3. RESULTS AND DISCUSSION

3.1. ZEIN COATINGS FROM ETHANOLIC SOLUTIONS

3.1.1. Plasticiser for zein

Pure zein films were very brittle due to the high glass transition temperature (Tg) (167 °C) (Fig. 1). Several substances were reported in the literature to act as plasticizers, however, the majority of them are not suitable for pharmaceutical purpose (Beck et al., 1996; Lai and Padua, 1998; Lai et al., 1997).

Plasticization resulted in an increase of flexibility, less shrinkage and lifting of the films (Table 3).

Table 3 Plasticiser evaluation for zein films from ethanolic solution

Plasticiser	Plasticiser content [% w/w]	Appearance	Flexibility	Shrinkage	Lifting	Stickiness
none	0	clear	-	-	-	-
Glycerol	10	clear	+++	-	-	++
Propylene Glycol	10	clear	+++	-	-	±
Sorbic acid	10	clear with crystals	+++	-	-	+
Tartaric acid	10	clear	+++	-	-	+
TEC	10	clear	+++	±	-	±
TBC	20	opaque	+	+	+	-
ATEC	20	opaque	+	-	+	-
ATBC	20	opaque	+	+	+	-
MCT (812)	20	opaque	-	+	+++	-
Castor oil	20	clear	-	+	++	++

(symbols: from ± to +++: increasing strength of the characterized parameter, -: no response)

Poor plasticization mostly correlated with shrinkage and in all cases showed a lifting of the films, which turned worse with decreasing plasticisation. The plasticising effect for zein was the strongest for glycerol and propylene glycol, as quantified by the reduction of Tg (Fig. 6A) and improved flexibility, determined as an increase in elongation (Fig. 6B). Propylene glycol was selected as the preferred plasticiser for coating, as glycerol resulted in sticky films already at 10% content (Table 3).

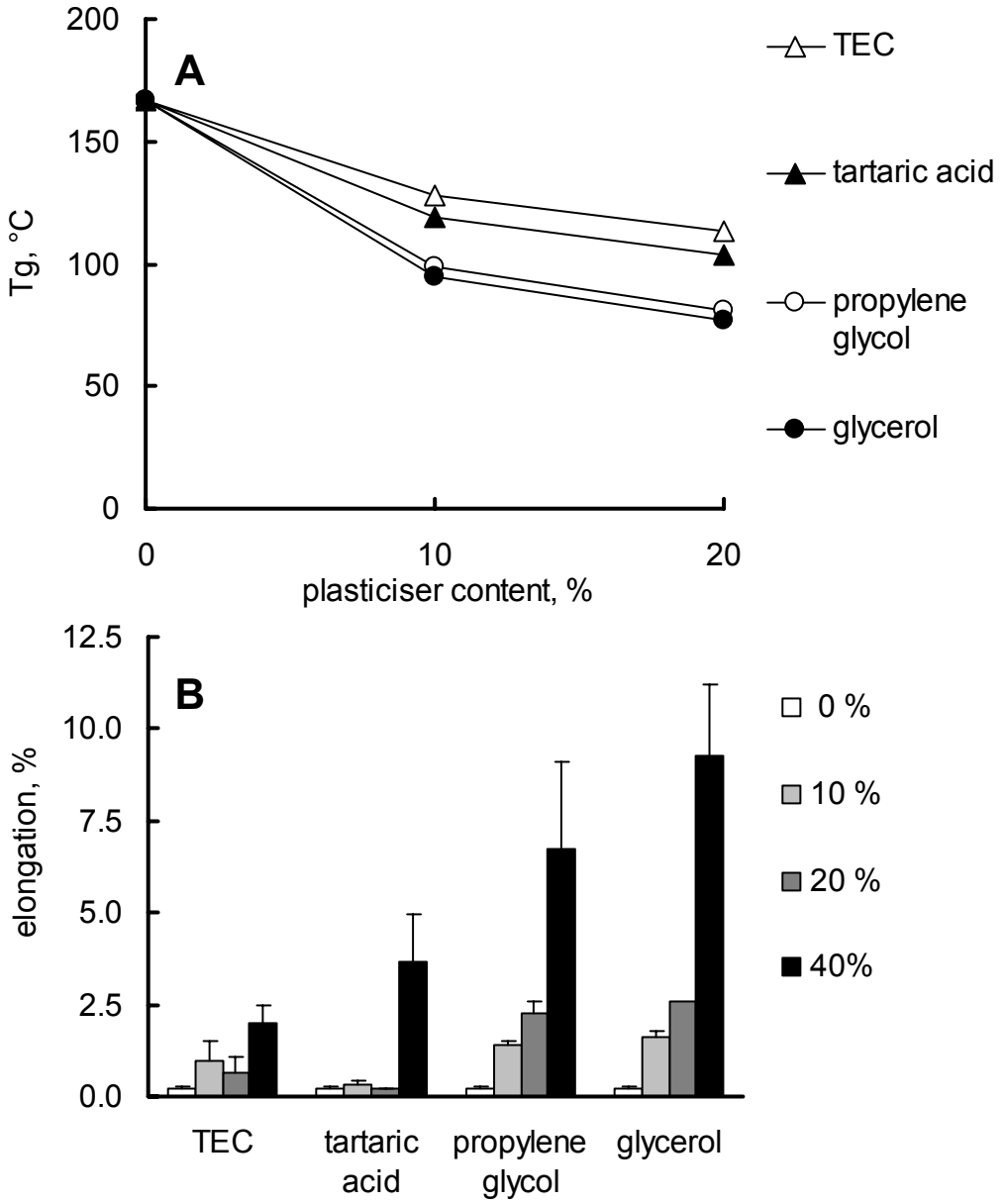

Figure 6 Evaluation of efficient plasticisers for zein: A: glass transition temperature (Tg) and B: mechanical properties of plasticised zein films in dependence of the plasticiser content

In general hydrophilic substances (e.g., glycerol and propylene glycol) had a higher plasticising efficiency for zein than more hydrophobic ones (e.g., MCT and castor oil). Improved plasticisation was related to stronger hydrogen bond forming capabilities (Beck et al., 1996).

3.1.2. Water as a plasticiser for zein

Water is an outstanding plasticiser for zein (Beck et al., 1996; Reiners, 1973). Zein films were very brittle in the dry state without plasticiser with an elongation less than 1%. They turned soft and very flexible in the hydrated state with decreased puncture strength and modulus and strong increase in elongation with increasing water content (Fig. 7). The improvement of flexibility was much more than for the plasticisers (Fig. 6B vs. Fig. 7).

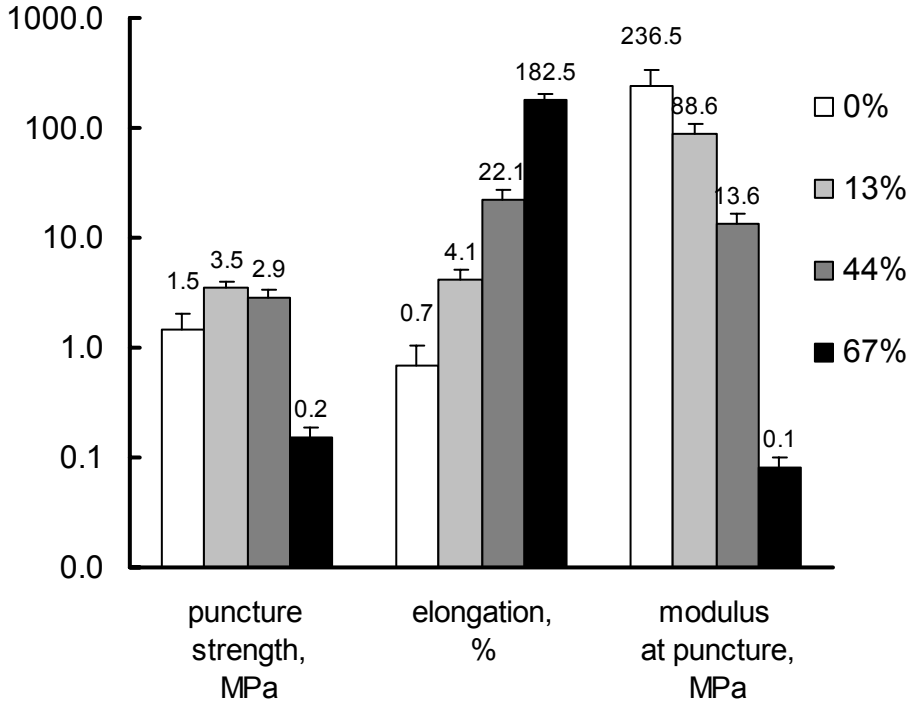

Figure 7 Effect of %- water content on the mechanical properties of zein films

Table 4 Weight fraction of absorbed water of zein films conditioned at different r.h.

% r.h.	Water weight fraction
100	0.44
85	0.13
74	0.10
22	0.05

The plasticising effect of water was also quantified by a decrease in the Tg of zein films after conditioning at different r.h. (Fig. 8). The moisture uptake increased and the Tg decreased with increasing r.h, with a dramatic jump between 85 and 100% r.h. (Table 4). The measured Tg correlated well to the values predicted by the Gordon-Taylor equation.

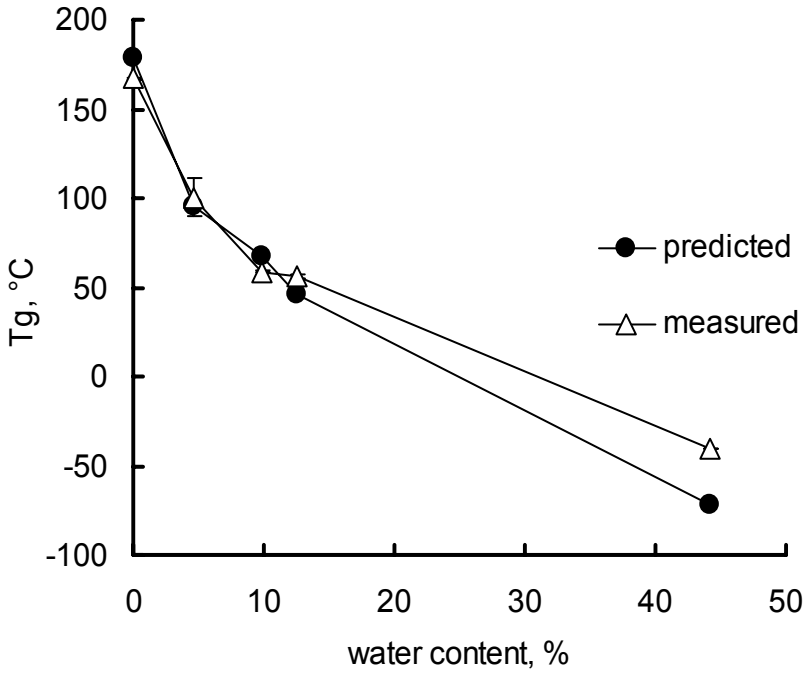

Figure 8 Measured vs. predicted Tg values by the Gordon-Taylor equation on dependence of the %-water content (w/w) of zein films

3.1.3. Drug release from coated pellets

Theophylline release from pellets coated with an ethanolic zein solution was rapid in pH 1.2 with > 90% release after 6 h (Fig.8). In pH 6.8 only 25% of the total drug was released after 8 h with a linear zero-order release kinetic.

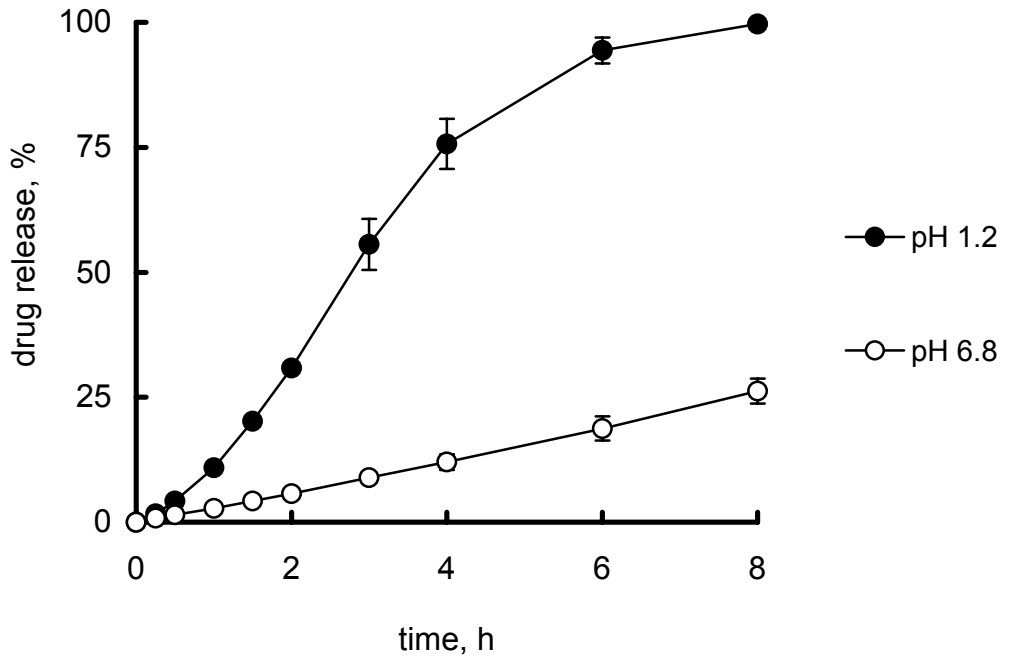

Figure 9 Theophylline release from zein coated pellets (coating level: 20%)

The difference in the drug release was attributed to a difference in the permeability of zein films in the different media, as the drug has a pH-independent solubility (Bodmeier and Paeratakul, 1990). The two media differed beside the pH also in their osmolality (pH 1.2: ~100 mosmol, pH 6.8: ~400 mosmol) and the ion type (pH 1.2: chloride buffer, pH 6.8: phosphate buffer).

The swelling of isolated films decreased with increasing osmolality (Fig. 10A), whereas it was independent of the pH, as long as the same ion type was present in the media (Fig. 10B). The media uptake in chloride buffer (pH 1.2) is enhanced compared to phosphate buffer (pH 6.8) at the same osmolality (Fig. 10C).

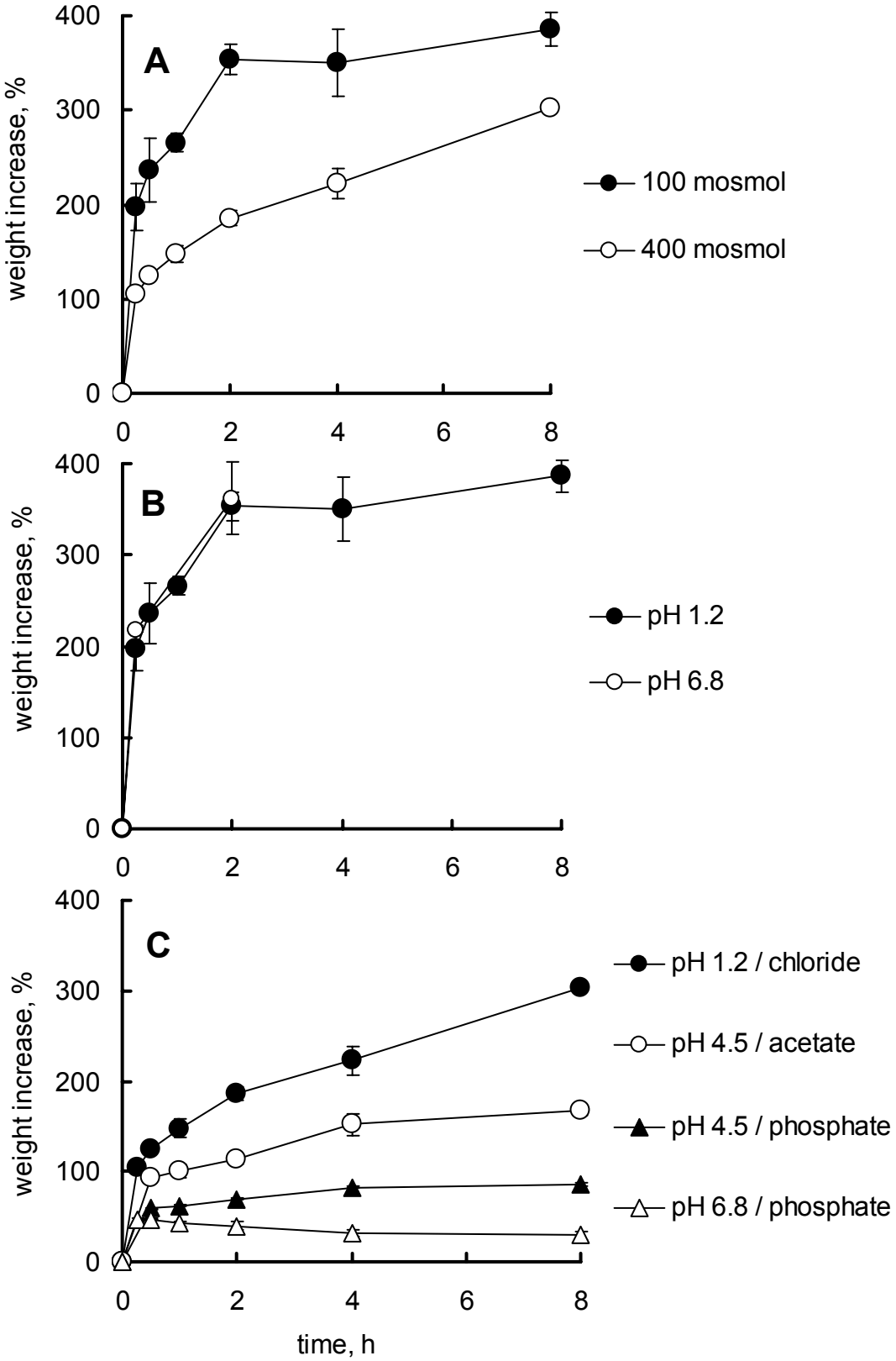

Figure 10 Effect of medium type on the weight increase of zein films A: 100 vs. 400 mosmol/kg (pH 1.2, chloride buffer); B: pH 1.2 vs. pH 6.8 (chloride buffer, 100 mosmol/kg); C: effect of ion type and pH (400 mosmol/kg)

The swelling behaviour of zein parallels the generally known ionic behaviour of proteins according the Hofmeister series (Hofmeister, 1888). The protein-ion interaction is thereby dependent on the kosmotropic or chaotropic character of the ion (Washabaugh and Collins, 1986). Ion binding to the protein surface and subsequent structuring of water at the protein surface produces a hydration barrier (Grigsby et al., 2001).

The swelling of zein films at pH 4.5 with two different ionic type buffers (acetate and phosphate) confirmed this hypothesis. The order of the swelling corresponds the Hofmeister series with chloride > acetate > phosphate (Fig. 10C). The difference in swelling in phosphate buffer at pH 4.5 and pH 6.8 is due the higher content of $H_2PO_4^-$-ions at pH 4.5, while at pH 6.8 the HPO_4^{2-}-ion content is increased. As the HPO_4^{2-}-ion has a more kosmotropic nature, the swelling is decreased.

3.1.4. Effect of zein batch-to-batch variability

A critical point in formulation development is the reproducibility. Natural products like zein are already from their origin prone to certain variability. Three zein batches were examined to compare the susceptibility of the drug release to the zein-batch variations. All examined batches were compliant to the USP-monograph for zein, although revealing differences already at organoleptic inspection (Table 5).

Table 5 Properties of different zein batches at organoleptic inspection

Batch	Batch-No:	Colour	Odour
1	181600	pale yellow	weak
2	131103	deep yellow	strong
3	0261-C	deep yellow	weak
4	0261-C (purified)	pale yellow	weak
5	263903	white	weak

Beside the protein zein, which is present at 88-96% in the product of grade F 4000 (Freeman Industries, 2004) there are also xanthophyll and corn oil residues, responsible for the appearance of the final product (Reiners, 1973; Swallen, 1941). The content of such ingredients may vary (e.g. for corn oil 2-5 % (Reiners, 1973; Sessa et al., 2003; Shukla and Cheryan, 2001)) and result in variability of the permeability and resulting drug release of zein coatings (Fig. 11). Higher corn oil contents lead to a more lipophilic film with a decrease in drug release.

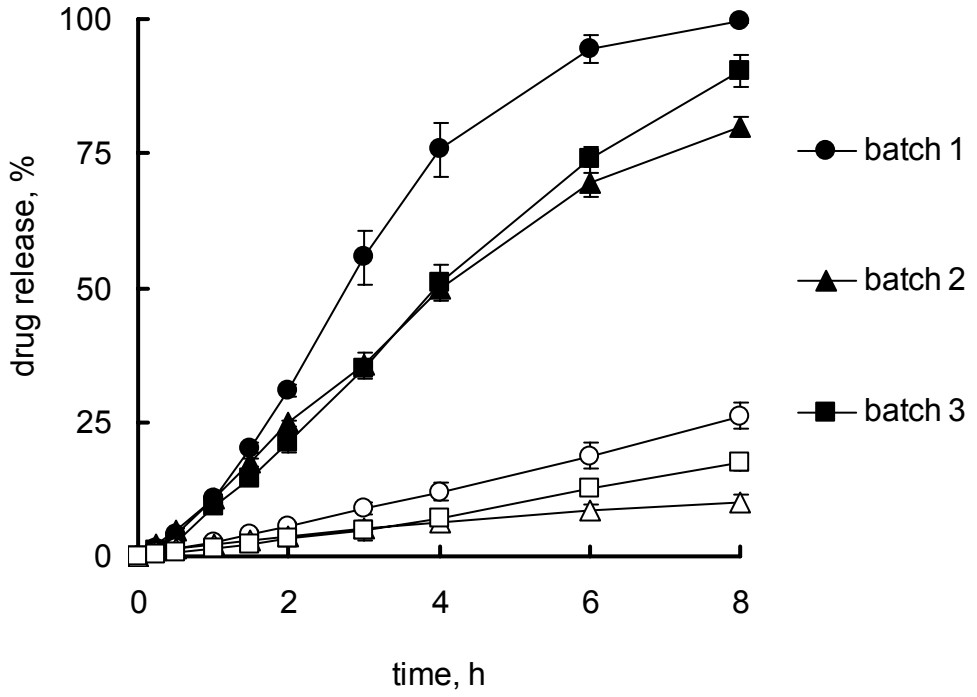

Figure 11 Comparison of drug release of pellets coated with different zein batches (closed symbols: pH 1.2, open symbols: pH 6.8; coating level: 20 %)

Purification by extraction with a lipophilic non-solvent such as petrol ether, hexane or benzene can reduce the lipid and corn oil content (Sessa et al., 2003; Shukla and Cheryan, 2001). The lower lipid and oil content of the purified zein resulted in an increased permeability and thus in an enhanced drug release of the coated pellets (Fig. 12).

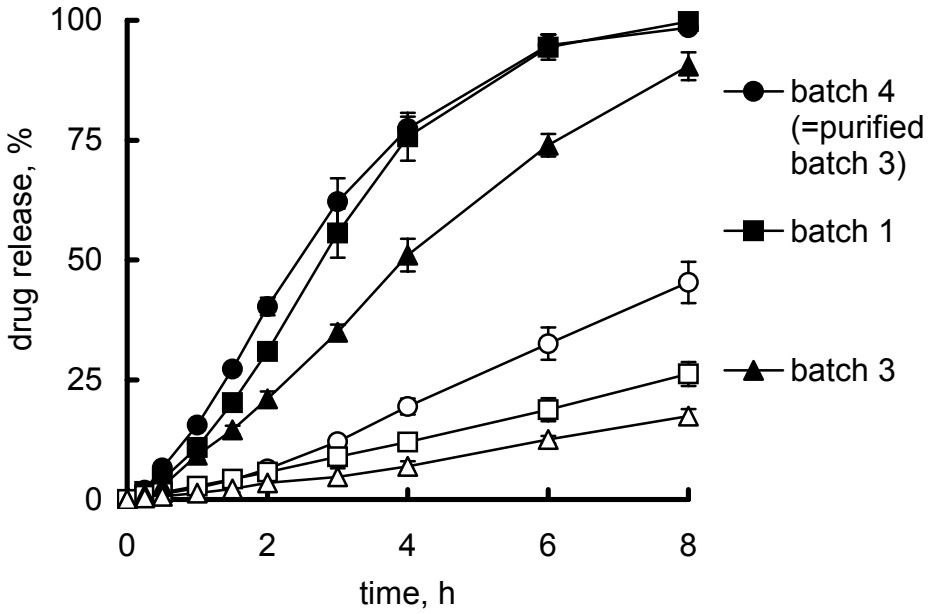

Figure 12 Effect of a purification step on the drug release of pellets coated with different zein (closed symbols: pH 1.2, open symbols: pH 6.8; coating level: 20 %)

No difference was found in the release from pellets coated with white zein (grade F 6000) compared to the regular grade zein (Fig. 13). Thus the xanthophyll content has no major impact on the drug release.

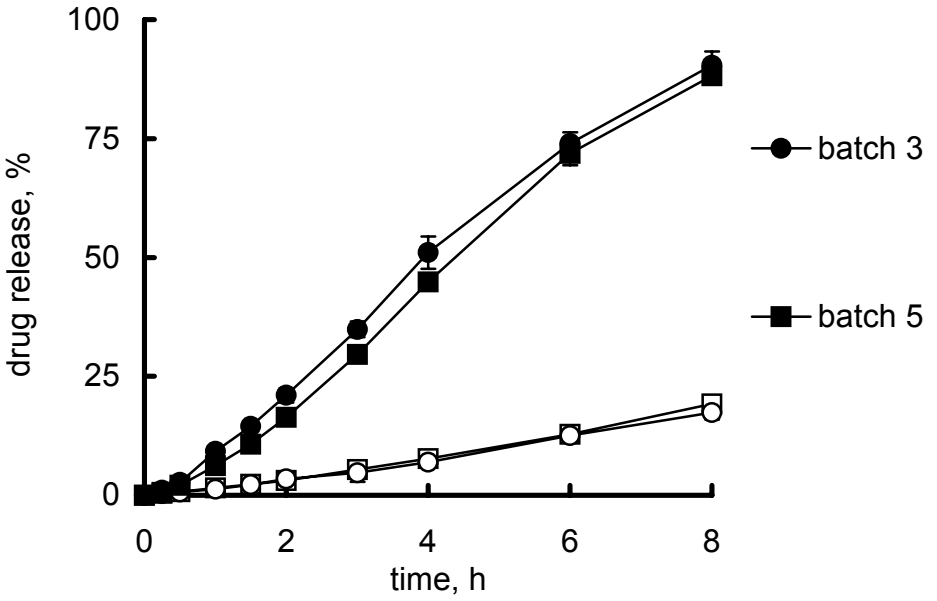

Figure 13 Comparison of drug release of pellets coated with white vs. yellow zein (closed symbols: pH 1.2, open symbols: pH 6.8; coating level: 20 %)

Based on the results of the drug release it is advisable to include the corn oil content into the product specification of zein.

3.1.5. Effect of proteolytic enzymes

Zein is a protein, thus its susceptibility to proteolytic enzymes was expected (Katayama and Kanke, 1992; O´Donnell et al., 1997). Drug release was performed in pH 1.2 in presence of pepsin and in pH 6.8 with pancreatin. The drug release with proteolytic enzymes was enhanced compared to the media without enzymes, however, the general release behaviour was maintained: fast release in gastric medium and retarded release under simulated intestinal conditions (Fig. 14).

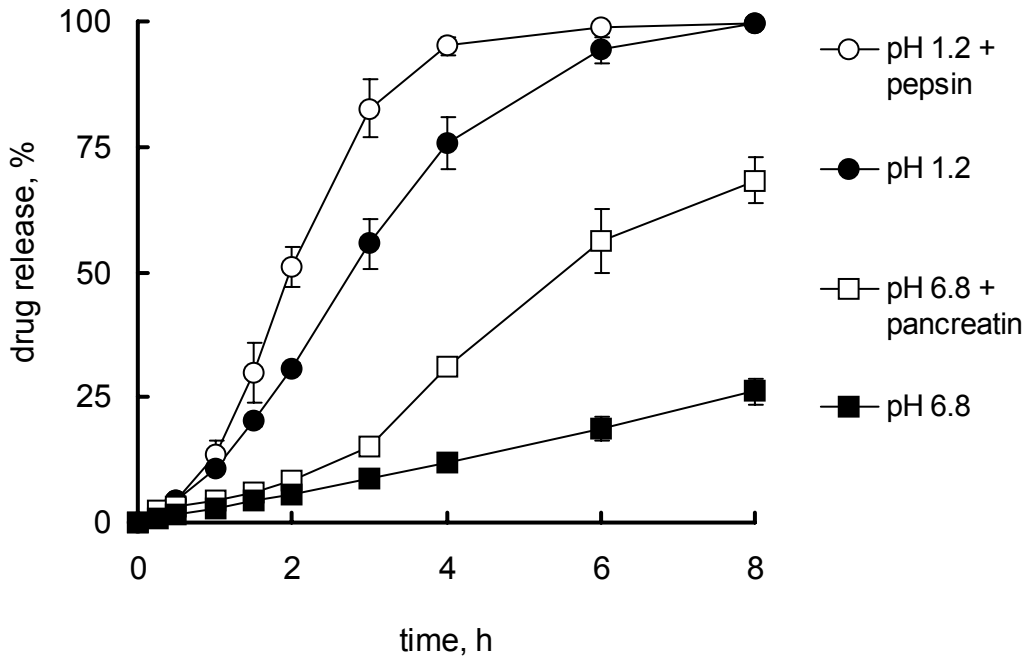

Figure 14 Influence of proteolytic enzymes on theophylline release from zein coated pellets (closed symbols: pH 1.2; open symbols: pH 6.8; coating level: 20%)

3.1.6. Drug solubility and osmotic pressure

The release of theophylline (slightly soluble) was compared to the release of CPM (freely soluble) to examine the influence of the solubility onto the release. CPM is a freely soluble drug (~550mg/ml in water) with pH-independent solubility. To exclude the influence of the osmotic pressure exerted by the sugar cores as the driving force for the faster release, drug-loaded pellets with theophylline were prepared in the same manner. The drug release was clearly enhanced for CPM compared to theophylline (Fig. 15).

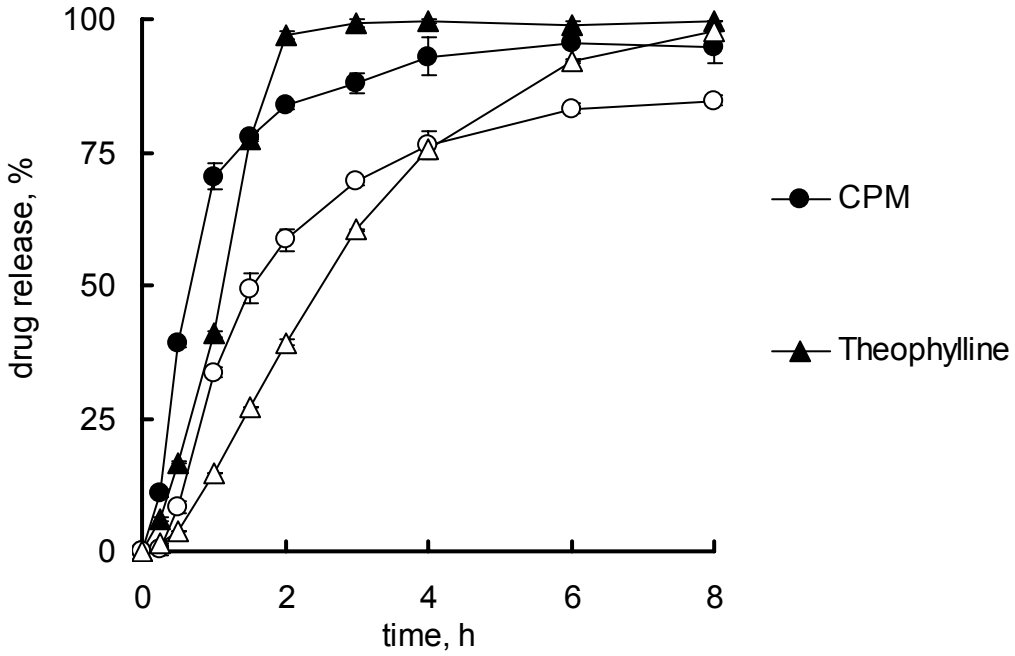

Figure 15 Influence of the drug solubility on the drug release from zein coated pellets: CPM vs. theophylline (closed symbols: pH 1.2; open symbols: pH 6.8; coating level: 20%)

In general the same tendency was followed as already seen for the theophylline matrix pellets with a faster release in pH1.2 and an extended release in pH6.8. As expected, the drug release from the osmotically active theophylline cores was also enhanced compared to the cores with no osmotic activity (Fig. 16).

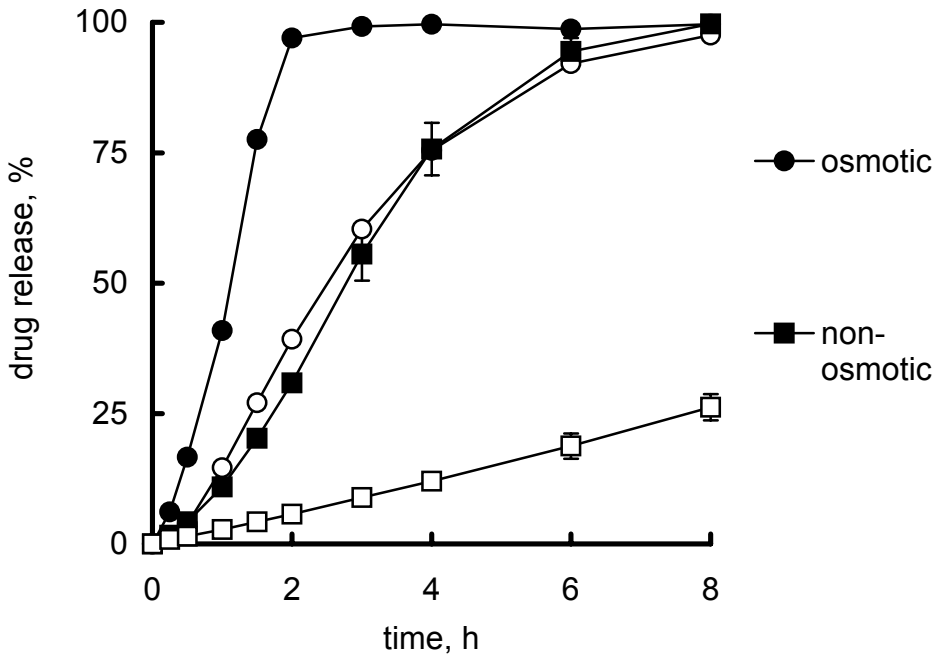

Figure 16 Influence of the core type on the drug release from zein coated pellets: osmotically active vs. non-active cores (closed symbols: pH 1.2; open symbols: pH 6.8; coating level: 20%)

3.1.7. Stability on storage

A pre-requirement for any pharmaceutical product to be marketed its stability on storage. Zein was appreciated for its stability especially against microbial attack (Reiners, 1973). Zein coated pellets showed excellent storage stability with no significant change in drug release even under accelerated conditions (40 °C / 75 % r.h.) for the observed time period of 24 months (Fig. 17).

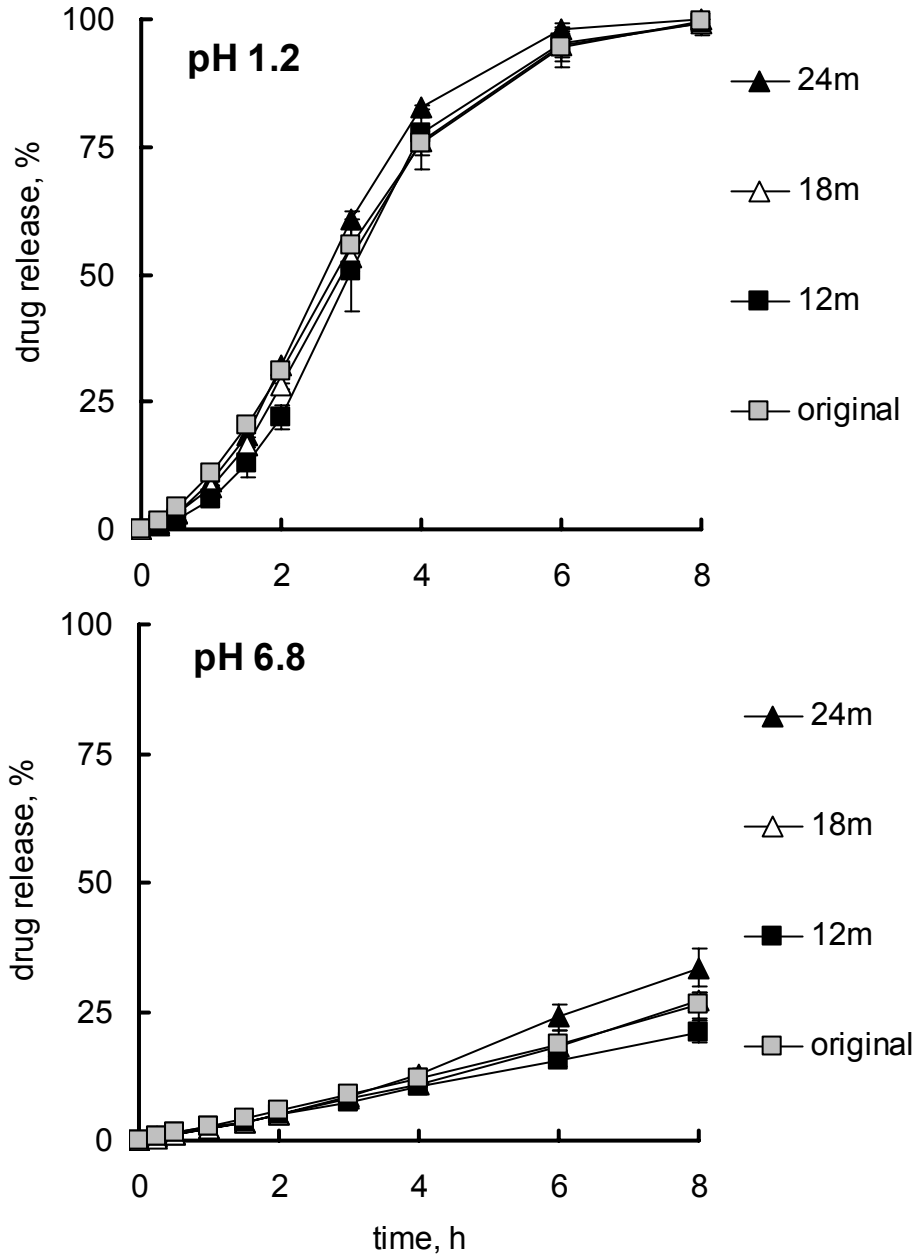

Figure 17 Stability on storage of the drug release from zein coated pellets (storage conditions: 40 °C/75 % r.h.; model drug: theophylline; coating level: 20%)

3.1.8. Moisture protection

Moisture protection is a critical issue, if hygroscopic or hydrolyse-sensitive drugs are used. Mostly HPMC or other hydrophilic polymers (e.g. PVA in Opadry® AMB) are used as moisture protective coatings. Zein was thus compared to HPMC for this purpose. The coating level

needs to be low enough, that the drug release is not further affected compared to the uncoated dosage form (Fig. 18).

Zein hindered the hydrolytic degradation of the moisture sensitive model drug acetylic salicylic acid with the same efficiency as HPMC (Fig. 19). At storage conditions with low moisture level (60 or 75 % r.h.) the coated tablets contained a higher amount of salicylic acid compared to the uncoated ones. This indicates, that by the water used during the coating process the degradation of the drug can be induced already. Thus, a moisture protective coating is only reasonable, if high r.h.-values are expected for the storage (>75 % r.h.). Otherwise the product quality of the coated dosage forms is inferior.

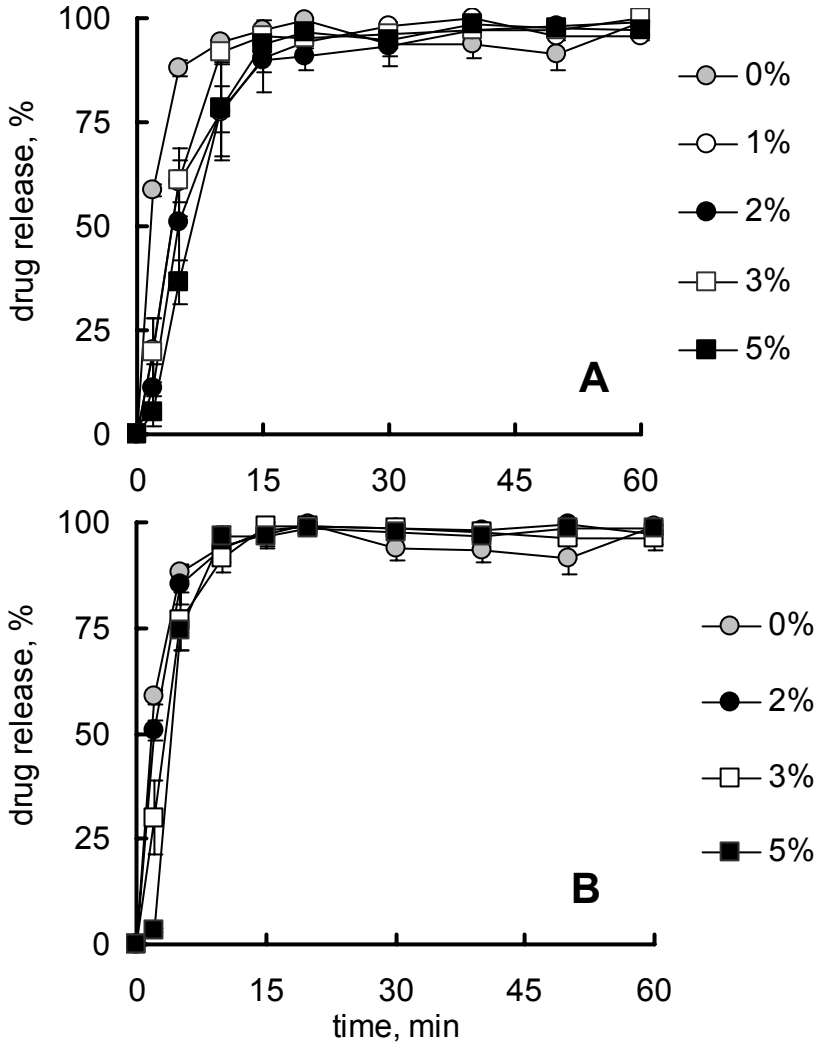

Figure 18 Effect of coating level on the drug release from tablets coated for moisture protection: A: zein and B: HPMC (model drug: ASS)

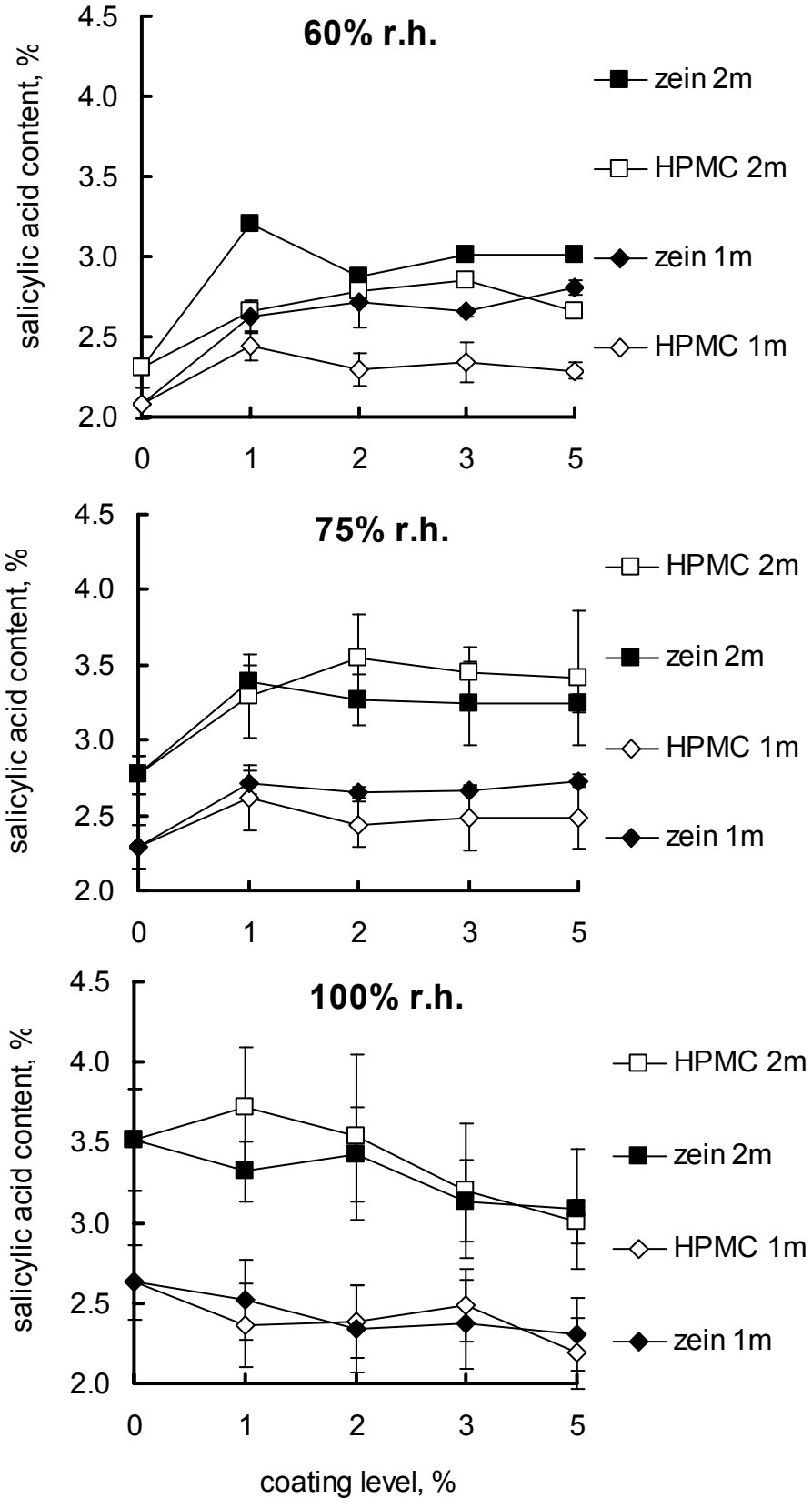

Figure 19 Effect of storage condition and the coating level on the salicylic acid content of zein coated ASS-tablets

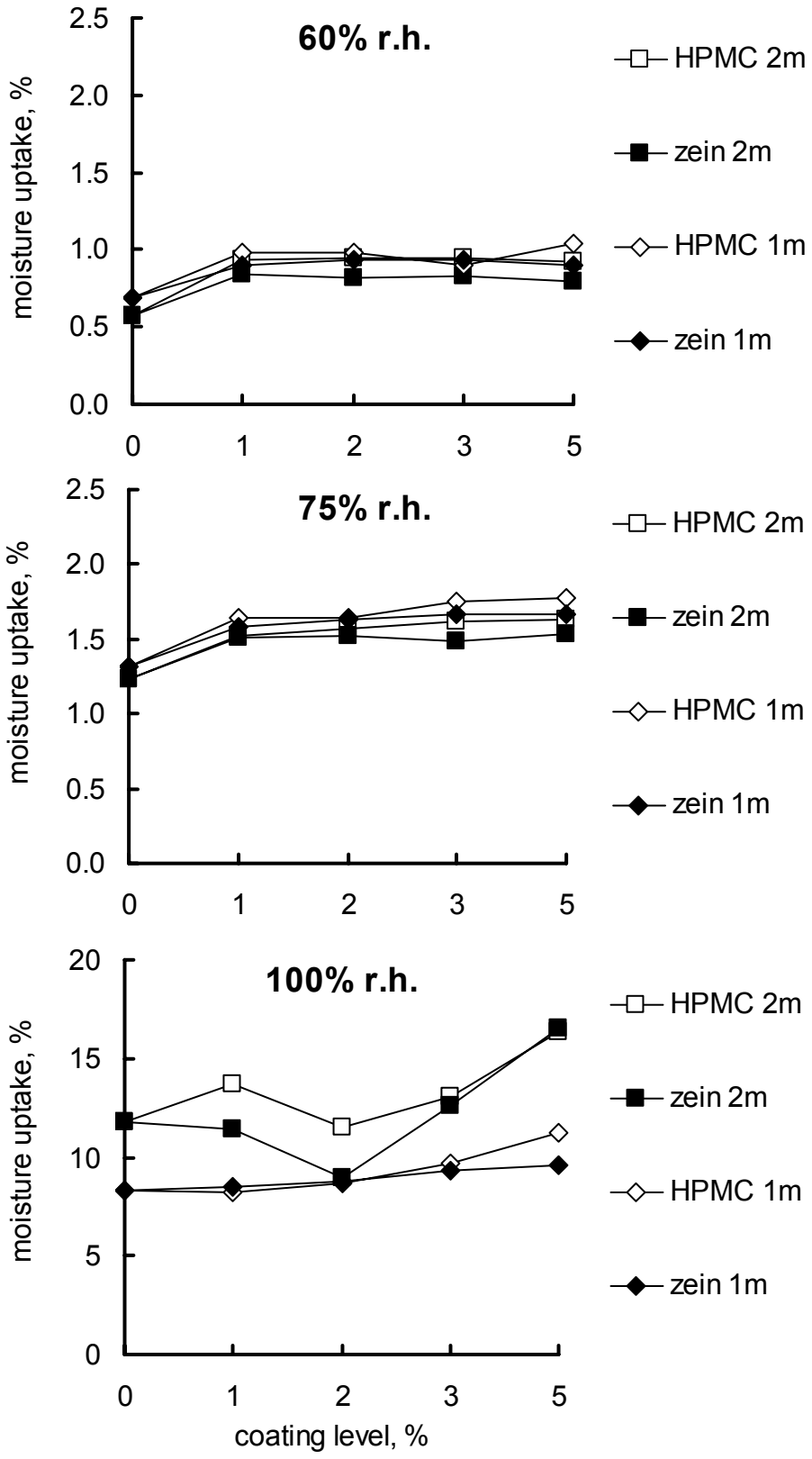

Figure 20 Effect of storage condition and the coating level on the moisture uptake of ASS-tablets coated for moisture protection

At 100 % r.h. the amount of the degradation product salicylic acid decreased with increasing coating level (Fig. 19). The moisture uptake of the coated tablets followed the opposite trend: with increasing coating level the moisture content increased (Fig. 20). The zein-coat is able to entrap the high amount of penetrating moisture and to hinder it from diffusing to the core. However, at 100 % r.h. the coatings swelled due to the high moisture uptake and the tablets got soft and could not maintain their shape even on slight mechanical stress.

3.1.9. Taste masking

Protective coatings for solid dosage forms are also used for masking the bitter or unpleasant taste of incorporated drugs. Thereby a sufficient retention of the taste should be achieved without affecting the instantaneous drug release profile, if the coating level of the polymer is low enough. Zein at a coating level of only 2% can suppress the bitter taste of the model drug paracetamol ten times longer than the standard polymer HPMC (table. 6) without retardation of the drug release (Fig. 21). It was even superior to shellac, which was reported to have a two times better performance compared to HPMC (Pearnchob et al., 2003b).

Table 6 Time until the perception of bitter taste of the model drug paracetamol in dependence of the polymer and coating level

Coating level, %	Time (± s.d.), s	
	Zein	**HPMC**
0	6 ± 3	
1	33 ± 13	16 ± 6
2	282 ± 177	32 ± 17
3	554 ± 356	39 ± 16

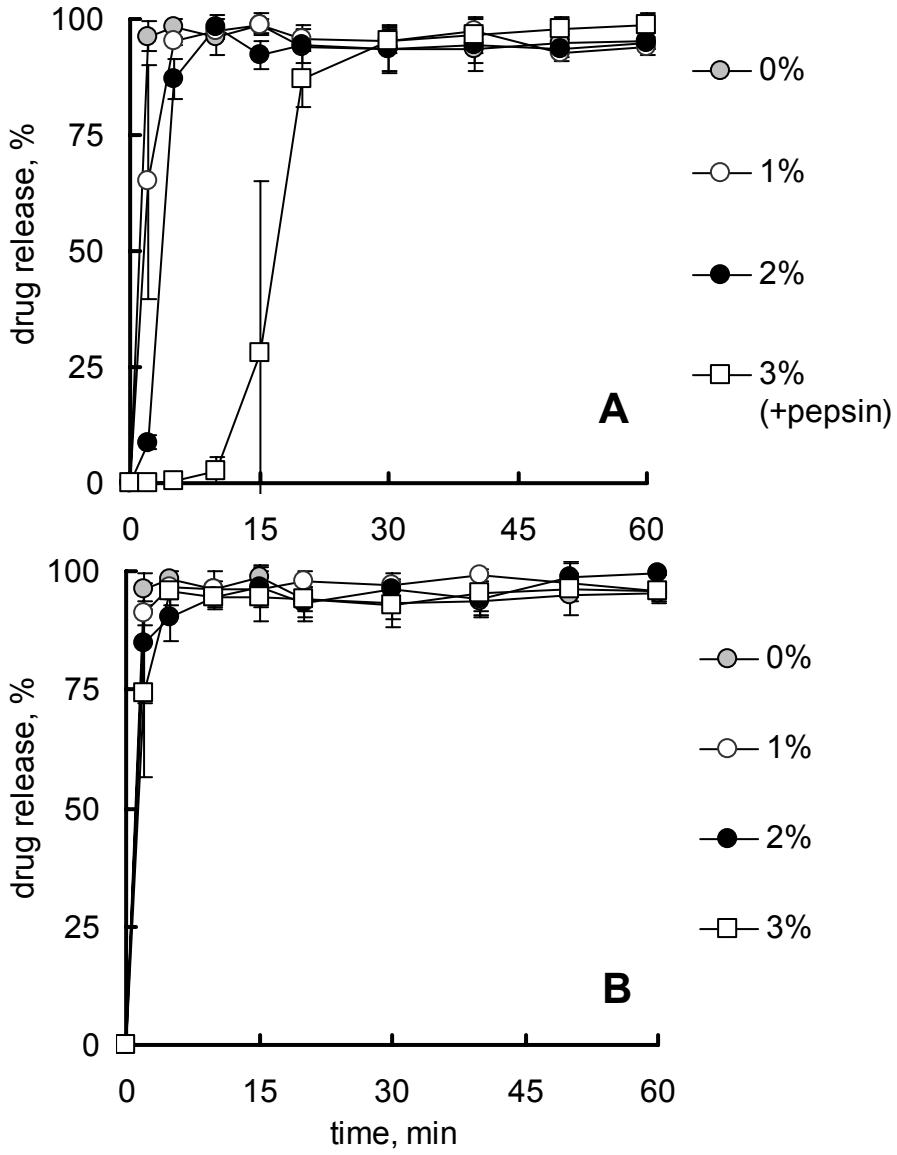

Figure 21 Effect of the coating level on the drug release of tablets coated for taste masking with A: zein and B: HPMC (model drug: paracetamol)

3.2. AQUEOUS ZEIN DISPERSIONS

Aqueous polymer dispersions are distinguished according the preparation method into latexes and pseudolatexes (Paeratakul, 1993). Latexes are prepared by emulsion polymerisation. The monomer is emulsified in water and polymerisation is started by an initiator. The molecular weight and particle size of the polymer can be controlled by the concentration of the initiator (Lehmann, 1997). However, this method is limited to polymers, which consist of water-immiscible monomers. The other polymers need to be emulsified as such into the aqueous phase and the resulting dispersion is called pseudolatex. The polymer is either melted or dissolved in an organic solvent before being emulsified into the aqueous phase (Colorcon, 2001; Lehmann, 1997). The solvent is usually a volatile water-immiscible organic solvent which is removed later by steam distillation. Alternatively, a water-miscible solvent can also be used, whereby the particle formation will take place spontaneously due to solvent exchange upon contact with the aqueous phase (Chang et al., 1990). Aqueous dispersions should have a high polymer content to keep the coating process time short. Usually a solids content of 15-30 % is recommended for the coating formulation (Colorcon, 2001; FMC, 1996).

The preparation of a zein aqueous dispersion by solvent exchange was described and investigated for extended release coatings (O´Donnell et al., 1997; Oshlack et al., 1994). However, zein dispersions of higher polymer content (>8 % w/v) resulted in agglomeration and lump formation.

3.2.1. Preparation methods for aqueous zein dispersions

Four different methods were evaluated and compared with regard to the resulting particle size (Table 7).

Table 7 Methods investigated for the preparation of aqueous zein dispersion

Method	Preparation
1	organic zein solution injected into the water phase by a syringe + needle (∅ 0.45mm)
2	organic zein solution injected into the water phase without needle
3	Organic zein solution + addition of water in 1 ml-steps → phase inversion after a certain water amount
4	Immersion of zein powder into the water → stirring → treatment with Ultra Turrax (5 min)

Method 1 and 2 were based on a solvent extraction method. An organic polymer solution was introduced into an aqueous phase at equal volume ratio. A needle was used to introduce the polymer solution directly into the aqueous phase for method 1, whereas for method 2 the organic phase was injected without a needle. Zein precipitated instantaneously in fine particles upon contact with the aqueous phase in both cases, resulting in a slightly yellow milky dispersion. However, the mean particles size was smaller for method 1 compared to method 2: 0.36 µm vs. 1.84 µm (Fig. 22). Obviously, when a needle was used (method 1) to introduce the organic polymer solution directly into the stirred aqueous phase the higher shear forces compared to method 2 resulted in smaller droplets and thus smaller particle size of the final dispersion.

Method 3 which is based on a phase inversion, started with an organic polymer solution. Water was added stepwise, until the polymer was precipitated. The mean particle size of the dispersion prepared by this method was 1.85 µm, thus comparable to method 2 (Fig. 22).

Method 4 was investigated as an alternative to circumvent the use of an organic solvent. Zein powder was directly dispersed into the aqueous phase. However a further step for particle size reduction was required (here: ultra turrax treatment). This process introduces a high amount of energy into the system, such that the aqueous phase warmed up despite cooling and resulted in sticking of the polymer. The particle size of the system prepared by method 3 was the biggest (129 µm) and far away from the acceptable colloidal range. As the smallest particle size was achieved by method 1, it was chosen as the standard preparation method.

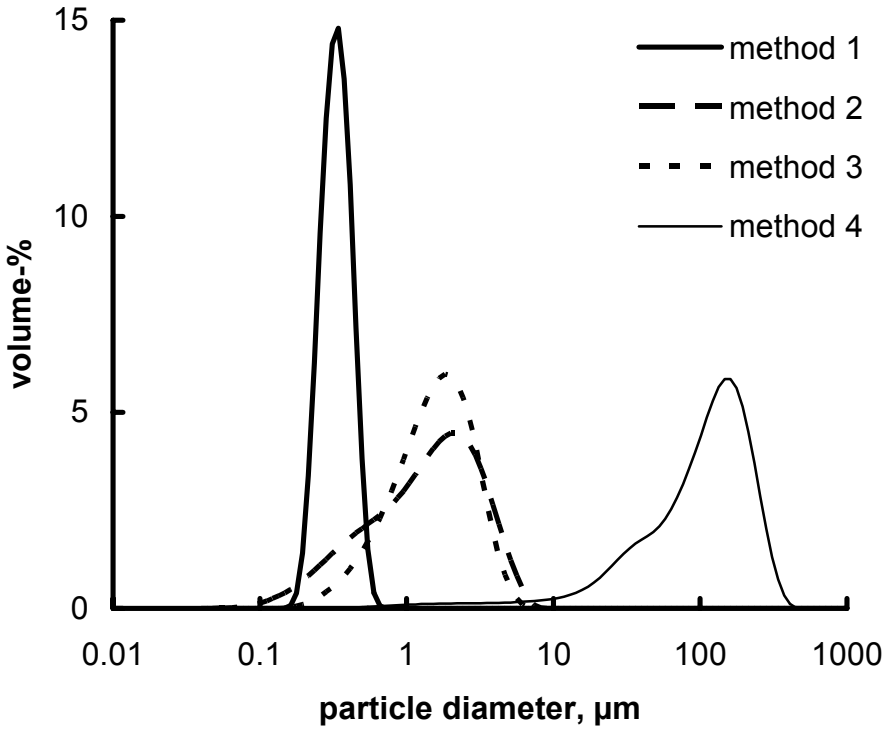

Figure 22 Particle size distribution of aqueous zein dispersions depending on the preparation method as measured by laser light scattering

3.2.2. Approaches to increase the polymer concentration

To increase the polymer concentration of the aqueous dispersion two approaches were examined:

a: During preparation of the dispersion the effect of additives in the aqueous phase and the exchange of the solvent used for the organic polymer solution were investigated.

b: After preparation steric stabilisation by additives or electrostatic stabilisation by appropriate zeta potential of the polymer particles were evaluated in order to prevent lump formation.

a. Parameters during preparation

The two phases, the aqueous water-phase and the organic polymer solution, used for the preparation of the dispersion by method 1 may affect the particle size and the highest possible polymer concentration of the zein dispersion.

Different additives, such as hydrophilic polymers (e.g. PEG) or surfactants (Tweens®, Pluronics®) were added to the water-phase before the introduction of the organic polymer solution (Table 8).

Table 8 Effect of additives in the aqueous phase during preparation on the particle size of the resulting zein dispersion

Additive	Surface tension, mN/m	Particle size, µm
none	78.2 ± 0.3	0.4
PEG 400	74.3 ± 0.9	0.4
Tween 20	-	9.3
Tween 80	43.0 ± 1.4	8.2
Pluronic F68	51.0 ± 1.5	0.4
Pluronic F127	40.6 ± 1.0	0.9

They were supposed to decrease the surface tension of the aqueous phase and lead to a decrease in particle size by facilitating the separation of the organic polymer solution into smaller droplets. However, a decrease in the surface tension did not result in a decrease of the particle size, moreover some surfactants destabilise the polymer particles and lead to agglomerates with increased particle size (Table 8).

Zein is insoluble in anhydrous alcohols, except methanol. To dissolve it in other aliphatic alcohols, a certain amount of water (20-30 %) needs to be added (Swallen, 1941). The solvents have different solubilising power for the polymer. The solvent powder of a given solvent for zein can be quantified by the critical peptisation temperature (CPT) (Manley and Evans, 1943; Swallen, 1941). This is the temperature, at which the polymer gets soluble in the solvent at any proportion, while below this temperature the polymer is insoluble and precipitates. Thus a lower CPT reflects an increased solvent power (Table 9).

Table 9 Critical peptisation temperature (CPT) of zein in different solvents / solvent mixtures and solubility parameters for the polar component (δ_p) of the solvents

Solvent system	**CPT*, °C**	**δ_p, MPa$^{1/2}$ ****
Water	-	16.0
MeOH (pure)	63	12.3
Acetone 80 % (v/v)	18	10.4
EtOH 80 % (v/v)	0	8.8
Isoprop. 80 % (v/v)	-13	6.1

* values estimated from Manley and Evans, 1943 and Swallen, 1941

** values from Barton, 1975

The solvent power correlated with the amount of water required to induce phase inversion of the organic zein solution to an aqueous dispersion, when prepared by method 3 (Table 10). With decreasing solvent power less water was necessary to induce the polymer precipitation.

This is related to the miscibility of the solvent with water, which was confirmed by the order of the solubility parameters (Table 9).

Table 10 Volume of water necessary for phase inversion of organic zein solutions (polymer concentration: 5 % w/v; volume of the organic polymer solution: 25 ml)

System	Polymer conc. org. phase, % w/v	First "clouds"	Milky dispersion	Mean particle size, µm	Polymer conc. aq. disp., % w/v
MeOH (pure)	5	10 ml	16 ml	1.88	4.34
Acetone 80%	5	15 ml	20 ml	2.47	3.04
EtOH 80%	5	22 ml	25 ml	1.85	2.88
	10	7 ml	23 ml	22.2	7.16
Isoprop 80%	5	28 ml	35 ml	2.05	2.92

The extraction rate of the organic solvent into the water phase affects the size and stability of the formed polymer particles. The comparison of the physical appearance (Table 11) and particle size (Fig. 23) of the dispersions prepared with methanol, acetone and isopropanol vs. ethanol, the solvent used in the standard setup, confirmed, that methanol resulted in an aqueous dispersion of the smallest particle size (0.15 µm).

Table 11 Effect of the solvent type on the appearance of the zein dispersion used for the organic phase (water: organic polymer solution ratio 1:1)

Solvent	Dispersion properties
Ethanol 80%	pale yellow
Acetone 80%	pale yellow; similar to Ethanol 80 %
Isopropanol 80%	deep yellow; immediate precipitation, lump formation
Methanol (pure)	pale yellow; shiny

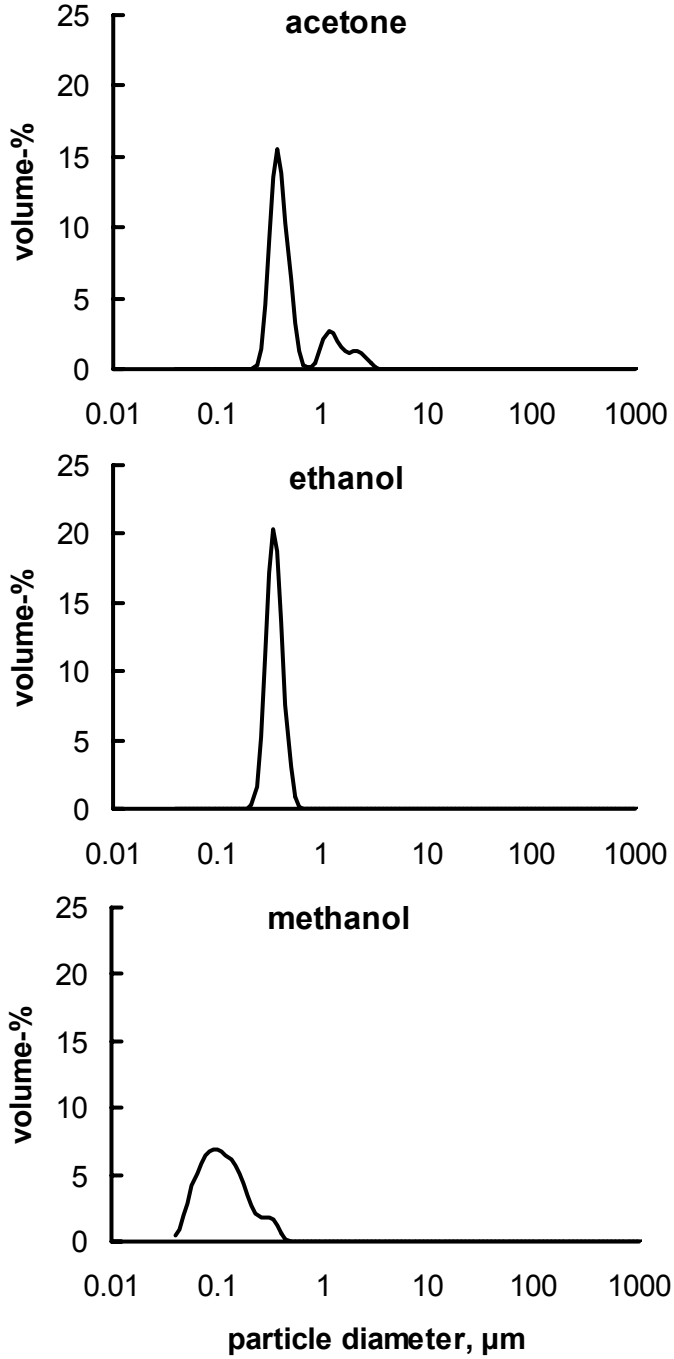

Figure 23 Particle size distribution of the particle size of aqueous zein dispersions after preparation with acetone, ethanol and methanol as the organic solvent

Isopropanol as the solvent with the strongest solvent power for zein from the investigated ones, resulted in agglomeration and lump formation at a phase ratio of 1:1. A higher water: organic solution ratio was necessary to obtain a stable dispersion of zein from isopropanol

solutions, as the water amount required to precipitated the polymer was higher than the volume of the organic solution (Table 10).

The results correlate to the CPT and to the Hansen-solubility parameter for the polar part δ_p in comparison to water (Table 9 vs 11). Methanol has the lowest solvent power for zein, but the solubility parameter δ_p closest to water. Thus solvent extraction rate decreased with increasing CPT and decreasing δ_p (isopropanol > ethanol > acetone >> methanol), resulting in a higher residual solvent content of the polymer particles. This leads to a plasticisation of the polymer with sticking and lump formation, as observed for isopropanol.

The preparation of an aqueous dispersion from isopropanol solutions was successful at a ratio of 2:1 water: organic polymer solution (Fig. 24). However, the particle size increased with increasing concentration of the polymer in the organic solution, as observed already for the dispersions prepared by method 3 (Fig. 24 vs Table 10).

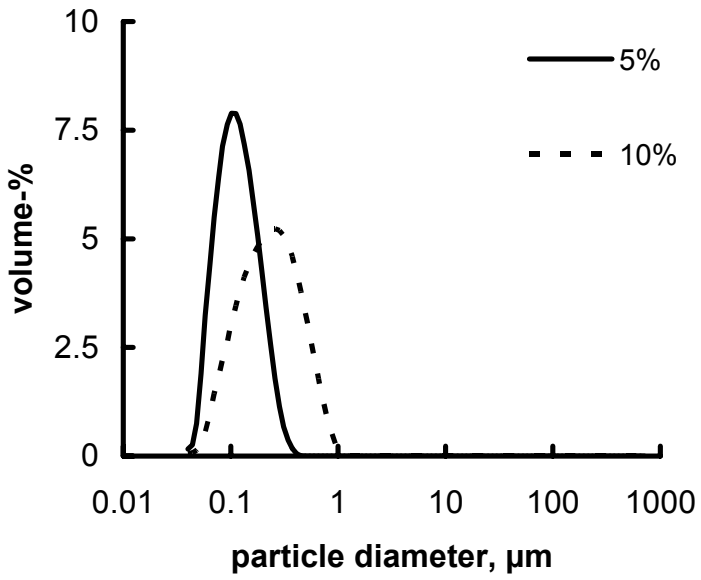

Figure 24 Effect of the polymer concentration of the organic solution on the particle size distribution of aqueous zein dispersions (preparation method 1; solvent: isopropanol 80 % v/v; phase ratio water: organic polymer solution: 2:1)

The influence of the batch-to-batch variability on the dispersion quality was evaluated with three different zein batches.

Zein powder has varying contents of impurities, as up to 2 % corn oil and 4-5 % carotinoid pigments (Sessa et al., 2003; Shukla and Cheryan, 2001). The employed zein batches revealed already differences at organoleptic inspection and thus the aqueous dispersions resulted in different physical appearance and particle size (Table 12). The processability and particle size correlated with the carotinoid content of the zein powders and to the glass-transition temperature (Tg) of each batch.

Table 12 Batch-to-batch variation of zein at organoleptic inspection and particle size of the aqueous dispersion prepared by method no. 1 from the zein batches

Batch	Batch-No:	Colour	Odour	Particle size (aq. dispersion), µm
1	181600	pale yellow	weak	0.36
2	131103	deep yellow	strong	lump formation
3	0261-C	deep yellow	weak	0.41 (main fraction) 1.2 (small fraction)
4	0261-C (purified)	pale yellow	weak	0.36
5	263903	white	weak	0.42

Zein receives its typical yellow colour due to its content of carotinoid pigments, mainly lutein and zeaxanthin (Sessa et al., 2003). The carotinoids absorb with a characteristic three-peak spectrum in the visible range (Fig. 25), thus the pigment content was quantified by the UV-absorbance in the visible range at the wavelength of the maximum absorbance of the carotinoid chromophore at λ = 445 nm.

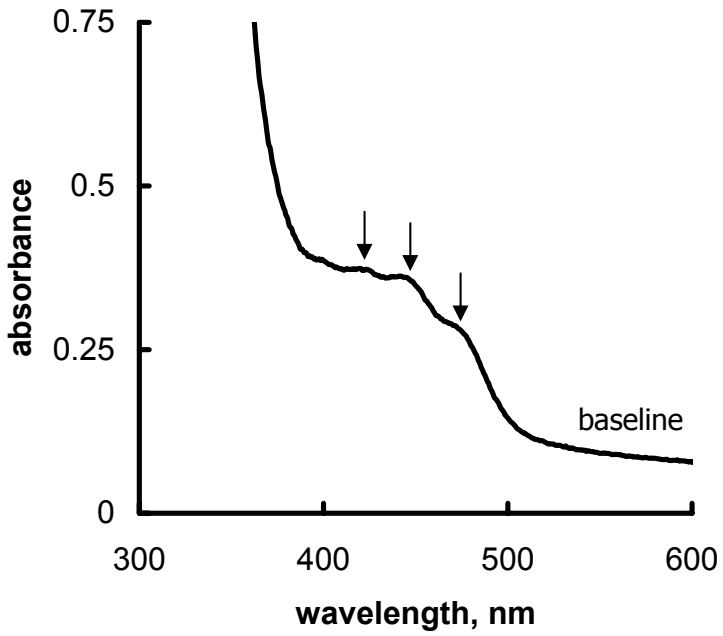

Figure 25 UV-visible spectrum of yellow zein dissolved in ethanol 80 % (v/v)

The increased pigment content was reflected in a stronger increase of the absorbance over the baseline and was associated to a decrease in the Tg (Table 13).

Purification with petrolether resulted in a zein powder of less intensive colour (batch 3 vs. 4) (Table 12). The increase in absorbance due to the pigments was lowered and the Tg increased compared to the initial batch (Table 13).

Table 13 Absorbance of zein solutions in 80 % (v/v) ethanol (λ=445nm) and Tg of the zein powders

Batch no.	**Total absorbance**	**Baseline absorbance**	**Absorbance increase** (total-baseline)	**Tg, °C**
1	0.4231	0.3737	0.0494	163.3 ± 1.6
2	0.0987	0.0358	0.0629	153.4 ± 1.1
3	0.5309	0.4703	0.0606	155.4 ± 0.4
4	0.1313	0.1068	0.0245	163.7 ± 1.9
5	0.0451	0.0371	0.008	171.5 ± 0.2

The decrease in the Tg with increasing colour intensity can be attributed to the plasticising effect of the carotinoid pigments, which, if exceeding a certain amount, lead to sticking and lumping, and hinder the proper preparation of the zein dispersion.

b. Stabilisation of the dispersion after preparation

In general a zeta-potential <-30 mV or >30 mV is recommended for the physical stability of dispersions. Electrostatic repulsion is then assured and agglomeration of the particles is prevented upon storage. This criterion is fulfilled for the dispersions at a pH below 4 and above 6.5 (Fig. 26). The aqueous dispersion of zein after preparation had a pH of 3.5, thus a further electrostatic stabilization was not necessary.

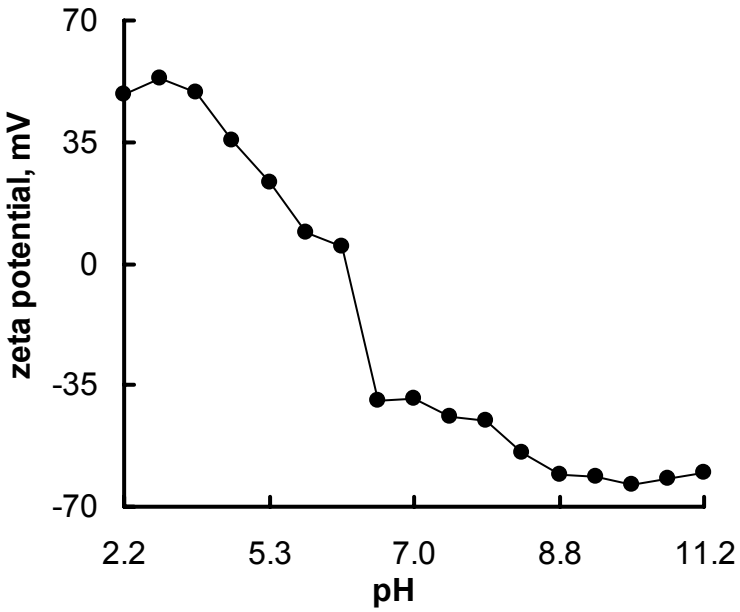

Figure 26 Zeta-potential of aqueous zein dispersions in dependence of the pH of the aqueous phase

Long chain additives were added to the aqueous zein dispersion after the preparation in order to achieve steric stabilisation of the particles and prevent lump formation at higher polymer concentrations (Table 14). However, it was not possible to maintain the original

particle size to a significantly higher polymer concentration than the already described ~10 % w/v.

Table 14 Influence of additives on the highest polymer concentration of aqueous zein dispersions with unchanged particle size distribution

Additive	Concentration, % (w/w) based on polymer	Polymer concentration, % (w/v)
SDS	~	immediate flocculation
Tween 80	6.6	11.1
Span 80	9.8	7.6
PEG 400	30.9	12.6
PEG 1500	30.9	8.2
AMG	30.3	8.7

3.2.3. Theoretical considerations

Since all attempts to increase the total polymer concentration of the system failed, a more general view and basic considerations were required. Zein is a polymer with very high swelling in aqueous media (Terebesi and Bodmeier, 2006a). Taking into account, that the swelling of small particles will be even enhanced due to the higher accessible surface and lower swelling hindrance related to spatial limitations, it can be assumed, that the zein-particles in the dispersions occupy a high volume. The volume fraction occupied by the polymer particles vs. the volume of the free surrounding aqueous medium of the zein dispersion was determined by centrifugation. The polymer particles occupied a volume fraction of 18 % (v/v) at a polymer concentration of 2.26 % (w/v), 49 % (v/v) at 5.16 % (w/v) and 76 % (v/v) at 9.15 % (w/v) polymer concentration (Fig. 27). In the most compact array of spherical particles of the same size, which may be a hexagonal or cubic lattice, the volume occupied by the particles is 74 % (v/v). In this arrangement, all particles

are in closest contact without being deformed. The volume fraction occupied by the particles increases proportionally to the polymer concentration, thus the highest polymer concentration in the most compact array at 74 % volume correlates to a polymer concentration of 8.7 % (w/v) (Fig. 27).

Slightly higher polymer concentrations can be achieved, due to the polydispers particle size distribution of the aqueous dispersion. However, these theoretical calculations explain the experimental results that agglomeration of zein aqueous dispersions starts at above 8 % (w/v) polymer concentration (Oshlack et al., 1994).

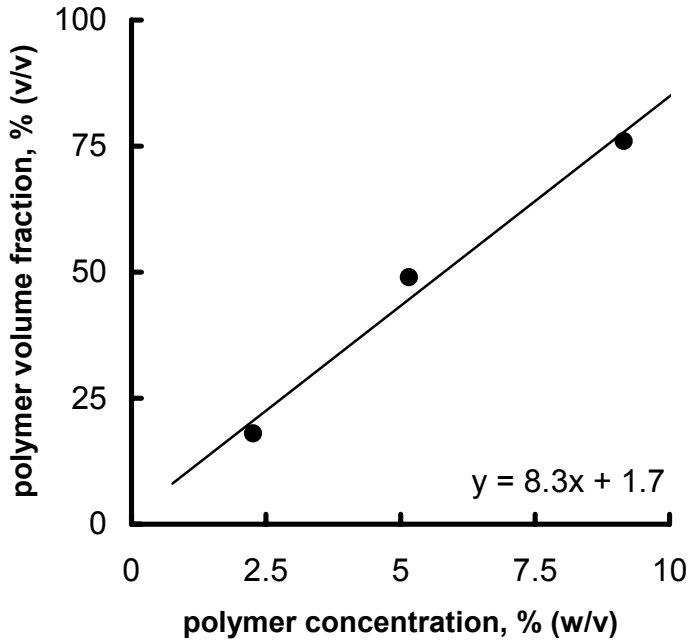

Figure 27 Volume fraction of the polymer in dependence of the polymer concentration and linear correlation

3.2.4. Redispersible powder formulations

Redispersible powder formulations were originally developed to achieve enhanced stability of aqueous dispersions against hydrolysis or microbial deterioration. Moreover, such formulations offer advantages concerning storage and shipping costs (Bodmeier, 1999). Lyophilisation and spray-drying are suitable methods to transfer aqueous polymer dispersions into redispersible dry powders (Lehmann, 1985).

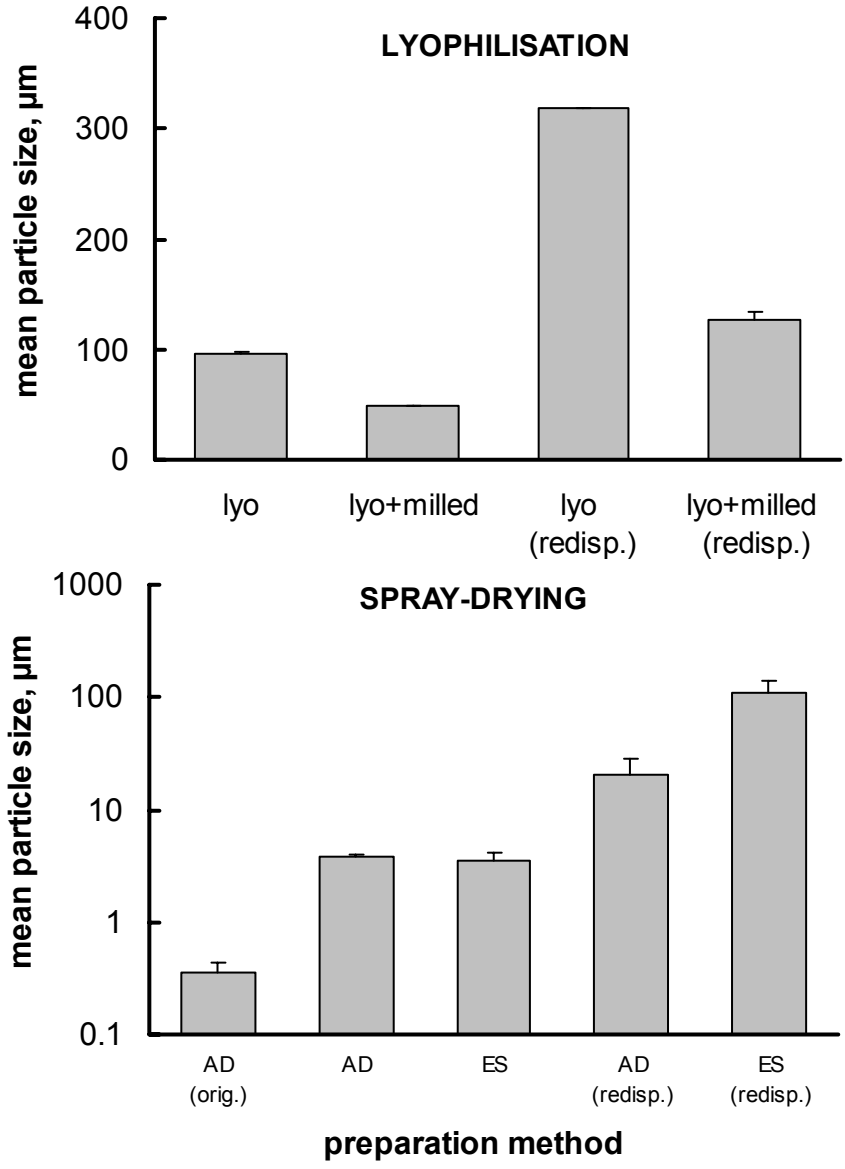

Figure 28 Particle size of zein powders prepared by lyophylisation or spray-drying from aqueous dispersions (AD) or ethanolic solutions (ES) of zein before and after redispersion in water

The zein powder obtained by lyophilisation had a much bigger particles size compared to the original aqueous dispersion, even after milling (48 vs. 0.36 µm) (Fig. 28).

This was related to an increase in the particle size induced by the freezing process. Freeze-thawing experiments confirmed the detrimental effect of the freezing of aqueous zein dispersions on the particles size (Fig. 29). The particle size increase during freezing was more dramatic at higher polymer content of the dispersion. Propylene glycol and tartaric acid were able to prevent particle agglomeration during freezing. Tartaric acid proved to be more effective already at low concentrations (5-10 %), whereas propylene glycol needed higher concentrations (20-30 %) (Fig. 30 vs 31).

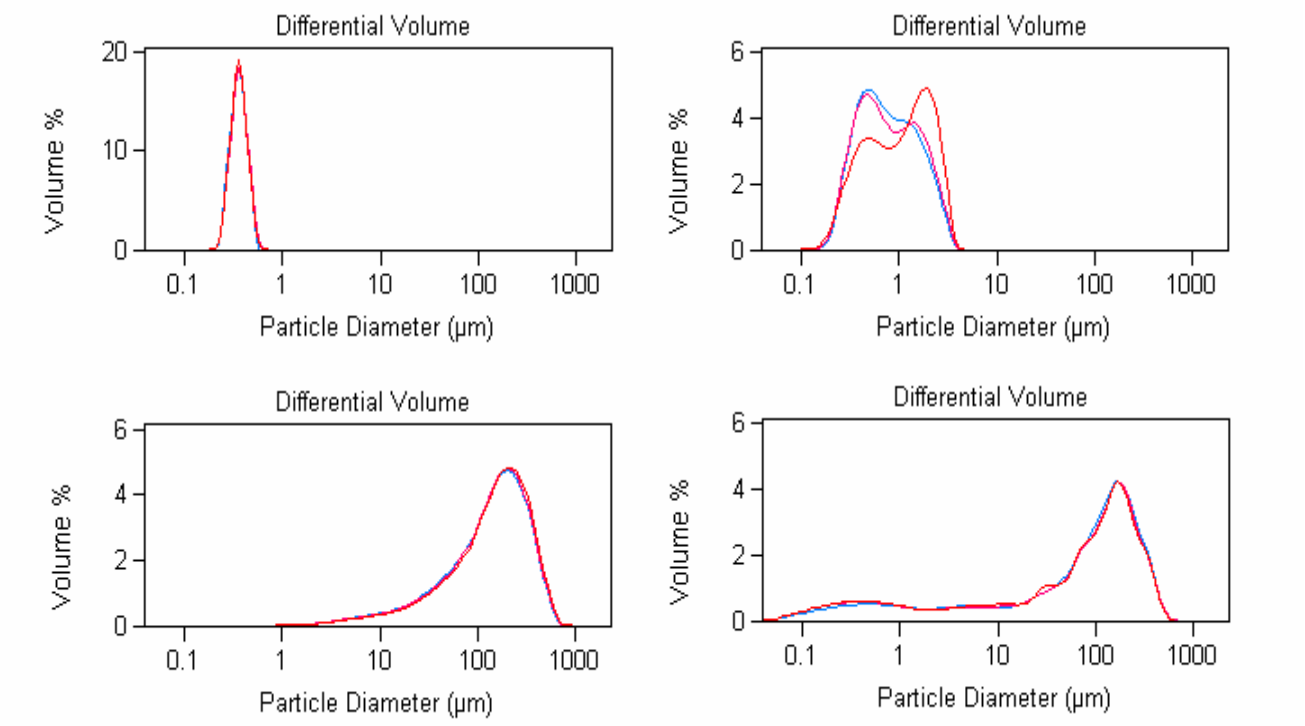

Figure 29 Effect of freezing on the particle size in dependence of the polymer concentration of aqueous zein dispersions: A: after preparation (no freezing); B: 2.4 %; C: 3.4 % and D: 9 % w/v polymer content

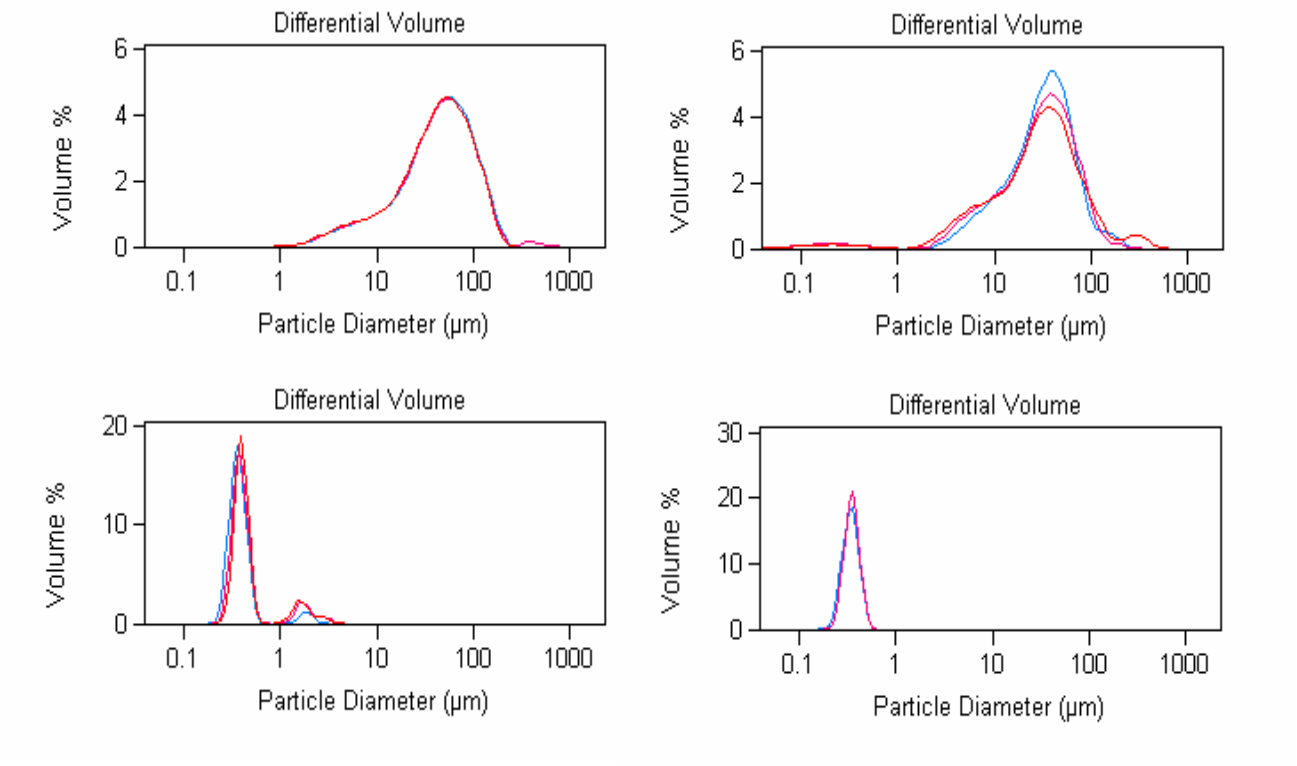

Figure 30 Stabilisation of the particle size with **propylene glycol** during freezing of aqueous zein dispersions at different stabiliser content: A: 5 %; B: 10 %; C: 20 % and D: 30 % based on the polymer (polymer content: 3.4 % w/v)

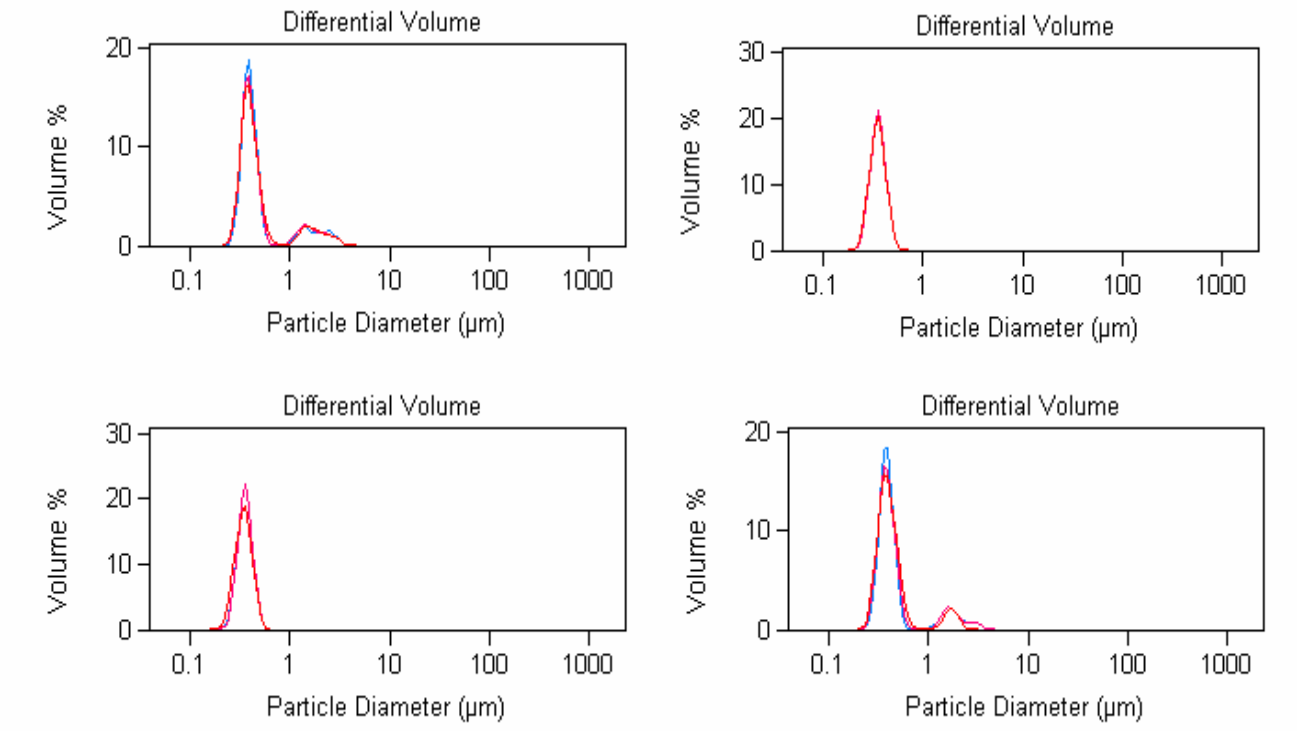

Figure 31 Stabilisation of the particle size during freezing with **tartaric acid** of aqueous zein dispersions at different stabiliser content: A: 5 %; B: 10 %; C: 20 % and D: 30 % based on the polymer (polymer content: 3.4 % w/v)

Spray-drying resulted in a dry powder of zein with a more than 10-fold increased particle size compared to the original aqueous dispersions (mean particle size: 3.9 vs. 0.36 µm) (Fig.28). The powder received from the spray-drying of an ethanolic solution resulted in a similar particle size as the spray-dried aqueous dispersion (mean particle size: 3.5 vs. 3.9 µm).

After redispersion of the powder in water, however, the particle size increased further due to the swelling of the polymer particles (Fig. 28). The swelling was less for the powder prepared from aqueous dispersions.

Changing the redispersion medium from water to phosphate buffer pH 7.4 further improved the redispersion such, that the major part of the particles returned into the range of the original particle size with only few agglomerates (Fig. 32). The mean particle size (0.97µm) was again in the colloidal size and was stable up to 1 h after redispersion without sedimentation.

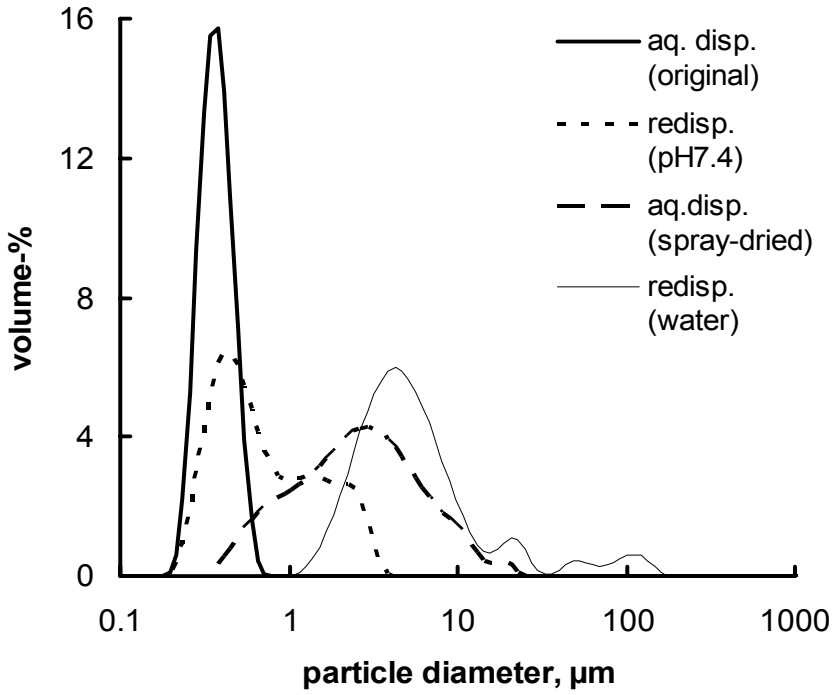

Figure 32 Particle size distribution of spray-dried aqueous zein dispersions as dry powder and after redispersion in comparison to the original dispersion (polymer concentration after redispersion: 10 % w/v)

The improved redispersion of zein at pH 7.4 is related to the amino acid composition and related charge of the polymer. Zein is soluble at high pH (>11.5) (Shukla and Cheryan, 2001; The Merck Index, 1989). This is attributed to the phenolic hydroxyl groups of the tyrosine amino acid residues (Shukla and Cheryan, 2001). The negative charges result in electrostatic repulsion and are responsible for the improved redispersion.

Milling is a process to achieve a particle size reduction without the use of organic solvent or water. However, the obtained powder had after the milling process a mean particle size around 60 µm and was further swelling after redispersion (Fig. 33).

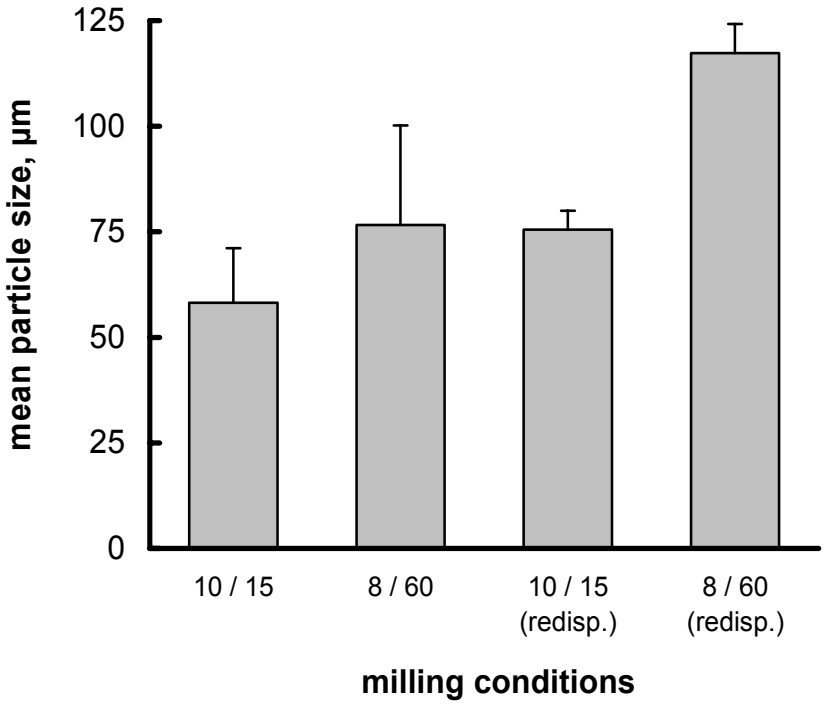

Figure 33 Particle size of zein powders prepared by milling before and after redispersion in water

Spray-drying is thus the most suitable process to receive a small particle size zein powder in the range of commercially available redispersible powders. It resulted in smaller particles size compared to powders obtained by lyophilisation of aqueous zein dispersions. The redispersion was further improved at pH 7.4 compared to the redispersion in water with the

mean particle size in the colloidal range. Milling resulted in a particle size much bigger than the required colloidal range. Thus a less effective coalescence can be expected, leading to a faster drug release compared to the aqueous dispersion as used after its preparation.

3.2.5. Plasticiser for aqueous zein dispersions

In general higher amounts of plasticisers were required to achieve a comparable film appearance and performance as for the films prepared from ethanolic solution (Table 15 vs. 3), as known in general for aqueous dispersions (Lippold and Monells Pages, 2001). However the same tendency could be found as for the zein-films prepared from organic solutions: the hydrophilic plasticiser exerted a stronger plasticising efficiency (Table 16).

Table 15 Effect of the plasticiser concentration on the physical properties, film appearance, flexibility, liftage and stickiness of zein films cast from aqueous dispersions (plasticiser: tartaric acid, drying at 40 °C)

Plasticiser conc. (w/w)	Appearance	Flexibility	Liftage	Stickiness
20	yellow transparent	-	-	-
30	yellow transparent	-	-	-
40	yellow transparent	+++	-	±

(symbols: from ± to +++: increasing strength of the characterised parameter, -: no response)

The major role of water as plasticiser for zein was confirmed once more (Beck et al., 1996; Reiners, 1973; Terebesi and Bodmeier, 2006a). Films containing the same amount of additive prepared at higher temperature lost their flexibility and turned brittle. This can be attributed to the loss of water and its plasticising effect by evaporation.

Table 16 Physical properties, film appearance, flexibility, liftage and stickiness of zein films cast from aqueous dispersions and drying at different conditions (plasticiser content: 40 % w/w, based on the polymer)

Room temperature (~25°C)

Plasticiser	Appearance	Flexibility	Liftage	Stickiness
None	white	-	++	-
Tartaric acid	yellow transparent	+++	-	-
Glycerol	white	-	+++	-
Propylene glycol	yellow transparent	+++	-	+

40°C

Plasticiser	Appearance	Flexibility	Liftage	Stickiness
None	yellow-white	-	++	-
Tartaric acid	yellow transparent	+++	-	±
Glycerol	yellow-white	-	+	-
Propylene glycol	yellow transparent	+++	-	+

60°C

Plasticizer	Appearance	Flexibility	Liftage	Stickiness
None	yellow + white spots	-	+	-
Tartaric acid	yellow transparent	-	-	-
Glycerol	opaque	-	-	-
Propylene glycol	yellow transparent	++	-	-

(symbols: from ± to +++: increasing strength of the characterised parameter, -: no response)

3.2.6. Drug release from coated pellets and stability on storage

Pellets coated with a zein aqueous dispersion followed the general release properties of zein-coatings with a rapid release in pH 1.2 compared to the slower release in pH 6.8 (Fig. 34A). The drug release decreased with increasing coating level, however, was much faster compared to the release from pellets coated with ethanolic zein solutions (Fig. 34B)

The faster drug release can be related to the higher plasticizer content of the films (30 % w/w) prepared from aqueous dispersions compared to those from ethanolic solutions (10 % (w/w) in ethanolic solutions, based on the polymer amount), resulting in a much higher permeability of the coating. This assumption is supported by the fact, that curing at 60 °C for 24 h did not result in a decrease of the drug release, thus the coalescence was completed during the coating process (Fig. 34C).

The stability of the drug release after storage for 18 months at ambient conditions was comparable to the initial release (Fig. 35), confirming the storage stability known already for the coatings prepared from ethanolic solutions.

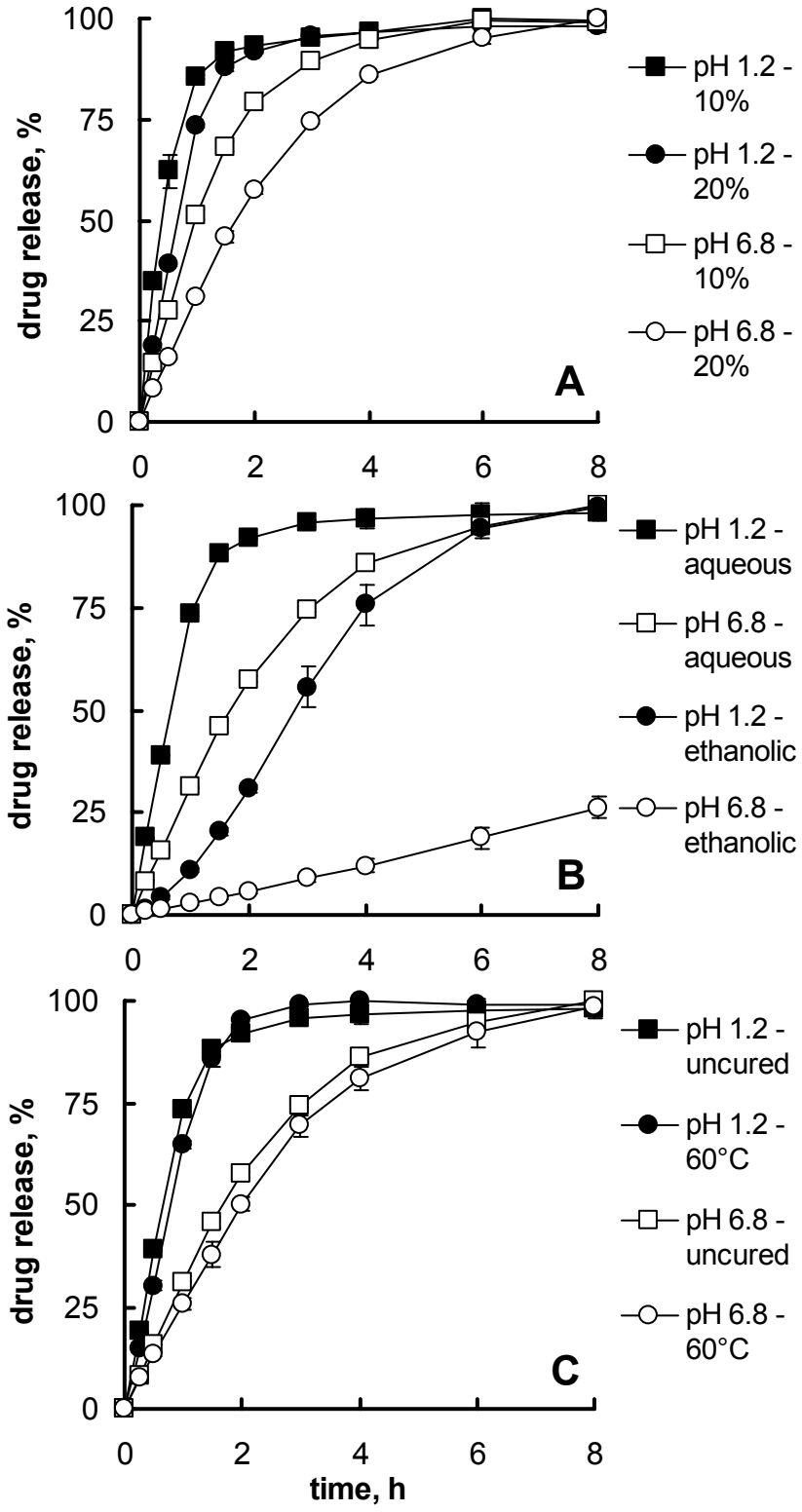

Figure 34 Drug release from pellets coated with an aqueous zein dispersion A: effect of the coating level and release medium, B: comparison to the release of pellets coated with ethanolic zein solution and C: effect of curing at 60 °C/24 h (coating level: 20 %)

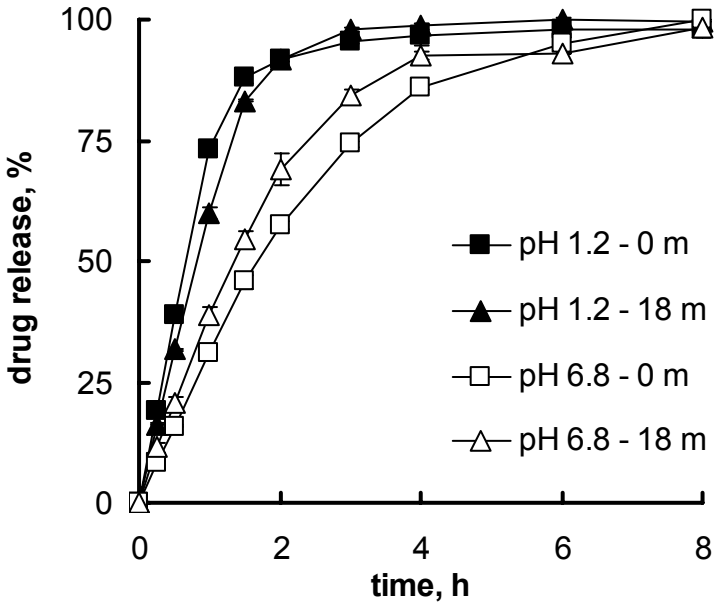

Figure 35 Stability on storage of the drug release of coated pellets (coating level: 20%, storage time: 18 months at ambient conditions)