L matrix and nearly no drug is released into a polymer insoluble aqueous medium.

As anticipated, microspheres prepared with Eudragit E (microspheres C and D) also showed pH-dependent drug release from the polymer matrix. In acidic medium praziquantel disintegrated nearly as fast as the pure drug: After 3 min already more than 50% drug had been released and after 5 min the microsphere matrix appears to have been completely dissolved as drug dissolution reached the level of the free drug. Praziquantel disintegration was nearly complete after 60 min with 94% dissolved drug. In phosphate buffer pH 6.8 the release was slower which of course was based on the fact that Eudragit E is insoluble above pH 5. However, if the results are compared to drug release from the Eudragit L microspheres in acidic medium, it has to be stated that the drug incorporation into the Eudragit E microspheres is not as good as in the case of Eudragit L: after 1 min already more than 6% and 18% praziquantel (microspheres C and D, respectively) and after 3 min more than 18% and 25% have been released into the dissolution medium. The first part of the drug dissolution is again evoked by drug particles on the surface of the microspheres which dissolve quite rapidly once in touch with the medium. However, compared to Eudragit L

microspheres the drug release in a polymer insoluble medium is much faster with Eudragit E as this polymer – although insoluble – swells and thus becomes permeable in water. Hence, drug disintegration from the matrix is enhanced. Another factor leading to faster drug release is the incomplete incorporation of praziquantel into the polymer matrix (see previous SEM chapter). This conclusion is supported by the even faster drug release from microspheres E. For these particles the incorporation of the active ingredient into the polymer matrix is even less pronounced resulting in a more rapid drug disintegration. Overall, these results gave a indication for a partial embedding of the drug in the polymer matrix, which might result in masking the taste of praziquantel by Eudragit E in form of microspheres.

2.3.6 Stability of microspheres

To detect any changes in the microspheres formulation during storage stability was tested at standard conditions: 25 °C/60% relative humidity (RH), 30 °C/65% RH and 40 °C/75% RH. Samples were kept for three and six months and were then characterized by dissolution studies.

For stability trials a new batch microspheres was needed. Unfortunately with the former experimental setup, no real microspheres were produced any more (see Chapter 2.3.2). But material was needed for the stability studies and there was no time for further improvement. Thus, the samples used for stability trials were not ideal, because a ground lump of the precipitated product was used (formulation microspheres D). In the dissolution diagrams it can be seen that praziquantel was released quite rapidly. The reason for this is the precipitation of drug and polymer separately, so no incorporation of praziquantel in the Eudragit E matrix took place but they exist in a sort of physical mixture which leads to a fast disintegration of the drug into the dissolution medium.

Dissolution data of the start formulation and the average values after three and six months are shown in Fig. 2.8. Between the three stability conditions no remarkable differences could be detected (standard deviation lower than 5%), so only the average of each time point is presented for clarity reasons. As described previously the drug release is not delayed much due to problems in manufacturing. After 3 min more than 60% are released which would be unfeasible for taste masking. Comparison between the start values and data after three months displays a slightly lower dissolution profile for the three months sample. The average amount released is about 5-10% lower than for the start value. But fluctuations were more pronounced in the stability samples so that the results are overlapping in their standard deviation and the reduction of drug release is thus not significant.

Results from the dissolution studies performed with samples which were kept for six months are also displayed in Fig. 2.8. The only noticeable difference between these values and the start value is the slower onset of the drug disintegration. This is caused by a different sample drawing method: the first dissolutions (initial and after three months) the samples were first drawn and then filtered which led to a tiny delay. Hence, particles which were drawn into the syringe could still release some

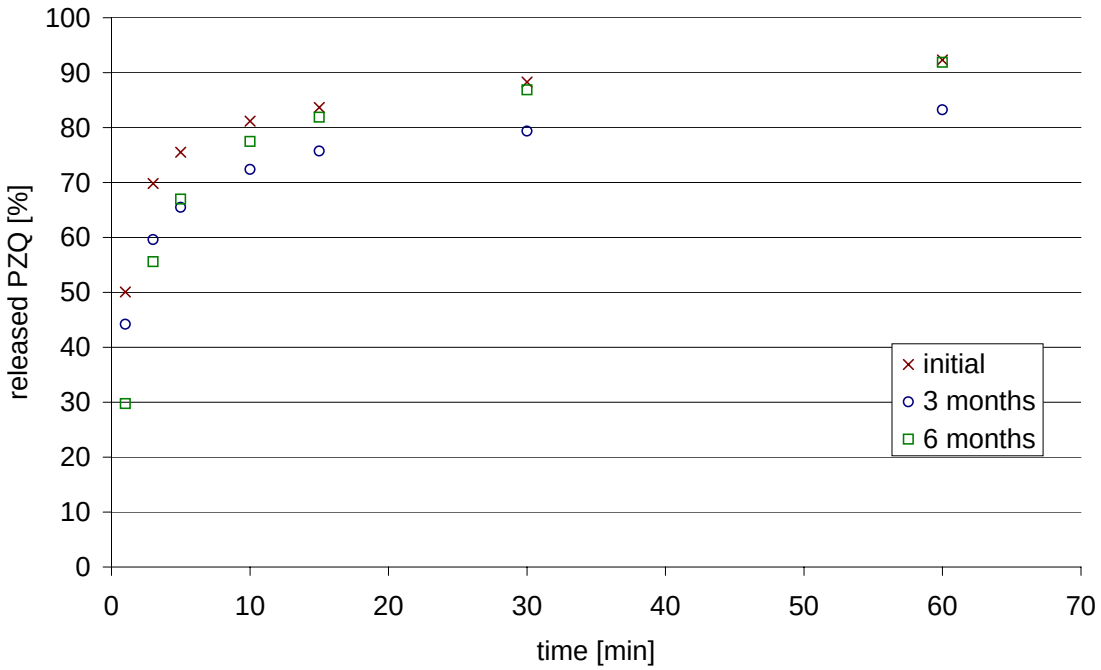

Figure 2.8: Dissolution of praziquantel from microspheres at start and after three and six months; test were run in triplicate; standard deviation did not exceed 5%.

drug and thus the values at the beginning are slightly higher than for the studies after six months. Here, the samples were drawn directly through the filter into the syringe so no further drug dissolution could occur. Altogether, no significant changes could be detect by dissolution studies after three and six months at the given storage conditions. This and especially the marginal differences between the three stability conditions leads to the assumption that temperature and humidity have no detectable effect on the stability of the Eudragit E microspheres. The only noticeable alteration was the smell: the rather fishy smell of the polymer was intensified during storage, but that does not seem to have any effect on the dissolution abilities. However, the reason for the pronounced smell could be interactions between the active ingredient and the polymer. This of course would not be desired in the end product because it might lead to change of modification or degradation of the drug. Another possibility could be residual solvent in the microspheres leading to composition of either polymer or active ingredient. But these parameters were not further investigated because the taste masking formulation with Eudragit E was abandoned due to bad results in the following acceptance tests (see Chapter 5.3.1).

2.4 Conclusions

Taste masking of praziquantel with Eudragit E is not easily achieved. Coacervation with Eudragit E by the applied techniques does not seem possible at all, which was

also ascertained by Eurand; they, too, did not reach satisfying results with their tested coacervation methods (NAH, 2000). In contrast to coacervation, better results were achieved with the solvent evaporation method. However, due to the properties of Eudragit E it proved to be rather tricky to obtain nicely formed microspheres and no sticky agglomeration of polymer and drug. But if all parameters are chosen correctly including type of dispersing agent, volume of solvent and non-solvent, small free flowing microspheres could be produced. These microspheres were nicely shaped and had an ideal size so that neither a sandy feeling in the mouth nor crushing of the particles by chewing were considered probable. Moreover, the microspheres showed a delayed drug release from the polymer matrix in the first minutes which was the main in vitro parameter for successful taste masking. One negative aspect, however, was the incomplete incorporation of the active ingredient into the polymer matrix which could be detected by SEM. But all things considered, the praziquantel Eudragit E microspheres were chosen for further acceptance tests in cats.

Other possible methods could have been tried for production of Eudragit E microparticles. Coating of inert pellets onto which praziquantel had been applied first might have been a feasible technique to obtain microcapsules. Yet, this process would have either involved spraying of organic solvents or otherwise would have been very time intensive if only water could have been used for the spraying solution. Microspheres could also have been produced by methods such as melt extrusion and spray drying. In both cases the active ingredient is incorporated in the polymer matrix. Nevertheless, in the case of melt extrusion the heat has to be adjusted carefully so that no chemical changes of the active ingredient such as decomposition happens. Another factor is that the drug might get dissolved in the polymer matrix which might lead to stability problems due to crystallization. Spray drying on the other hand could have aroused the same problems as in the case of coating: Either spraying of an organic solution would have been necessary or if water was used, the process would have been quite time and cost demanding.

Further optimization of the produced microspheres might have been achieved by a mixture of Eudragit E and L. The ratio of the polymers would have had to be adjusted so that no sustained release effect would have been reached but still drug disintegration from the polymer matrix was delayed at the beginning in neutral medium. A mixture of Eudragit L and E would also have had the advantage of the polymer matrix being harder than in the case of pure Eudragit E. Other alterations could have included decrease of temperature, so that the evaporation of the solvent from Eudragit E matrix is slowed and thus the polymer film is not disrupted or use of another solvent.

However, no further production techniques were tried with praziquantel and Eudragit E because the microspheres did score a poor result in the acceptance tests performed with cats (see Chapter 5.3.1). The reason for this might be on the one hand incomplete incorporation of the drug into the polymer matrix. Another equally or even more important factor is the formulation the particles were tested in: the taste masked formulations were incorporated in three different paste bases (water, Miglyol

and polyethylenglycol) which were used for acceptance tests. As Eudragit E is soluble in Miglyol and swellable in water it could only be tested in PEG. Yet, praziquantel is partially soluble in this base and thus diffusion of the active ingredient from the polymer matrix into the paste base most probably occurred. This of course eliminated the taste masking effect and led to an absolute unsatisfying result in the acceptance test. But having established the paste bases due to former palatability trials a revision was not feasible. Hence, as any Eudragit microparticles would thus have had to be tested in a PEG base in which the active ingredient is soluble and which is additionally least liked by the cats, this taste masking method was not investigated further.

Chapter 3

Inclusion complexation with cyclodextrins

3.1 Short introduction

β-cyclodextrin was used to develop methods for inclusion complexation of praziquantel. It was shown in previous studies that this cyclodextrin is the most suitable of the three naturally occurring cyclodextrins (α, β and γ-cyclodextrin) for inclusion of praziquantel since it shows the best complex formation and most acceptable solubility and dissolution profiles (Becket et al., 1999).

The possibility of complex formation was investigated with phase solubility studies from which the stability constant of the formed complex and the complexation efficiency of the cyclodextrin with the active ingredient was calculated. Additionally, different complex preparation techniques were applied including physical mixture, co-precipitation by solvent method, kneading and spray drying (partially according to El Arini & Leuenberger (1996)). The ratio of drug and cyclodextrin was always set at a 1:1 molar ratio system which has led to the best results in an experiment regarding different praziquantel/cyclodextrin ratios (El Arini & Leuenberger, 1996). The thus formed inclusion complexes were examined regarding their free and included drug amount from which the inclusion yield for each preparation method could be determined.

Moreover, several investigative methods were performed for further characterization of a complex formation between β-cyclodextrin and praziquantel such as differential scanning calorimetry, infrared spectroscopy and nuclear magnetic resonance. The last mentioned technique was further used to research the steric orientation of the drug in the cavity of β-cyclodextrin. Finally, the stability of a complex prepared by solvent method was studied. The samples were kept for up to nine months and characterized by dissolution studies.

3.2 Materials and methods

3.2.1 Materials

The drug substance praziquantel was obtained from PCAS, Limay, France. β-cyclodextrin and polyvinylpyrrolidone (PVP) K30 were purchased from Fluka AG, Buchs, Switzerland and hydroxypropyl-β-cyclodextrin was obtained from Wacker Chemie AG, Burghausen, Germany. The solvents ethanol absolute and acetonitrile were used from Merck Inc. Darmstadt, Germany. Phosphate buffer solution pH 6.8 was purchased from Fluka AG, Buchs, Switzerland and Solutol HS 15 was provided by BASF, Ludwigshafen, Germany. Purified water was prepared with ELGA Maxima ana (Labtec Services AG, Wohlen, Switzerland).

The praziquantel enantiomers were separated by Carbogen Amcis AG, Bubendorf, Switzerland. The used column was Chiralpak AS, 20 μm, and as eluent methanol was used. The flowrate was set at 450 ml min^{-1} and detection took place at 220 nm. Separation of the enantiomers was achieved with a purity of 100% for $(-)$-praziquantel and 99.9% for $(+)$-praziquantel.

3.2.2 Complex formation

A physical mixture of praziquantel and β-cyclodextrin was prepared by trituration in a laboratory mortar. The amount of drug and cyclodextrin necessary for a 1:1 molar ratio inclusion (1.8175 g praziquantel, 0.5 g β-cyclodextrin) were weighed into a mortar and triturated with a pestle for 30 min. Because the drug amount differed greatly in several samples the complex was once again mixed in a mortar for 30 min which led to an improvement in drug allocation. Content measurement was performed by UV spectroscopy at 263 nm (Spectrophotometer Lambda 2, Perkin Elmer AG, Schwerzenbach, Switzerland).

Furthermore praziquantel-β-cyclodextrin inclusion complexes were prepared using the solvent method according to El Arini & Leuenberger (1996). 1.8175 g β-cyclodextrin were dissolved at 70 °C in 40 ml water. Praziquantel (0.5 g) was separately dissolved in 7 ml 95% ethanol and added dropwise to the β-cyclodextrin solution under continuous stirring, which was kept for another 30 min at 70 °C. After this time the solution was slowly cooled to room temperature and afterwards kept in the fridge overnight to obtain a clear supernatant above the precipitate. The filtered product was dried under reduced pressure at room temperature and afterwards put under an extractor hood until constant weight. For the complex obtained by the kneading method the same amount of praziquantel and β-cyclodextrin were used. Praziquantel was dissolved in 8 ml absolute ethanol and triturated with β-cyclodextrin in a mortar for 45 min, using 6 ml water to ensure a partial dissolution of β-cyclodextrin. After the liquids had been evaporated the complex was pre-dried (200 mbar, 1 h) and left under an extractor hood for complete drying. The drug content of the complexes was determined photometrically; additionally, the complex prepared by solvent method was analyzed with HPLC (method see Chapter 2.2.4.

Praziquantel-β-cyclodextrin complexes were also prepared by spray drying. For that, the same molar ratio of drug substance and β-cyclodextrin were dissolved in a sufficient amount of water. In the second trial, PVP was added (0.1% w/v) to enhance the complexation efficiency of β-cyclodextrin and thus reduce the amount of water needed to dissolve praziquantel. The spray dryer (Büchi mini Spray Dryer B-191, Büchi Labortechnik AG, Flawil, Switzerland) was operated under the following conditions: inlet temperature 130 °C, outlet temperature 75 °C, spray flow rate 450 Nl h^{-1}, aspirator rate 75% and pump rate 30%. Content measurements and determination of the complexed amount of drug in the collected product were performed by HPLC.

3.2.3 Determination of complexed drug amount

β-cyclodextrin and thus its inclusion complexes are insoluble in solvents like ethanol absolute and acetonitrile. This can be used to determine the amount of complexed drug in these inclusion complexes: the precipitated complex is suspended in pure acetonitrile so that the free drug is dissolved, but the complexed drug stays undissolved in the β-cyclodextrin cavity (Van Hees et al., 2002). The drug amount determined with this method can be compared to the total drug amount in the complex and thus the free/complexed drug ratio can be calculated.

The complexed amount of praziquantel in the β-cyclodextrin complexes prepared by physical mixture, solvent method and spray drying were determined. The substances were weighed into volume flasks, filled up with pure acetonitrile and sonicated for 10 min. The samples were then filtered and measured both photometrically at 263 nm and by HPLC.

3.2.4 Phase-solubility studies

Phase-solubility studies were performed according to the method of Higuchi & Connors (1965) and El Arini & Leuenberger (1996). From the results, both the stability constant after Higuchi and Connors and the complexation efficiency after Loftsson were determined (see Chapters 1.2.3 and 1.2.4) (Higuchi & Connors, 1965; Loftsson et al., 2005). The same amounts of praziquantel (racemic drug or separated enantiomers) well in excess of its solubility (100 mg) were dispersed in 50 ml water. Increasing amounts of β-CD or HP-β-CD, and also PVP if tested, were added to the volume flasks. These were closed tightly and stirred for five days at 37 °C on a magnetic stirrer (RT 15 power IKAMAG®, Ika-Werke GmbH, Staufen, Germany). The samples were filtered warm (0.45 μm filter, Millipore Millex-HV, Billerica, USA), diluted with water and measured spectrophotometrically at 263 nm.

As it is often stated in literature that simple addition of a polymer alone does not enhance the complexation efficiency of cyclodextrin (Loftsson et al., 1999; Loftsson & Fridriksdottir, 1998; Ribeiro et al., 2003) two additional studies with β-CD and PVP were performed: in the first attempt, the aqueous suspensions of drug, β-CD and PVP as prepared previously were given in glass vials, sealed and autoclaved for 40 min at

120 °C. In another experiment the suspensions were also mixed in glass vials, sealed and treated in an ultrasonic bath at 70 °C for 60 min. Following both treatments, the phase-solubility studies were carried on as previously described.

3.2.5 Differential scanning calorimetry

Furthermore, differential scanning calorimetry (DSC) was performed in order to detect inclusion complexation between β-cyclodextrin and praziquantel using a differential thermoanalyzer, model DSC7 from Perkin-Elmer. 5 mg of each sample – physical mixture, complexes obtained by solvent and kneading method and for reference the pure substances praziquantel and β-cyclodextrin – were weighed into a gold pan, the heating rate was 10 °C min^{-1} over a temperature range of 0-200 °C (0-160 °C for physical mixture).

3.2.6 Infrared spectroscopy

Fourier transform infrared spectroscopy (IR) was performed both in solid state and dissolved in methylene chloride with the Vertex 70 from Bruker Optics GmbH (Fällanden, Switzerland). Spectra were taken from the single substances praziquantel and β-cyclodextrin, the physical mixture (1:1 weight ratio), the complex obtained by the solvent method and a complex precipitated from aqueous solution, both in a 1:1 molar ratio.

3.2.7 Nuclear magnetic resonance

Nuclear magnetic resonance (H-NMR) spectra of praziquantel, β-cyclodextrin and the complex prepared by solvent method were recorded on a Bruker DPX-400 spectrometer (Bruker Optics GmbH, Fällanden, Switzerland). Samples were prepared in deuterium oxide and chemical shifts are reported in parts per million relative to deuterium oxide at 4.79 ppm.

3.2.8 Stability measurements

To investigate the effect of storage on the chemical stability of the praziquantel-β-cyclodextrin complex prepared by solvent method, this substance was kept at standard conditions: 25 °C/60% relative humidity (RH), 30 °C/65% RH and 40 °C/75% RH. Samples were kept for three, six and nine months and were then characterized by dissolution studies. They were performed using the paddle method according to USP 30 specification. As dissolution apparatus a Sotax AT 7 (Sotax AG, Allschwil, Switzerland) was employed. 900 ml medium consisting of phosphate buffer pH 6.8 containing 5% (w/w) Solutol HS15 was used and the complex was weighed to reach a maximum concentration of 100 ppm praziquantel. Paddle speed was set at 100 rpm and temperature at 37 $\pm$ 0.5 °C. Samples were taken after 1, 3, 5, 10, 15 and 30 min, filtered and the drug content was determined by HPLC (see Chapter 2.2.4).

3.3 Results and discussion

3.3.1 Determination of the total and included drug amount

The in different ways prepared praziquantel-β-cyclodextrin-complexes – physical mixture, kneading and solvent method and spray drying – were analyzed both for their total drug amount in water and for their complexed praziquantel fraction in acetonitrile.

The drug content of the physical mixture was measured photospectrometrically at 263 nm after dissolution in water. The discrepancy of drug concentration in different samples was very low after several mixing steps with an average content of 19.6% praziquantel. This being slightly lower than the theoretical amount (21.6%) one reason for it might be insufficient mixing or loss of substance during production, especially as praziquantel tends to charge and thus to agglutinate or stick to the wall of the mixing vessel. Determination of the uncomplexed fraction in acetonitrile gave a praziquantel concentration of 18.6% which results in an included drug amount of 5.2%. Drug complexation in simple dry mixing is normally only possible if the cyclodextrin still has its crystal water molecules which can be exchanged with the drug molecules (Szejtli & Szente, 2005). Besides, the hydrophobic nature of praziquantel can lead to an insufficient complexation by dry mixing. Additionally, this type of complexation needs a lot of time and can be enhanced a little by applying force. The preparation of the physical mixture in a mortar might thus lead to partially complexation due to the grinding of the product with a pestle. But with an included drug amount of only 5.2% a real inclusion complexation cannot be really assumed.

The drug content of the complexes prepared by kneading and solvent method were measured photospectrometrically at 263 nm and with HPLC with good conformity. For several batches prepared by the solvent method the praziquantel content was between 21.4 and 22.0% and the average drug content with the kneading method was 21.5%; both correspond very well with the theoretically determined amount (21.6%). The marginal higher drug amount can be explained by the better water solubility of β-cyclodextrin compared to praziquantel so that a tiny part of the cyclodextrin might remain dissolved in the supernatant. Determination of the included praziquantel yielded about one third of the total drug amount for both complexing techniques: 32% for the complex prepared by solvent method and 33% for the one produced by kneading. These values lead to the assumption that real inclusion complexes of praziquantel in β-cyclodextrin are formed. If the drug were not really included in the cavity of the cyclodextrin but existed as a molecular mixture it would get dissolved in acetonitrile and thus the value for the complexed amount would be less. So if additionally the rigidity of the drug and the moderate affinity to β-cyclodextrin are considered (El Arini & Leuenberger, 1996) the determined inclusion yield has to be rated even higher. The included amount, of course, is only valid for the solid state as in solution the complex exhibits a dynamic equilibrium between free dissolved drug and inclusion complex.

Thus, a complex was prepared by spray drying from an aqueous solution (1:1

molar ratio) to investigate the included yield of this product. Because the water amount needed for dissolution of drug and cyclodextrin was quite high, polyvinylpyrrolidone (0.1% w/v) was added in the second spray drying batch in anticipation of enhancing the complexation efficiency of β-cyclodextrin and thus reducing the water amount needed to dissolve the whole amount of the active ingredient. The total drug content of the spray dried complex without PVP was 17.8% which is slightly less than the theoretical amount (21.6%). One reason for this difference might be degradation products of the active ingredient, yet, only 1.2% could be detected. Another possibility might be residual moisture in the final product although it was kept in a desiccator until constant weight. The included drug amount was determined to be 68.5% which is very good and more than twice as high as in the case of the complex prepared by solvent method. As the complex is prepared from an aqueous solution of active ingredient and β-cyclodextrin it can be assumed that – if both substances are completely dissolved – in equilibrium more than two thirds of praziquantel molecules are included into the cavity of β-cyclodextrin in a 1:1 molar ratio. The reason for this discrepancy between the complexes prepared by solvent method and spray drying is thus most probably the complete dissolution of both drug and cyclodextrin in the case of spray drying. During the solvent method preparation the active ingredient is dissolved in ethanol and β-cyclodextrin in water. At the end of the process all ethanol has evaporated (process temperature 70 °C) and the drug occurs in water both dissolved and suspended as well as included in the cyclodextrin cavity either in solution or as precipitate. There is a constant equilibrium between dissolved free drug, cyclodextrin and the complex which precipitates once its solubility limit is exceeded. Hence, more complex can be formed in solution in a sort of balance reaction which might go on over quite some period of time. The process, however, was slowly cooled down after 30 min to allow the rest of the complex to precipitate so that it could be collected by filtration. Thus, the balance reaction in solution was determined after a couple of hours. It might have been possible to achieve a higher inclusion yield if the procedure had been carried on for a longer period of time. But this was not investigated further as the spray drying already yielded satisfying results and additionally would have been a more convenient method for upscaling.

In order to improve the spray drying process PVP was added to the spraying solution as it was thought to be able to reduce the water quantity needed to dissolve the drug completely by enhancing the complexation efficiency of β-cyclodextrin. This was also achieved, yet only to a small extent, and both free and included drug amount of the obtained product were determined. These measurements revealed some odd numbers in comparison to the spray dried product without PVP: the total drug amount was measured with 9% (theoretical content 11.6%) and in acetonitrile an included amount of 10.3% was determined. Yet, it was discovered that PVP is soluble in acetonitrile and hence, if PVP is participating in complex formation (either binary PZQ-PVP or ternary PZQ-CD-PVP complexes) they might become soluble, too. Thus, the real amount of included drug in the β-cyclodextrin cavity cannot be determined for this spray dried product but it indicated that PVP might somehow

form ternary complexes with praziquantel and β-cyclodextrin. Another alternative might include binary complexes between praziquantel and PVP without participation of β-cyclodextrin. If these complexes were more stable than those of praziquantel and β-cyclodextrin they might rather be formed in the aqueous spraying solution and hence would dissolve during the content measurement in acetonitrile. But nonetheless, the most probably assumption is the formation of ternary praziquantel-β-cyclodextrin-PVP non-inclusion complexes, especially if several literature data are considered (Duan et al., 2005; Loftsson et al., 1994b; Mura et al., 2001).

3.3.2 Phase-solubility studies

Because phase-solubility studies are very important for characterization of drug-cyclodextrin complexes a series of phase-solubility studies was performed with the racemic praziquantel, its separated enantiomers, different cyclodextrins (β-cyclodextrin and hydroxypropyl-β-cyclodextrin), and with polyvinylpyrrolidone to examine its influence on the complexation efficiency of cyclodextrins. From the obtained diagrams both stability constant and complexation efficiency were calculated.

The phase-solubility diagram for praziquantel with β-cyclodextrin is shown in Fig. 3.1. As can be seen, the solubility of praziquantel increases linear with rising β-cyclodextrin concentration. This fits the A_L-type diagram according to Higuchi (see Chapter 1.2.3). With the slope (0.26) and the intercept (0.97) the stability constant K=371 M^{-1} was calculated according to Equ. 1.6. As mentioned in Chapter 1.2.3, stability constants for cyclodextrin complexes most often lie between 50 and 2000 M^{-1}, with an average value of 490 M^{-1} for β-CD (Connors, 1995). Therefore, the praziquantel-β-cyclodextrin complex lies within this range but slightly below the average constant for β-CD complexes. Thus, this complex does not seem to be very stable possibly due to the rigidity of praziquantel. The solubility of praziquantel was increased more than fivefold with the highest tested cyclodextrin concentration.

The stability constant calculated from the data above corresponds well with data described in literature. El Arini & Leuenberger (1996) reported a value of 368 M^{-1} and de Jesus et al. (2006) described a fivefold increase in the solubility of praziquantel at 25 °C.

Several studies on the inclusion complexes between praziquantel and β-cyclodextrin have been published (El Arini & Leuenberger, 1996; de Jesus et al., 2006; Becket et al., 1999). Becket et al. (1999) studied the interaction of praziquantel with all three natural occurring cyclodextrins and found that β-CD forms the most stable complexes with praziquantel. However, in their work the phase-solubility diagram of praziquantel and β-CD are described as a B_S-type diagram, which is in contrast to the results obtained by other working groups (El Arini & Leuenberger, 1996; de Jesus et al., 2006). In their work, a clear A-type phase diagram is described with the argument that the solubility of praziquantel is enhanced up to the solubility limit of β-CD. This limit leads to a plateau in the diagram which lead Becket et al. (1999) to the propose of a B-type diagram. Furthermore, de Jesus et al. (2006) found out using NMR and ROESY that praziquantel and β-CD form inclusion complexes of a 1:1 ratio (see also

Chapter 3.3.5). This also fits into the theoretical data gained from the phase-solubility diagram.

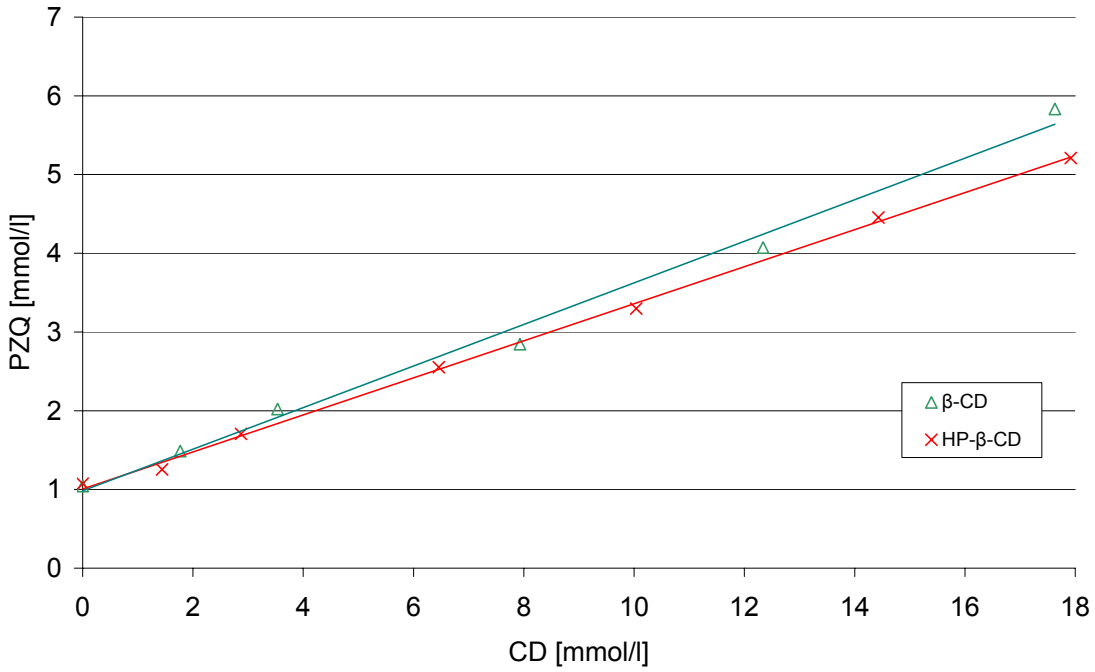

Figure 3.1: Phase-solubility diagram of praziquantel with β-cyclodextrin or hydroxypropyl-β-cyclodextrin in water at 37 °C; duplicate measurements

Additionally to the stability constant, the complexation efficiency according to (Loftsson et al., 1999) was determined. From the slope (0.26) a value of CE=0.36 was calculated. Along the lines of Equ. 1.8 this means that about two out of every seven β-cyclodextrin molecules form a complex with praziquantel if a 1:1 molecular ratio is assumed. The value calculated from data from the article of El Arini & Leuenberger (1996) would give a complexation efficiency of 0.34 and thus confirm the data from this study. A CE of 0.36 is quite good for β-cyclodextrin complexes as the average complexation efficiency of cyclodextrin is 0.30 (Loftsson et al., 2005).

Moreover, phase-solubility studies were performed with HP-β-CD to examine if this cyclodextrin with a higher water solubility is able to build more stable complexes with praziquantel and thus enhance the drug solubility even further. Its phase-solubility diagram with praziquantel is displayed in Fig. 3.1. From the slope (0.235) and the intercept (1.0068) the stability constant and complexation efficiency were calculated and gave values of 305 M^{-1} and 0.30, respectively. These parameters are slightly lower than for the system praziquantel/β-CD, for which a stability constant of 371 M^{-1} and a CE of 0.36 was determined. These lower values might be mainly due to the larger HP-β-CD molecule compared to β-CD – the substituted hydroxypropyl side chain takes up more space and thus might lead to a steric hindrance for inclusion complexation of drugs. This could particularly be the case for praziquantel which is a

rather rigid molecule and is only partially included in the cyclodextrin cavity (de Jesus et al., 2006; El Arini et al., 1998). Therefore, this might be the foremost reason for a lower stability constant and CE than β-CD.

Further studies were made to examine if both stability constant and complexation efficiency of β-CD and HP-β-CD could be enhanced upon addition of PVP K30. Water soluble polymers such as PVP or hydroxypropyl methylcellulose enhance the complexation efficiency of cyclodextrins mainly by improving the complexing abilities and the solubility of the cyclodextrin and of the drug-cyclodextrin complex (Loftsson & Fridriksdottir, 1998; Loftsson et al., 1994b; SigurÐardóttir & Loftsson, 1995). It is often stated in literature that this enhancement is not reached by simple mixing of active ingredient, cyclodextrin and polymer, but is only accomplished if the aqueous solution containing drug, polymer and cyclodextrin is heated up to at least 120 °C for 20-40 min (Loftsson et al., 1999; Loftsson & Fridriksdottir, 1998; Ribeiro et al., 2003). However, in other publications it could be shown that such a heat treatment is not necessary and an improvement in the complexation efficiency is also achieved by simple addition of the polymer to an aqueous solution of drug and cyclodextrin (Mura et al., 2001; Valero et al., 2004). Hence, three different phase solubility studies were performed with β-CD and 0.1% (w/v) PVP: 5 days at 37 °C to be able to compare these results directly with the previous test without PVP, autoclaving at 120 °C for 40 min and sonication for 60 min at 70 °C, both followed by 5 days of stirring at 37 °C. Studies with HP-β-CD and PVP were only conducted at 37 °C without further treatment.

	slope	intercept	stability constant [Mol^{-1}]	complexation efficiency
β-CD	0.2649	0.9709	371	0.36
β-CD + 0.1% (w/v) PVP	0.2807	0.9775	399	0.39
β-CD + 0.1% (w/v) PVP; sonicated	0.2764	0.8475	451	0.38
HP-β-CD	0.2352	1.0068	305	0.31
HP-β-CD + 0.1% (w/v) PVP	0.2599	1.0654	330	0.35

Table 3.1: Phase-solubility data of praziquantel and cyclodextrins

First it has to be mentioned that the trials in which the samples were autoclaved did not lead to any satisfying results. After autoclaving every sample was completely dissolved – even those without any cyclodextrin. During subsequent stirring a precipitate occurred in nearly all vials, yet the composition of this precipitate in the samples with cyclodextrin could not be determined: It might either have been pure active ingredient or a complex with β-cyclodextrin. Thus, an increased solubility of praziquantel might not have been the true value to draw conclusions on complex formation

with β-cyclodextrin as part of this complex might have already precipitated. Besides, the samples containing only praziquantel and PVP without cyclodextrin showed a very high drug solubility with nearly three times as high as without treatment. One reason for that might by supersaturation of the suspension with active ingredient. Moreover, the values varied quite much between identical probes so that this experiment could not be used for further interpretation. Therefore, another trial was started in which the samples were only sonicated at 70 °C for 60 min. The results for this study along with those of β-cyclodextrin and HP-β-CD and PVP are displayed in Fig. 3.2 and Tab. 3.1.

As can be seen the addition of 0.1% PVP to the aqueous drug-cyclodextrin suspensions without any further treatment led only to a slight improvement in both the stability constant and the complexation efficiency. In both cases, β-CD and HP-β-CD with PVP without heat treatment, the stability constant only rose marginally from 371 M^{-1} to 399 M^{-1} for β-CD and for HP-β-CD it increased from 305 to 330 M^{-1}. The complexation efficiency was also only enhanced by about 10% in both cases (from 0.36 to 0.39 for β-CD and for HP-β-CD from 0.31 to 0.35). Nonetheless, the addition of a water soluble polymer did result in an increase both in the stability of the drug-cyclodextrin complex and also in the complexation efficiency for the two tested cyclodextrins with praziquantel.

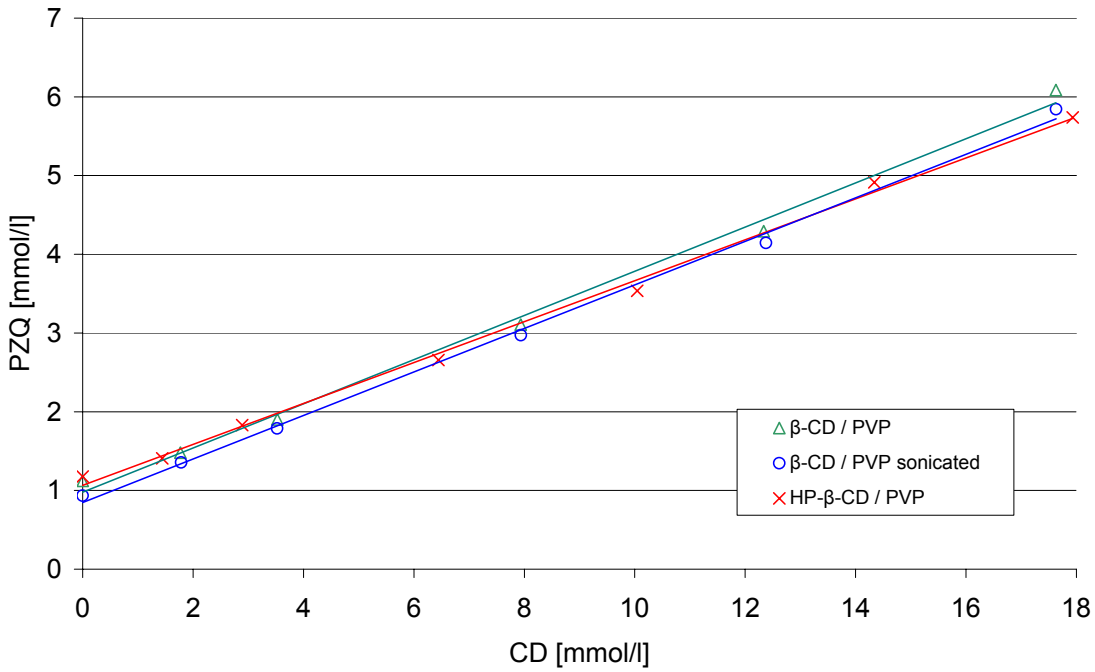

Figure 3.2: Phase-solubility diagram of praziquantel with β-cyclodextrin or hydroxypropyl-β-cyclodextrin and 0.1% (w/v) PVP in water at 37 °C untreated or previously sonicated; duplicate measurements

Hence, it was tested if the complexation efficiency of β-CD with praziquantel and PVP could be further enhanced by heat treatment. As written above, autoclaving

did not work for the system praziquantel, β-CD and PVP so that sonication of the samples for 60 min at 70 °C was tested instead. However, no significant improvement of complex formation parameters could be observed with this handling. The slope and thus the complexation efficiency is nearly identical to the study containing PVP without any treatment (0.38 in contrast to 0.39) whereas the stability constant is slightly higher (451 M^{-1} to 399 M^{-1}). Yet, this is only due to the lower intrinsic solubility in the studies with sonication and the thus lower intercept value. Apart from that it can be seen that the curve progression is very close to parallel to the one without heat treatment so that the increase in solubility is nearly the same for both studies. Therefore it is indicated that indeed the complexation efficiency is a more meaningful parameter to judge the complexing abilities of cyclodextrins. Moreover, it can be assumed that sonication does not have a pronounced enhancing effect on the interactions between β-cyclodextrin and praziquantel in the presence of 0.1% PVP.

But overall it can be stated that PVP does have a positive influence on complex formation between β-cyclodextrin and praziquantel. The main reason for this effect is attributed to PVP interacting with the drug-cyclodextrin complex although the exact nature of the mechanisms is still unknown. Yet, it has been shown that polymers increase the complexing abilities of cyclodextrin (Loftsson et al., 1994b; Loftsson, 1998) and enhance drug availability in cyclodextrin solutions (SigurÐardóttir & Loftsson, 1995). It is assumed that PVP stabilizes the complexes through participation, thus building ternary non-inclusion complexes with drug and cyclodextrin. These complexes are often characterized by hydrogen bonds between polymer and cyclodextrin and/or drug, and a more negative free energy consequently leading to an increase in the stability constant (Loftsson & Masson, 2004).

Another possibility could be that PVP forms complexes with praziquantel alone thereby increasing its solubility without the participation of cyclodextrin. Yet, this thought is not deemed too possible considering two main factors: first of all, the concentration of PVP is the same in all samples, hence an increase in drug solubility by complex formation with the polymer should thus happen always in the same quantity. This would then enhance the solubility of praziquantel more in percentage in the first samples containing no or little cyclodextrin than in the probes with a higher cyclodextrin concentration. Therefore, the slope of the phase solubility diagram would be less steep as the values at the beginning would be higher. Furthermore, no significant difference could be seen between the intrinsic solubility (i.e. without cyclodextrin) in the phase solubility studies containing either no or 0.1% PVP. If a complex formation between active ingredient and PVP were of importance for the conducted phase solubility studies, there should be an increase in the intrinsic praziquantel solubility with PVP. It might be thoroughly possible that PVP is able to complex praziquantel, however, in these studies with this low polymer concentration it is not very probable if the aforementioned aspects are considered.

Summarizing the above, it can be stated that the addition of PVP to aqueous samples containing praziquantel and cyclodextrin led to a small increase in drug solubility and hence to an improvement in complex stability and complexation efficiency.

Yet, this effect is not very pronounced with about 10% increase in both constants. The overall drug solubility is also not significantly increased through addition of PVP. In average, the addition of water-soluble polymers leads to an 70% increase in the cyclodextrin solubilization of drugs (Loftsson & Masson, 2004). It can further be concluded that heat treatment does not have a positive effect on praziquantel solubility in the presence of β-cyclodextrin and PVP. However, the enhanced complexation efficiency by polymers was mainly to be used for the oral paste (see Chapter 5.3.3) to be able to reduce the cyclodextrin amount. As it would not have been convenient to autoclave the paste during preparation this procedure was not tracked further.

Enantiomers often show different properties than their racemates such as solubility, melting behavior and efficacy. Besides, as the stereospecific behavior of cyclodextrins is well known from their use in chiral HPLC columns to separate racemates, it seems to be obvious that drug complexation with cyclodextrin can be different for enantiomers compared with the racemic drug. In literature, this is well described for some drug substances, for example ibuprofen and terbutaline (Nunez-Aguero et al., 2006; Kim & Park, 1998). Moreover, studies with praziquantel enantiomers have already shown different stability constant values for the racemate and the enantiomers (El Arini et al., 1998). Thus, phase-solubility studies with both separated enantiomers were performed as well.

Fig. 3.3 shows the phase-solubility diagram for racemic praziquantel and its $(-)$- and $(+)$-enantiomers. In all cases it can clearly be seen that the solubility increases with increasing cyclodextrin concentration so that an A-type phase solubility diagram according to Higuchi can be assumed. Although the differences between the enantiomers seem not that major, the stability constants calculated from slope and intercept show clearly different values (see Tab. 3.2).

Racemic praziquantel shows overall a slightly lower solubility than its enantiomers. This might be due to different crystallinity because of re-crystallization of the enantiomers after separation. But although this parameter is different, racemic praziquantel and its $(+)$-enantiomer do not vary much in their stability constants and thus seem to be able to form equally stable complexes. $(-)$-PZQ on the other hand shows a very high intrinsic solubility, but the solubility is not enhanced as much as that of its partner $(+)$-enantiomer, so that the stability constant is considerably lower. This indicates that β-cyclodextrin exhibits stereoselective behavior and prefers $(+)$-PZQ over $(-)$-PZQ, but interestingly does not seem to mind between $(+)$-PZQ and its racemic drug if only the stability constant is considered.

Though, if the complexation efficiency is regarded, differences can be seen between these two substances. Here, $(+)$-PZQ shows a much higher value (CE=0.45) than racemic praziquantel and $(-)$-PZQ which possess the same complexation efficiency (CE=0.36). According to Loftsson, the complexation efficiency is better suitable to judge the interaction between drug and cyclodextrin because it does not need the intrinsic solubility of the drug, which particularly is a critical parameter for drug concentrations lower than 1 mg/ml, but is only calculated with the slope of the phase-solubility diagram (Loftsson et al., 2005). So from that point of view (+) PZQ does

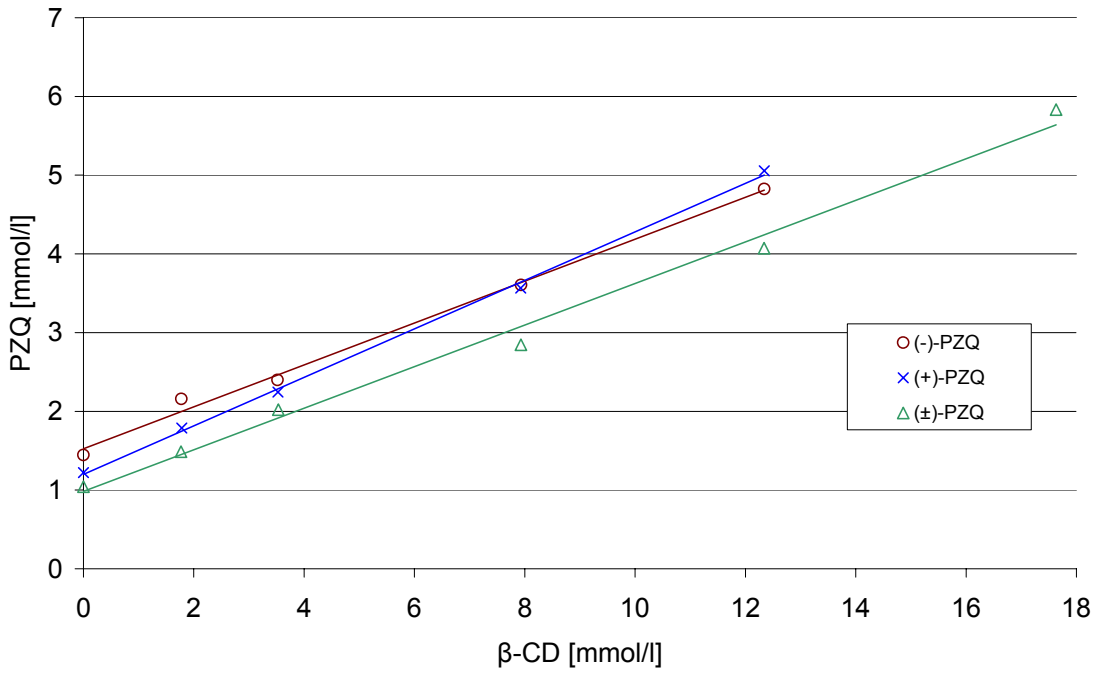

Figure 3.3: Phase-solubility diagram of β-cyclodextrin and racemic ($\pm$)-PZQ, ($-$)-PZQ and ($+$)-PZQ in water at 37 °C; duplicate measurements

	Slope	Intercept	Stability constant [Mol^{-1}]	Complexation efficiency
Racemic PZQ	0.2649	0.9709	371	0.36
($+$) PZQ	0.308	1.1998	371	0.45
($-$) PZQ	0.2661	1.5257	238	0.36

Table 3.2: Phase-solubility data of β-cyclodextrin with racemic praziquantel and its enantiomers

seem to be more capable of forming complexes with β-cyclodextrin or in other words more β-cyclodextrin molecules take part in complex formation with ($+$)-PZQ than with the racemic drug and the ($-$)-enantiomer.

Hence, β-cyclodextrin indeed seems to show a stereoselective behavior with praziquantel with a higher affinity to the ($+$)-enantiomer than to the racemic drug or ($-$)-praziquantel. This observation can also be supported if the filtered phase-solubility samples are cooled and left standing for one day at room temperature: in the samples with racemic and ($-$)-praziquantel with high β-cyclodextrin concentration precipitation presumably of the complex occurs, whereas in the case of ($+$)-praziquantel no precipitate can be seen. One reason for that could be that more cyclodextrin molecules might take part in complex formation so that the complex is better soluble and thus does not precipitate from aqueous solution.

3.3.3 Differential scanning calorimetry

The DSC thermograms obtained for praziquantel, β-cyclodextrin and complexes prepared by physical mixture, kneading and solvent method are shown in Fig. 3.4 to 3.8. Praziquantel exhibits a characteristic fusion peak at 140 °C with a high enthalpy (89.4 J g^{-1}), no other peaks due to polymorphism appeared under the above described conditions. Reported melting ranges for praziquantel are from 136-142 °C (USP, 2007) and 136-140 °C (Cioli & Pica-Mattoccia, 2003). β-cyclodextrin displays an endothermic peak at 182 °C which is attributed to dehydration of the crystal water in the cavity of the cyclodextrin.

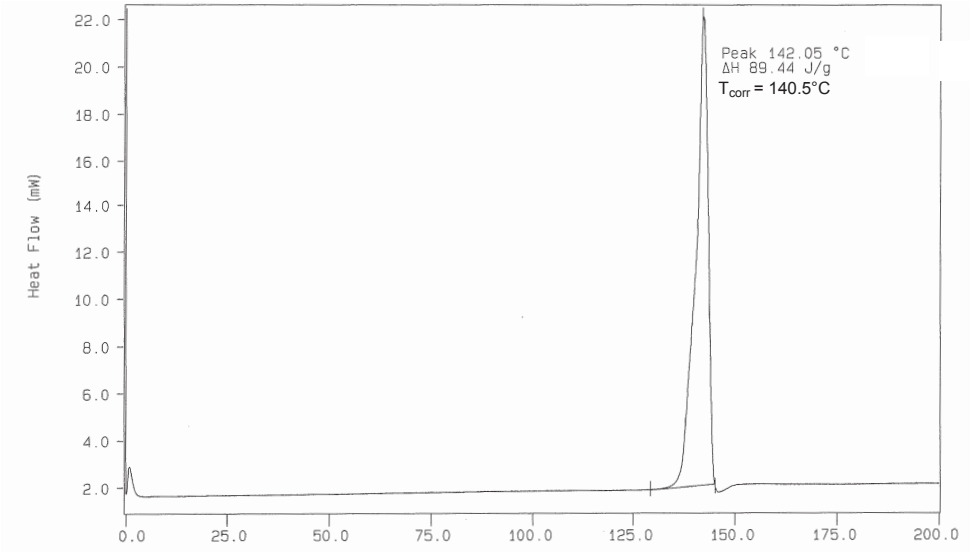

Figure 3.4: DSC-thermogram of praziquantel (x-axis shows temperature)

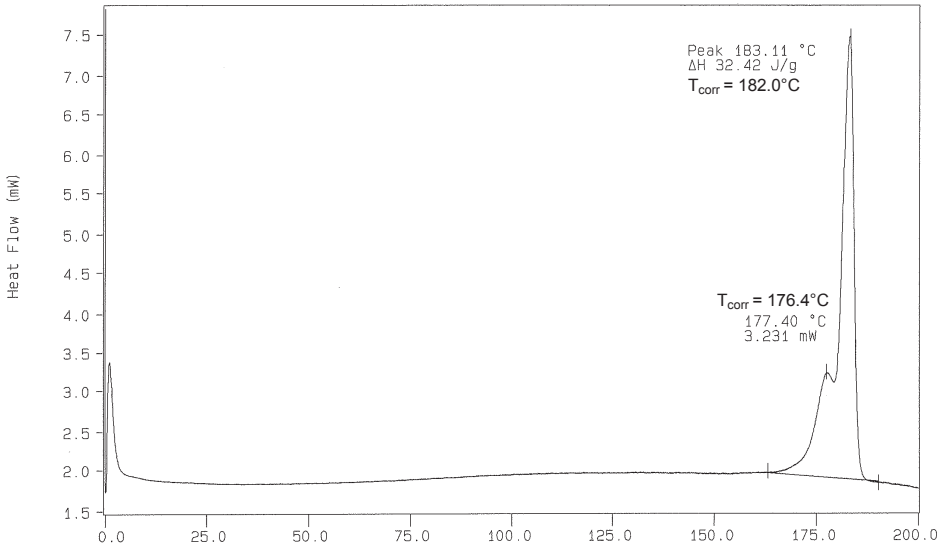

Figure 3.5: DSC-thermogram of β-cyclodextrin (x-axis shows temperature)

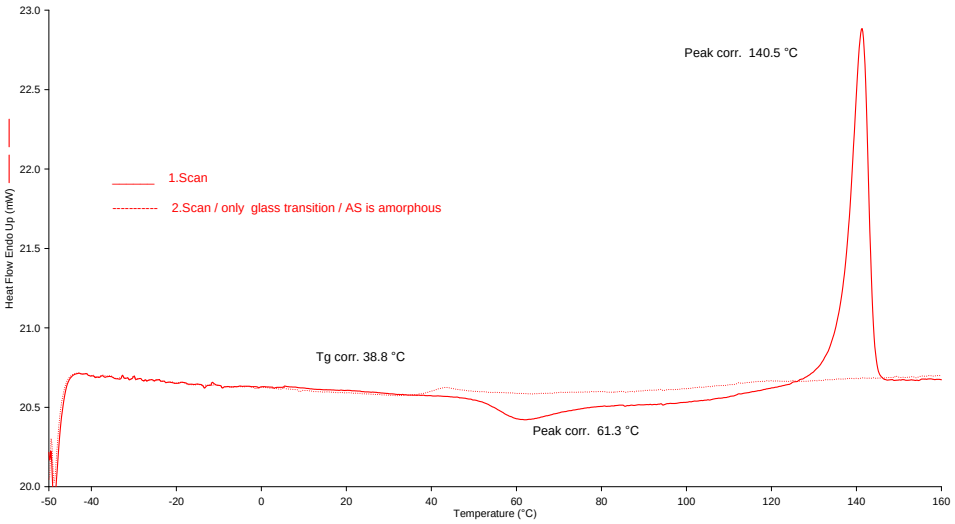

Figure 3.6: DSC-thermogram of a praziquantel/β-cyclodextrin physical mixture prepared by grinding

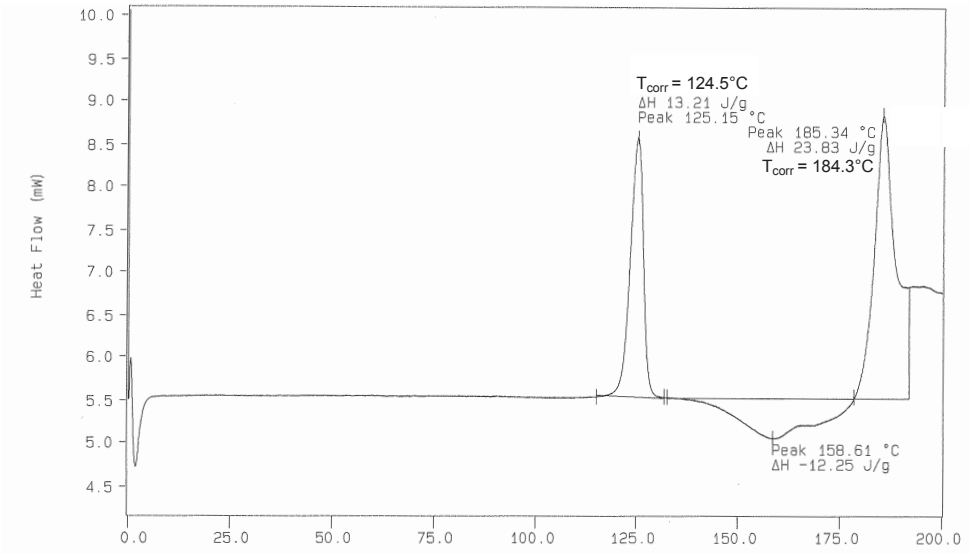

Figure 3.7: DSC-thermogram of praziquantel-β-cyclodextrin complexes prepared by solvent method (x-axis shows temperature)

Two physical mixtures of praziquantel and β-cyclodextrin were further investigated which had been prepared either by grinding in a mortar or single mixing in a turbula mixer for 10 minutes. Regrettably, in these measurements it was only heated up to 160 °C so that no peak appears for β-cyclodextrin. In the thermogram of the sample prepared by grinding a small exothermic peak at 61 °C and a broadening of the praziquantel peak at 140.5 °C can be seen. Both phenomena might be explained by the formation of a complex: Small seed crystals of the complex might have been produced through the force during grinding and these crystals could have led to further complexation during heating. The thus formed complex might be the reason for the broadening of the praziquantel peak. In the thermogram of the physical mixture

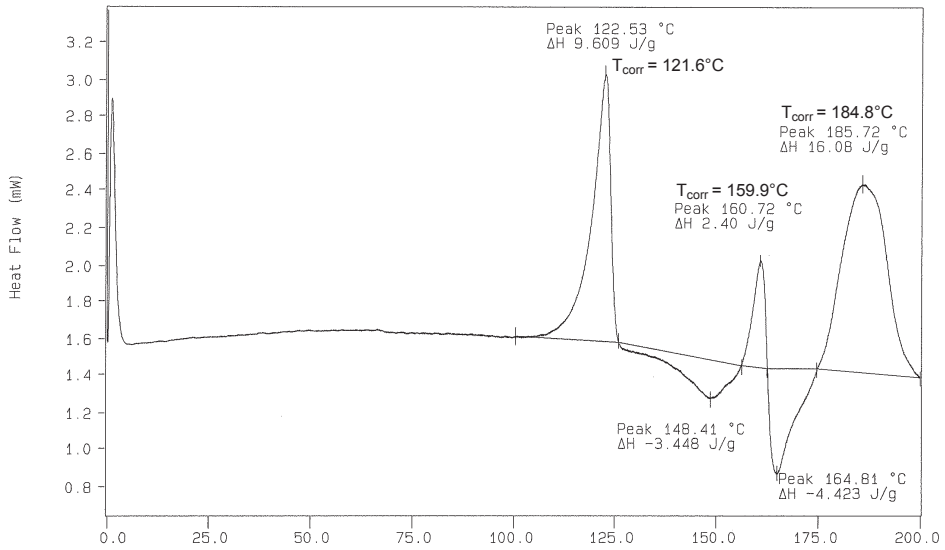

Figure 3.8: DSC-thermogram of praziquantel-β-cyclodextrin complexes prepared by kneading method (x-axis shows temperature)

prepared by simple mixing no such peaks appear but a single characteristic fusion peak of praziquantel can be seen (data not shown). This shows that no complex is obtained by simple mixture and confirms the theory of complex formation through grinding.

The thermogram of the praziquantel-β-cyclodextrin inclusion complexes obtained by the solvent method shows two narrow endothermic peaks at 124 °C and 184 °C. The latter might be due to uncomplexed β-cyclodextrin so that dehydration of the included water molecules of β-cyclodextrin does still take place. An indication for the building of inclusion complexes might be the peak at 124 °C which can neither be attributed to praziquantel nor β-cyclodextrin. If an inclusion complexation has been achieved a new substance – the praziquantel-β-cyclodextrin-complex – has been formed which shows its own physical and chemical properties and might therefore exhibit a quite different peak from its "parent molecules". This consideration is further supported by the DSC thermogram of the complex prepared by kneading method because there a similar peak appears at 122 °C which also indicates the formation of a substance with new physical properties. In addition, the thermogram also exhibits an endothermic peak at 185 °C which, as described before, might be the crystal water of uncomplexed β-cyclodextrin. Three other peaks can be seen in this thermogram, another small endothermic peak at 160 °C and two small and broad exothermic peaks at 148 °C and 164 °C. These substances might be by-products which arise from the manufacturing step using ethanol as a solvent. Praziquantel seems to be partially instable in ethanol since a strange and different smell appeared after a few days. This might be the reason for these additional peaks, the exothermic thereby arising probably mainly from re-crystallization of a substance. Unfortunately, no literature evidence could be found on this decomposition.

Based on these results an inclusion complexation of praziquantel in β-cyclodex-

trin by solvent and kneading method can be assumed with high probability. Moreover, a slight complexation can also be achieved in a physical mixture by grinding.

3.3.4 Infrared spectroscopy

For further characterization of praziquantel-β-cyclodextrin complexes infrared spectroscopy was used. Although it is known that this method is of limited use for investigation of cyclodextrin inclusion complexes (Frömming & Szejtli, 1994) and praziquantel-β-cyclodextrin-complexes have already been analyzed by Becket et al. (1999), IR tests were performed nevertheless to see if any shifts or bands indicated a complex formation.

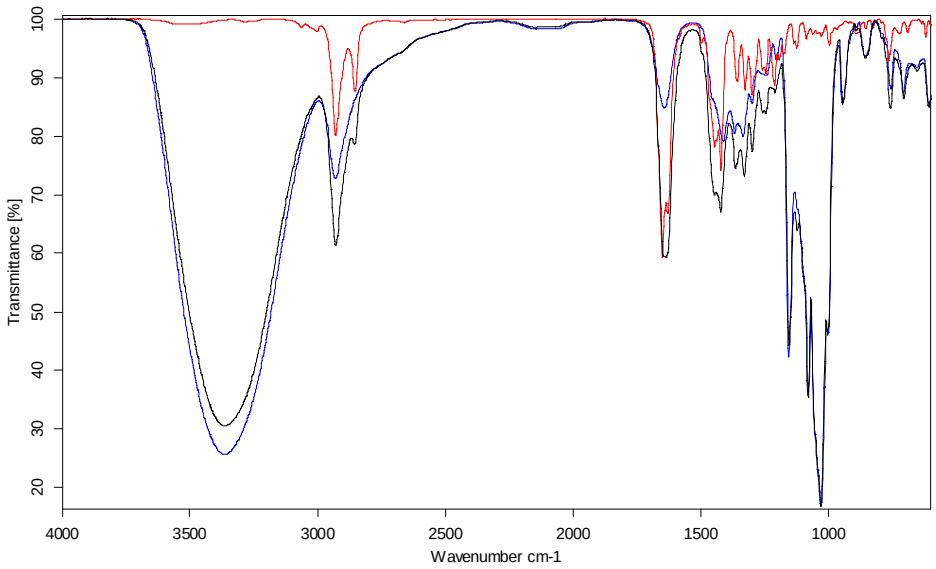

Figure 3.9: FTIR-spectrum in transmission: red: praziquantel; blue: β-cyclodextrin; black: isolated complex prepared by solvent method

Spectra were taken from three different complexes: The first sample was the isolated complex obtained by the solvent method and one sample was precipitated from an aqueous drug-cyclodextrin solution by evaporation of the solute. The drug to cyclodextrin ratio of these two samples was 1:1 molar. The third sample was a physical mixture prepared by simple mixing in a turbula; here the drug to cyclodextrin ratio was 1:1 in weight. IR-spectra were obtained in solid state with a FTIR-microscope and also dissolved in dimethylsufoxide (DMSO). The spectra are displayed in Fig. 3.9 to 3.11. As can be seen all samples are at least a mixture of praziquantel and β-cyclodextrin because the examined sample show no direct identicalness with the references, especially β-cyclodextrin. Small changes in the form of the bands attributed to praziquantel appear in the spectra of the two samples prepared with solvent, especially of the C=O bands (see Fig. 3.9). This is most probably due to changes in crystallinity because of precipitation of the crystalline drug in an amorphous form. In the complex prepared by solvent method both amorphous and crystalline forms occur whereas in the sample precipitated from aqueous solution no crystalline form

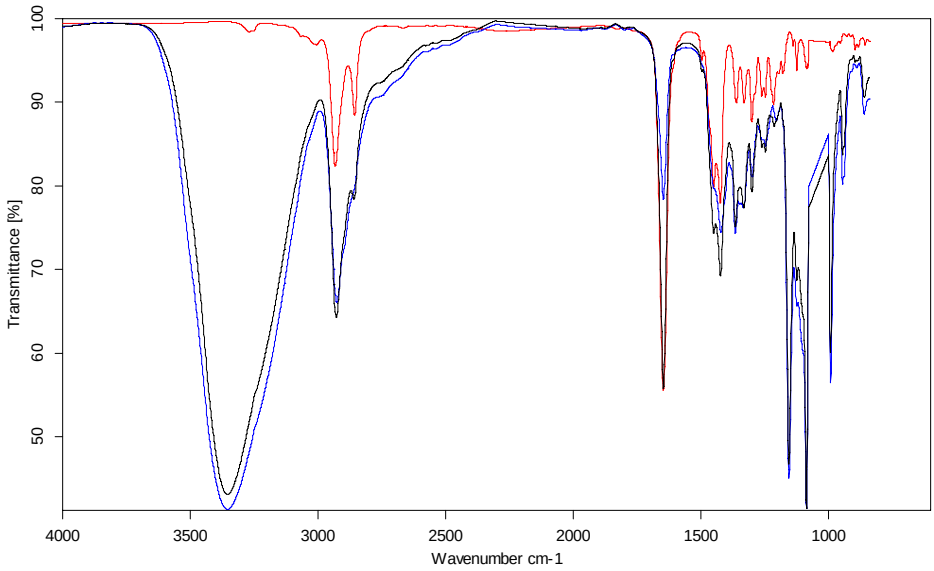

Figure 3.10: IR-spectrum in solution: red: PZQ; black: isolated complex prepared by solvent method; blue: complex precipitated from aqueous solution

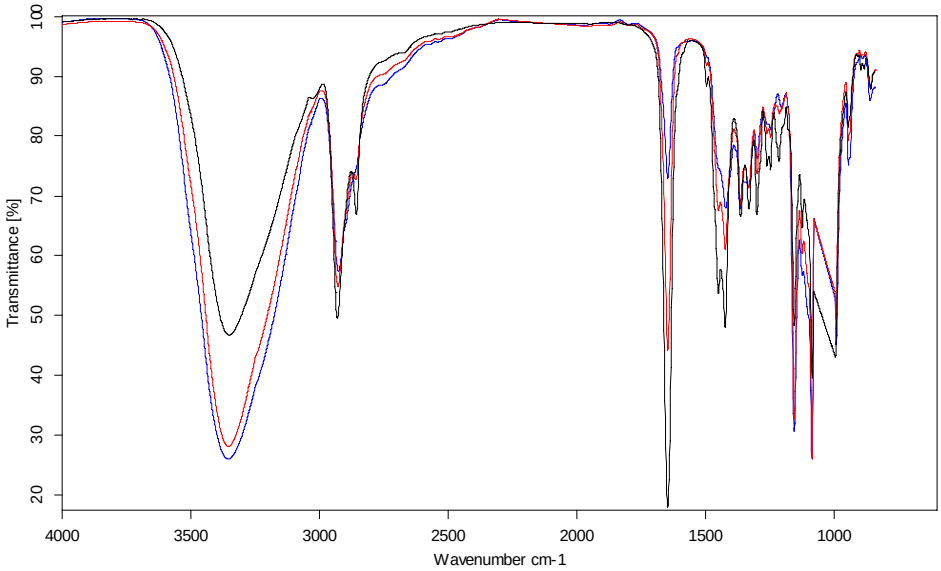

Figure 3.11: IR-spectrum in solution: red: isolated complex prepared by solvent method; blue: complex precipitated from aqueous solution; black: physical mixture

can be detected any more. This statement can further be supported by a DSC thermogram of the precipitate from aqueous solution in which no peak could be detected for praziquantel (data not shown).

Moreover, it becomes evident that the praziquantel concentration is different in the three samples: While the physical mixture has the highest drug amount the peaks characterizing praziquantel are least pronounced in the sample precipitated from aqueous solution. This of course is due to the fact that the physical mixture was prepared with a 1:1 weight ratio and the other two samples had a 1:1 molar ratio

which equals a mass ratio of about 1:3.6. Yet, in the precipitated sample the praziquantel amount is less than in the complex prepared by solvent method for manufacturing reasons: This sample was gained from a solution of praziquantel and β-cyclodextrin in a molar ratio 1:1. Although it was mixed for several days, the solubility of praziquantel in water is quite low and hence its concentration in the crystallized product is much less than β-cyclodextrin.

All in all, the spectra of the samples prepared from solution are more similar to β-cyclodextrin which is provoked by the higher amount of cyclodextrin, thus alterations in the spectrum which might be due to complex formation are usually covered by the cyclodextrin (Frömming & Szejtli, 1994). For any mixture nearly the same spectrum is obtained apart from small differences due to changes in crystallinity. Hence it can be concluded that a potential inclusion complex formation between praziquantel and β-cyclodextrin can indeed not be detected with IR-spectroscopy.

3.3.5 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) measurements were employed for a more detailed characterization of the intermolecular interactions between praziquantel and β-cyclodextrin in the complex. From the results conclusions could be drawn regarding a real inclusion of the drug into the cyclodextrin cavity and also the stoichiometry and geometry of this interaction.

The obtained spectrum of the two-dimensional ROESY (rotating frame Nuclear Overhauser Effect spectroscopy) experiments is presented in Fig. 3.12. The chemical shifts displayed on the y-axis are attributed to β-cyclodextrin and the shifts on the x-axis to praziquantel. For the active ingredient, the shifts between 7.45 and 7.43 ppm could be assigned to the aromatic hydrogen atoms (de Jesus et al., 2006; Schepmann & Blaschke, 2001). The reported shifts for β-cyclodextrin at 4.0 and 3.9 ppm belong to the hydrogen atoms inside the cavity, i.e. H3 and H5 (Schneider et al., 1998; Pinto et al., 2005; Chen et al., 2006). Nuclear Overhauser Effects (NOE) could be detected between these hydrogen atoms thus resulting in the peaks shown in the ROESY spectrum. The presence of these intermolecular cross-peaks indicated an inclusion complex between praziquantel and β-cyclodextrin in which the aromatic part of praziquantel (isochinoline ring) is inserted in the cyclodextrin cavity. Additionally, no further interactions could be detected which led to the assumption of a complex with 1:1 stochiometry as has already been shown by phase solubility studies (see Chapter 3.3.2).

The assumption of a true inclusion complex can be supported by two facts: first of all, the complex has to be quite stable so that the cross-peaks can be detected at all. Moreover, with ROESY interactions between two atoms have to be in the range of less than 0.3 nm to be detectable (Guenther, 1983). So, if the drug were not truly inserted and hence within the proximity of the hydrogen atoms inside the cavity of β-cyclodextrin no spectrum could be obtained. From this it can be concluded that a real inclusion complex is formed where the aromatic ring of praziquantel is inserted into the β-cyclodextrin ring.

This result has already been published by de Jesus et al. (2006). With support

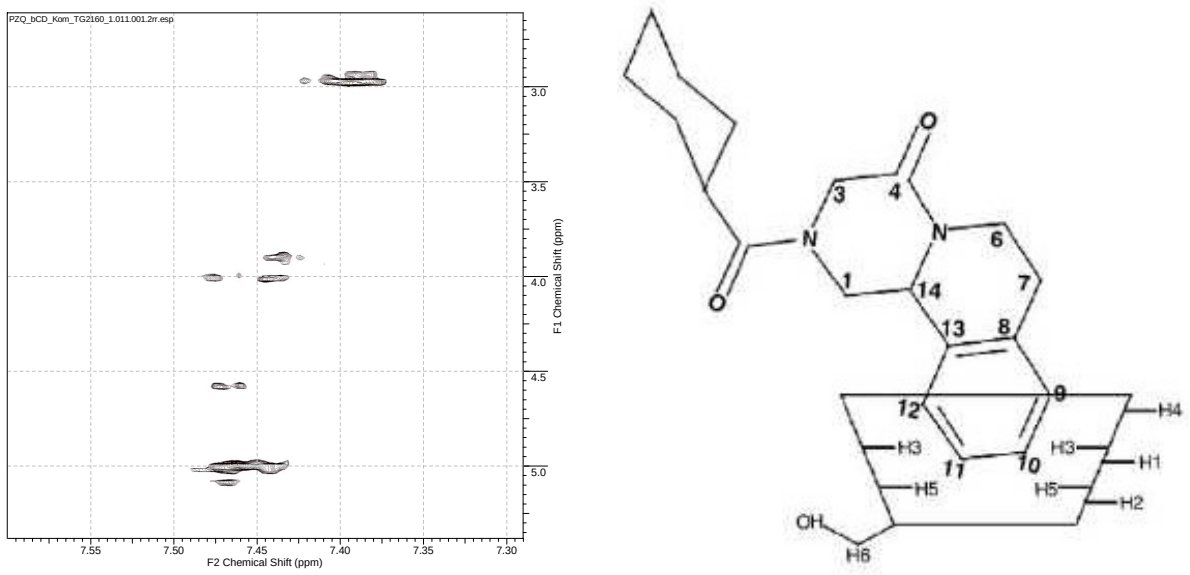

Figure 3.12: ROESY spectrum of PZQ/β-CD complex in D_2O (left); assumed geometry of the PZQ/β-CD complex as presented by de Jesus et al. (2006, right).

from molecular modeling calculations, they suggested the exact geometry of praziquantel β-cyclodextrin complex with the aromatic part of the drug being inserted into the wider opening of the cyclodextrin ring ("tail" opening) (see Fig. 3.12). However, the attribution of the cyclodextrin hydrogen atoms inside the cavity (H3 and H5) as presented in their article in the ROESY spectrum give the impression of being interchanged. In our study, H3 could be attributed to a peak at higher shifts than H5 (4.02 ppm to 3.91 ppm). This finding can be supported with data from other literature (Schneider et al., 1998; Pinto et al., 2005; Chen et al., 2006). de Jesus et al. (2006) on the other hand assigned the hydrogen atom H3 to lower values as H5 and concluded from these data the exact geometry of the praziquantel-β-cyclodextrin complex as described above. While the statement of the aromatic part of the drug being included in the cavity can be confirmed by our measurements, the second assumption of the exact orientation within the cavity cannot be verified. Yet, from our results and the above described findings it can in turn not be argued that the inclusion geometry of the drug molecule is inverse, i.e. into the narrower part of the cyclodextrin cavity.

So, from NMR studies a true inclusion complex formation between praziquantel and β-cyclodextrin can be concluded. In this complex, the aromatic part of the praziquantel molecule is inserted in the cyclodextrin cavity in a 1:1 stoichiometry.

3.3.6 Stability of cyclodextrin complexes

Stability of the praziquantel-β-cyclodextrin complex was tested and dissolution studies were performed to investigate possible changes during storage. Samples were kept at 25 °C/60% RH, 30 °C/65% RH and 40 °C/75% RH for three, six and nine months. Although changes in the properties of cyclodextrin complexes due to temperature or humidity might not be detected with dissolution studies, they were performed never-

theless. Yet, as the complete drug amount is released during dissolution an assumption could be made regarding change of drug content in the cyclodextrin complex during storage due to factors such as degradation.

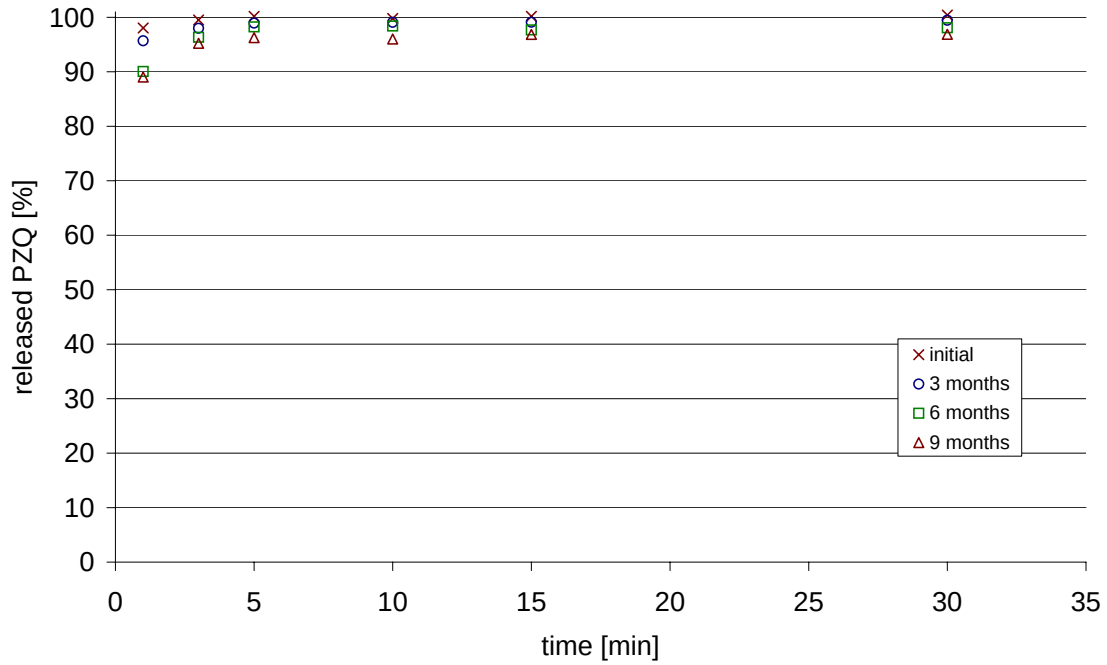

Figure 3.13: Dissolution of praziquantel from β-cyclodextrin complexes at start and after three, six and nine months; tests were run in triplicate; standard deviation did not exceed 5%.

Results of the dissolution studies from samples stored for three, six and nine months as well as the initial values are displayed in Fig. 3.13. No significant differences appeared between the three different storage conditions so that the values are averaged for each time point. As expected, dissolution of praziquantel took place very rapidly and was finished after three to five minutes in all samples. This of course is a result of complexation with β-cyclodextrin as this is mostly used for solubility enhancement and the formed inclusion complex is much better water soluble than praziquantel alone. All tested samples show a nearly equal dissolution rate with only negligible differences. These appear mainly at one and three minutes between the initial values and those after three months on the one hand and the values after six and nine months on the other. The reason for this is a change in the sample drawing method: in the first dissolution studies (initial and after three months) the samples were first taken and subsequently filtered which led to a tiny delay. So particles which had not dissolved yet could have been drawn into the syringe and dissolve there. Hence, the values at the beginning are slightly higher than for the studies after six and nine months. Here, the samples were drawn directly through the filter into the syringe so no further drug dissolution could occur. Another noticeable, though minor change is the reduction of the total dissolved drug amount: while in

the initial studies 100% active ingredient had been dissolved, this was reduced 1% on average after each measured time point; so after three months 99% praziquantel had been dissolved and after six and nine months this value was 98% and 97%, respectively. However, these variations can be neglected if analytical measurement errors are taken into consideration. Additionally, fluctuations between different samples at the same time points exist and thus the values partly overlap in their standard deviation. Finally, no drug degradation could be observed in aqueous pastes with praziquantel and β-cyclodextrin after up to twelve months (NAH, 2007), so that the variances observed by dissolution studies can be ignored. Consequently, the β-cyclodextrin inclusion complex with praziquantel seems to be stable during the tested time period and conditions.

3.4 Conclusions

Complexation of praziquantel with β-cyclodextrin seems to be possible and hence hopes are high that this might have a positive effect on the bitter taste of the drug. The characterization of the newly built substance, the inclusion complex, only delivers results regarding their physical and chemical properties which might lead to assumptions on the taste masking abilities, but the final test for confirmation of acceptance can only be performed with cats.

From literature it is known that β-cyclodextrin is the most suitable natural occurring cyclodextrin for complexation of praziquantel (Becket et al., 1999). Therefore, this cyclodextrin and one of it derivatives, hydroxypropyl-β-cyclodextrin, were characterized by phase-solubility studies in respect of their ability to form complexes with the active ingredient. Moreover, the influence of a water-soluble polymer, polyvinylpyrrolidone, on complex formation was tested. It could be shown that praziquantel has a moderate affinity to β-cyclodextrin, yet it is higher than to hydroxypropyl-β-cyclodextrin, which might be due to steric hindrance because of the voluminous substitute. From the appearance of the curves a 1:1 molar complex could be concluded for both cyclodextrins and drug solubility was increased nearly six-fold with β-cyclodextrin. Addition of 0.1% PVP led to a yet small increase in both stability constant and complexation efficiency. This was achieved even without heat treatment which is often stated necessary for a complexation enhancement. Phase solubility studies performed with the separated praziquantel enantiomers indicated a stereospecific behavior of β-cyclodextrin with a higher affinity to (+)-praziquantel than to its (−)-enantiomer or racemate.

Different methods were applied for the formation of a complex between praziquantel and β-cyclodextrin. A simple physical mixture did – as expected – not lead to any satisfying results regarding drug complexation, however, preparation in a mortar by grinding seems to have formed at least a small amount of complex. On the other hand, inclusion complexes were obtained by kneading, precipitation from solution (solvent method) and spray drying. It could be demonstrated that with the kneading and solvent methods an included drug amount of approximately 30% could be

achieved whereas this rate was even higher for spray drying with 68%. This is a pleasing outcome especially if the rigidity of the drug molecule is considered and confirms the results from the phase solubility tests assuming the formation of real inclusion complexes. The complex formation by kneading and solvent method was further examined by DSC measurements. In the thermograms a new peak was detected which could be attributed to the creation of a new substance with its own physicochemical properties. Infrared studies could not be utilized for investigation of cyclodextrin complex formation, as apart from differences in crystallinity, no changes in the spectra could be detected.

NMR-studies were employed to closer investigate the interactions between drug and cyclodextrin molecules especially in respect of their geometry in the inclusion complex. With the help of ROESY it could be demonstrated that a true inclusion complex is formed between praziquantel and β-cyclodextrin in which the aromatic part of the active ingredient is inserted into the cyclodextrin cavity. Moreover, a 1:1 stochiometry of this complex could be shown again. However, the exact orientation of the cyclodextrin in this complex (so into which side of the cyclodextrin ring the praziquantel molecule was included), could not be concluded from the obtained data.

Stability of the inclusion complex prepared by solvent method was studied for up to nine months at different temperatures. Although the samples were only characterized by dissolution studies, it could be evidenced that no drug degradation or other changes, which might have an impact on drug content and dissolution behavior, occurred.

An indication of complex formation is given by nearly all of the above described studies. Hence, the possibility of a taste masking effect of β-cyclodextrin seems to be feasible. The most suitable preparation method for this complex would be spray drying as this technique can easily be used to produce larger batch volumes. Besides, the use of organic solvents is not required as might be in the case for other production methods to dissolve the active ingredient. However, it has to be kept in mind that separation of a drug-cyclodextrin complex which is then added to a pharmaceutical product requires the registration process of a new chemical entity for the complex. This is very time and cost-intensive and thus most often undesired. Therefore, the direct addition of cyclodextrin to the product and a resulting in situ complex formation with the drug is the most convenient way to employ cyclodextrins in pharmaceutical products. Yet, of course this is only possible in an aqueous environment such as a solution, suspension, emulsion – or a paste. Hence, this in situ complex formation was tested in an aqueous paste regarding oral acceptance by cats (see Chapter 5.3.3). The results were very promising so that there was no need for further investigations concerning the separate production of the praziquantel-β-cyclodextrin-complex.

Chapter 4

Lipid embedding

4.1 Short introduction

Lipid embedding was tried for praziquantel using Precirol ATO 5, an atomized glyceryl dipalmitostearate which can be used for taste masking due to its chemical and physical properties, especially melting point and viscosity (Gattefossé, 2005). In order to achieve a taste masked formulation different methods such as fluid bed coating or melt granulation can be applied. In this study only melt embedding was tried. Praziquantel is partially soluble in hot Precirol so hot melt coating would not be feasible because dissolved drug particles might re-crystallize in the lipid coating and therefore exhibit bitter taste again, thus making the coating superfluous. Two different manufacturing processes were applied, simple melt suspension prepared by hand and spray congealing of a melt mixture on an industrial scale. For improvement of the dissolution rate Aerosil was incorporated into the lipid matrix. Physico-chemical characterization of the lipid particles was performed using particle size distribution measurements, scanning electron microscopy and hot stage microscopy. With differential scanning calorimetry and infrared spectroscopy further investigative methods were applied. Moreover, to investigate drug disintegration from the lipid matrices dissolution studies were performed. Different media were used to examine the effects of drug release from the lipid. Finally, the lipid particles prepared by hand consisting of only praziquantel and Precirol were put on stability. Samples were then characterized by DSC, IR and dissolution studies in order to detect any changes that might occur over a certain time under special storage conditions.

4.2 Materials and methods

4.2.1 Materials

The drug substance praziquantel was provided by PCAS, Limay, France. Precirol ATO 5 was kindly donated by Gattefossé AG, Luzern, Switzerland. Aerosil 200 was supplied by Degussa AG, Düsseldorf, Germany. Phosphate buffer pH 6.8 was purchased from Fluka AG, Buchs, Switzerland. Solutol HS 15 was obtained from BASF,

Ludwigshafen, Germany and soybean lecithin (Lipoid S 100) was provided by Lipoid GmbH, Ludwigshafen, Germany. Purified water was prepared with ELGA Maxima ana (Labtec Services AG, Wohlen, Switzerland).

4.2.2 Development of lipid particles

Different lipid particles were prepared with two methods; the exact compositions of the formulations can be seen in Tab. 4.1. For the hand made particles (TG 2159 and TG 2159/2 and 3) the lipid was melted in a beaker standing in a water bath previously heated to 70 °C. After obtaining a clear melt the drug and Aerosil, if used, were homogenously suspended in it to a creamy consistence. This melt suspension was then poured onto glass plates and rapidly spread in a thin layer as the lipid solidifies very fast once outside the water bath. This procedure was repeated several times to achieve a good yield. The glass plates were kept in a freezer over night so that on the next day the formulation could be peeled off and ground in a laboratory mortar. Afterwards the lipid particles were passed through a sieve (315 μm) in order to achieve homogeneously small particles. Drug content of the lipid particles was measured with the HPLC method described in Chapter 2.2.4.

Component	TG 2159	TG 2159/2	TG 2159/3	Brace 25%	Brace 37.5%
Praziquantel	25%	25%	25%	25%	37.5%
Aerosil 200	-	2%	3%	-	-
Precirol ATO 5	75%	73%	72%	75%	62.5%

Table 4.1: Compositions of lipid particles

Another preparation method was performed by the company BRACE GmbH, Alzenau, Germany. During this process the drug/lipid mixture containing either 25 or 37.5% (w/w) praziquantel was melted at 150 °C and then sprayed through vibrating nozzles with a diameter of 200 μm. Due to the vibration, the liquid beams were constricted into discrete segments. The spherical droplets were allowed to solidify below -10 °C during falling. The obtained particles were supposed to have a size distribution between 300-450 μm. Content and impurities were determined by HPLC.

4.2.3 Particle size distribution

Particle size was determined by the polydisperse method using the Mastersizer X (Malvern Instruments Ltd., Malvern, UK) with a dry powder feeder unit, a range lens of 1000 mm and a beam length of 10 mm. Measurements were run in triplicate and results are reported as volumetric mean diameter D(4, 3).

4.2.4 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to study the size, morphology and especially the surface of the lipid particles produced by hand and obtained from Brace. The samples were sputtercoated with gold and examined using a Philips XL 30 ESEM (Philips Electron Optics, Eindhoven, The Netherlands) at 3 kV accelerating voltage. Different magnifications were applied to gain overall and detailed impressions.

4.2.5 Hot stage microscopy

Microscopic observations of morphological features and changes during heating were carried out using a polarizing optical microscope (Zeiss Axioskop, Carl Zeiss AG, Jena, Germany) equipped with a Linkam THMS 600 temperature stage connected to a TMS 94 temperature programmer (Linkam Scientific Instruments Ltd, Waterfield, UK). A small amount of each sample (pure drug and lipid, microparticles and physical mixtures) was placed on a glass slide and heated at 10 °C min^{-1} in the temperature range of 25-140 °C.

4.2.6 Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a differential thermoanalyzer, model DSC7 from PerkinElmer Inc. (Waltham, USA). 5 mg of each sample – freshly prepared lipid particles, particles which were kept for 9 months at 40 °C/75% RH and for reference the pure substances praziquantel and Precirol ATO 5 – were weighed into a gold pan with a heating rate of 10 °C/min over a temperature range from −50 to 160 °C.

4.2.7 Infrared spectroscopy

Fourier transform infrared spectroscopy was performed both in solid state and dissolved in methylene chloride with the Vertex 70 from Bruker Optics GmbH (Fällanden, Switzerland). Spectra were taken from the single substances praziquantel and Precirol ATO 5, freshly prepared lipid particles and particles which were kept for 9 months at 40 °C/75% RH.

4.2.8 Dissolution studies

Drug dissolution was performed according to USP 30 specification. Two different media were tested: Phosphate buffer pH 6.8 containing 5% (w/w) Solutol HS15 as surfactant and purified water with 1% (w/w) soybean lecithin (Lipoid S 100). Lecithin was used as surfactant to examine its influence on drug dissolution from the lipid matrix. 900 ml medium were used and the particles were weighed to reach a maximum concentration of 100 ppm praziquantel. Paddle speed was set at 100 rpm and temperature at 37 ± 0.5 °C. Samples were taken after 1, 3, 5, 10, 15, 30 and 60 min, filtered and the drug content was determined by HPLC (method see Chapter 2.2.4).

4.2.9 Stability measurements

Stability with the lipid particles were conducted to detect any changes in the formulation which were characterized by dissolution studies. The samples were stored for three, six and nine months at 25 °C/60% RH, 30 °C/65% RH and 40 °C/75% RH. The dissolution studies were performed as described in the previous chapter with phosphate buffer pH 6.8 containing 5% Solutol HS15 as surfactant.

4.3 Results and discussion

4.3.1 Development of lipid particles

Two different manufacturing procedures were used for preparation of praziquantel-Precirol ATO 5 particles. The first method included a manually preparation of the lipid particles. A melt suspension was spread onto glass plates and the obtained product was ground in a mortar. The melt was kept in the water bath at 70 °C only long enough to achieve a homogeneous dispersion of drug in the molten lipid. Yet, a small amount of active ingredient might have become dissolved as it is partially soluble in Precirol. The particles prepared by this technique appeared more or less as a fine white powder with no perceptible smell of praziquantel but rather of Precirol which has a marginal sweet smell of milk. Drug content of the lipid particles gave an average praziquantel yield of 24.7% for different batches which is very close to the theoretical value (25%).

The particles prepared by the company Brace were produced from a complete melt mixture through a vibrating nozzle system. The obtained product consisted of spherical and free flowing microspheres. However, they should have had a desired size of 100 and 200 μm and a drug loading of 25 and 37.5%. While the last goal was achieved with an average drug content of 25.2 and 37.7%, respectively, the size requirements could not be fulfilled as the viscosity of the lipid melt was too high for those small nozzles. Instead, particles with a specified size distribution between 300 and 450 μm were produced which in fact were much bigger than the lipid particles manufactured manually. Another noticeable difference to the hand made lipid particles was the remarkable pink color of the particles obtained by this preparation method. No degradation products of the active ingredient could be found so that the reason for this might be the high production temperature: the lipid drug mixture had to be heated up to 150 °C to reach the needed viscosity for the production procedure. This was beyond the melting temperature of praziquantel (melting ranges for praziquantel are described at 136-142 °C (USP, 2007) and 136-140 °C (Cioli & Pica-Mattoccia, 2003)) which leads to the conclusion that praziquantel was also molten and hence during solidification a solid solution might have been produced. As praziquantel is also partially soluble in Precirol ATO 5 this might have led to the bright pink color. In contrast to these particles, the hand made lipid particles had only been heated up to 70 °C so that a suspension of solid praziquantel in molten Precirol was obtained. Thus, the largest drug fraction was not dissolved but dispersed in the lipid

matrix and no coloration could be seen.

4.3.2 Particle size distribution

The size distribution of the different lipid particles was examined for further characterization, especially in respect of drug dissolution from the lipid matrices which is strongly dependent on the surface area and thus on the size of the microparticles. The results of particle size measurements are displayed in Tab. 4.2.

formulation	mean diameter	D (v, 0.1)	D (v, 0.9)
TG 2159	137 μm	38 μm	269 μm
TG 2159/2	193 μm	34 μm	400 μm
TG 2159/3	189 μm	35 μm	384 μm
Brace 25%	408 μm	347 μm	471 μm
Brace 37.5%	388 μm	326 μm	444 μm

Table 4.2: Particle size distribution of lipid particles

The size of the lipid particles prepared by hand containing only praziquantel and Precirol was very small with an average of 137 μm. Size distribution lay between 38 and 269 μm for 80% of the particles which is a quite large range but can be explained be the manufacturing process: the particles are ground in a mortar by hand and thus no homogenous particle size can be achieved as already small particles might be milled even further, especially if they are frozen. This had to be the case because otherwise the lipid became soft and no milling effect could be realized. About 3% were bigger than 350 μm which is mainly caused by particle agglomeration as the particles were passed through a 315 μm sieve. Yet, as the particle size is calculated assuming a spherical shape it might be possible that some irregular shaped particles were estimated to be bigger than they actually were.

The lipid particles produced by hand with 2 and 3% Aerosil 200 had a slightly higher mean diameter (193 and 189 μm, respectively) and also a wider size distribution: Lipid particles containing 2% Aerosil ranged between 34 and 400 μm and those with 3% Aerosil were between 35 and 384 μm. The wider size distribution which is the main factor for the higher average size was most probably evoked by a more pronounced agglomeration of the individual particles. The reason for this might be the incorporation of Aerosil into the lipid matrix. This substance has a very large volume and surface area and therefore the particles might get stuck more easily than without Aerosil.

Particle size of the lipid particles manufactured by the company Brace was supposed to lie between 300 and 450 μm. An average size of 408 μm was determined for the particles containing 25% praziquantel and the particles with 37.5% active ingredient were 388 μm in average size. So both formulations were within the specified spectrum, yet at the top which was a bit disappointing because smaller particles were

desired. On the other hand, the size distribution of these lipid particles was much narrower compared to the hand made particles (see Tab. 4.2). For particles with 25% drug it lay between 347 and 471 μm and lipid particles containing 37.5% active ingredient had a size distribution between 326 and 444 μm. This is due to the manufacturing process through a vibrating nozzle system; with this nozzle system droplets of a size defined by the nozzle diameter and the viscosity of the suspension are created, which solidify to form the microspheres.

4.3.3 Scanning electron microscopy

For further characterization of the lipid particles scanning electron microscopy (SEM) was employed. Pictures of two lipid formulations prepared by hand or by Brace with different magnifications are shown in Fig. 4.1 and 4.2. Here, the differences between the hand made product and the particles prepared by Brace became obvious again: while all hand made formulations have a quite large size distribution and a non-uniform appearance with oddly shaped particles and agglomerates, the Brace microspheres are nearly completely round and seem to be much more homogeneous and smoother. This, of course, once again results from the manufacturing process. But if the preparation of the hand made formulations is considered with breaking the solidified melt in a mortar, the particles seen in the micrograph are rounder and not as angular as could have been expected. The reason for this might be the softening of the lipid during grounding in the mortar. While this is true for the particles without Aerosil, those containing Aerosil are slightly more irregular and show a higher degree of agglomeration (pictures not shown). As stated above for the results of the higher size distribution, this might be due to the incorporation of this voluminous substance, which, because of its large surface, might induce the observed agglomeration. The product from Brace on the other hand is very homogeneous showing only spherical particles. Small irregularities of the complete spherical shape can be seen, yet they could be a result of electrostatic charge during microscopy. Compared to the hand made particles, these are quite large which has already been demonstrated by particle size analyses and could of course also be observed by the human eye.

While the surface of the Brace particles seems to be quite plane on lower magnification, on closer examination it can be seen that it is not completely smooth but consists of small leaf-shaped crystallites. These structures appear in all lipid particles, although they seem to be most pronounced in the hand made ones. In these formulations the small plates are present as nearly separate elements whereas the surface of the Brace particles is more homogeneous as these crystallites seem to be molten together and hence are better linked between each other. One reason for this leaf-like formation might be the fast solidification of the lipid melt. The lipid crystallizes very rapidly and thus might build small plates from single seed crystals which are not connected to each other but sort of crystallize next to each other. This assumption could be supported by pictures from Precirol particles which were also prepared by melt solidification (Pongjanyakul et al., 2004; Craig, 2004; Albertini et al., 2004). Another possible explanation for the lipid scurf might be the so-called "blooming". This

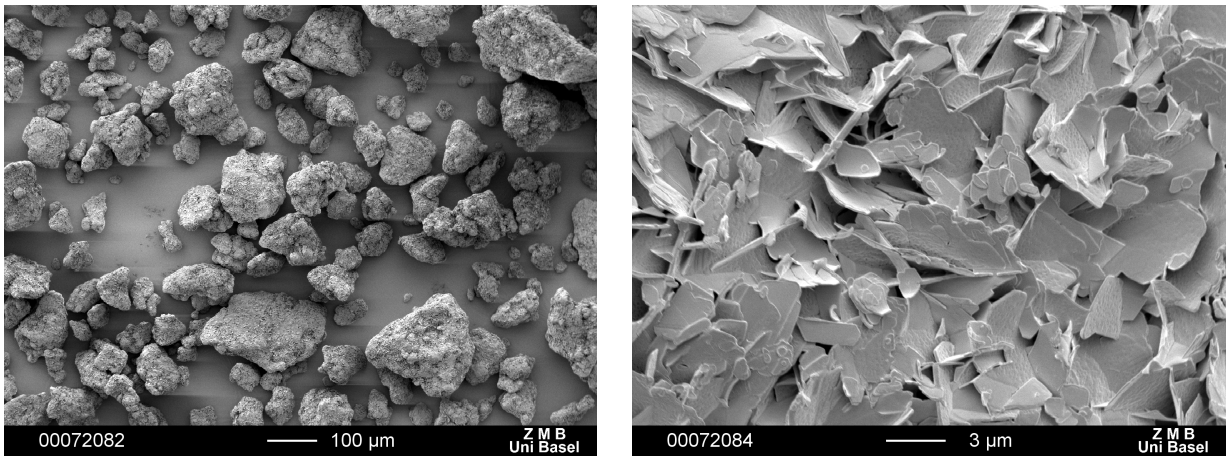

Figure 4.1: Micrographs of lipid particles prepared by hand (TG 2159).

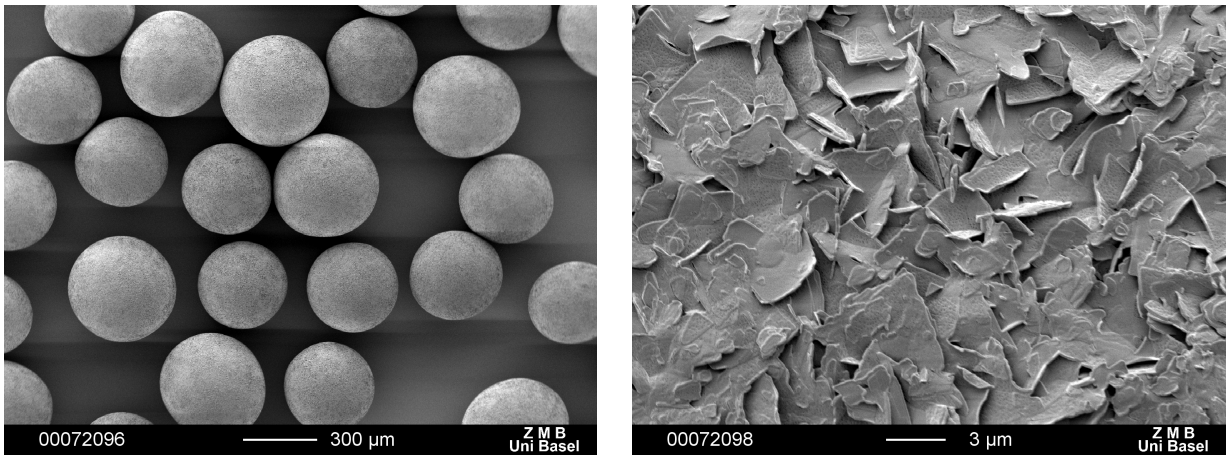

Figure 4.2: Micrographs of lipid particles prepared by Brace (37.5% PZQ).

describes the development of large crystals on storage of fat systems and is generally associated with crystal alterations due to polymorphic changes (Khan & Craig, 2004). As Precirol is well known to be found in different polymorphic forms (Hamdani et al., 2003; Reitz & Kleinebudde, 2007; Evrard et al., 1999) this might be a possibility for the formation of this leaf-like structure. Moreover, the particles were not investigated straight after production but were stored for a couple of months at ambient temperature. Hence, the rough characteristic of the surface could also be attributed to polymorphic changes.

Interestingly, small dots can be seen in the largest magnification on the surface of the particles without Aerosil (both hand made and Brace). A possible explanation for this phenomena might be drug crystals either crystalline or in solid solution. This assumption could be supported by the fact that these dots are more pronounced on the surface of the Brace particles containing 37.5% praziquantel. In the particles with Aerosil the drug crystals might be covered by that substance and might hence not be detectable.

Overall it can be stated from these micrographs that the appearance of the lipid

particles is indeed as expected: while the hand made particles are inhomogeneous with a wide size distribution the Brace particles are regular and spherical shaped. Surprising was the leaf-shaped surface of all particles, however, this is identical for all products and thus most probably characteristic for the lipid matrix. Furthermore, it could be ascertained that the overall surface of the hand made lipid particles is much larger than that of the Brace ones. This of course is a result of the smaller particle size but also of the more heterogeneous appearance in contrast to the spherical Brace products.

4.3.4 Differential scanning calorimetry

Differential scanning calorimetry relies on the principle that solid-state modifications are characterized by different melting points and melting enthalpies (Jaspart et al., 2005). Hence it was used to characterize the differently prepared lipid particles in respect of crystallinity, stability or interactions between lipid and active ingredient. The thermograms of the examined particles and also of the pure substances are shown in Fig. 4.3 to 4.7. Pure praziquantel has a strong endothermic peak at 140 °C for a single crystalline modification (see Chapter 3.3.3, Fig. 3.4), which is in agreement with the melting point of the racemic form of the drug (El Arini et al., 1998; Liu et al., 2004; De La Torre et al., 1999). Precirol is found in different polymorphic forms which becomes obvious in Fig. 4.3: the raw untreated material exhibits a single, yet rather broad endothermic peak at 60.3 °C. This is in good correspondence with data found in literature (Hamdani et al., 2003; Evrard et al., 1999). However, if the heated sample is cooled rapidly and then re-heated, other modifications appear in form of two other peaks at lower temperature. During storage the lowest melting endotherm is lost and an increase in the height and sharpness of the higher melting endotherm is observed (Hamdani et al., 2003; Evrard et al., 1999). Moreover, in the study of Evrard et al. (1999) in Precirol samples stored at 40 °C for ten days another, yet small peak could be detected in the DSC thermogram which might be attributed to further changes in the modification of Precirol.

In the thermograms of all praziquantel-Precirol particles the characteristic peak of the crystalline drug disappeared from which could be concluded that the modification of the active ingredient changed due to incorporation into the lipid matrix or during heating in the DSC measurements. Moreover, only one distinct peak can be seen in the thermograms of the lipid particles which is close to the peak of Precirol followed by a small endothermic area up to ca. 110 °C. There might be several explanations for this phenomena: one reason might be that praziquantel and Precirol result in an eutectic mixture thus leading to a decrease in melting temperature (Leuner & Dressman, 2000). The eutectic temperature would in this case lie near the melting range of Precirol and hence probably also at a high ratio of Precirol to praziquantel. Another explanation could be that the active ingredient incorporated into the lipid matrix was either in a solid solution state or in an amorphous or disordered-crystalline phase in a solid dispersion so that it cannot be detected by DSC (Albertini et al., 2004; Mainardes et al., 2006). A further possibility might be that the active in-

gredient is dissolved in the lipid matrix during the heating of the DSC measurement as praziquantel is partially soluble in Precirol. It could thus be thoroughly possible that all mechanisms are the cause of this occurrence: Precirol and praziquantel could form an eutectic mixture, due to manufacturing part of the active ingredient is either dissolved in the lipid matrix or in an amorphous form and the rest of the crystalline drug substance dissolves in the molten lipid during heating.

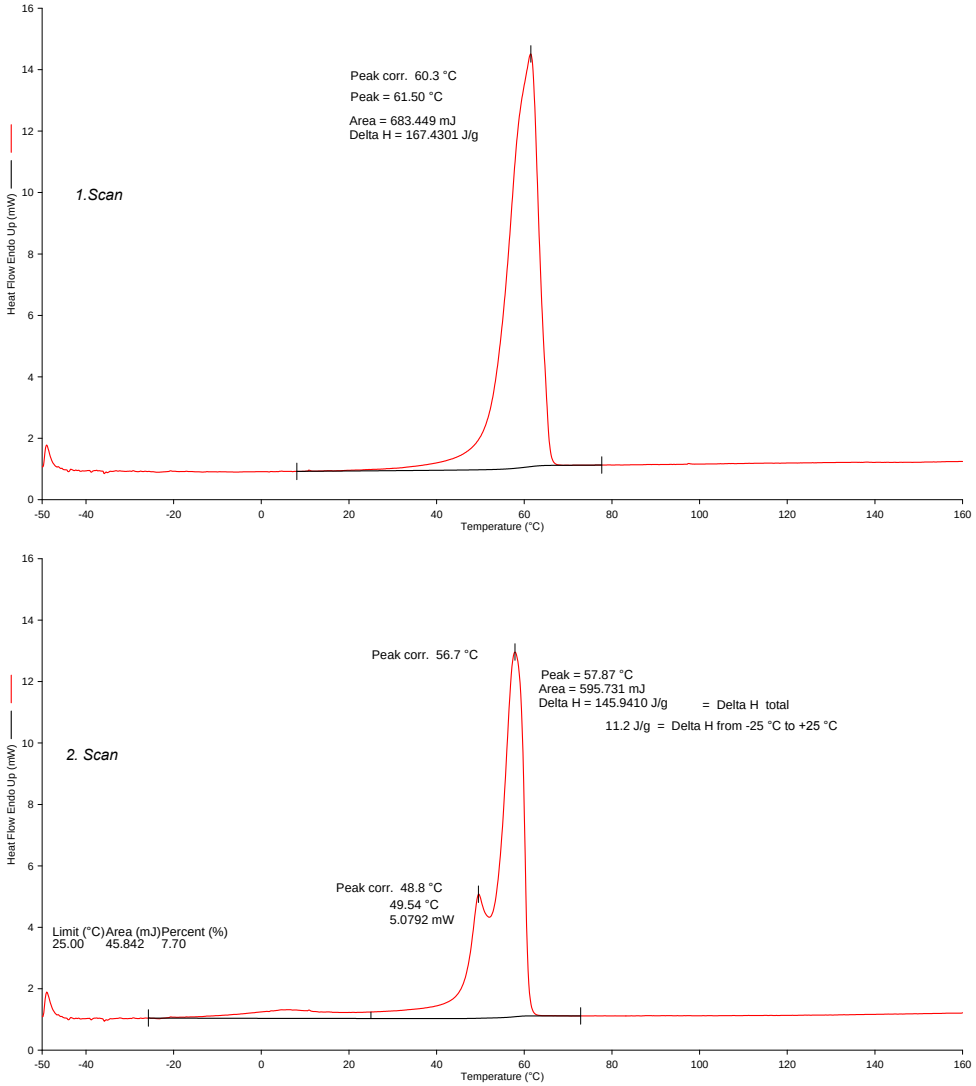

Figure 4.3: DSC thermogram of Precirol ATO 5; second scan (lower plot) was performed straight after the first scan.

With HSM (see following chapter) it could be observed that after melting of Precirol drug crystals were present which disappeared during further heating. This might be attributed to two of the above described mechanisms: either the active ingredient was dissolved in the lipid melt or, because of the formation of an eutectic mixture, the melting point of praziquantel was lowered and thus it melted earlier. Of course it is also possible that both phenomena occurred and the effects are overlapping. However, both incidents are supported by the small endothermic area between

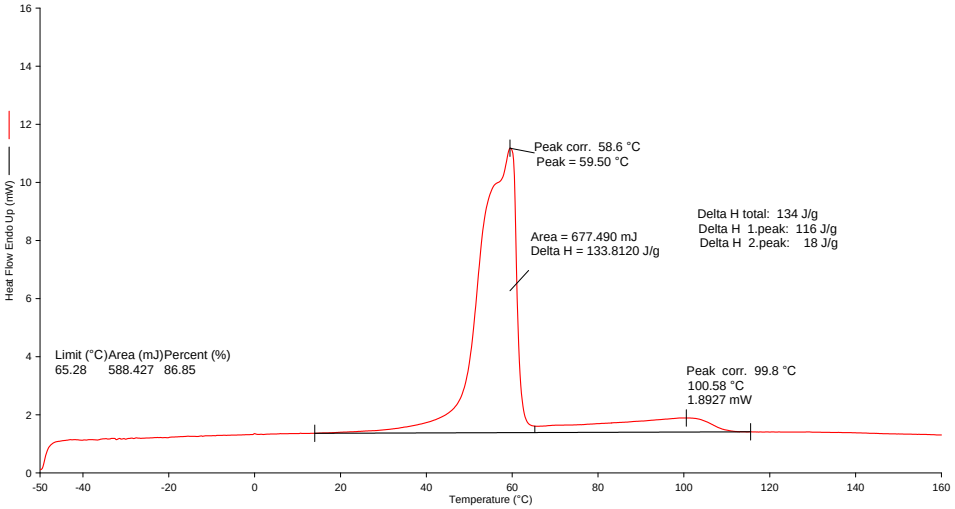

Figure 4.4: DSC thermogram of fresh lipid particles prepared by hand.

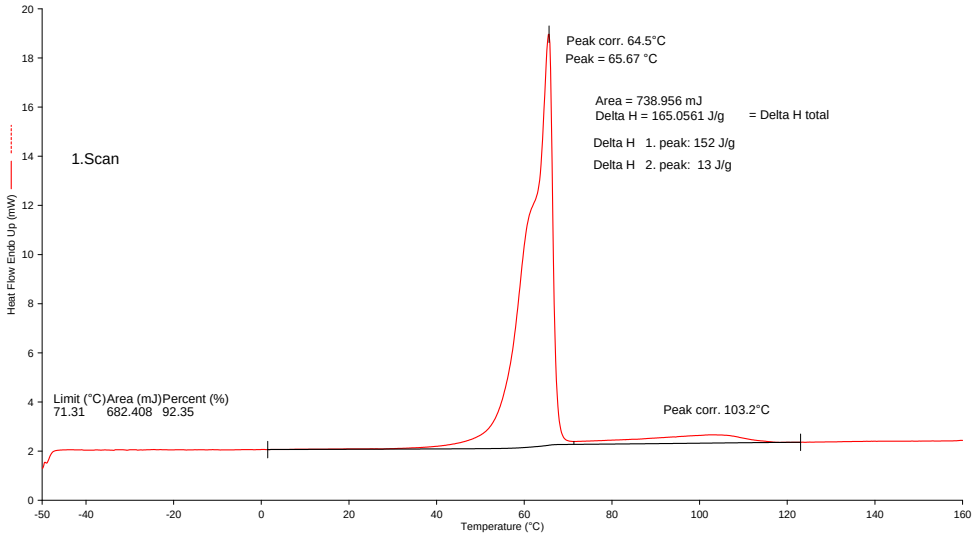

Figure 4.5: DSC thermogram of hand made lipid particles stored for nine months at 40 °C.

ca. 75 to 110 °C, which is detected in all probes. This is hence most probably assigned to residual crystalline praziquantel either dissolving in the molten lipid or melting itself. As this peak appeared in all samples it can be concluded that at least a small amount of praziquantel is in crystalline form in the lipid matrix in all tested particles.

Two samples of the lipid particles prepared by hand were investigated: More or less freshly prepared substance (stored at room temperature for one month) and one sample which had been stored for 9 months at 40 °C so that any changes occurring during storage could be detected (as they should be most pronounced in a probe stressed at 40 °C over a longer period of time). The thermograms of these samples are displayed in Fig. 4.4 and 4.5. Differences can be seen with a higher temperature onset, an overall higher peak temperature, a more pronounced shoulder in the first peak and

also a greater enthalpy in the case of the stored probe. On the first thought this might be attributed to precipitation of drug crystals in the lipid matrix during storage. While in the fresh sample part of the drug might be present molecularly dispersed in form of a solid solution, this fraction might decrease over time due to re-crystallization (Craig, 2004; Vasconcelos et al., 2007; Bodmeier et al., 1990). However, if the stability data from dissoluion studies both from the pure particles or incorporated in a paste (see Chapters 4.3.8 and 5.3.5) are regarded this effect becomes less probable: in both dissolution studies a faster drug disintegration occurred if the probe was stored at 40 °C. An explanation of this phenomena might be that at that temperature the lipid becomes soft (see Fig. 4.3) and hence, more drug can be dissolved in the matrix. Yet, this would not explain the more pronounced peak in the thermogram as more drug crystals should thus be present in a molecularly dispersed state and hence no energy would be needed to further dissolve these molecules. However, it might be possible that due to further drug dissolution in the matrix the ratio of drug to lipid in the eutectic mixture is shifted towards more drug which then might lead to an increase in the overall melting temperature. Moreover, the small endothermic area between ca. 75 to 110 °C which could be attributed to the remaining praziquantel melting or dissolving in the lipid is less than in the freshly prepared sample which leads to the conclusion that more active ingredient is already molten or dissolved in the carrier up to about 70 °C.

Of course, changes in the thermogram of a lipid due to treatment or storage could also be attributed to changes of its modification (Jannin et al., 2006; Hamdani et al., 2003; Evrard et al., 1999; Jaspart et al., 2005). Lipids are complex substances and their modification are often easily changed by many factors such as time, temperature or interaction with other substances. Hamdani et al. (2003) observed a shift in the melting range towards higher temperatures in aged Precirol samples (two weeks at 40 °C). Evrard et al. (1999) investigated Precirol samples stored for ten days at 40 °C and here a second peak appeared in the DSC which neither fit to the modifications of freshly solidified Precirol detected between 45 °C and about 58 °C nor to its stable modification. Therefore it could also be possible that the differences between the thermograms of the fresh and stored samples are evoked by changes in lipid modification.

In contrast to the samples of the lipid particles prepared by hand, those manufactured by Brace exhibit one clear peak at about 56 °C and also the further endothermic area up to about 110 °C to 120 °C (see Fig. 4.6 and 4.7). Here, the assumption of the drug being either in an amorphous form or molecularly dispersed in a solid solution in the lipid matrix can be adopted. This would most probably be due to the manufacturing process: as described earlier, the samples produced by Brace were heated beyond the melting temperature of praziquantel and kept there for a certain period of time. Thus, a solution of molten drug and lipid was formed. During cooling the drug might have either solidified molecularly dispersed in the lipid as praziquantel is partially soluble in Precirol or it precipitated in an amorphous form from the melt. Of course, both phenomena might have occurred simultaneously. However, with a drug concentration of more than 25% it can be presumed that the solubility limit of

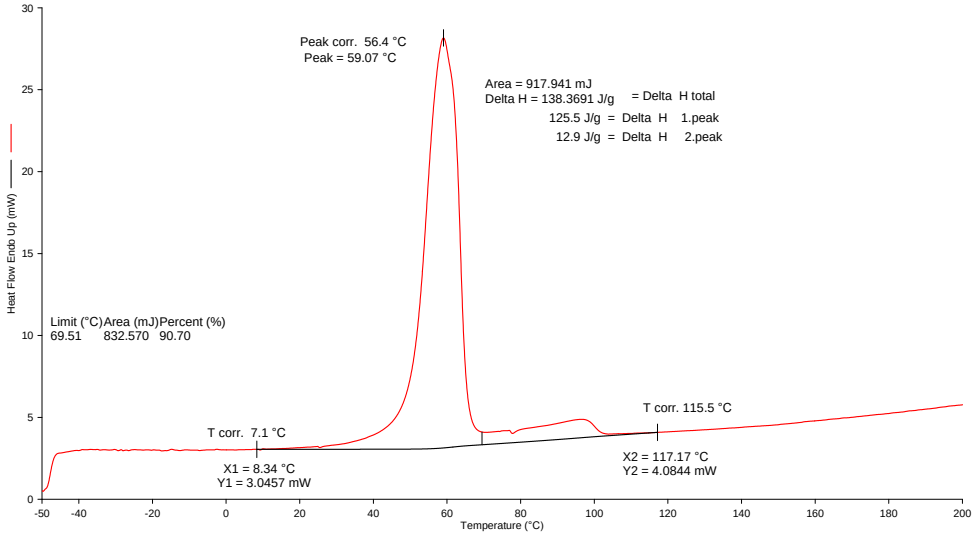

Figure 4.6: DSC thermogram of the Brace lipid particles containing 25% PZQ

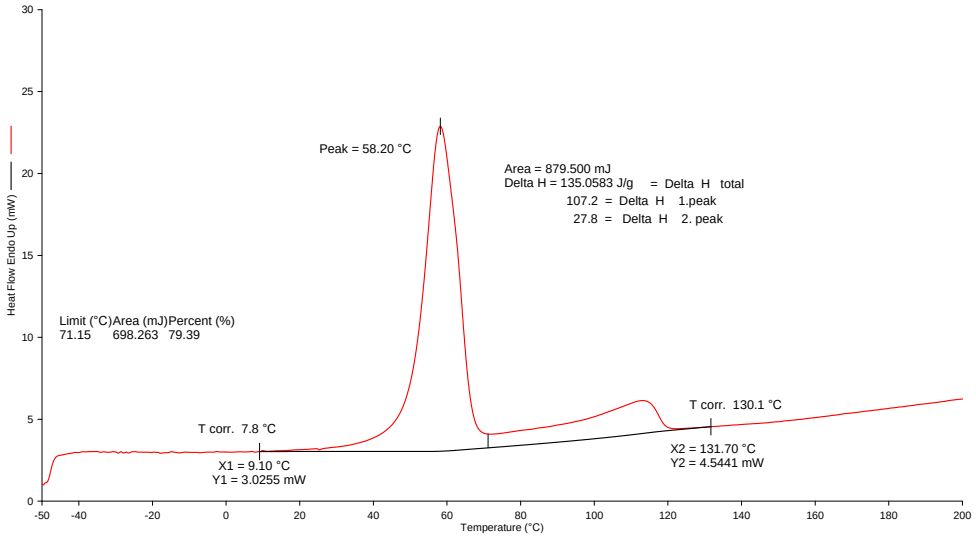

Figure 4.7: DSC thermogram of lipid particles prepared by Brace with 37.5% praziquantel

praziquantel in Precirol was exceeded and hence, even if all drug was at one stage dissolved in the lipid, some part precipitated again, most probably in a crystalline form. This fraction is then either dissolved or molten at higher temperatures thus leading to the endothermic area after the main peak. This assumption that the endothermic area up to about 120 °C is exhibited by residual praziquantel melting or dissolving can be supported by the differences between the two Brace samples: in the probe containing 37.5% active ingredient the endothermic value for this area is larger and the peak also goes up to higher temperatures. It can thus be concluded that more drug is present in a crystalline state in the lipid matrix and more energy is needed to either dissolve or melt this amount.

From the DSC measurements it can still not definitely be stated if praziquantel and Precirol form an eutectic mixture and/or if the active ingredient is dissolved in the lipid at least partly during manufacturing and also during heating in the DSC. However, assumptions can be made especially if results from HSM are taken into consideration (see following chapter): at least some part of the active ingredient is present in crystalline form in the lipid and hence is molten or dissolved in the lipid during heating, thereby exhibiting an endothermic peak. As to the rest of the drug no clear statement can be made if it is either molecularly dispersed in the lipid thus forming a solid solution or in an amorphous state – in both cases it cannot be detected by DSC and thus no differentiation can be made. However, on the basis of the differences in the manufacturing procedure it can be assumed with high probability that in the case of the Brace particles more drug is present in a solid solution or even amorphous due to precipitation – the latter on the other hand is not very probable for the hand made particles. Here during manufacturing the suspension was only heated up to 70 °C so that most of the drug was suspended and not molten as in the case of the process used by Brace. Thus, the active ingredient was not in a liquid form and it is hence highly unlikely that it transformed from a stable crystalline state into an amorphous one. So it can be hypothesized that in the particles prepared by hand most of the drug is present in crystalline form as a solid dispersion and that only a small part of it might be molecularly dispersed in the lipid. It is yet possible that this fraction can be increased if the particles are stored at 40 °C as the lipid softens and thus more drug molecules might get dissolved – of course only up to the solubility limit of praziquantel in Precirol. The substance manufactured by Brace also contains crystalline fractions of praziquantel which might have re-crystallized from a solid solution or an amorphous state. This was either driven by exceeding the solubility limit of praziquantel in Precirol or due to transformation from an energetic unfavored state to a stable one. The remaining drug amount is hence either molecularly dispersed in a solid solution state or occurs amorphous. Further characterization of the drug modification in the lipid matrix would have been possible by x-ray diffractometry, however, this was outside the scope of this work.

4.3.5 Hot stage microscopy

To confirm the assumptions from DSC measurements, physical changes in the samples during heating were monitored performing hot stage microscopy (HSM) studies. It could be shown that in all manufactured samples at least part of the drug was present in crystalline form in the lipid matrix (see Fig. 4.8). As observed in the pure sample, melting of Precirol started in nearly all investigated probes around 53 °C and was finished at about 60 °C. After melting of the crystalline Precirol, drug crystals could be detected which disappeared in the lipid during further heating and was completed by about 120 °C. However, it became not definitely clear whether this phenomena is evoked by drug dissolving in the lipid or melting due to formation of an eutectic with Precirol. The latter mentioned process might be supported by the observation that drug crystals vanished in the lipid melt only during further heating;

if the probes were kept at constant temperature no movement of particles could be detected. However, another possible explanation could be a temperature dependent solubility of praziquantel in Precirol or a combination of both incidents.

By analysis of the particles prepared by hand compared to those manufactured by Brace clear differences could be seen in the appearance of the drug crystals: in the hand made formulation the drug crystals were identical to that of pure praziquantel appearing in rather large needle-shaped crystals. Due to the size of the crystals the disappearance of the drug in the lipid took a bit longer compared to the Brace sample containing 25% active ingredient in which very fine, almost filigree drug crystals arranged in a nearly regular structure were found. This confirms the hypothesis that these crystals are formed by precipitation from a supersaturated (solid) solution which is formed during the manufacturing procedure by a melt mixture. This assumption is further supported by the fact that more drug crystals could be detected in the probe prepared by Brace containing 37.5% praziquantel (pictures not shown). In this case it also took longer (up to 130 °C) until all drug particles had disappeared. This occurrence might also be used to support the assumption of formation of an eutectic mixture so that a higher temperature is needed to dissolve the higher amount of praziquantel.

A small difference in the melting behavior could be detected between a fresh hand made sample and one put on stability for nine months at 30 °C: while in the first case the lipid had completely melted at 60 °C and thus drug crystals could be detected in the melt separately this was not the case for the latter mentioned sample. Here, thick crystal aggregations could still be observed at 60 °C (pictures not shown). However, it could not clearly be determined whether these crystals can be attributed to not fully melted Precirol or to the existence of more drug in crystalline form due to precipitation during storage. This incident did not occur in the sample stored at 40 °C which behaved as the freshly prepared probe so that hence the hypothesis of drug precipitation at 30 °C might be supported. However, it has to be considered that with HSM only a very, very small fraction of the substances are investigated and thus occurring differences should not be overestimated.

Finally, physical mixtures of praziquantel and Precirol with 5%, 10% and 25% active ingredient were examined regarding their melting behavior (pictures not shown). The mixture containing 25% drug behaved similar to the lipid particles prepared by hand except for the occurrence of separate drug crystals which melted at 140 °C. The mixture containing 5 and 10% active ingredient were observed between two glass plates so that the separate crystals were included into the lipid melt. It could be noticed that in the mixture with less drug all crystals had disappeared at lower temperature (about 85 °C) while in the case of 10% praziquantel the sample had to be heated up to approximately 100 °C until all drug had vanished. This supports both assumptions of a temperature dependent solubility or the forming of an eutectic mixture. Of course, both phenomena might occur simultaneously. Nevertheless, from this it might be assumed that the solubility of praziquantel in molten Precirol at 60 °C (and hence also in solid Precirol) is lower than 5%.

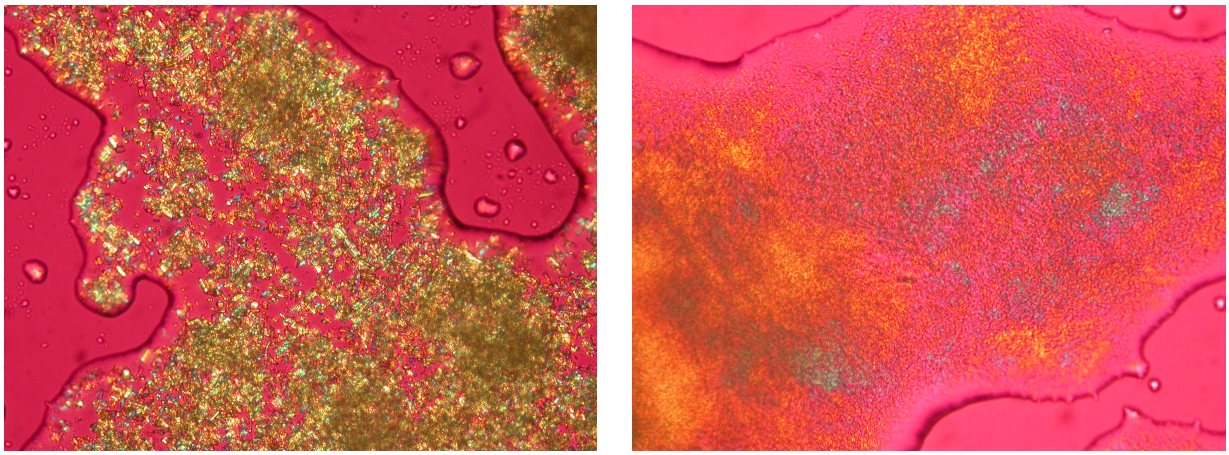

Figure 4.8: Microscopic pictures of lipid particles prepared by hand (TG 2195; left) and those produced by Brace (right); drug content 25%; pictures were taken after melting of Precirol (60 °C).

Interestingly, after melting of pure praziquantel, the recover of crystal structure at room temperature was delayed whereas Precirol solidified in another crystalline modification than fresh substance at about 50 °C down to 40 °C. This is in accordance with the findings during a second heating in DSC where other peaks could be detected for Precirol leading to the assumption of a different modification (see previous chapter).

With the HSM studies the presumptions gained from DSC measurements could be approved. It could be shown that at least part of the active ingredient is present in crystalline form in all lipid samples and that this fraction dissolves or melts in the lipid during further heating. Moreover, it could be demonstrated that the drug crystals appear in different forms in the samples prepared by hand compared to those manufactured by Brace. This supports the theory that in the case of Brace particles drug substance precipitated from a solidified melt mixture probably due to exceeding of solubility. In addition, small differences in melting behavior and crystallinity could be detected between fresh samples and those put on stability. However, it could still not definitely be stated if the disappearance of the drug crystals in the lipid melt during heating is due to (temperature dependent) dissolution or melting or a combination of both phenomena.

4.3.6 Infrared spectroscopy

Infrared (IR)-spectra were measured both in solid state in transmission and in dichloromethane solution. The spectra of the samples pure praziquantel and Precirol, the particles prepared by hand right after manufacturing and after 9 months at 40 °C/75% RH and the particles produced by Brace containing 25% drug are displayed in Fig. 4.3 to 4.6. The hand made particles did not seem to change in their modification detectable by IR during storage as the spectra of the two measured samples are nearly identical. On the other hand, small differences especially in the fingerprint

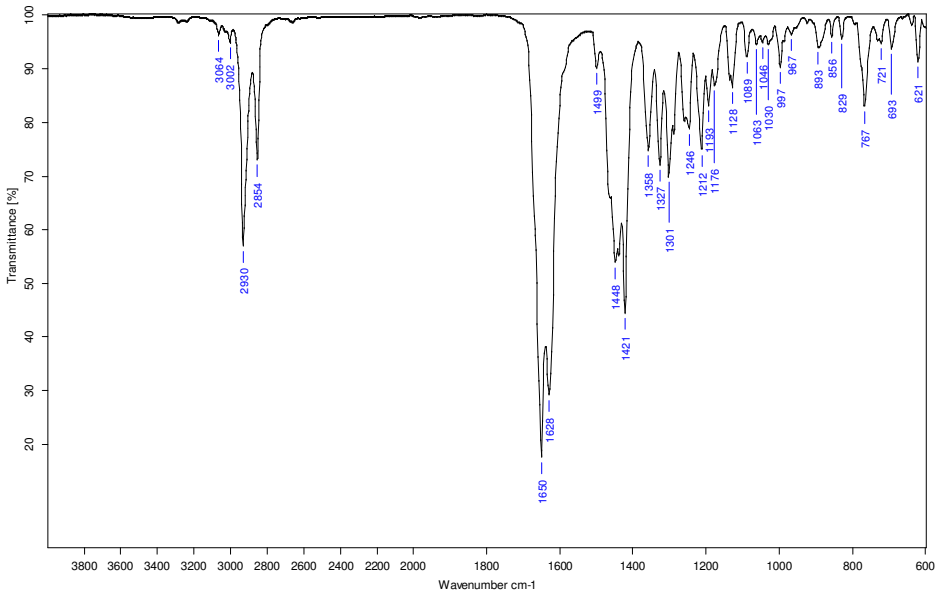

Figure 4.9: IR-spectrum of praziquantel; FTIR in transmission

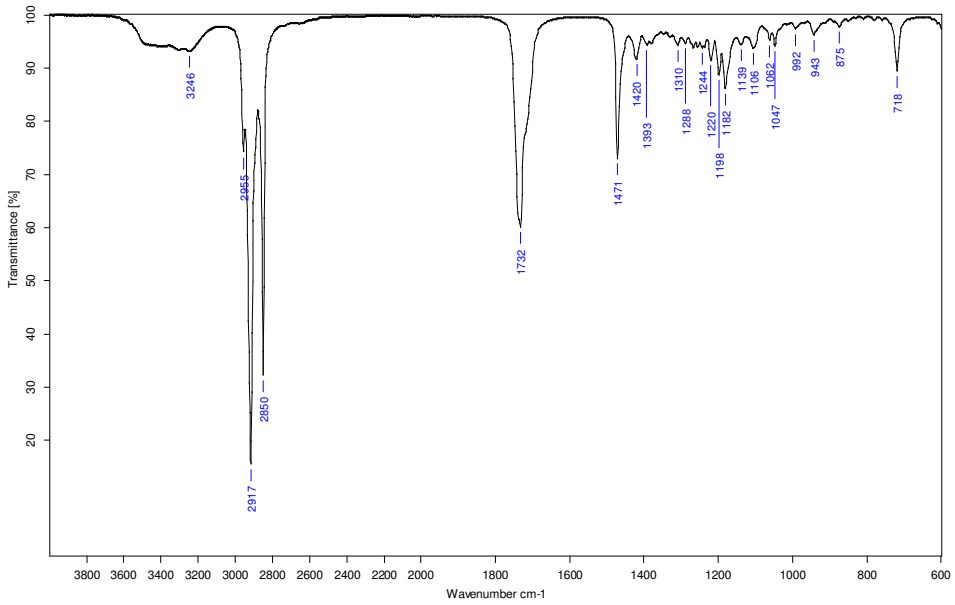

Figure 4.10: IR-spectrum of Precirol ATO 5; FTIR in transmission

area can be detected between these particles and the Brace sample when measured in solid state. As nearly no discrepancy is seen between all particles in solution, it can be assumed that the dissimilarity in solid state is a result of differing drug crystallinity. This, of course, most probably arouse from the manufacturing process as described in the previous chapters. Part of the active ingredient in the substance prepared by Brace most probably occurs either in a molecularly dispersed and/or amorphous form; the crystalline part precipitated from a solid solution. In contrast to this, the particles prepared by hand solidified from a suspension and hence although some fraction might

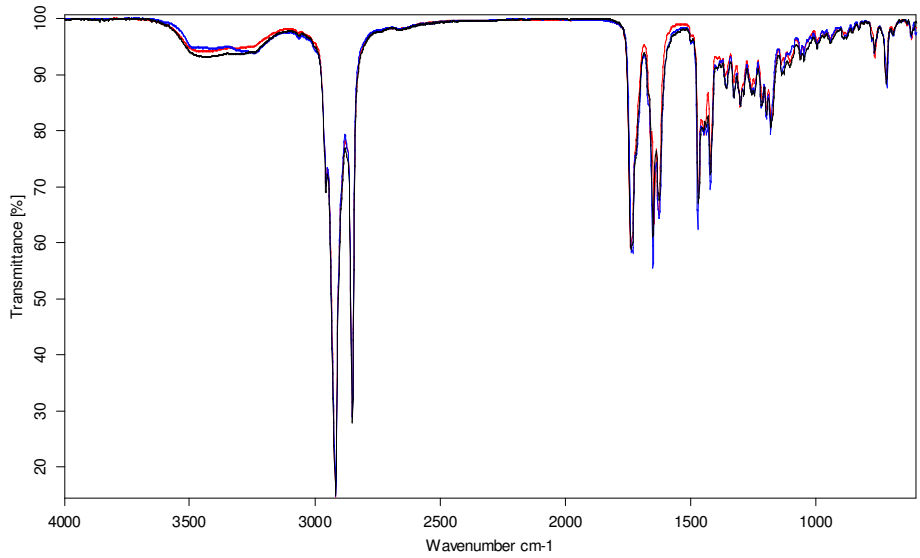

Figure 4.11: FTIR-spectra in transmission: black: hand made particles straight after production; blue: hand made particles after 9 months at 40 °C; red: Brace particles; drug content in all samples 25%

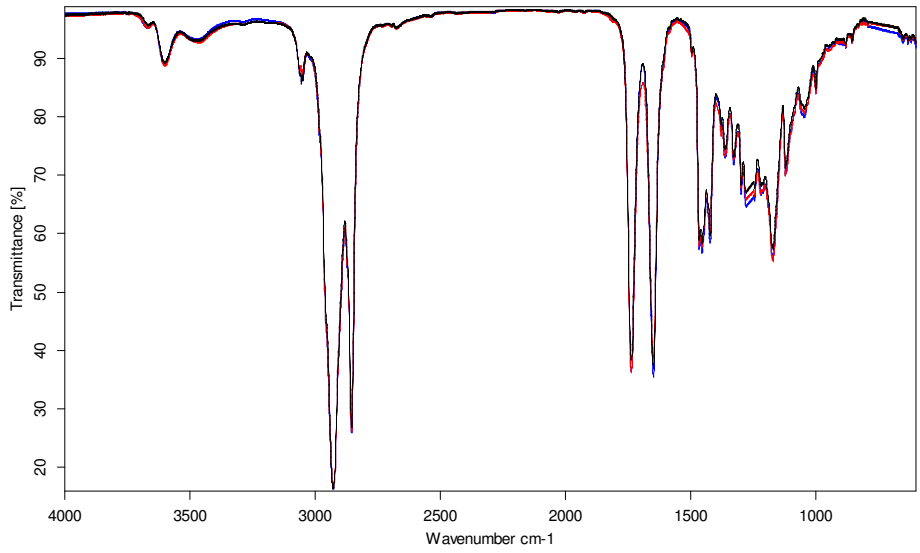

Figure 4.12: IR-spectra in solution: black: hand made particles straight after production; red: hand made particles after 9 months at 40 °C; blue: Brace particles; drug content in all samples 25%

be dissolved in the lipid most drug molecules are present in crystalline form in a solid dispersion. These discrepancies are most likely the reason for the differences in the IR-spectra in solution.

4.3.7 Dissolution studies

Different dissolution studies were performed to characterize the disintegration behavior of praziquantel from the Precirol matrix. The hand made particles containing no or a small amount (2 or 3%) Aerosil 200 were studied in respect to an enhanced dissolution rate upon the inclusion of a hydrophilic substance. Moreover, it was investigated whether the use of Lecithin as a surfactant has an influence on drug disintegration from the lipid matrix. Finally, the dissolution profiles of the particles prepared by the different manufacturing methods were compared.

The drug dissolution profiles of the lipid particles prepared by hand with and without Aerosil are displayed in Fig. 4.13. It can clearly be observed that embedding in Precirol led to a delayed drug release compared to pure praziquantel. After 5 min about 25% active ingredient had disintegrated and this amount had risen to nearly 60% after one hour. Yet, this is quite rapid for an insoluble matrix, which normally leads to a sustained release; however, this is most probably due to the overall large surface as the particles are quite small and also irregular shaped (see Chapter 4.3.3). Another possibility might be the slight drug solubility in Precirol so that dissolution could be enhanced, especially at 37 °C where Precirol might soften and hence, more praziquantel might get dissolved in the matrix. It was found that the dissolution profile follows a square root of time relationship (R^2=0.96) thus indicating a Fickian diffusion-controlled mechanism (Ozyazici et al., 2006). This assumption is supported if the properties of both matrix and active ingredient are regarded as Precirol is an inert, non-swellable matrix (Jannin et al., 2006; Marchaud et al., 2006; Savolainen et al., 2002). Other factors influencing drug disintegration from an inert lipid matrix would be erosion and digestion (Jannin et al., 2006; Marchaud et al., 2006; Voigt, 2006). While the first mechanism mentioned is of minor importance for these microparticles, digestion will play an essential role in vivo. When the matrix is digested drug disintegration and hence also absorption should occur faster and a biphasic run of the dissolution curve should be observed. This occurrence would be desired as no sustained release formulation was intended but the major drug amount (70%) should be released after one hour (NAH, 2003). This requirement was just not achieved with the particles prepared by hand containing only Precirol and praziquantel.

For that reason, the addition of a hydrophilic substance was tested to enhance the dissolution profile. 2 and 3% Aerosil 200 were employed as this was shown to have the best characteristics needed for a taste masking formulation (Albertini et al., 2004). It can be seen in Fig. 4.13 that drug dissolution was slightly slower at the beginning but after about 20 to 30 min the dissolved drug amount rose above the level of the formulation without Aerosil. This is most probably due to the swelling properties of Aerosil: With its hydroxyl groups Aerosil is able to form hydrogen bonds with the aqueous dissolution medium, thus absorbing water on the surface. Due to this gelation ability drug release is a bit lower at the start as dissolution medium penetrating into the lipid particles is absorbed by Aerosil and the drug starts to dissolve therein. After a while though, the active ingredient diffuses from this gel layer into the dissolution medium, which then results in an improved dissolution rate. Another possibility

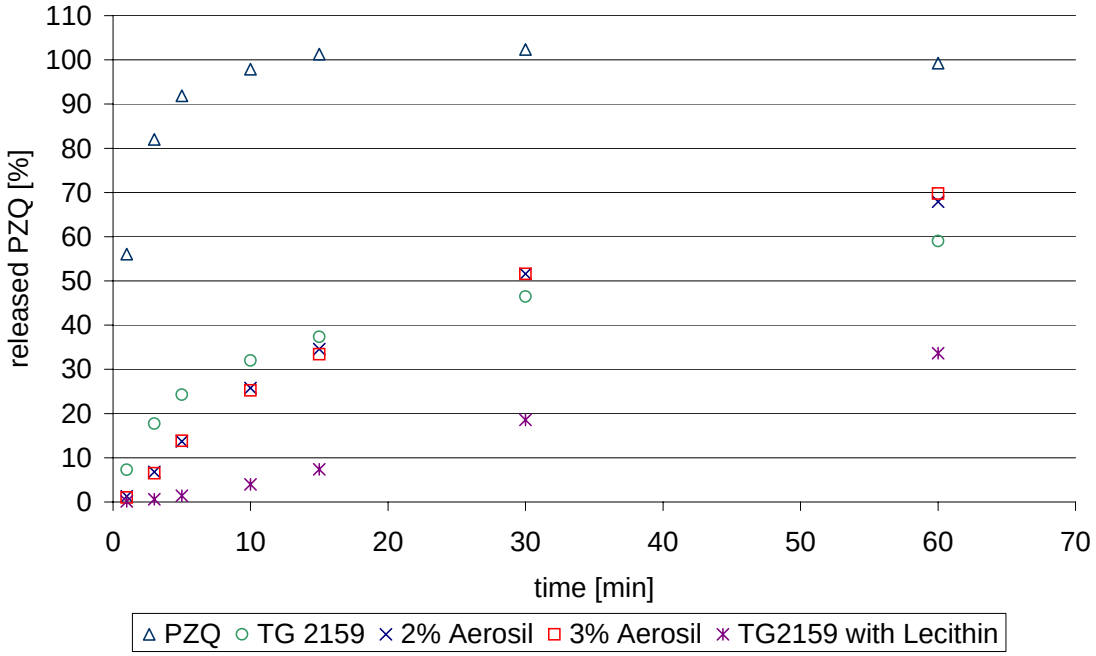

Figure 4.13: Dissolution profile of praziquantel and hand made lipid particles in phosphate buffer pH 6.8 + 5% (w/w) Solutol HS15 or 1% (w/w) Lecithin; test were run in triplicate; standard deviation did not exceed 5%

for the marginal slower disintegration start might be the slightly larger particle size of the formulations containing Aerosil compared to the one without (see Chapter 4.3.2). No significant differences could be detected between the two formulations containing 2 or 3% Aerosil – even after 120 min in both cases 85% drug had been released. It would have been expected that a higher amount of Aerosil would have led to a faster drug dissolution due to its gelation abilities. A possible explanation might be that with 2% Aerosil an optimal swelling has already been reached and further addition of Aerosil does not influence this property anymore.

So overall, it can be stated that praziquantel dissolution from a Precirol matrix is in vitro mainly induced by diffusion and hence, drug disintegration is enhanced by incorporation of a hydrophilic substance into the lipid matrix. In vivo, digestion of the lipid would also play a role so that drug dissolution is supposed to be faster.

Surfactants have a great influence on drug dissolution. As praziquantel is embedded in a lipid matrix is was thought that a lipid derived natural occurring tenside might also be appropriate to investigate drug disintegration from this lipid matrix. For these tests lecithin was chosen which is present in vivo in bile and might thus be better suitable to simulate in vivo conditions. So praziquantel dissolution from the lipid particles was tested with 1% lecithin as surfactant. The obtained curve is shown in Fig. 4.13. Compared to the results from the other test media containing 5% surfactant (Solutol HS15) drug dissolution with lecithin is slower; after 60 min just 34% active ingredient had disintegrated and after 120 min this amount had only risen

to 50%. It might have been expected that drug dissolution from a lipid matrix could have been faster in the presence of lecithin due to its properties as it might be able to include the lipid molecules into micelles upon the formation of an emulsion.

However, it has already been demonstrated that praziquantel dissolution was slower in media with lecithin (milk, fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF)) compared to dissolution in USP medium containing 0.2% sodium laurylsulfate (Dinora et al., 2005). Although these tests were conducted with tablets the same effects might be present in case of the lipid particles. Nonetheless, it could be assumed that drug absorption would be higher in the presence of food as dissolution was faster in FeSSIF than in FaSSIF and milk. This is in accordance with another study where the influence of lipids and carbohydrates on the absorption of praziquantel was examined (Castro et al., 2000). It was found that by administration of a high lipid or a high carbohydrate diet the maximum plasma levels increased 243 and 515%, respectively. The mechanism by which the bioavailability of praziquantel is enhanced by lipids and carbohydrates is yet unknown. It could be due to an increase of splanchic blood flow, to changes in luminal metabolism or to an inhibition of the synthesis of certain enzymes (Dinora et al., 2005). Besides, several investigations on oral bioavailability of poorly soluble drugs from lipid matrices have demonstrated that drug absorption from these formulations can be enhanced (Bowtle, 2000; Charman, 2000). So both effects might play a role in the absorption of praziquantel from the Precirol particles so that bioavailability could be higher than would be expected from in vitro dissolution data. However, simulation of all occurring mechanisms would be very difficult, if not impossible with in vitro dissolution studies. So, from these data it can be assumed that the use of lecithin as surfactant slightly underestimates drug disintegration in vitro as more complex mechanism are of importance for in vivo bioavailability. Yet, praziquantel being embedded in a lipid matrix might lead to an increase in drug absorption in vivo as lipids seem to have a positive influence on praziquantel bioavailability.

In Fig. 4.14 drug dissolution from the particles prepared by hand from a melt suspension and by spray congealing from a complete melt is displayed. It can clearly be seen that the active ingredient dissolves much faster from the hand made lipid particles compared to the particles produced by Brace. Of course, the main reason for that is the smaller particle size and irregular shape which leads to an overall larger surface area for the same sample amount. The general surface area would be about three times larger if a spherical shape for both products is assumed; however, the form of the hand made particles is far from being spherical thus the surface area is even greater (see Chapter 4.3.3). Additionally, drug particle diffusion is strongly dependent on the diameter of the formulation. With increasing size, diffusion is reduced inversely proportional to the squared diameter (Voigt, 2006). As the particle sizes between the hand made formulation and the one prepared by Brace differ by about a factor of three (see Chapter 4.3.2) this means that diffusion is approximately nine times lower in case of the larger Brace particles. Furthermore, it was demonstrated with SEM that the surface of all lipid particles is not completely plane but consists

of separate plates so that water can easily penetrate into the particles (see chapter 2.3.3). Yet, the surface of the Brace formulations is smoother than that of the particles prepared by hand so that the degree of water permeation would be more pronounced for the latter. This smoothness of the surface might be another reason for the more delayed drug release from the Brace particles: normally, forming a matrix with the active ingredient, a burst effect might have been expected as drug molecules should also be located on the surface of the lipid particles thus leading to a faster release at the beginning. However, as this is not the case another explanation seems probable: during solidification, the lipid most probably crystallized from the surface to the core of the microparticles. The praziquantel molecules might hence have been translocated at the solid-liquid interface of the crystallizing matrix so that finally they are enclosed in the core of the particle surrounded by lipid. This phenomena is known from steel and graphite processing for purification. The described crystallization process then results in a form of coating where the drug particles are surrounded by a lipid layer and hence, drug dissolution would be sustained.

If the manufacturing process of the Brace particles is regarded after which most probably at least a small part of the drug should be present in form of a solid solution, drug dissolution should be faster than in the case of particles prepared from a melt dispersion. However, this factor seems to be of minor importance for drug dissolution if all of the above described incidents are considered.

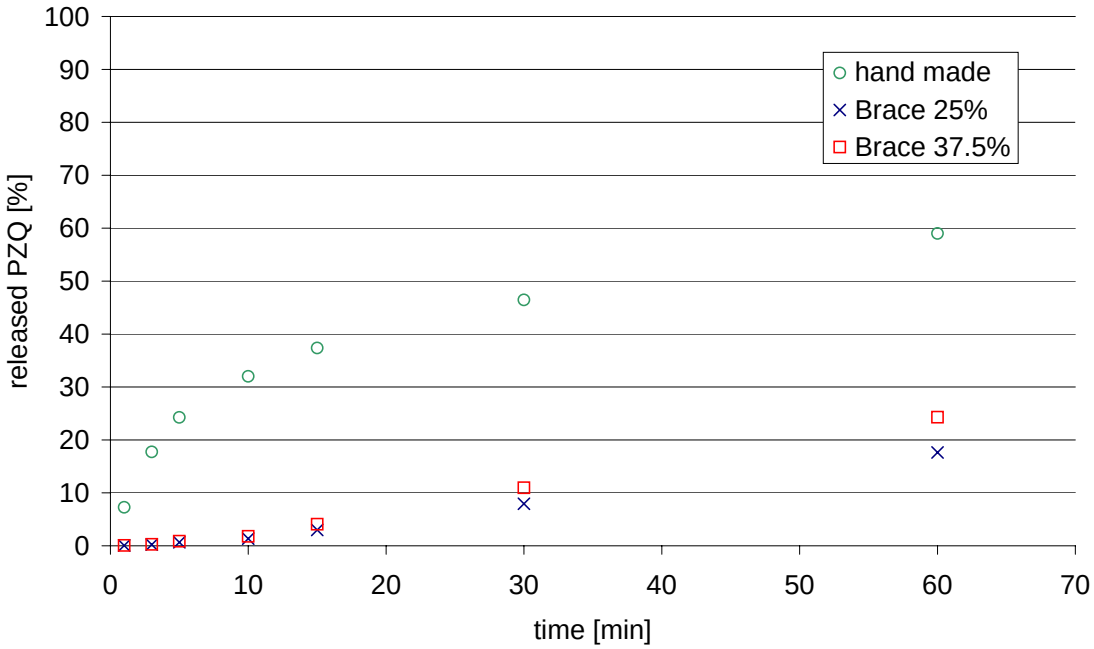

Figure 4.14: Dissolution profile of hand made lipid particles (PZQ content 25%) and particles manufactured by Brace (PZQ content 25% and 37.5%) in phosphate buffer pH 6.8 + 5% (w/w) Solutol HS15; test were run in triplicate; standard deviation did not exceed 5%

Finally, it can nicely be seen that drug disintegration is dependent on drug loading and thus on the formation of pores. Dissolution of praziquantel from the Brace particles containing 37.5% active ingredient became faster after 15 min compared to the particles with 25% drug. This is due to drug molecules getting dissolved upon the penetration of dissolution medium and migrating from the matrix thereby building pores. The more drug particles are incorporated into the lipid matrix the more pores are formed with time and hence, drug dissolution becomes faster (Jaspart et al., 2005).

Concluding, it became clear that the manufacturing technique has a great influence on drug dissolution from the lipid matrix. Yet in our case, this was most of all due to the different production temperature and size of the obtained particles. Thus, no direct comparison of the two applied production procedures is possible. It would have been very interesting to evaluate lipid particles prepared by spray congealing with the same parameters (especially regarding process temperature and particle size) as in case of the hand made formulation. Unfortunately, this was not possible with the available equipment.

4.3.8 Stability of lipid particles

The stability of the active ingredient in the lipid matrix was characterized by dissolution studies. Samples were stored for three, six and nine months at 25 °C/60% RH, 30 °C/65% RH and 40 °C/75% RH and then examined. The results are presented in Fig. 4.15. As discussed previously, embedding in Precirol ATO 5 evidently leads to a delayed release of praziquantel which would in its pure form show a dissolution of more than 60% after 5 min. By forming a solid dispersion with a water insoluble and non-swellable matrix, drug disintegration from the matrix is prolonged.

It can clearly be seen that the dissolution alters dependent on storage time and temperature. While the active ingredient disintegrates slower with time if stored at 25 °C and 30 °C, the dissolution rate becomes faster for the sample kept at 40 °C. The reason for the delayed release observed at the two lower temperatures could lie in the different molecular state of the drug in the matrix: during manufacturing a small amount of drug could have dissolved in the molten lipid as praziquantel is partially soluble in Precirol. Hence, straight after production part of the active ingredient might be molecularly dispersed in the matrix forming a solid solution and/or at least appear in an amorphous form, so that upon contact with the dissolution medium drug particles are easily dissolved and diffuse from the carrier into the dissolution medium. During storage at the two lower temperatures which are below the melting range of Precirol, the either dissolved or amorphous drug particles might re-crystallize to their energetically favored crystalline structure (Craig, 2004; Vasconcelos et al., 2007; Bodmeier et al., 1990). Hence, more drug molecules are present as a solid dispersion where the crystalline particles first have to get dissolved in the penetrating dissolution medium so that drug disintegration evidently takes longer (Savolainen et al., 2002, 2003). The assumption of drug precipitation in the lipid matrix might also be supported by the fact that the decrease in the dissolution rate can only be detected up to six months. After this point, no further reduction can be perceived which might

lead to the notion that either the complete drug substance has precipitated and hence a (more or less) stable solid dispersion has been formed or an equilibrium between dissolved and dispersed parts has been reached. In both cases no alteration of the dissolution profile would occur during further storage which is the case in this study for other three months.

While at the two lower temperatures a slight decrease in the dissolution rate is observed, this is not the case for the sample stored at 40 °C. Here, drug dissolution increased over the tested nine months. This could be attributed to softening of the lipid matrix at that temperature (see Chapter 4.3.4) which might have led to two different effects: on the one hand, the particle size of the material used was presumably smaller than in the original probe as the sample agglutinated as one cluster. For dissolution studies, the substance had to be scraped off and hence, smaller particles were probably created which then led to a slightly faster drug dissolution. Another possibility might be that through the softening of Precirol more praziquantel could be dissolved in the matrix. Hence, more drug might be present in a molecularly dispersed state which then leads to a faster disintegration into the dissolution medium. This assumption is supported if dissolution data from the pastes containing lipid embedded praziquantel stored for six or nine months at 40 °C are regarded (see Chapter 5.3.5). Here, a faster dissolution rate is observed which most probably is caused by softening of the lipid and resulting diffusion of the active ingredient into the paste base. Thus, for the pure lipid particles probably both above described effects – reduced particle size and increased dissolved drug amount – are of importance for the enhanced drug dissolution.

Of course, it cannot be excluded that changes in the dissolution profile can also be evoked by modification of the lipid matrix. Precirol can appear in different polymorphic forms and thus alter during storage (Jannin et al., 2006; Hamdani et al., 2003; Evrard et al., 1999; Jaspart et al., 2005). It might, for example, build new crystallite micelles in the matrix thereby including the drug which then could lead to a delayed release at the lower temperatures. In other studies on the stability of various lipid based formulations an increase in drug dissolution could be observed on storage at 40 °C (Bowtle, 2000; Remunan et al., 1992; Sutananta et al., 1995). This was mostly attributed to changes in the microstructure of the carrier.

In conclusion, an influence of storage conditions on praziquantel dissolution from a Precirol matrix can be taken for granted. If stored below the softening range of Precirol, drug disintegration was delayed, which might be due to further crystallization of the active ingredient in the lipid matrix. On the other hand, storage at 40 °C led to an increase in the dissolution rate. This might have been evoked by softening of the lipid and following dissolution of drug crystals in the matrix. Of course, a modification of the microstructure of the lipid is a further possibility for the observed alterations.

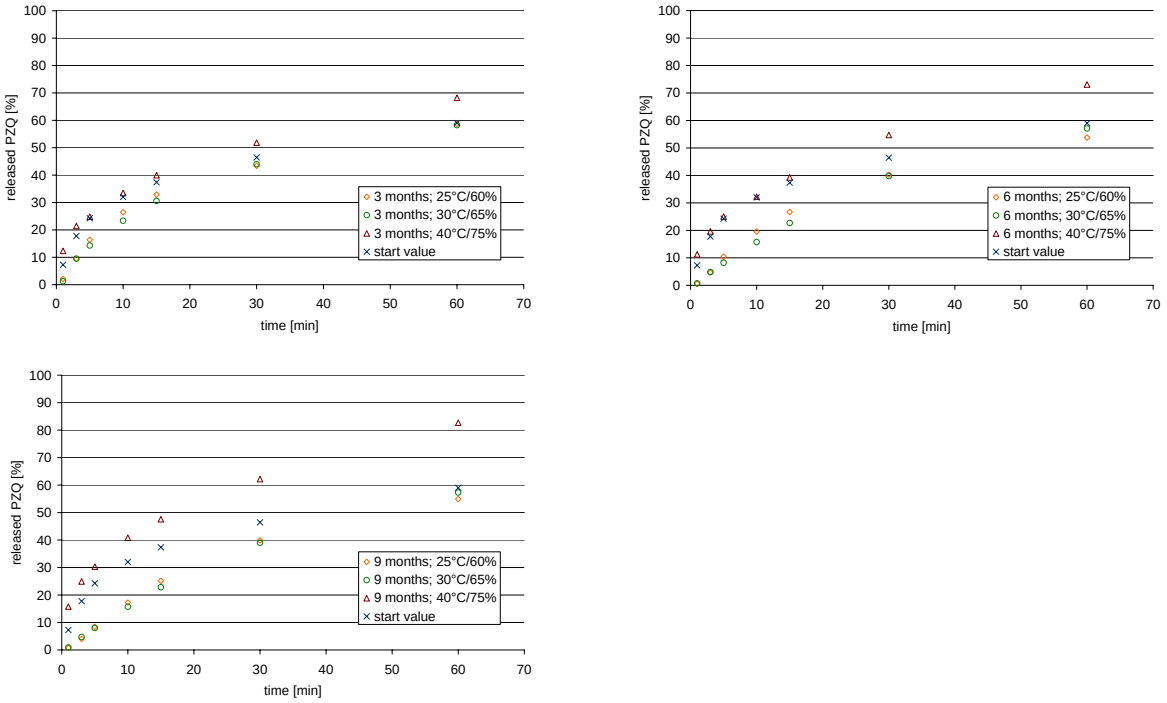

Figure 4.15: Dissolution of praziquantel from Precirol embedding at start and after three, six and nine months at set temperature and humidity; tests were run in triplicate; standard deviation did not exceed 5%.

4.4 Conclusions

The embedding of praziquantel into a Precirol matrix seems to be a feasible technique to achieve a taste masking effect. Drug disintegration from those lipid particles is sustained at the beginning and the smell of praziquantel could also be concealed by Precirol.

The preparation of the lipid particles was approached in two different ways: on a small scale particles were produced by hand in the laboratory by dispersing praziquantel in molten Precirol and grinding of the solidified product. The second production technique, spray congealing by BRACE GmbH, was primarily applied to be able to produce a larger batch size conveniently and also to achieve a smaller particle size leading to enhanced drug dissolution. With the first technique small, but irregular shaped microparticles in the range of 137 μm were obtained which showed a promising dissolution profile regarding taste masking. The Brace particles on the other hand were completely spherical but unfortunately could not be produced in the small sizes desired as the viscosity of the melt was too high for these small nozzles. So the particles were about three time larger (408 μm) than the particles prepared by hand. Resulting from this and also from the small surface area due to the spherical shape the dissolution rate was very slow and not feasible for the needed purpose.

Characterization of the lipid particles was further performed with DSC, IR and HSM. It could be demonstrated that the crystalline state of the active ingredient is

slightly different in the formulation prepared by hand compared to the one from Brace. This is most probable due to the different manufacturing procedures: in the first case the particles were produced from a melt suspension so that the drug particles are mostly dispersed in the solid lipid matrix, yet, a small amount might have dissolved as praziquantel is partially soluble in Precirol. The particles from Brace, on the other hand, solidified from a complete melt mixture and hence, some amount might have stayed molecularly dispersed or became amorphous while part of the drug re-crystallized again.

Stability problems arising from crystallization phenomena are often reported for lipid formulations prepared from melts either due to modification of the lipid itself or because of precipitation of the active ingredient. The lipid particles prepared by hand were thus put on stability at different temperatures and investigated by dissolution studies. It could be shown that storage at 40 °C increased praziquantel disintegration from the lipid matrix whereas at 25 °C and 30 °C drug dissolution was slightly reduced. The second occurrence might be explained with drug precipitating with time while in the first case the lipid became soft and hence more drug molecules could dissolve in the matrix. Besides, changes in the modification of the lipid might also be responsible for these incidents.

Drug dissolution from the lipid microparticles should match the requirements for the cat tablets in order to show bioequivalence (NAH, 2003). With the hand made formulation this specification (at least 70% released after 60 min) was just not attained. It was therefore tested if by incorporation of a hydrophilic substance this feature could be improved. The addition of Aerosil 200 led to an increase in the dissolution after about 20 min which would be ideal for taste masking: at the beginning drug disintegration was even slightly reduced due to swelling of Aerosil and the resulting dissolution of the drug in this gel layer. After a while though, the active ingredient started to diffuse from this layer into the dissolution medium which led to an enhanced dissolution just matching the requirements. The incorporation of a hydrophilic substance was thus successful for increasing praziquantel dissolution from a Precirol matrix.

This thought might be an approach for further improvement of drug dissolution from a Precirol matrix for taste masking purposes. It could already be demonstrated that the addition of other hydrophilic substances such as poloxamers, mannitol, hydroxypropyl methylcellulose, polyethylene glycol or polyvinyl pyrrolidone could lead to a faster dissolution rate (Jannin et al., 2006; Albertini et al., 2004; Savolainen et al., 2002, 2003; Parab et al., 1986). A combination of hydrophilic substances and/or a higher quantity might lead to an even increased drug release from the lipid matrix. Yet, the taste masking effect still has to be considered so drug dissolution should not be too rapid at the beginning. However, as demonstrated this could thoroughly be achieved by the addition of hydrophilic substances as they swell or have to get dissolved at the start themselves and thus slightly delay drug dissolution. Another opportunity might be the incorporation of a flavor into the lipid matrix which might also enhance the dissolution rate but additionally could have a positive effect on taste

masking.

Of course, an increase in dissolution rate could also be achieved by a smaller particle size. Preparation methods employed in the production of solid lipid nanoparticles such as solvent emulsification/evaporation, homogenization and sonication might be used (Mehnert & Mader, 2001). Yet, it has to be considered that nearly all of these preparation techniques require solvents and hence, the advantage of a convenient solvent-free method would be lost. Moreover, a production with these techniques at larger scale would be a challenging task. Therefore, other manufacturing methods should by evaluated. The spray congealing performed by Brace was a first step in this direction, however, no satisfying results matching our needs were obtained. A further possibility might be the use of another base matrix with a lower viscosity so that it could be proceeded through narrower nozzles.

Finally, further investigations would be necessary regarding characterization of the modifications of both drug and lipid, especially concerning changes during storage. Feasible methods for this might be X-ray diffractometry or Raman spectroscopy. However, as the lipid particles were not the first choice for further development following the acceptance tests with the pastes (see Chapter 5.3.2) there was no immediate need for further investigations. Apart from that, changes in lipid modifications are very complex and thus the determination of the occurring mechanisms that are important would be extremely difficult.

Chapter 5

Milbemax Exact Dose Oral Pastes

5.1 Short Introduction

5.1.1 How it came to be a paste

A market research was performed with cat owners and companion animal veterinarians in order to chose the optimal route of administration for a dewormer for cats. The participants were given six formulation choices and had to rank the individual formulations. The ranking took into consideration issues such as ease of administration, exact dosing, safety (for owners and cats), acceptance by the patients and costs. An oral paste was judged to have a good applicability for several reasons: first of all, a paste is more viscous than a liquid and cats are expected to like the paste consistency as it is more like food than medicine. Furthermore, filling the exact dose of paste needed into a syringe and applying the paste directly into the mouth, assures that cats are getting the correct dose. In addition, owners hope that the palatability is good so that cats will accept the medication as a treat and take it willingly. Finally, there are several offering options if the cats might not take the medication in the first step: either direct into the mouth or mixed and disguised in food or as a treat to lick of the finger. Another alternative might also be to apply the paste onto the cat's paw or fur to have it licked off in a step of selfcleaning. One request voiced by vets mainly was to increase the active ingredient concentration in the paste due to difficulties of administering a high paste volume (maximum 1 ml). Overall, the oral paste got the best rating in comparison with the other formulation concepts (see Tab. 5.1). Therefore, the development of a dewormer paste for cats was carried on.

As owners hoped for a palatable formulation, another advantage of an oral paste is the possibility to include the active ingredient in an already taste masked form. However, this can be a challenging task considering that these formulations have to be stable in the pastes. The taste masking agents thus must be inert in the paste bases so that no leaking of the active ingredient occurs. In an aqueous paste, for example, a normal coating e.g. a cellulose or methacrylate, could not be used as this would either dissolve or swell over time and hence release the drug into the paste. Therefore, only taste masking agents compatible with the paste bases can be employed. Nevertheless, another opportunity to achieve a very good acceptance in a simple way in pastes is

Concept	Owners	Vets
#1: Small Pill	4.1	1.8
#2: Oral Paste	1.9	2.5
#3: Supersoft Pill	2.6	3.6
#4: Liquid Pump	3.5	3.5
#5: Thin Strips	3.3	4.4
#6: Mini Pills	5.7	5.5

Table 5.1: Rank of each formulation concept in order of preference: 1=highest, 6=lowest

the addition of a flavor for taste improvement.

5.1.2 Acceptance tests

In vitro tests for bitterness have been developed in recent years (Takagi et al., 2001; Zheng & Keeney, 2006). These tests are based on transforming taste information into a pattern composed of the electronic signals of the lipid membranes potentials. This so-called e-Tongue can be used for evaluation of taste masking efficiency and comparison of taste intensities. Up to now the current experience base is limited and more research has to be done to clarify if it can be used solely to evaluate the bitterness of a substance. Besides, it is not known if the e-Tongue could also be used for flavored pastes. The flavors themselves might have an impact on the taste information and the results could thus be falsified. Moreover, this system has only been used to evaluate the bitterness as it is tasted in human mouth. The taste receptors in an animal's mouth are quite different as in humans; cats, for example, seem not to be able to taste sweet – they are unable to distinguish between water and sucrose solution (Bradshaw et al., 1996; Thombre, 2004). Finally, cats are individuals and can have very different taste sensation. Therefore, it was preferred to test the acceptance of the taste masked pastes with cats directly.

Previous to tests with active ingredient, three different placebo pastes were tested with cats at the Centre de Recherche St. Aubin (CRA), Novartis Animal Health Inc. to choose the best paste formulation for further development. With water, miglyol and polyethylene glycol (PEG) three different paste bases were tested. The paste formulations were composed according to Table 5.2 and contained additionally to the bases thickening agents and preservatives. The results from these tests showed that cats slightly preferred the aqueous paste to the oily one. The PEG paste was least accepted and also evoked minor salivation, but the acceptance for all bases were at least satisfactory and thus these formulations were used for further paste development.

The marketed product will contain two active ingredients, praziquantel and milbemycin oxime (MO). Praziquantel is known for its bitter taste and therefore needs to be taste masked. To assure that MO does not affect the acceptance of the formulation pastes containing only MO were also tested for their acceptance. It was shown

Aqueous		Miglyol		PEG	
0.10%	Methylparaben	0.10%	Methylparaben	0.10%	Methylparaben
0.02%	Propylparaben	0.02%	Propylparaben	0.02%	Propylparaben
0.50%	Propylene glycol	0.50%	Propylene glycol	0.50%	Propylene glycol
30.00%	Glycerin anhydrous	2.50%	Aerosil 200	5.00%	Aerosil 200
2.50%	Avicel RC591	10.00%	Myvaplex 600P	20.00%	PEG 300
64.38%	Water demin.	84.38%	Miglyol 812	71.88%	PEG 600

Table 5.2: Placebo paste base formulations

that there is no influence of MO on the compliance and hence taste masking is only needed for praziquantel.

So, the taste masked formulations developed earlier (microspheres with Eudragit E, inclusion complex with β-cyclodextrin and lipid embedding with Precirol) were incorporated into an appropriate paste base and acceptance tests were performed with cats.

5.2 Materials and methods

5.2.1 Materials

The drug substance praziquantel was provided by PCAS, Limay, France and milbemycin oxime was supplied by Sankyo Co., Tokyo, Japan. β-cyclodextrin was purchased from Fluka AG, Buchs, Switzerland and hydroxypropyl-β-cyclodextrin was obtained from Wacker Chemie AG, Burghausen, Germany. Polyvinylpyrrolidone (PVP) K30, methylparaben, propylparaben, propylenglycol, glycerin anhydrous, polyethylene glycol (PEG) 300 and 600, benzoic acid and hydrochloric acid were provided by Fluka AG, Buchs, Switzerland. Sodium benzoate and benzyl alcohol were purchased from Riedelde Haën AG, Seelze, Germany. Avicel RC591 (micro-crystalline cellulose) was obtained from FMC BioPolymer, Newark, USA. Myvaplex 600P (concentrated glyceryl monostearate) was provided by Eastman Chemical Company, Kingsport, USA. The Artificial Beef Flavor was supplied by Pharma Chemie Inc, Syracuse, USA and the Malt Extract was obtained from Wander, Neuenegg, Switzerland. Aerosil 200 was provided by Degussa AG, Düsseldorf, Germany and titan dioxide was purchased from Kronos Titan GmbH, Leverkusen, Germany. Iron oxide red was supplied by Ellis&Everard Ltd., London, United Kingdom. Miglyol 812 was obtained from Hänseler AG, Herisau, Switzerland. Phosphate buffer solution pH 6.8 was purchased from Fluka AG, Buchs, Switzerland and Solutol HS 15 was provided by BASF, Ludwigshafen, Germany. Purified water was prepared with ELGA Maxima ana (Labtec Services AG, Wohlen, Switzerland).

5.2.2 Preparation of pastes

Aqueous		Miglyol		PEG	
Taste masking agents:		Taste masking agents:		Taste masking agents:	
• β-CD • Precirol • Beef 10% • Malt 10%		• β-CD • Precirol		• Eudragit E • none	
2.50%	PZQ	2.50%	PZQ	2.50%	PZQ
0.10%	Methylparaben	0.10%	Methylparaben	0.10%	Methylparaben
0.02%	Propylparaben	0.02%	Propylparaben	0.02%	Propylparaben
0.50%	Propylene glycol	0.50%	Propylene glycol	0.50%	Propylene glycol
30.00%	Glycerin anhydr.	2.50%	Aerosil 200	5.00%	Aerosil 200
2.50%	Avicel RC591	10.00%	Myvaplex 600P	20.00%	PEG 300
ad 100%	Water demin.	ad 100%	Miglyol 812	ad 100%	PEG 600

Table 5.3: Pastes for the first acceptance tests with taste masked praziquantel; PZQ amount in all 2.5%; β-CD was added as complex prepared by solvent method, Precirol embedded lipid particles were prepared by hand, Eudragit E microspheres from formulation E were used

Component	β-CD/Beef	β-CD/Malt	Precirol/Beef	Precirol/Malt
[%w/w]	TG 2130/12	TG 2130/13	TG 2130/14	TG 2130/15
Praziquantel	2.5[a]	2.5[a]	2.5[b]	2.5[b]
Benzoic acid	0.10	0.10	0.10	0.10
Sodium benzoate	0.10	0.10	0.10	0.10
Benzyl alcohol	0.50	0.50	0.50	0.50
Glycerin anhyd.	30.00	30.00	30.00	30.00
Avicel RC591	6.00	6.00	6.00	6.00
Flavor	10.00 Beef	10.00 Malt	10.00 Beef	10.00 Malt
Water demin.	ad 100.0	ad 100.0	ad 100.0	ad 100.0

[a]In form of β-cyclodextrin complex, [b]In form of lipid embedding

Table 5.4: Combined formulations with taste masked praziquantel and a flavor

The first formulations of all pastes were prepared by first dissolving the preservatives (methylparaben and propylparaben) in propylene glycol at 50 °C. This mixture was then added to the three different bases. For the oily pastes, Myvaplex 600P was previously dissolved in Miglyol 812 at 70 °C and the PEG bases (PEG 300 and 600) had

also been mixed together. After addition of the preservatives, the remaining excipients were given to the suspension and it was mixed until a homogeneous mixture was obtained. The aqueous pastes were stirred additionally for 15 min on a magnetic stirrer (Ika-Werke GmbH, Staufen, Germany) after the addition of Avicel RC591. Finally, the active ingredient pure or in a taste masked formulation was suspended in the pastes which were homogenized for 10 min with an Ultra-Turrax (Ika-Werke GmbH, Staufen, Germany). Lipid embedded praziquantel (see Chapter 4.2.2), praziquantel-β-cyclodextrin complex prepared by solvent method (see Chapter 3.2.2) and Eudragit E microspheres (see Chapter 2.2.3) were used as taste masked formulations. The exact compositions (%w/w) of the pastes are shown in Tab. 5.3.

In the next round of acceptance tests, combinations of the best previous tested formulations were used. All pastes were water based and contained one taste masked formulation, namely lipid particles or β-cyclodextrin complex, and either artificial beef flavor or malt extract as flavor. The preservative system was changed in the meantime to sodium benzoate and benzoic acid which were dissolved in water at 50 °C. Additionally, the amount of Avicel was increased to 6% due to viscosity issues. Apart from that, the pastes were prepared as previously described for aqueous pastes. The recipes can be seen in Tab. 5.4.

Component [%w/w]	TG 2113/16	TG 2113/17	TG 2113/18	TG 2113/19	TG 2120/4	TG 2120/5
MO	2.0	2.0	2.0	2.0	-	-
PZQ	5.0[a]	5.0	5.0	5.0	-	-
Cyclodextrin	18.3[a] β-CD	18.3 β-CD	5.0 β-CD	5.0 HP-β-CD	5.0 β-CD	5.0 β-CD
Sodium benzoate	0.4	0.4	0.4	0.4	0.4	0.4
Benzyl alcohol	1.0	1.0	1.0	1.0	1.0	1.0
Glycerin anhyd.	5.0	5.0	5.0	5.0	5.0	5.0
Avicel RC591	6.0	6.0	6.0	6.0	6.0	6.0
Color	0.1 iron oxide red	0.1 iron oxide red	0.1 iron oxide red	0.1 iron oxide red	0.1 iron oxide red	1.0 titanium dioxide
PVP	-	-	1.0	1.0	1.0	1.0
Beef flavor	10.0	10.0	10.0	10.0	10.0	10.0
HCl 0.01 M	ad 100.0	ad 100.0	ad 100.0	ad 100.0	ad 100.0	ad 100.0

[a] PZQ in form of separated β-CD-complex

Table 5.5: Paste formulations of cyclodextrins variation

The following acceptance tests were performed with the pastes displayed in Tab. 5.5 using only cyclodextrins as taste masking agents. Here, the preparation of

the pastes was changed so that the drug-cyclodextrin complex should be formed in situ during the manufacturing of the paste. In addition to β-cyclodextrin in different concentrations, hydroxypropyl-β-cyclodextrin was also tested. Furthermore, a higher praziquantel concentration (5%) and colorants were used. Moreover, 0.01 M hydrochloric acid was used as base to avoid the extensive dissolution of benzoic acid at 50 °C. Thus, only sodium benzoate was dissolved in HCl and the equilibrium of the preservative system adjusted itself at pH 4-5.5. Additionally, the glycerin amount was reduced to 5% as palatability tests with placebo pastes showed no difference between formulations with 30% and 5% glycerin. This reduction was desired to have a higher water amount in the paste because of complexation issues. Finally, polyvinylpyrrolidon was used to enhance the complexation efficiency of cyclodextrin (see Chapter 1.2.4). So the paste preparation was performed as follows: the preservatives sodium benzoate and benzyl alcohol were dissolved in hydrochloric acid. Subsequently, Avicel was dispersed and the resulting suspension was stirred for 15 min. Following that, glycerin and afterwards cyclodextrin and PVP were added and the suspension was stirred on a magnetic stirrer for 30 min. Afterwards, praziquantel was added and it was agitated for 60 min to enable complex formation between cyclodextrin and praziquantel. Flavor and colorant were both mixed in at the end and the paste was homogenized for 10 min with an Ultra-Turrax. For comparison, a paste with the separated praziquantel-β-cyclodextrin-complex prepared by solvent method was also tested.

	Aqueous		β-CD in Miglyol
Enantiomers:	Taste masking agents:		Taste masking agent:
• (−) PZQ • (+) PZQ	• β-CD (2.5%) + PVP(1%) • PVP (1%)		• β-CD (2.5%)
2.50%	Praziquantel	2.50%	Praziquantel
0.10%	Methylparaben	0.10%	Methylparaben
0.02%	Propylparaben	0.02%	Prpylparaben
0.50%	Propylene glycol	0.50%	Propylene glycol
30.00%	Glycerin anhydr.	2.50%	Aerosil 200
2.50%	Avicel RC591	10.00%	Myvaplex 600P
ad 100%	Water demin.	100%	Miglyol 812

Table 5.6: Paste formulations of the last tested pastes containing separated praziquantel enantiomers, solely PVP, in situ complex formation with β-CD and β-CD in oil

The final round of acceptance tests were conducted with the praziquantel enantiomers and different praziquantel and PVP and/or cyclodextrin compositions. The formulations are shown in Tab. 5.6. The paste recipes of the first acceptance tests were used to be able to compare the results directly with each other without flavor or colorant. As it is known that enantiomers can taste differently than their racemate or

their partner enantiomer (Lee & Williams, 1990; Bassoli et al., 2000) this was tested for praziquantel. Besides, it was examined if only PVP might be able to mask the taste of praziquantel as it is assumed to form complexes with praziquantel (El Arini & Leuenberger, 1998). Moreover, in situ formation of the drug-β-cyclodextrin complex in the aqueous paste base with PVP was tested without beef flavor as this in situ complex formation had previously only been tested in combination with a flavor. Finally, the in situ formation of the complex was also evaluated in oil, where its formation should not be possible as cyclodextrin is insoluble in Miglyol but should be dissolved to be able to include its guest molecules. These formulations were tested on eight cats which had not participated in the former studies.

5.2.3 Acceptance tests in vivo

The tests were performed at the Centre de Recherche St. Aubin (CRA), Novartis Animal Health Inc.. For these tests either six, eight or twelve cats which were identified by a subcutaneous tag were first acclimated to the planned study conditions for one week. They were administered twice 1 ml of a highly palatable oral nutritive paste available on the market (Nutriplus-Gel®, Virbac AG, Glattbrugg, Switzerland) via a syringe, which was also used as positive control. Absolute denial of oral paste administration of the positive control led to exclusion from the experiment. Moreover, one weighing and daily health checks were conducted and only cats which were healthy and passed the physical examination took part in the study. All pastes were evaluated in all animals and in between two administrations, Nutriplus-Gel® was given to preclude a potential systemic reluctance due to some bitterness memorization from previously tested formulations.

Throughout the study conduct, 1 ml of all tested oral paste formulations was administered similarly. Initially, a small amount of paste was proposed on the lips/nose of the animal. The acceptance was rated as follows: high acceptance (3 points) is reached if the cat spontaneously licks and swallows the paste, and the complete dose is administered without inserting the syringe into the mouth. Satisfactory acceptance (2 points) is achieved when the paste is not spontaneously licked, but the syringe must be inserted in the cat's mouth and the dose is administered entirely. The cat might shake its head and chew during and after administration, but neither hypersalivation nor vomiting over the post-dose 5 minutes is accepted. For a poor acceptance (1 point), the criteria are the same as for satisfactory acceptance, but hypersalivation and/or excitation might be observed and vomiting can occur within 5 minutes after paste application. The worst rating, failed acceptance (0 points), is given if the dose cannot be administered at all due to incidents such as head withdrawal or scratch attempt or if the paste is entirely rejected after insertion into the mouth. This might also involve hypersalivation and vomiting.

5.2.4 In vitro dissolution tests

Drug dissolution from the pastes displayed in Tab. 5.7 was performed using the paddle method according to USP 30 specification. As dissolution apparatus a Sotax AT 7 (Sotax AG, Allschwil, Switzerland) was employed. The medium (1000 ml) consisted of purified water containing 0.2% (w/w) Solutol HS15. 0.4 g of the pastes containing 2.5% praziquantel and 0.8 g of the pastes with 5% praziquantel were filled into syringes according to tablet specification (NAH, 2003) and weighed before and after insertion into the medium to determine the real drug amount in the sample. Tests were run in six fold. Paddle speed was set at 100 rpm and temperature at 37±0.5 °C. Samples were taken after 15 and 60 min, filtered (0.45 μm filter, Millipore Millex-HV, Billerica, USA) and the drug content was determined using the HPLC method described in Chapter 2.2.4.

Component [%w/w]	TG 2113/17	TG 2130/17	TG 2130/18	TG 2130/19	TG 2113/18	TG 2130/20	TG 2130/21
MO	2.0	-	-	-	2.0	-	-
PZQ	5.0	5.0	5.0	2.5[a]	5.0[a]	2.5[b]	5.0[b]
taste masking agent	18.3 β-CD	5.0 β-CD	5.0 HP-β-CD	7.5[a] Precirol	15.0[a] Precirol	9.2[b] β-CD	18.3[b] β-CD
Sodium benzoate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Benzyl alcohol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glycerin anhyd.	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Avicel RC591	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Color	0.1 iron oxide red	0.1 iron oxide red	0.1 iron oxide red	1.0 titanium dioxide	0.1 iron oxide red	1.0 titanium dioxide	0.1 iron oxide red
PVP	-	1.0	1.0	-	-	-	-
Beef flavor	10.0	10.0	10.0	10.0	10.0	10.0	10.0
HCl 0.01 M	ad 100.0	ad 100.0	ad 100.0	ad 100.0	ad 100.0	ad 100.0	ad 100.0

[a]PZQ in form of Precirol embedding, [b]PZQ in form of separated β-CD-complex

Table 5.7: Paste formulations for dissolution studies

5.3 Results and discussion

5.3.1 First acceptance tests of taste masked formulations

In the first testing round pastes with different taste masked praziquantel formulations or flavors were evaluated. According to their properties they were incorporated into the three paste bases. The concentration of praziquantel was 2.5% in all pastes. The

Eudragit E microspheres (see Chapter 2.2.3) could only be included in a PEG base because Eudragit E is soluble in miglyol and swellable in water. The praziquantel-β-cyclodextrin-complex obtained by solvent method (see Chapter 3.2.2) and the hand made lipid particles with Precirol ATO 5 (see Chapter 4.2.2) were both incorporated into water and miglyol pastes. These bases were chosen because they showed the best results in the placebo tests, and to compare the taste masked formulations in two bases. Besides, β-cyclodextrin is partially water soluble and thus further inclusion complex formation of β-cyclodextrin with free praziquantel could happen in the aqueous paste. In addition, water based pastes with only flavor as taste masking agent were tested to study the effect of the simple addition of a flavor: artificial beef flavor and malt extract were used for this issue. Other taste masked formulations from another group were also tested, including hot melt coating with PEG 8000 and granulation with Marcoat/HPMC or Kollicoat SR30D as taste masking agents.

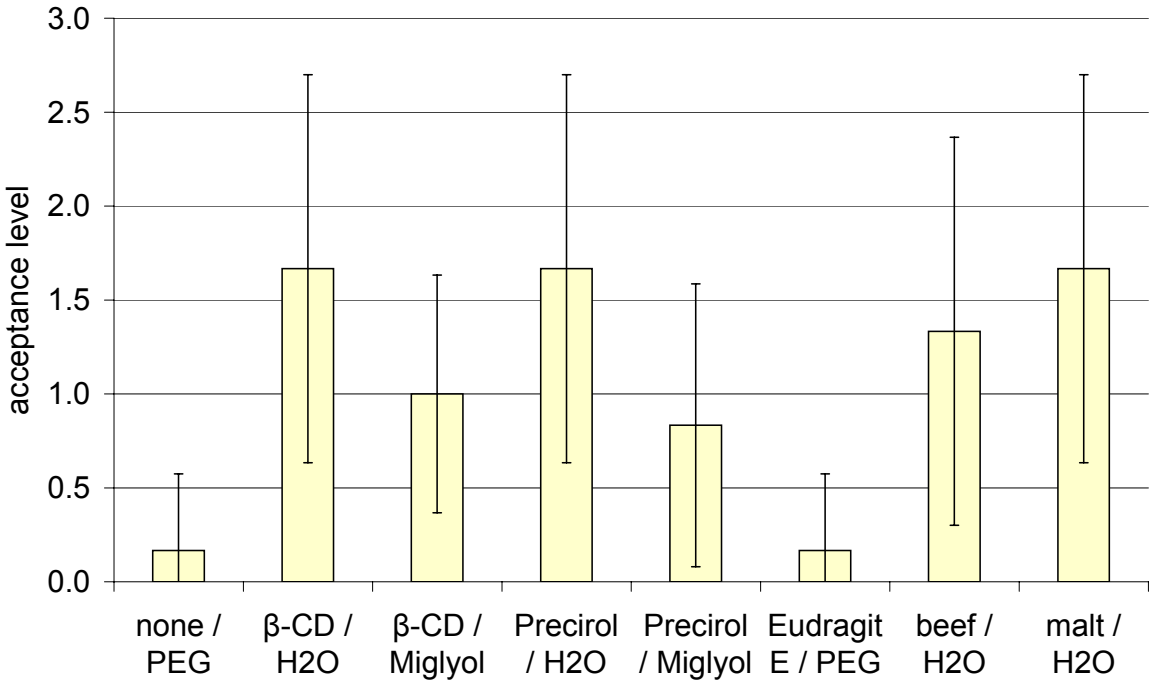

Figure 5.1: Acceptance tests of taste masked praziquantel pastes; indicated are taste masking agent and paste base; PZQ amount 2.5%; number of cats: 6; mean$\pm$stdev

Results of these acceptance tests with taste masked praziquantel or a flavor are shown in Fig. 5.1. The pastes were applied to six cats whereby the first four formulations were tested on a group of six cats and the last four formulations were tested on another group of six cats. As can be seen the formulations based on water were accepted best. Both taste masking agents which were tested in water and miglyol, β-cyclodextrin and Precirol, showed clearly better values in water. Both formulations reached the same average of 1.67 with a standard deviation of 1.03 in water whereas their values for miglyol were rather low: 1.00 for β-cyclodextrin and 0.83 for Precirol.

In all cases slight salivation was recorded for 33% of the cats. Excitation was only needed for the oily pastes (33% for β-cyclodextrin and 50% for Precirol); the aqueous pastes were taken by the cats more willingly so that no animation was required. This is another indicator that the aqueous pastes are superior in acceptance compared to the pastes based on miglyol.

Surprisingly, the flavor pastes reached good results, too: the malt extract equaled the average of β-cyclodextrin and Precirol and the beef flavor paste was also accepted quite good by the cats (average 1.33). Similarly, salivation occurred in 33% and stimulation was necessary in 33% of all cases for the beef flavor and 17% for malt. This is slightly astonishing because the pastes containing flavor should smell much better and thus should be taken more willingly by the cats than those without, even if they do not taste better in the end. However, 17% means that only one cat out of six needed to be animated to take the paste so this variation does not seem to be significant.

For comparison a worst-case paste was also tested: praziquantel is partially soluble in PEG and, because only dissolved substances elicit taste (Szejtli & Szente, 2005; Nanda et al., 2002), this base was chosen as a negative control to experience the reaction of cats to dissolved and unmasked praziquantel. Reactions were unambiguous: nearly all cats (eleven out of twelve) were rated a zero accompanied with severe salivation (see Fig. 5.2).

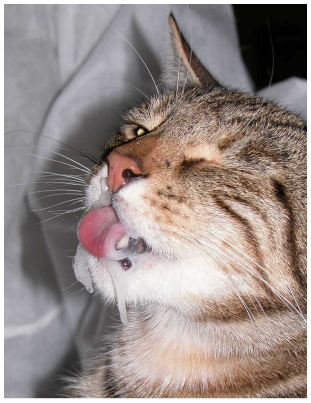

Figure 5.2: Salivating cat after administration of negative control

As can be seen the Eudragit E microspheres containing praziquantel which were also tested in a PEG base are nearly equaling the "worst case scenario". The average acceptance is 0.17 ± 0.41 which means that 5 cats were rated with failed acceptance (0 points) and only one cat showed poor acceptance (1 point). Severe salivation was recorded for 5 cats (83%) and excitation was needed for one half of the cats. The reason for this failed acceptance might be the good solubility of praziquantel in PEG which leads to drug leaking out of the microspheres into the base. This is possible because the active ingredient is not entirely coated with Eudragit E, but only suspended homogeneously in the microsphere matrix and thus can diffuse into the surrounding paste. As a result the concentration of dissolved praziquantel might be at its solubility limit as it is in the negative control paste. This and the reason that cats prefer other paste bases to PEG led to the very bad result. Therefore, this method was not tested any further.

Taste masked formulations of praziquantel from another group included hot melt coating with PEG 8000 tested in a PEG paste and granulation with Marcoat/ HPMC or Kollicoat SR30D which were incorporated in an oily paste. All these taste masking methods did not reach satisfying results (data not shown). Consequently, these methods were abandoned for further development of the paste.

The four water based pastes (β-cyclodextrin, Precirol, malt extract and beef flavor) were further tested with the other six cats which had not been used for the

respective pastes in the first tests. β-cyclodextrin showed the same acceptance level as before but with a lesser deviation whereas the level rose for the flavored pastes and Precirol. The malt and beef flavor were accepted much better and showed an average of 2.17 and 2.00, respectively. The acceptance level for Precirol also showed a slight increase (1.83).

To optimize the acceptance of the pastes combinations of the best taste masked formulations with a flavor was tested in the next acceptance round.

5.3.2 Acceptance tests of combined pastes

All water based pastes showed rather good results in the first round including either taste masked formulations or flavor. In order to achieve better acceptance of the pastes, combinations of the taste masked formulations and flavors were tested. The four pastes were presented to six cats with each two β-cyclodextrin pastes and two Precirol pastes tested on the same cats. Due to results from microbiological stability tests the conservation system was changed to benzoic acid and sodium benzoate. However, this did not have any impact on the acceptance.

Results are shown in Fig. 5.3: all combinations show a satisfactory acceptance level around a ranking of two. Moreover, none of the tested formulations was rejected completely by the cats, which means no zero ratings were reported and salivation was reduced. The least accepted formulation of these four was Precirol embedded praziquantel combined with malt extract (average 1.83 ± 0.41). This was only a slight improvement compared to the acceptance of Precirol alone tested on 12 cats where it also reached a level of 1.83, but with a higher standard deviation (0.83) as one cat was rated zero. The pastes flavored with beef showed the same average for both taste masking technologies (2.00) but β-cyclodextrin showed a higher variety in the acceptance level: two cats each were rated three and one, respectively, leading to a higher standard deviation (0.89) compared to Precirol (0.63) where only one cat each was rated with three and one. The combination of β-cyclodextrin and malt extract received the best ranking (average 2.17 ± 0.75).

The combinations of the taste masking technologies and flavors in the paste formulations showed an improvement in compliance: not only the average acceptance was increased but also a reduction of the fluctuations was observed. No pastes were rated by any cat with a failed acceptance and as nearly no hypersalivation was recorded, acceptance was in most cases at least satisfactory.

For classification of the reached acceptance level for the praziquantel pastes two commercial pastes containing an active ingredient were tested as well. Fubenol (Biokema SA, Crissier, Switzerland) and Banminth (Pfizer AG, Zürich, Switzerland) are known to be both well accepted by cats. In our tests they received an average of 2.42 and 2.08, respectively (data not shown). These results are not significantly different ($p>0.05$) from the tested praziquantel pastes. This was another indication for the good acceptance of the tested combinations of taste masked praziquantel with flavor.

All praziquantel pastes did not differ significantly from each other, so that all combinations could have been further developed. However, focus was laid on beef

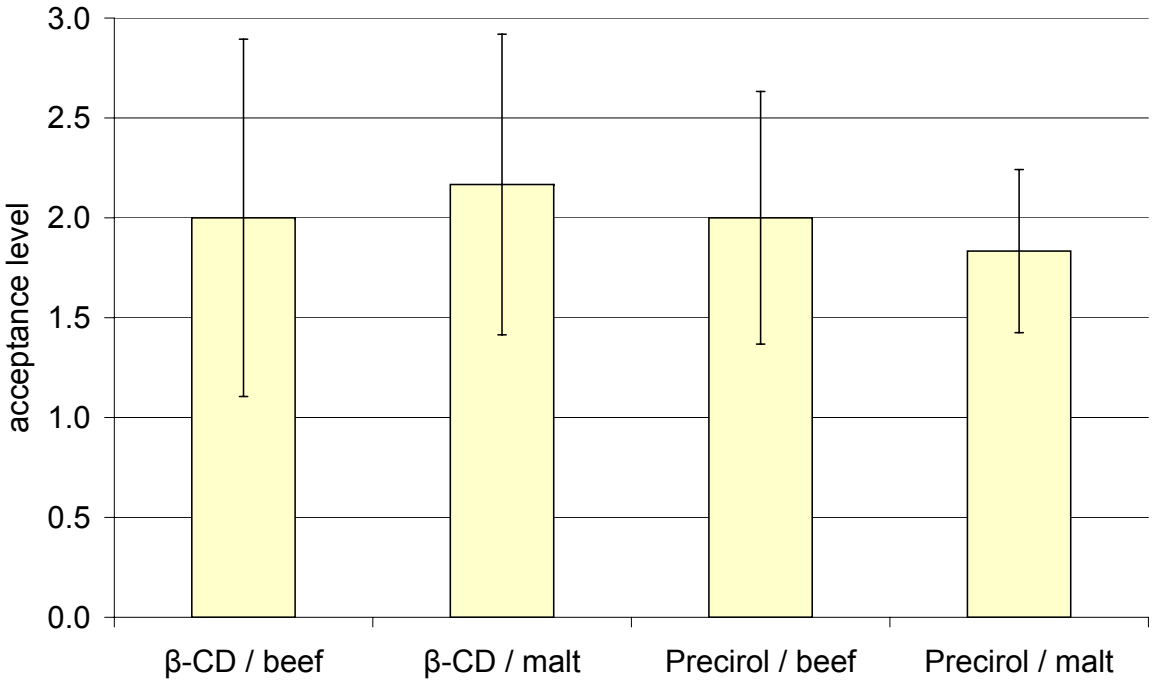

Figure 5.3: Acceptance tests of paste combinations (β-cyclodextrin and Precirol each with beef and malt flavor) in aqueous base; PZQ amount 2.5%; number of cats: 6; mean$\pm$stdev

flavored pastes for registration reasons. Besides, β-cyclodextrin was chosen for further development. This was done primarily because the preparation of the taste masked formulation with cyclodextrin was thought to be less laborious compared to the lipid embedding. But this can only be assumed if the drug-cyclodextrin complex did not have to be prepared and isolated separately but could be produced in situ in the paste base. Consequentially, acceptance tests were conducted with in situ formed praziquantel-β-cyclodextrin complexes.

5.3.3 Acceptance of cyclodextrin paste versions

Taste masking with β-cyclodextrin included up to now rather extensive steps with production and isolation of the complex. The paste being aqueous based these steps might be avoided by an instant (in situ) complex formation of the active ingredient with cyclodextrin in the paste which should happen after a while to a certain extent. This complexation will not be complete for the entire amount of praziquantel because only a small quantity of both praziquantel and β-cyclodextrin are dissolved in the paste. But the dispersed part is not responsible for the taste sensation and therefore the dissolved and complexed amount of praziquantel might be sufficient for taste masking. Additionally, there is always an equilibrium between free and complexed drug which might be the same if the complex is prepared beforehand or is formed in the paste. Moreover, the isolated praziquantel-β-cyclodextrin complex might be

regarded as a new chemical entity and would thus have to be registered completely as such. As a result, a one pot process was used for in situ complexation in the aqueous base: cyclodextrin was partially dissolved in the base and praziquantel was added to the suspension which was stirred for another 60 min.

For the separated inclusion complex a molecular ratio of 1:1 was chosen as recommended in literature (Becket et al., 1999; El Arini & Leuenberger, 1996). One molecule of active ingredient can thus be complexed by one molecule of β-cyclodextrin. This ratio means that the weight proportion is about 1:3.6 praziquantel to cyclodextrin. The concentration of praziquantel in the pastes is either 2.5% for small cats and 5% for large cats. Staying with a molecular ratio of 1:1 this would mean that about 9% and 18% β-cyclodextrin would be needed, respectively. The solubility of β-cyclodextrin is rather low with 2 g/100 ml at 25 °C. Consequently, only a small amount of β-cyclodextrin is dissolved in the paste and is able to form an inclusion complex with praziquantel. Although there is a equilibrium between suspended, dissolved and complexed drug and cyclodextrin, this happens only up to the solubility limit of the cyclodextrin. So addition of cyclodextrin above its own solubility level is not really necessary. Hence the amount of β-cyclodextrin was reduced to a 1:1 weight ratio with praziquantel. This has also the advantage of lower costs with β-cyclodextrin being rather expensive.

In addition to β-cyclodextrin, hydroxypropyl-β-cyclodextrin (HP-β-CD), a cyclodextrin derivate with better water solubility, was tested. This cyclodextrin shows a much higher water solubility than β-cyclodextrin (>60 g/100 ml to 2 g/100 ml, respectively) and might therefore complex more active ingredient in the aqueous pastes. The HP-β-CD:PZQ ratio was set at 1:1 in weight for comparison with the β-cyclodextrin 1:1 weight ratio paste. Although HP-β-CD would be completely dissolved in a 1:1 molecular ratio this would lead to a very high amount of HP-β-CD in the paste (22.3% for 5% PZQ, molecular weight of used HP-β-CD: 1395 g mol^{-1}). But in this case, the whole amount of praziquantel would still not be complexed. This huge amount of HP-β-CD would further lead to a decrease of water in the paste and thus to a considerable increase in viscosity. Finally, HP-β-CD is quite expensive, so a weight ratio of 1:1 with praziquantel was chosen.

In the literature several water soluble polymers such as polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) are described to enhance the complexation efficiency of cyclodextrins (see Chapter 1.2.4). For the pastes with in situ complex formation PVP was added in order to improve the complexation efficiency of the used cyclodextrins. Additionally, PVP can be used to adjust viscosity which is of advantage for the development of the pastes as well as its properties as wetting agent.

The previous tests were conducted with a praziquantel concentration of 2.5%. In this acceptance round the higher concentration (5% active ingredient) was tested to investigate if the higher amount of praziquantel is equally accepted as the 2.5%. Moreover, a colorant (iron oxide red) was used in the paste. Different colorants were chosen for the two pastes: titanium dioxide for the lower concentration and iron oxide

red for the paste intended for big cats. To exclude an impact of these colorants on the paste acceptance placebo pastes with the colorants were also tested.

Finally, a paste well established on the market (Nutriplus-Gel®; acceptance level three) was used as reference for the taste masking effect of the systems previously tested. The paste consists of soybean oil, nutrients, vitamins and flavors. Praziquantel was incorporated into the paste in a concentration of 5% and this paste was also given to the cats and compared with the results from the praziquantel taste masked and flavored pastes.

Results of this round of acceptance tests with twelve cats are displayed in Fig. 5.4. The paste with the isolated β-cyclodextrin inclusion complex showed a slightly lower acceptance compared to the 2.5% paste in the previous test (see Fig. 5.3). But when the high amount of active ingredient is considered the level was also admissible with an average of 1.83 ± 0.58. The paste with the same praziquantel-β-cyclodextrin ratio, but prepared in a one pot process was slightly lower ranked with an average level of 1.67 ± 0.65. Compared to this the in situ complex formation with a reduced amount of β-cyclodextrin (weight ratio 1:1) got the best ranking of all cyclodextrin pastes (average 1.92 ± 0.51). These results showed that in the paste with higher amount of cyclodextrin, in situ complex formation is not more pronounced than in the paste with lower amount of β-cyclodextrin. One could have expected the higher amount of β-cyclodextrin to be able to include more praziquantel molecules and thus enhance the acceptance level. But due to the limited solubility of β-cyclodextrin only a small amount of β-cyclodextrin is dissolved and this should be the same in both pastes. Consequently, both pastes should show almost the same acceptance level as it is the case. The acceptance is not significantly different between the two one pot processes with β-cyclodextrin.

HP-β-CD has the same acceptance as the β-cyclodextrin inclusion complex although its standard deviation is slightly lower (1.83 ± 0.39). This result was lower than anticipated because of the high solubility of HP-β-CD. It was expected to be able to complex more praziquantel molecules and thus show a higher level of acceptance. However, although HP-β-CD is dissolved completely in the paste, it is not able to complex all praziquantel molecules due to its rather low complexation efficiency (see Chapter 3.3.2), so that the remaining drug amount could still elicit a bad taste when part of it is dissolved in the cat's mouth. This suspended praziquantel amount might also be the reason for the overall nearly equal acceptance of the pastes and the maximum acceptance level two.

All in all, the acceptance tests with the different cyclodextrin-praziquantel combinations reached satisfying levels. The cats were not utterly disgusted by the taste (no zero ratings) and only slight excitation (zero to two cats per formulation) and salivation (in three to four cases in each run with 12 cats) were recorded.

However, salivation might have been evoked by the colorant. The placebo paste with iron oxide red did show about the same acceptance level as the cyclodextrin pastes: It reached an average of 1.83 ± 0.39 which is even slightly lower than the best cyclodextrin paste. Furthermore, salivation was reported in three cases as well as

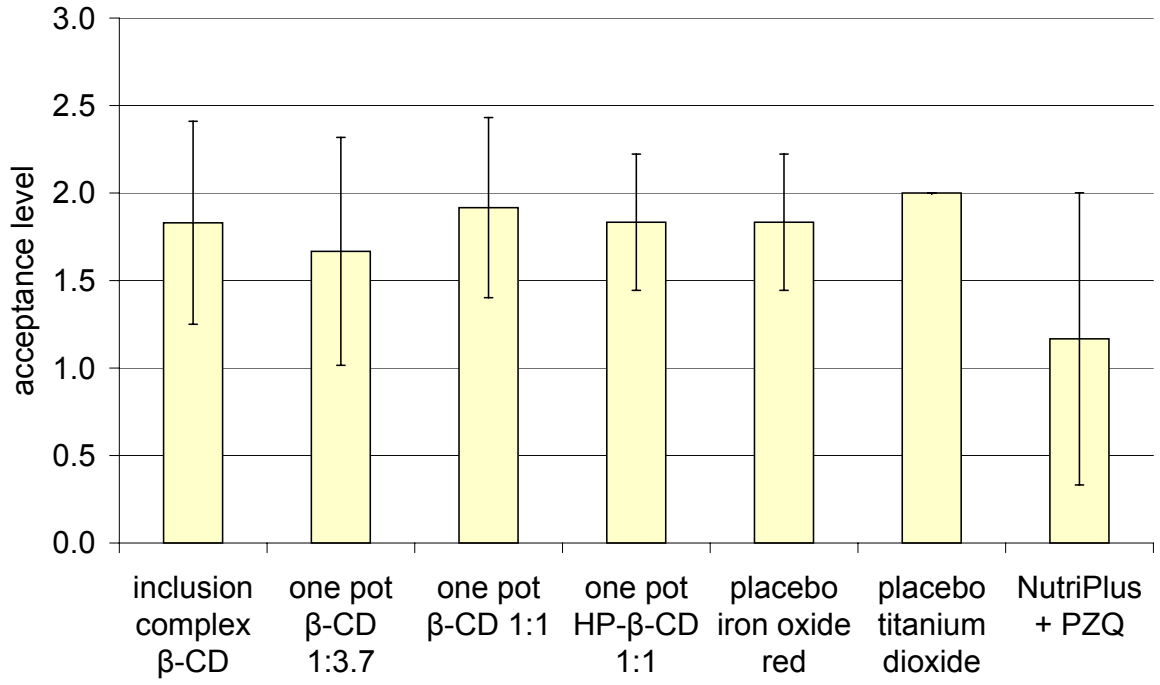

Figure 5.4: Acceptance tests of different cyclodextrin pastes (β-cyclodextrin and HP β-CD in different ratios); PZQ amount 5%; number of cats: 12; mean$\pm$stdev

staining of cats fur, owners' clothes and surroundings. The cats don't seem to take pleasure in iron oxide red and because of its ability to stain the cat and the clothes it will be replaced by another colorant in further development. With titanium dioxide no such troubles were reported. All cats were rated with a two and no salivation or any other negative incidents were observed. It seems as if the highest acceptance level reached with the placebo formulation is a satisfactory one, so the cyclodextrin results have to be regarded even higher as they are near to the maximum reachable acceptance level! To illustrate this, another paste was evaluated for its acceptance: Nutriplus-Gel® is a nutrition paste for cats which is very much appreciated by cats (acceptance level three). During the preceding tests, it was given to the cats to preclude a potential systemic reluctance due to some bitterness memorization from previously tested formulations. However, for this test praziquantel was incorporated into Nutriplus-Gel® in a concentration of 5%. The rather low acceptance level (average 1.17$\pm$0.83) differs significantly ($p<0.05$) from the best cyclodextrin result (one pot process 1:1). However, it has to be considered that Nutriplus-Gel® is a paste based on soybean oil and could thus not be directly compared with the aqueous pastes. But as proven, this oily base has absolutely no negative impact on the compliance of the paste which has the best possible acceptance. Moreover, praziquantel is insoluble in soybean oil in contrast to miglyol where it is partially soluble, so these two bases cannot be compared with each other. Besides all oily pastes tested contained only a taste masked formulation without any flavor so no direct comparison is possible with

Nutriplus-Gel® containing praziquantel as this paste contains a lot of nicely tasting excipients such as cod liver oil, meat extract and vitamins.

Hence, it can be thoroughly assumed that the results from the acceptance tests of the aqueous cyclodextrin pastes and Nutriplus-Gel® with praziquantel can be balanced against each other. So it could be demonstrated that the taste masking ability of the tested cyclodextrin system is very good for praziquantel and the simple incorporation of the bitter active ingredient in a well tasting paste is not sufficient to mask its taste.

5.3.4 Testing of enantiomers and β-cyclodextrin/PVP pastes

In the final round of acceptance tests the separated praziquantel enantiomers were tested. It is known that enantiomers can have different properties than their racemate or their partner enantiomer. This can include pharmacokinetic parameters such as absorption and metabolism but also physicochemical properties like solubility and also and especially taste (Lee & Williams, 1990; Bassoli et al., 2000). Therefore, acceptance tests were performed with the pure praziquantel enantiomers to check if there might be any difference in taste perception. Furthermore, it was tested if a taste masking effect is also reached by PVP alone without cyclodextrin as it is assumed that PVP is also able to form complexes with praziquantel (El Arini & Leuenberger, 1998). For the sake of completeness, in situ complex formation with β-cyclodextrin and PVP was also tested in a paste containing no flavor as all previous in situ complex forming tests were performed with beef flavor. Finally, it was tested if in situ complex formation between praziquantel and β-cyclodextrin was also possible in an oily paste in which β-cyclodextrin is insoluble.

Results of this acceptance round are displayed in Fig. 5.5. During paste preparation differences between the enantiomers regarding smell and also taste could already be noticed. This observation was confirmed by the cats in the acceptance tests: the pastes containing the praziquantel enantiomers reached significant different values ($p<0.05$) with an average of 1.88 ± 0.64 for $(-)$-praziquantel and 1.00 ± 0.93 for its $(+)$-enantiomer. Additionally, no failed acceptance (zero rating) was reported for $(-)$-praziquantel whereas in the case of $(+)$-praziquantel three cats were rated zero. Salivation and excitation were the same for both pastes with 13% and 75%, respectively. It can thus be said that differences in perception of the two praziquantel enantiomers exist with the $(-)$-enantiomer being more appreciated by cats. The scored acceptance of $(-)$-praziquantel is equal to the results from the earlier tests with β-cyclodextrin or Precirol as taste masking agents or incorporation of a flavor. No significant differences could further be noticed from the results of a combination of a taste masking agent and a flavor. So it might also be possible to use the $(-)$-enantiomer of praziquantel in the paste and hence renounce taste masking. However, if additionally a taste masking agent such as β-cyclodextrin and/or a flavor were employed an even higher acceptance might be reached which, of course, would be highly desirable. Another advantage of the use of the $(-)$-enantiomer might be that fewer side effects such as emesis and diarrhea might occur as these reactions are mainly evoked by $(+)$-

praziquantel (Andrews, 1985; Blaschke & Walther, 1985). Furthermore, it might be possible that due to the use of an enantiomer less substance is needed if its efficacy is higher than that of the racemate. In literature, it can be found that (−)-praziquantel is more effective against schistosomas (Andrews, 1985; Andrews et al., 1983; Liu et al., 1988; Shu-Hua & Catto, 1989; Xiao et al., 1998); however, no data are available on its efficacy against cestodes apart from a short statement that the efficacy of (−)-praziquantel is a bit higher than that of its (+)-enantiomer (Andrews et al., 1983). Unfortunately, no in vitro efficacy trials could be initiated due to the lack of an in vitro cestode model. In vivo studies in cats were not performed due to animal welfare. So no detailed data on the efficacy of the enantiomers on cestodes were available and tests were performed with 2.5% active ingredient in both cases. Concluding, it can be stated that the acceptance of (−)-praziquantel is better than its partner enantiomer and it might be even higher if additionally a taste masking agent and a flavor were used. Moreover, if a dose reduction were possible due to a higher efficacy this might also result in an enhanced acceptance level. Yet, it has to be kept in mind that enantioseparation of a racemate is very complex and expensive. Moreover, the usage of an enantiomer in a new product would require a completely new registration including efficacy and safety trials as it would be regarded as a new chemical entity. Hence, a less laborious and expensive method for achievement of a good acceptance is preferred.

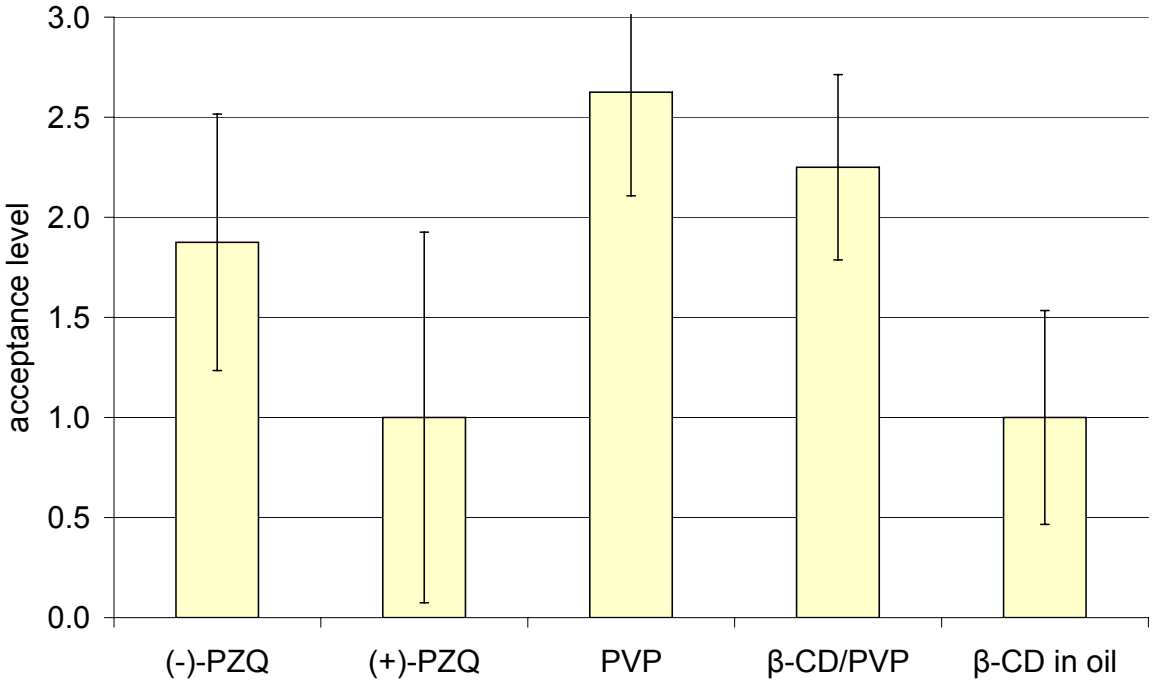

Figure 5.5: Acceptance tests of aqueous pastes containing PZQ enantiomers, PVP and β-CD with PVP (in situ) and an oil based paste with β-CD; PZQ amount 2.5%; number of cats: 8; mean$\pm$stdev

In situ complex formation between β-cyclodextrin, PVP and praziquantel was up

to now only tested in the presence of a flavor and reached a satisfactory acceptance level. To examine if the taste masking effect is also that distinct without flavor a paste containing only praziquantel, β-cyclodextrin and PVP was tested. It could be shown that the results were equal or, as the case may be, even slightly better with an average acceptance of 2.25 ± 0.46. However, these differences are not significant ($p > 0.05$), especially if it is considered that in the pastes with beef flavor iron oxide red was also present which was not really liked by cats, so that even placebo pastes only reached a satisfactory acceptance (see previous chapter). It can thus be concluded that taste masking due to in situ complex formation with β-cyclodextrin and PVP is as pronounced in the absence of a flavor as it is in its presence.

PVP was included in the former tested pastes to enhance the complexation efficiency and thus the taste masking effect of β-cyclodextrin on praziquantel. However, as PVP is thought to form complexes with praziquantel itself (El Arini & Leuenberger, 1998) its influence on acceptance was tested in a separate paste containing only PVP without β-cyclodextrin. The result was indeed very good and it nearly scored a high acceptance with an average of 2.63 ± 0.52. This was the best level reached in all acceptance tests and led to the supposition that PVP is indeed able to complex the active ingredient in some form and hence mask the taste of praziquantel. Still, the result is not significantly different ($p > 0.05$) from the previous discussed paste forming an in situ complex with β-cyclodextrin and PVP. Nevertheless, it seems to be thoroughly feasible to achieve an equally good taste masking effect if PVP is solely used in an aqueous paste. It might further be possible that the acceptance could be enhanced by the addition of a flavor. This would mean that β-cyclodextrin might not be needed in the paste to achieve a satisfactory acceptance level and hence, production costs and preparation time of the pastes would be less. Yet, this theory would have to be supported by further acceptance tests as this test series was only performed on eight cats.

Complex formation between cyclodextrin and its guest molecule is mostly only achieved if the cyclodextrin is dissolved. However, inclusion into the cyclodextrin cavity can also happen in solid state by exchange of crystal water against guest molecule; yet, this is quite laborious as either a long mixing time or force or a combination of both is necessary. β-cyclodextrin is insoluble in Miglyol whereas praziquantel is soluble therein. It was thus tested if the addition of β-cyclodextrin to an oily paste in a 1:1 weight ratio with praziquantel results in an enhanced acceptance due to in situ complex formation. Administration of this oil based paste was difficult as the viscosity was too high for easy application. Excitation and salivation were thus recorded in 50% and 38% of all cases, respectively. The paste only reached a poor acceptance level with an average of 1.00 ± 0.53. This was significant less ($p < 0.01$) than an in situ complex formation between β-cyclodextrin and praziquantel in an aqueous paste. Still, the result is the same as if a separated β-cyclodextrin/drug complex is incorporated into an oily paste (see Chapter 5.3.1). It might be possible that a certain quantity of active ingredient is included into the cyclodextrin cavity as praziquantel is present in dissolved state in Miglyol. It could thus be exchanged against crystal water from

the cyclodextrin cavity; however, this is quite unlikely to happen as the release of water into the oily paste base would not result in an energetically more favorable state. Besides, nearly all oily pastes tested containing praziquantel have achieved a poor acceptance – no matter if the drug was incorporated in a taste masked form (β-cyclodextrin or Precirol, and from another group a combination of shellac and HPMC or Kollicoat SR30D (data not shown); see Chapter 5.3.1) or just added to a highly palatable oily paste (see Chapter 5.3.3). What is more, even in the placebo tests a slightly better result was scored for the aqueous paste than for an oily one. It might hence be concluded that this poor acceptance level is generally reached if an oily paste with praziquantel is applied. Therefore based on these results, it cannot be stated if an in situ complex formation between β-cyclodextrin and praziquantel occurs in an oily paste which might lead to a taste masking effect.

5.3.5 Dissolution studies

Dissolution studies with different pastes have been performed to assure sufficient drug release from the pastes. The dissolution rate should be equaling the standards of the tablets already on the market which require release of 30% and 70% active ingredients after 15 min and 60 min, respectively. This is an obligation for the prove of bioequivalence so that the paste does not have to be registered as completely new product but can be marketed as a follow-up product of the tablets.

Several pastes were tested for drug disintegration containing either cyclodextrin or Precirol as taste masking agent (recipes see Chapter 5.2.4). In the case of cyclodextrin the separated complex with β-cyclodextrin as well as a complex formed in situ with both β-cyclodextrin in different ratios and hydroxypropyl-β-cyclodextrin were tested. All pastes had a praziquantel concentration of 5%; the separated complex was additionally also tested with 2.5% active ingredient. In one paste with in situ complex formation milbemycin oxime was added to exclude any influence of this substance on praziquantel dissolution. Taste masked formulations with Precirol were added in form of particles prepared by hand and those manufactured by Brace containing either 25% or 37.5% praziquantel. The latter ones mentioned were only tested in a praziquantel concentration of 2.5% in the paste whereas the hand made lipid particles were tested in both concentrations (2.5 and 5%). In addition, pastes containing lipid embedded praziquantel were tested after storage at 25 °C, 30 °C and 40 °C for six and nine months to investigate any occurring drug leakage from the lipid matrix in this time.

The results are displayed in Fig. 5.6. Samples were only drawn at two time points (15 and 60 min) as these dissolution studies were mainly performed to ensure equal drug disintegration from the pastes compared to the tablets already on the market. Moreover, it was tested if the drug might leak out of the taste masked formulations with Precirol and for that, samples at those two time points were sufficient. It can clearly be seen that the cyclodextrins enhance the solubility of praziquantel very efficiently: from all pastes the complete drug amount had dissolved after 15 min already independent of drug loading, in situ complex formation or isolated com-

plex and type of cyclodextrin. Moreover, the addition of milbemycin oxime does not have any influence on praziquantel release in the presence of β-cyclodextrin. Hence, the requirements for drug dissolution from pastes containing cyclodextrin are easily achieved. As expected, drug disintegration from pastes prepared with lipid embedded praziquantel is much slower. Here, differences can again be noticed between the formulation prepared by hand and the Brace particles although they are not as pronounced as in case of dissolution from the pure lipid particles. The pastes containing the hand made lipid particles released the active ingredient faster with 41% for the paste with 5% active ingredient and 49% in the case of 2.5% drug whereas the value for the Brace particles was at 24% and 29% for 25% and 37.5% drug loading, respectively. After 60 min, the released drug amount of the pastes containing the hand made particles nearly reached the requirements with 63% and 68% for 5% and 2.5% drug concentration, respectively. In contrast to this, drug release from the pastes with Brace microparticles was significant less with 37% and 39% for 25% and 37.5% drug loading, respectively. The reasons for these discrepancies are, of course, again the size and shape variations between the particles manufactured by different techniques as those prepared by Brace are much larger and also have a smoother surface, thus resulting in an overall considerable smaller surface area (see Chapter 4.3.3). Drug disintegration from the paste with the hand made particles is in the range of drug dissolution from the pure lipid particles with only a slightly faster dissolution in the case of the paste. This might be due to an improved wettability of the particles by incorporation in the paste. Another option could be that drug disintegration is enhanced by other paste excipients such as the beef flavor which contains substances that might act as emulsifier. A further possibility might be drug leakage from the lipid particles into the paste. Yet, as the differences are not that pronounced, this theory is not very probable. In contrast to this, variations are quite major for the Brace particles between drug dissolution from the particles incorporated into the paste and those alone. While only less than 5% praziquantel had been released from the pure particles after 15 min, this amount was increased much in the case of the pastes up to 24% and 29%. After 60 min, differences in the released drug quantity were not as distinct, but still nearly twice as much praziquantel had dissolved from the pastes than from the separate particles. Besides, only marginal differences could be observed between the Brace particles with differing drug loading incorporated into the pastes. As discussed previously, a possible reason might be enhanced wettability of the particles in the paste, an influence of other paste excipients or drug leakage. However, the main cause for the enhanced drug release is most probably a crushing of the particles during paste homogenization with the Ultra Turrax. So, the microparticles became smaller which resulted in faster drug dissolution rate. This effect would be more pronounced for the Brace particles due to their large size compared to the particles prepared by hand. As drug disintegration from the pure Brace particles is very low, these effects might have a higher impact on drug dissolution than in the case of the hand made particles.

To further investigate a possible drug leakage from lipid particles into the paste base drug dissolution studies were performed with pastes containing hand made

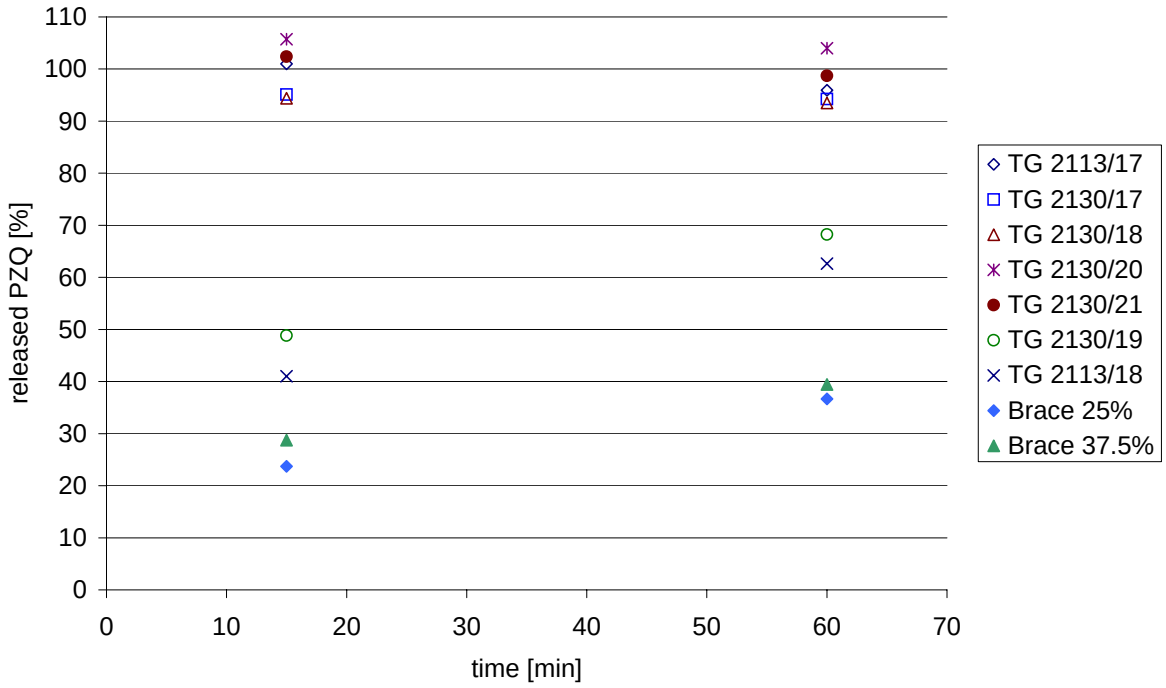

Figure 5.6: Drug dissolution from pastes containing taste masked praziquantel; tests were run in six fold

lipid particles with a praziquantel concentration of 2.5%, which had been put on stability for six or nine months at 25 °C, 30 °C and 40 °C (formulations from the first acceptance tests containing either only Precirol particles (TG 2130/5; 9 months) or a combination of lipid particles and malt flavor (TG 2130/15; six months); see Chapter 5.2.2). The dissolution data are shown in Fig. 5.7. Due to a malfunction of the clock the first samples of the formulation TG 2130/15 were taken after 18 min instead of 15 min. Still, it is clearly observed that drug dissolution from the pastes stored at 40 °C is much more pronounced with more than 70% released after 15 min than it is in the case of the pastes stored at 25 °C and 30 °C. Drug release from these pastes is in the range of a freshly prepared paste containing lipid particles prepared by hand (see Fig. 5.6). Besides, no differences in drug dissolution behavior occurred between the two storage durations. These results are in good accordance with the dissolution studies performed with pure lipid particles put on stability (see Chapter 4.3.8). There, a faster drug dissolution from the samples stored at 40 °C in contrast to those at 25 °C and 30 °C was also reported. Hence, it can be concluded that the increased drug release from the paste is due to the higher storage temperature and effects arising from it. The main argument might be that the lipid particles soften at that temperature as the melting range of Precirol already starts below 40 °C. The phenomenon of increased drug dissolution might then be explained on the one hand by more praziquantel dissolving in the lipid matrix as it is partially soluble in Precirol thereby forming a solid solution. On the other hand drug diffusion from the lipid

particles into the paste base could take place which would also lead to a faster drug disintegration. Of course it could be possible that both incidents occur. If more praziquantel were dissolved in the paste it might be possible that the taste masking effect is reduced and resulting from that a lower acceptance might be achieved. Yet, this can only happen if the pastes are kept at or above 40 °C for a longer period of time. Storage of pastes at ambient temperature on the other hand should have no negative effect on the acceptance, as in this case no increased drug diffusion into the paste base is supposed to happen.

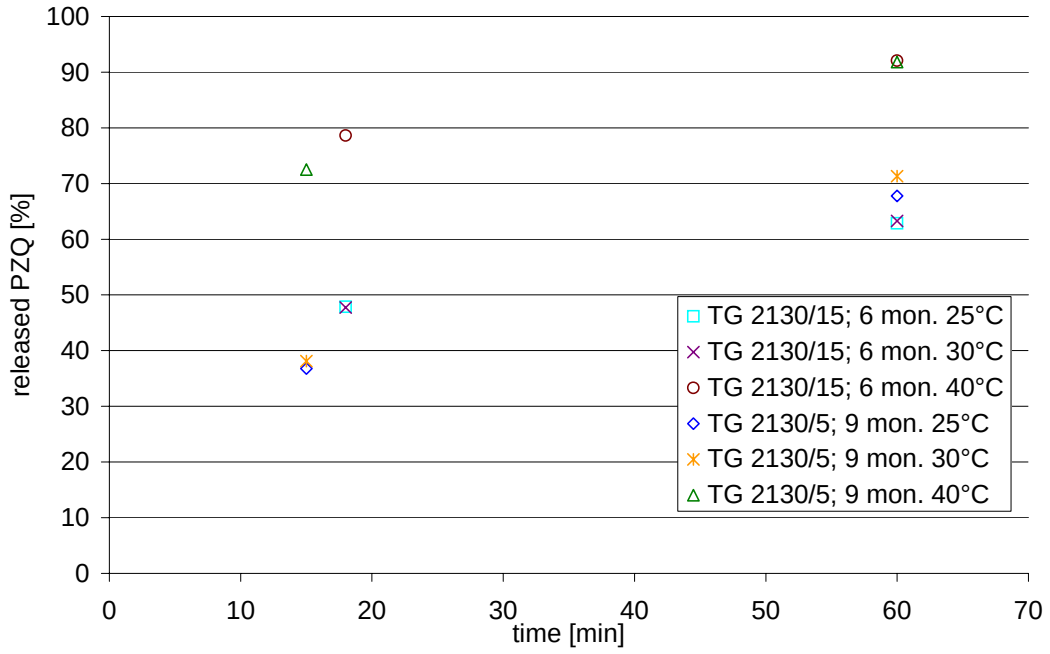

Figure 5.7: Dissolution of praziquantel from pastes containing lipid particles stored at specified conditions; duplicate measurements

In conclusion it can be stated that dissolution of praziquantel from aqueous pastes containing cyclodextrin is very rapid and no problems arise in fulfilling the dissolution requirements (NAH, 2003). In the case of lipid embedding this is not as easily achieved. Of course, drug disintegration from the particles in the paste is dependent on particles size as can be seen in the differences between the particles prepared by hand and those manufactured by Brace. Yet, dissolution from the small hand made particles is still slightly below the requirements. It might be possible to increase the dissolution rate by incorporation of hydrophilic substances into the lipid matrix (see Chapter 4.3.7). However, it might thoroughly be probable that this could then lead to a diffusion of praziquantel from the lipid matrix into the paste which might hence result in a reduced acceptance. Another possibility to rise drug dissolution could be reduction of particle size. This might be achieved by a different preparation method such as an emulsion method. Finally, it was observed that drug

disintegration from pastes containing lipid embedded particles was enhanced if the pastes were kept at 40 °C for more than six months. This might be due to drug diffusing from the lipid matrix into the paste base. Yet, if the pastes are stored at ambient temperature no drug leakage could be noticed even after nine months.

5.4 Conclusions

The acceptance tests with cats were an invaluable tool to test the previous developed taste masked formulations of praziquantel. Satisfying results were reached in most cases especially if an aqueous paste was employed.

The Eudragit E microspheres could only be incorporated into a PEG based paste as the polymer is soluble in Miglyol and swellable in water. But as praziquantel is soluble in PEG it most probable diffused from the microspheres into the paste which then led to a miserable result in the acceptance tests. The achieved level was nearly identical with a PEG paste containing praziquantel in pure unmasked form. So for that reason taste masking with Eudragit E was not tracked further.

Taste masking with β-cyclodextrin and Precirol resulted in a satisfactory acceptance in an aqueous paste; however, in an oily one the acceptance level was only poor. This might be due to a better solubility of praziquantel in Miglyol compared to water so that a larger part of the active ingredient is dissolved in the base and thus leads to a lower acceptance. Consequently, focus was laid on aqueous pastes for further development. The solely addition of a flavor (malt or beef) to an aqueous paste without any taste masking agents also led to quite good results. Therefore, a combination of taste masked praziquantel and flavor was tested. It could be demonstrated that this combination resulted in an overall satisfactory acceptance with a narrow deviation and no complete failed acceptance which was an improvement to the previously tested pastes containing only a taste masking agent or a flavor.

Nearly all taste masked formulations of praziquantel include separate preparation of these substances. These procedures can be quite extensive and therefore, another, less complex, production option was desired. For the aqueous pastes, it was thought possible that complex formation between praziquantel and β-cyclodextrin could also occur directly (in situ) in the paste. Acceptance tests performed with pastes containing in situ formed complexes with β-cyclodextrin and hydroxypropyl-β-cyclodextrin in combination with beef flavor also showed satisfactory acceptances. They were equaling the results of placebo pastes of the same formulations and two other commercial pastes containing active ingredients not known to taste especially unpleasant. Hence, the results from the aqueous pastes with in situ formed drug-cyclodextrin complexes and beef flavor have to be regarded even higher. Moreover, it could be shown that the simple incorporation of praziquantel in a highly palatable paste available on the market was not successful in achieving good acceptance. Due to segregation incidents after scale up which seemed to have resulted from interactions between the beef flavor and Avicel RC591 the formulation was changed to xanthan gum as thickening agent. In addition, iron oxide red was not used any longer as it

was not liked by cats and stained the surroundings. However, these changes did not have any negative effect on the cats' acceptance.

In the last round of acceptance tests, the two separated praziquantel enantiomers were investigated in an aqueous paste without any flavor. Differences between these substances were significant with (−) praziquantel being more liked by the cats – it nearly reached a satisfactory acceptance level. It might thoroughly be possible that this level might be heightened by the addition of a flavor. Moreover, if this enantiomer were more effective against cestodes a lower drug amount would be needed which might also have a positive effect on the acceptance. Yet, it has to be considered that enantioseparation is a very expensive and extensive procedure and therefore, other less laborious methods are preferred.

Polyvinylpyrrolidon (PVP) was added to the previous cyclodextrin pastes to enhance the complexation efficiency of cyclodextrin and thus have a positive influence on the acceptance. As PVP is thought to form complexes with praziquantel itself acceptance tests were performed with aqueous pastes containing only PVP. The results were very good and almost reached the best acceptance level possible. A further increase might again be achieved by the combination with a flavor. Still, the results were not significantly different from a paste containing β-cyclodextrin and PVP. However, if these good results from eight cats were supported by additionally acceptance tests it might thoroughly be possible to renounce the use of β-cyclodextrin which would then lead to lower production costs.

The most important step for praziquantel absorption belonging to class II drugs of the biopharmaceutical system is the dissolution from the formulation. For that reason, dissolution studies with pastes containing taste masked praziquantel were performed. It could be shown that this criteria is of no concern in the case of cyclodextrins as these substances also act as solubilising agent and hence the complete drug amount had disintegrated after 15 min. Precirol embedded praziquantel on the other hand did not achieve such good results and the dissolution requirements were just not fulfilled. Still, they were quite closely missed and as it could be shown that the rate of drug disintegration is dependent on particle size and surface area this might be a possibility for further improvement.

The stability of lipid embedded praziquantel in an aqueous paste was further examined regarding drug diffusion from the lipid matrix into the paste base. It could be demonstrated that no drug leakage occurred when the pastes were stored below the melting range of Precirol (25 °C and 30 °C). However, if the storage temperature was higher (40 °C) a softening of Precirol probably happened which resulted in a significant increase in drug disintegration. This might either be due to praziquantel diffusing into the paste base from the softened lipid matrix or praziquantel dissolving in Precirol to a greater extent.

Overall, it can be concluded that the objective of a well accepted praziquantel paste was achieved with two of the three tested taste masking methods. The acceptance level of the best paste containing β-cyclodextrin was equaling the results from placebo pastes and also that of pastes already established on the market.

Final remarks and outlook

The objective of this work was to mask the taste of a bitter active ingredient for use in a veterinary product for cats. This can be a challenging task as cats are known to be especially fastidious and their mechanism of taste perception is not yet as well understood as for humans. Still, the aim of this study could be achieved in form of a well accepted oral paste containing praziquantel, a pharmaceutical ingredient known to be very bitter, and taste masking agents.

Three different approaches were employed to achieve an improvement in the taste of praziquantel. The most often used and easiest technique to mask the taste in human health is coating. However, this was judged to be unfavorable as the animals might crush the coating during chewing and as a result, perceive the bitter taste. Therefore, other methods were applied using incorporation into a specific polymer, a lipid or complex formation with cyclodextrins. The challenge was further increased as the developed taste masked formulation should be incorporated into a semisolid oral dosage form. So, the drug formulations containing taste masked praziquantel had to be stable in this semisolid paste especially with respect to drug leakage from microparticles into the base.

Satisfying results were obtained with embedding of the drug into a lipid matrix in form of microparticles and an inclusion complex formation with β-cyclodextrin. Matrix formation with a lipid (Precirol ATO 5) led to a delayed drug release from the microparticles which was the main criteria for successful taste masking. But on the other hand, a retarded drug form was not desired. Due to incorporation of a hydrophilic substance into the lipid matrix drug disintegration could be altered to better match the needs for a taste masked formulation: drug release was slightly decreased in the first minutes so that no active ingredient might get dissolved in the animals' mouth. Yet, after about 20 min, drug disintegration from the microparticles was enhanced and hence, no retarded effect was to be expected. Moreover, digestion of the lipid would occur in vivo and thus the drug dissolution process is supposed to be faster. For evaluation of the taste masking abilities of cyclodextrin dissolution studies were no useful tool as the taste masking effect of these substance is not based on delayed drug release but on inclusion of the drug molecule into the hydrophobic cavity of the cyclodextrin ring. Several investigations (phase solubility studies, determination of the included drug amount, DSC and NMR) indicated the formation of a true inclusion complex between praziquantel and β-cyclodextrin. As a result, a taste masking effect was hoped for. Incorporation of the active ingredient into an

Eudragit E matrix by solvent evaporation method was additionally tested. This polymer was chosen as it is insoluble in the neutral medium of the mouth but dissolves rapidly in the acidic environment of the stomach and hence, no sustained drug release would be the case. Dissolution studies showed a slightly delayed drug release in a polymer insoluble medium. Yet, with scanning electron microscopy an insufficient incorporation of the drug into the polymer matrix could be observed.

So, for in vivo acceptance tests the three above described taste masking formulations were incorporated into different oral pastes according to their properties. On the basis of acceptance tests, water, oil and PEG were chosen as paste bases. The Eudragit E microspheres could only be evaluated in a PEG base as the polymer is soluble in Miglyol and swellable in water. But praziquantel being soluble in PEG unacceptable results were achieved and therefore, this taste masking method was abandoned. The praziquantel-β-cyclodextrin-complex and the lipid microparticles were both tested in aqueous and oily pastes. Better results were scored in water with a satisfying acceptance level. Upon addition of a flavor (artificial beef flavor or malt extract) this value could even be enhanced a little and no complete detest of the paste was reported any more. Further, it could be shown that an in situ complex formation between active ingredient and β-cyclodextrin is possible in the aqueous paste as an equally good acceptance result was scored. This in situ complex formation was desired in respect of ease of production and registration. Thus, an aqueous paste containing β-cyclodextrin and beef flavor as taste masking agents was chosen for further development.

Additionally, the separated enantiomers of praziquantel were tested for their acceptance in cats as it is known that enantiomers can not only have different efficacies but can also vary in other properties. This statement was emphasized with $(-)$-praziquantel scoring significantly higher acceptance values than its $(+)$-enantiomer. A satisfying acceptance level was achieved even without the addition of a flavor. Hence, if a flavor were used an improvement of this result might be accomplished. This might also be the case upon addition of β-cyclodextrin as a complex formation between these two substances could also be demonstrated. Moreover, it could be possible that $(-)$-praziquantel is more effective than the racemate and as a result, less amount of active ingredient would be necessary which might lead to a better acceptance. Though, it has to be kept in mind that chiral separation of a pharmaceutical ingredient is very extensive and expensive. Besides, the use of an enantiomer would be regarded as a new chemical entity and thus require a completely new registration procedure. For these reasons, a paste containing the $(-)$-enantiomer of praziquantel was not developed further yet and focus was laid on the aqueous paste with β-cyclodextrin and beef flavor.

The acceptance tests described in this study were performed with cats adopted to such procedures. Hence, these results might be regarded as a first indication and should be supported by additional tests. So, for further evaluation of the pastes' acceptance on "normal" cats and their owners a large study is currently running with more than 170 cats. The pastes are ranked according to their ease of application by the pet owner and willingness to be taken by the cats. For comparison, a placebo

beef flavored tablet is also tested on the same group of cats; yet, only half of the cats were also given the tablet as the study is mainly conducted for evaluation of the paste acceptance.

The intermediate results are very promising: for the paste the results for both the ease of administration and the acceptance by the cats exceeded those for the placebo tablet by far. Nearly two thirds of all cats swallowed the whole amount of the paste immediately whereas this results was only reported for half of the cats in case of the tablet. Moreover, the tablet was completely rejected (i.e. the cat spat out the complete tablet or the whole amount of paste) in 37.5% of all cases while this occurrence was only marginal for the paste with less than 6%. Thus, it can be claimed that an oral paste containing praziquantel in a taste masked form is much more liked by cats than a placebo beef-flavored tablet. Additionally, the paste was also rated better applicable by the pet owners: more than 60% evaluated the ease of application as very easy or quite easy. For the tablet, this was only recorded for about 45% of all cases while one third of the pet owners reported that it was impossible to administer the tablet to their cat at all. The paste, on the other hand, could not be given to less than 5% of all cats. Hence, from the cats owners assessments the statement of an overall better accepted oral paste in comparison to a tablet could be supported.

To sum it all up, a paste containing praziquantel, β-cyclodextrin and a flavor is very well accepted by cats and is mostly immediately swallowed. This is a very pleasing result especially with respect to the lower acceptance values obtained for a flavored placebo which is often rejected. Moreover, the consistence of a paste seems to be preferred to a tablet as even a paste containing praziquantel achieves better results than a placebo tablet. This advantage is also confirmed by the cat owners who rated the ease of administration of this paste much higher than the placebo tablet. So in conclusion, it can be stated that the goal of a taste masked formulation for cats containing praziquantel was truly achieved.

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Curriculum Vitae

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