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Thermodynamic approach to protein microencapsulation into poly(D,L-lactide) by spray drying

B. Gander[a,*], P. Johansen[a], Hô Nam-Trân[b], H.P. Merkle[a]

[a]*Department of Pharmacy, ETH, Winterthurerstr. 190, 8057 Zürich, Switzerland*
[b]*School of Pharmacy, Institute of Pharmaceutical Analysis, University of Lausanne, 1015 Lausanne, Switzerland*

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Abstract

Microencapsulation was studied by a thermodynamic approach taking quantitatively into account the molecular interactions between polymer, solvent and the aqueous protein phase. Bovine serum albumin was microencapsulated into poly(D,L-lactic acid) by spray drying using single solvents and binary solvent mixtures. The use of binary solvent mixtures allowed systems with adjustable solubility parameters to be designed. Microsphere characteristics like entrapment efficiency, burst release after 24 h and surface morphology were investigated and proved to be highly dependent on the polymer solvent system. Hildebrand or partial Hansen solubility parameters (δ, δ_d, δ_p, δ_h) proved to be insufficient for predicting microsphere properties, although low or moderate hydrogen-bonding solvents and solvent mixtures were found to be generally appropriate, whereas strongly H-bonding solvents gave poor quality microspheres. Similarly, water miscible solvents were shown to give often unsatisfactory products. A more powerful tool for optimizing a microencapsulation process is estimating polymer-solvent-drug interactions by using δ_d, δ_p, and the Drago parameters E (electrostatic) and C (covalent) of the components involved. Entrapment efficiency is increased and burst release reduced if polymer-drug interaction is dominant and polymer-solvent, drug-solvent interactions are reduced. This thermodynamic approach provides a rational basis for optimizing microencapsulation processes.

Keywords: Biodegradable microspheres; Poly(D,L-lactide); Thermodynamics; Solubility parameters; Interaction energies

1. Introduction

Biodegradable microspheres based on poly(D,L-lactic acid) (PLA) as well as poly(D,L-lactic-*co*-glycolic acid) (PLGA) have become very attractive parenteral delivery systems for peptides and proteins (Cohen et al., 1991; Bodmer et al., 1992; Gander et al., 1995b), hormones (Sanders et al., 1985; Ruiz and Benoit, 1991; Okada et al., 1994), vaccines (O'Hagan et al., 1991; Esparza and Kissel, 1992; Almeida et al., 1993; Alonso et al., 1993; Gander et al., 1993; Mestecky et al., 1994) and other active agents. Great effort has been devoted on improving microencapsulation

* Corresponding author: Tel: +41-1-2576012; Fax: +41-1-2625459.

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technologies, and the scientific as well as the patent literature covers numerous aspects of process parameters. However, all of these investigations adopted a rather empirical strategy to examine the consequences of changing operational parameters (Jalil and Nixon, 1990a,b; Arshady, 1991; Aftabrouchad and Doelker, 1992; Pavanetto et al., 1994). The few studies considering a more fundamental approach are limited to purely qualitative physico-chemical aspects. In a solvent evaporation process, Bodmeier and McGinity (1988) used different polymer solvents and showed that drug entrapment and other PLA-microspheres properties tend to depend on the Hansen partial solubility parameters. On the other hand, Gander et al. (1995b) showed that the characteristics of spray dried BSA-loaded PLA-microspheres can be correlated only to some extent to physico-chemical parameters such as solubility parameters, surface and interfacial tension, and vapour pressure of the solvent. For coacervation, Wu et al. (1994) described the effect of solvent/non-solvent pairs on properties of ethyl cellulose microcapsules using a critical interaction parameter. The results suggest that microspheres properties must depend on the interaction capacity of all components forming the system, i.e. polymer, solvent, drug and water. Indeed, the components of a microencapsulation mixture represent a thermodynamic system with different interactions occurring simultaneously. Therefore, more generally valid concepts of microencapsulation should be based on the thermodynamic interaction energy balance.

The present investigation focuses on the influence of various solvents on different properties of PLA-microspheres loaded with bovine serum albumin (BSA) used as a model protein. As our approach should be of practical relevance, all solvents taken into consideration were environmentally and toxicologically acceptable. Therefore, the commonly used chlorinated solvent dichloromethane was only used as reference and should be exchanged for less harmful organic solvents.The main object was to design solvent systems on the basis of interaction energies between solvent, polymer and aqueous BSA phases, which produce microspheres with optimum properties, i.e. high loading efficiency and low burst release. In a first step, single polymer solvents and solvent mixtures were selected on the basis of the Hansen solubility parameters (Hansen, 1969). Second, a refined selection of single polymer solvents was made according to calculated interaction energies of solvent-polymer, solvent-water, and polymer-water by using four interaction capacity parameters, i.e. two partial solubility parameters of Hansen (δ_d, δ_p), and the E (electrostatic) and C (covalent) parameters of Drago (Drago et al., 1971, 1993).

2. Theory

Microencapsulation by spray-drying generally involves the three components polymer, drug and solvent (or solvent mixture). It can be easily recognized that product characteristics such as drug encapsulation efficiency, burst release of drug and residual solvent greatly depend on the molecular interactions between the components. These interactions can be illustrated graphically by a schematic triangular model, as shown in Fig. 1.

According to Hansen (1969) the interaction capacity of a compound can be estimated by a set of

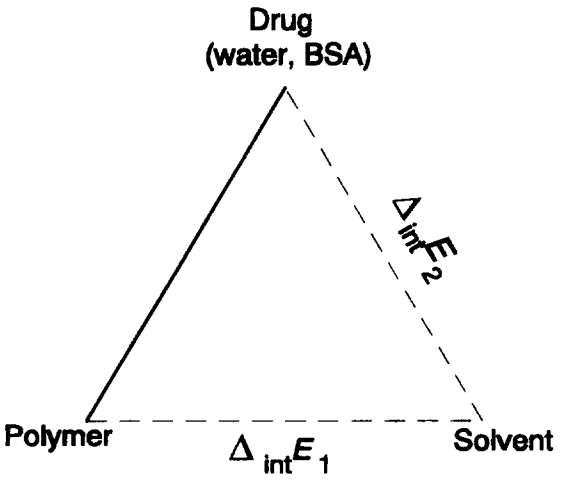

Fig. 1. Schematic model of intermolecular interactions taking place during BSA microencapsulation into PLA by spray drying. Good drug encapsulation is expected if the drug-polymer interaction (continuous line) dominates over the polymer-solvent and drug-solvent interactions (dashed lines).

three partial solubility parameters: δ_d (dispersive), δ_p (polar), and δ_h (hydrogen bonding). The first two parameters are good descriptors for the dispersive and polar interactions, while δ_h is considered to be a very poor one for the hydrogen bonding interaction. Marks and Drago (1975) introduced the parameters E (electrostatic) and C (covalent) to quantify the hydrogen bonding capacity of a Lewis acid or a Lewis base. Although these parameters are semi-empirical, they are, for the time being, the best descriptors of the interaction enthalpy of hydrogen bonding (Hô, 1994).

The interaction energy, $\Delta_{int}E_1$, between a solvent A and a polymer B, wherein the polymer is dissolved, can be expressed by the equation proposed by Hô (1994):

$$\Delta_{int}E = -2{}^AV\,{}^A\delta_d\,{}^B\delta_d - 2{}^AV\,{}^A\delta_p\,{}^B\delta_p - ({}^AE{}^BE + {}^AC{}^BC) \quad (1)$$

where ${}^AV$ is the molar volume of the solvent (cm^3/mol) (with ${}^AV \approx {}^A\bar{V}$), δ_d, δ_p are the Hansen partial solubility parameters ($MPa^{1/2}$) for dispersive and polar forces, respectively, and E and C are the Drago parameters ($kJ^{1/2}/mol^{1/2}$) for electrostatic and covalent contributions, respectively.

In the case of poor mixing between two components, such as occurs between water and water-insoluble polymers or between water and organic solvents which are only partly miscible with water, an adsorption type interaction must be considered. The corresponding interaction energy, $\Delta_{int}E_2$, is calculated by:

$$\Delta_{int}E = -{}^AV\,{}^A\delta_d\,{}^B\delta_d - {}^AV\,{}^A\delta_p\,{}^B\delta_p - ({}^AE{}^BE + {}^AC{}^BC) \quad (2)$$

For simplicity, parameters for pure water were used in this work although the presence of BSA should modify the properties of the aqueous component. When the solvent is miscible with water, Eq. (1) must be used.

For this work, we have made the hypothesis that for good drug encapsulation into the polymer, drug-polymer interaction must dominate over the interactions of drug-solvent and polymer-solvent. For given drug-polymer components, this interaction should become dominant through weakening the interactions with the solvent, illustrated by the dotted lines in Fig. 1. In other terms, if the sum of interaction energies $\Delta_{int}E_1$ and $\Delta_{int}E_2$ is low, the encapsulation efficiency should be high.

When a single polymer solvent is replaced by a solvent mixture, the interaction energies cannot be calculated with the above equations. At present, no appropriate models exist for solvent mixtures. In these cases, Hildebrand's total solubility parameter for the mixture, δ_{mix}, has to be considered:

$$\delta_{mix} = \Phi_A\delta_A + \Phi_B\delta_B \quad (3)$$

where Φ_A and Φ_B are the mole fractions for component A and B, respectively, and δ_A and δ_B are the corresponding Hildebrand parameters. The mole fractions can be calculated by

$$\Phi_A = \frac{n_AV_A}{n_AV_A + n_BV_B} \quad (4)$$

and

$$\Phi_B = 1 - \Phi_A \quad (5)$$

where n_A and n_B are the number of moles, and V_A and V_B the molar volumes (cm^3/mol) for A and B, respectively.

3. Materials and methods

3.1. Materials

Poly(D,L-lactic acid) (PLA), Resomer® R202 ($M_n = 7700$; $M_w = 15\,600$, as determined in our laboratory) was purchased from Boehringer Ingelheim (D-Ingelheim) and bovine serum albumin (BSA) was obtained from Fluka (CH-Buchs) (product No. 05477). The analytical grade solvents used for PLA were from Fluka (CH-Buchs), and their relevant physico-chemical characteristics are summarized in Table 1.

3.2. Microencapsulation

Microspheres were prepared by typically dispersing 2 g of a 2.0% (w/w) aqueous BSA solution in 40 g of 5.0% (w/w) solutions of PLA, using the

Table 1
Selected physicochemical characteristics of the polymer solvents

Solvent	*K*p (°C)	δ^a	δ_d^a	δ_p^a	δ_h^a	H-bond[b]
Dichloromethane (DCM)	40	20.3	18.2	6.3	6.1	p
Methylal	42	17.5	15.1	1.8	8.6	m
Acetone	56	20.0	15.5	10.4	7.0	m
Tetrahydrofuran (THF)	66	19.4	16.8	5.7	8.0	m
1,3-Dioxolane	76	23.2	14.8	11.3	13.9	m
Ethyl acetate (EtAc)	77	18.1	15.8	5.3	7.2	m
Cyclohexane[c]	80	16.8	16.8	0	0.2	p
Isopropanol[c]	82	23.5	15.8	6.1	16.4	s
Dimethyl carbonate	90	20.2	15.9	6.6	10.6	m
Isobutanol[c]	99	22.2	15.8	5.7	14.5	s
1,4-Dioxane	100	20.5	19.0	1.8	7.4	m
Toluene	111	18.2	18.0	1.4	2.0	p
Nitroethane	115	22.7	16.0	15.5	4.5	p

[a] δ is the Hildebrand solubility parameter, and δ_p, δ_d and δ_h are the Hansen partial solubility parameters $[MPa]^{1/2}$ representing the polar, dispersive and hydrogen bonding cohesive forces (taken from Barton, 1983).[b] p, m and s represent poor, moderate and strong hydrogen bonding forces.[c] Solvents used only in mixtures with ethyl acetate.

organic solvents or solvent mixtures listed in Tables 1 and 3, respectively. The W/O-mixture was homogenized by ultrasonication (Model Vibra Cell, Sonic and Materials., Danbury, USA) twice for 30 s each at output control 7 under ice-cooling. Subsequently, the obtained emulsion was spray dried in a Büchi 190 laboratory spray dryer (Büchi, CH-Flawil) at a rate of 2–5 ml/min. The process parameters were the following: flow of pressurized air: 400–500 Nl/h; aspiration: −40 mbar; inlet temperature: 37–80°C (depending on the solvent); outlet temperature: 33–60°C (depending on the solvent).

To break up any agglomerates or remove any BSA adsorbed on the microspheres' surface, the product was washed in 0.1% aqueous poloxamer F68 solution and distilled water, and sieved through a 100 μm sieve. After drying under vacuum at room temperature for 24 h, the spheres were washed in hexane, thereby removing any adsorbed water, and dried again under vacuum for 12 h.

3.3. Particle morphology and size

Microspheres shape and size were examined by visible light microscopy, scanning electron microscopy (SEM) and laser light scattering (Mastersizer X, Malvern Instruments, Worcestshire, UK). For SEM, the microspheres were mounted on a double faced adhesive tape, sputtered with platinum and viewed in a Hitachi S-700 scanning electron microscope.

3.4. Protein loading

BSA was extracted from the accurately weighed (50 mg) microspheres by first dissolving the polymer in dichloromethane. After filtration on a 0.2 μm membrane filter (RC 58, Schleicher and Schuell, D-Dassel), the protein was eluted from the filter with 0.067 M phosphate buffer, pH 7.4. The filter was washed three times with 3 ml buffer, and the combined solution assayed fluorometrically (Fluoromax, Spex, Edison, NJ, USA).

3.5. In vitro release

Release studies were conducted in rotating 4-ml glass vials at 37°C. Microspheres were accurately weighed (50 mg) into the vials and 4 ml of 0.067 M phosphate buffer, pH 7.4, were added. Prior to incubation the suspensions were sonicated for 15–30 s in a sonication bath, at 25 kHz. After 24 h, the samples were collected and centrifuged for 15 min at 3500 rev./min. The concentration of BSA

in the supernatant was subsequently measured fluorometrically at 37°C.

4. Results and discussion

4.1. Solvent selection

Single organic solvents for PLA were selected to cover a wide range of boiling points, i.e. 40–115°C, and solubility parameters, i.e. 16.8–23.5 $MPa^{1/2}$ (Table 1). More interestingly, the solvents also show varying polar and hydrogen bonding cohesive energies, two parameters which should greatly influence the behaviour of PLA in solution, as PLA is a polar molecule and a Lewis base. Dichloromethane (DCM) was used as reference for studying new individual solvent systems. Some of the solvents were selected for their low calculated interaction capacity with the polymer and with water (see below).

With the solvent mixtures, three different strategies were followed. The first group of solvent mixtures was characterized by a total Hildebrand solubility parameter comparable with DCM, i.e. EtAc/isopropanol (58.8:41.2) and EtAc/isobutanol. In the second group, the volume fraction of solvent with polar cohesive energy was reduced by mixing with a non-polar solvent, i.e. EtAc/cyclohexane. In the third group, proton donors and proton acceptors were mixed providing mutual 'neutralization' of the hydrogen bonding capability, i.e. EtAc/DCM and DCM/nitroethane. On a qualitative basis, these mixtures were expected to provide insight into the interaction between BSA and PLA.

4.2. Microencapsulation process

The solvent systems used for PLA substantially influenced both the manufacturing process and the quality of the microspheres. In the production process, the stability of the W/O-emulsions varied. Methylal, 1,3-dioxolane, and the mixtures EtAc/alcohols and EtAc/cyclohexane (61:39) all required additional sonication at some point before or during the spray drying process to maintain the mixtures homogeneous. The spheres produced with the higher boiling solvents nitroethane and toluene showed a similar behaviour. With all other solvent systems, good W/O-emulsion stability and satisfactory powder flow in the cyclon was achieved. Generally, the total yield was in the order of 50–70% after sieving and drying; for the higher boiling solvents, i.e. nitroethane and toluene, the yield was only 8–40%.

4.3. Particle morphology and size

Microencapsulation of BSA by spray drying generally resulted in spherical particles of about 1–10 μm in size. This size range was found by SEM and confirmed by laser light scattering. The particle size distribution was uniform, with a mean diameter of about 3.4 μm. The SEM micrographs showed regularly shaped microspheres with a non-porous surface. With DCM, marked surface indentations were visible. Ethyl acetate, dimethyl carbonate, toluene and nitroethane all gave very satisfactory particle morphology. By contrast, the water miscible solvents acetone, methylal, THF, and 1,4-dioxane produced a substantial part of coalesced microspheres with irregular morphology. These results with the single solvents confirm our previous findings (Gander et al., 1995b). With the solvent mixtures, particles with regular, spherical morphology and a smooth surface were obtained.

4.4. Protein loading

Drug incorporation efficiency is one of the key criteria for optimizing microencapsulation techniques. As presented in Table 2, BSA incorporation efficiency depends greatly on the polymer solvent used. Ethyl acetate and dichloromethane proved to be appropriate solvents, providing efficiencies of 65–75% (w/w). For ethyl acetate, this efficiency appears to depend greatly on the loading level.

Microencapsulation with toluene, nitroethane and dimethyl carbonate resulted in intermediate loading efficiencies, i.e. 41–58%. The first two, however, were expected to give higher incorporation rates due to low interactions solvent-PLA

Table 2
BSA loading, loading efficiency and burst release (after 24 h) from PLA microspheres prepared with a single polymer solvent

Solvent	Loading(%)	Loading efficiency ± S.D. (%)	Burst release ± S.D.(%)
Dichloromethane[a]	1.28	64.9 ± 5.3	9.2 ± 0.1
Dichloromethane[a]	3.77	66.8 ± 2.9	36.4 ± 1.1
Methylal	0.21	10.8 ± 0.5	45.0 ± 1.0
Acetone	0.49	25.0 ± 1.0	41.8 ± 1.3
THF	0.69	35.0 ± 1.0	16.6 ± 0.3
1,3-Dioxolane	0.20	10.2 ± 1.5	50.3 ± 1.3
Ethyl acetate[a]	1.44	72.3 ± 1.8	8.6 ± 1.0
Ethyl acetate[a]	3.22	56.6 ± 1.3	33.6 ± 0.3
Dimethyl carbonate	0.81	41.2 ± 4.3	60.9 ± 1.0
1,4-Dioxane	1.30	67.8 ± 3.3	12.3 ± 0.4
Toluene	1.16	58.8 ± 1.9	27.2 ± 1.2
Nitroethane	0.88	44.8 ± 2.6	16.5 ± 0.3

[a]Microspheres prepared to examine the effect of BSA loading.

and solvent-water (BSA), as discussed below. This discrepancy might be due to the higher boiling points of these solvents leading to a less dense surface structure of the microspheres. From the SEM micrographs, however, no surface pores were visible.

Finally, methylal, acetone, THF and 1,3-dioxolane gave low encapsulation efficiencies. This was anticipated since these compounds are highly or even entirely miscible with water, thereby having the potential to precipitate the protein and/or compete for adsorption on the polymer. In light of this, the surprisingly high loading efficiency of nearly 70% attained with 1,4-dioxane cannot be explained.

The experiments with the solvent mixtures provided highly relevant results (Table 3). The mixtures of ethyl acetate and alcohol having the same total solubility parameter as DCM resulted in very low loadings. This might be attributed to a partial precipitation of BSA in the presence of alcohol. Isobutanol, however, gave even lower efficiencies, although it is less water-miscible than isopropanol and therefore was expected to cause less pronounced precipitation of BSA. With the 80:20 mixture of ethyl acetate and isopropanol, a rather inconsistent result was obtained. The incorporation rate for this mixture was expected intermediate between pure ethyl acetate and the 58.8:41.2 mixture, but only about 5% was found. At present, no rational explanation can be provided, but the result provides clear evidence that an optimum ratio of individual components in a mixture must exist. The effect of protein loading with the two EtAc/isopropanol mixtures was negligible (Table 3), as found for the single solvent DCM.

Two different mixtures of ethyl acetate and cyclohexane, a polymer non-solvent, were used to examine the influence of reduced volume fraction of the solvent with polar cohesive energy contribution. The findings partly support our hypothesis that a reduced volume fraction of the polar solvent in a mixture may weaken the solvent-polymer interaction, whereby the drug-polymer interaction becomes more dominant, as illustrated in Fig. 1. With the EtAc/cyclohexane 81:19 mixture, the highest loading of all solvent systems studied was obtained (Table 3). However, addition of cyclohexane seems to have limitations since spray drying of the 61:39 mixture resulted in more than 50% reduction of the loading efficiency. This confirms the observation made above for the ethyl acetate/isopropanol mixture that the ratio of individual components in a solvent mixture is crucial for obtaining good quality microspheres.

The third approach was based on a possible neutralization mechanism of hydrogen-bonding forces by mixing organic solvents with proton donor and proton acceptor properties. DCM, a proton donor, was mixed with the acceptors nitroethane and ethyl acetate, respectively. The

Table 3
BSA loading, loading efficiency and burst release (after 24 h) from PLA microspheres prepared with polymer solvent mixtures

Solvent mixture[a]	δ^{b}_{mix}	Loading (%)	Loading efficiency ± S.D. (%)	Burst release ± S.D. (%)
EtAc/isopropanol (58.8:41.2)[c]	20.3	0.14	28.8 ± 5.2	11.6 ± 0.6
EtAc/isopropanol (58.8:41.2)[c]	20.3	0.55	27.9 ± 3.4	40.3 ± 1.2
EtAc/isopropanol (58.8:41.2)[c]	20.3	0.64	32.2 ± 1.0	23.1 ± 1.4
EtAc/isopropanol (58.8:41.2)[c]	20.3	1.43	30.0 ± 2.5	62.3 ± 1.9
EtAc/isopropanol (80:20)[c]	19.3	0.03	5.2 ± 3.0	54.3 ± 1.8
EtAc/isopropanol (80:20)[c]	19.3	0.09	4.7 ± 0.3	69.3 ± 3.5
EtAc/isopropanol (80:20)[c]	19.3	0.24	5.0 ± 1.0	65.9 ± 5.4
EtAc/isobutanol (50.1:49.9)	20.3	0.08	4.1 ± 0.1	Not measured
EtAc/cyclohexane (81:19)	17.9	1.56	79.4 ± 5.8	31.6 ± 0.7
EtAc/cyclohexane (61:39)	17.6	0.68	34.8 ± 0.9	47.1 ± 0.6
DCM/nitroethane (71.6:28.4)	21.2	1.49	75.9 ± 3.8	4.6 ± 0.1
DCM/nitroethane (38.6:61.4)	21.7	0.29	14.8 ± 0.8	30.8 ± 0.5
DCM/EtAc (49:51)	19.0	1.24	76.2 ± 4.4	21.1 ± 0.5
DCM/EtAc (65.8:34.2)	19.3	1.21	62.3 ± 1.8	19.5 ± 1.3

[a]Solvent ratios are expressed in weight percentage.[b]δ_{mix} was calculated by Eq. (3).[c]These batches were prepared to examine the effect of BSA loading.

DCM/nitroethane mixture 71.6:28.4 (molar ratio 2.2:1) seems to confirm a synergistic effect as loading efficiency is higher as compared with the individual solvents. The DCM/EtAc mixtures 49:51 (molar ratio 1:1) and 65.8:34.2 (molar ratio 2:1) also indicate favourable properties, even though the effect is less prominent. With the DCM/nitroethane mixture 38.6:61.4 (molar ratio 1:1.8), an inconsistent finding was observed as only 15% of the theoretical amount of protein was incorporated. This strongly emphasizes the importance of a finely adjusted volume ratio in solvent mixtures.

4.5. *In vitro release*

Protein release from a biodegradable polymer matrix such as PLA microspheres is a complex process, composed of (i) the release of material located near the particle surface, (ii) diffusion in macropores or in the polymer phase itself, and (iii) the more delayed release taking place due to degradation of the polymer matrix. In this study, we were mainly interested in examining the first stage of protein release, the so-called burst release. If the protein concentration near the surface of the microspheres is high, a more pronounced burst release will be observed. High protein concentration near the microsphere surface may be expected when strong interactions between the polymer solvent and the protein occur during manufacturing of the spheres, whereby the solvent carries along the protein towards the surface during evaporation. As biodegradable microspheres are commonly used for controlled drug release over several weeks or months, the burst release should be moderate (< 10%) and controlled.

The data in Tables 2 and 3 show that for most of the solvent systems, burst release is inversely proportional to the loading efficiency. Clearly, the solvent systems giving the highest incorporation rate tend to give the lowest burst release; DCM, EtAc and 1,4 dioxane prove to be the superior solvents. These three solvents provide acceptable loading efficiency combined with a moderate burst release in the order of 10%. The increased burst release from the formulations with higher actual BSA loading (EtAc and DCM; Table 2) is in agreement with results in the literature (Bodmer et al., 1992; Alonso et al., 1993).

Dimethyl carbonate and the two acetals produced microspheres with a very pronounced burst. As the SEM micrographs did not reveal any surface pores, this high burst might be attributed to BSA transported by the organic solvent towards the microspheres' surface during the

evaporation process. These particular solvents are characterized by a relatively high hydrogen bonding capacity (Table 1). A quite important burst release was also observed with toluene, which, in contrast to the aforementioned acetals and carbonate, interacts only very weakly through hydrogen bonding and polar forces. For toluene, the SEM micrographs reveal indeed that the particles are relatively porous.

Among the solvent mixtures, EtAc/alcohol produced microspheres with very high burst release. The results suggest that strong hydrogen-bonding solvents interact intensely with the protein, thereby transporting the protein towards the microspheres' surface during the solvent evaporation in the manufacturing process. With the EtAc/isopropanol mixture (58.8:41.2), an increasingly important burst release is observed from the microspheres with increasing actual loadings.

The intended reduction of the polar and hydrogen bonding forces through lowering the volume fraction of EtAc in the mixture with cyclohexane did obviously not lead to improved release characteristics. Compared with pure EtAc, the mixtures with cyclohexane (81:19 and 61:39) gave a 3-fold increase of the burst release. Here, however, a higher final loading seems to give a lower burst. We can speculate that, in the final step of particle drying, cyclohexane as a non-solvent for the polymer would possibly not diffuse readily through the polymer matrix but remain as residual in the microspheres. Consequently, this might create a more porous matrix morphology.

The microspheres prepared from the solvent mixtures consisting of a Lewis acid (DCM) and a Lewis base (nitroethane, ethyl acetate) gave, with one exception, a burst release which lies above that observed when single solvents were used. The DCM/nitroethane mixture (71.6:28.4) produces spheres, however, with excellent release properties and, as mentioned previously, high loading efficiency. This particular mixture consisting of 2 moles of DCM and 1 mole of nitroethane appears to fulfil best our requirements for good quality microspheres, i.e. high loading efficiency and low burst release. Although the assumed mutual neutralization between Lewis acid and Lewis base had generally a favourable effect on the loading efficiency, this was not generally the case for the burst release.

4.6. Evaluation of thermodynamic parameters

The results presented in Tables 2 and 3 show the wide range of solvents suitable for successful spray drying of PLA. Table 2 illustrates this in terms of increasing boiling point. Solvents with boiling points ranging from 40 to 110°C produce good quality microspheres. There is, however, clear evidence that poor quality particles are obtained from solvents which are highly water miscible such as, the acetals, acetone, and THF. In this respect, 1,4-dioxane represents a striking exception which cannot be explained for the time being.

Fig. 2 illustrates the pattern of BSA encapsulation efficiency plotted versus the Hildebrand solubility parameter δ (17.5–23.2 $MPa^{1/2}$), and the Hansen hydrogen bonding δ_h (2.0–13.9 $MPa^{1/2}$) and polar δ_p (1.4–15.5 $MPa^{1/2}$) cohesive parameters. For the Hildebrand solubility parameter, the borderline solvents methylal and 1,3-dioxolane do not reflect the real boundary of the suitable solubility parameter range. These two solvents are particular compounds as they exhibit relatively strong H-bonding interactions (Table 1). Overall, the irregularity of the profile demonstrates that the Hildebrand parameter does not represent an adequate tool to optimize solvent systems for protein microencapsulation.

The weakness of the Hildebrand parameter lies in the fact that it does not discriminate between dispersive, polar and hydrogen-bonding forces involved in the drug-polymer-solvent interactions. For aliphatic polyesters, which obviously interact greatly through polar and hydrogen bonding forces, the Hildebrand parameter is clearly not appropriate.

A more discriminative picture on the type and energy of interactions should be expected when the Hansen partial solubility parameters are considered, which distinguish between dispersive, permanent dipole-dipole, and H-bonding contributions. The plot representing the H-bondings (Fig. 2) tendentiously shows the superiority of low or moderate H-bonding solvents. This

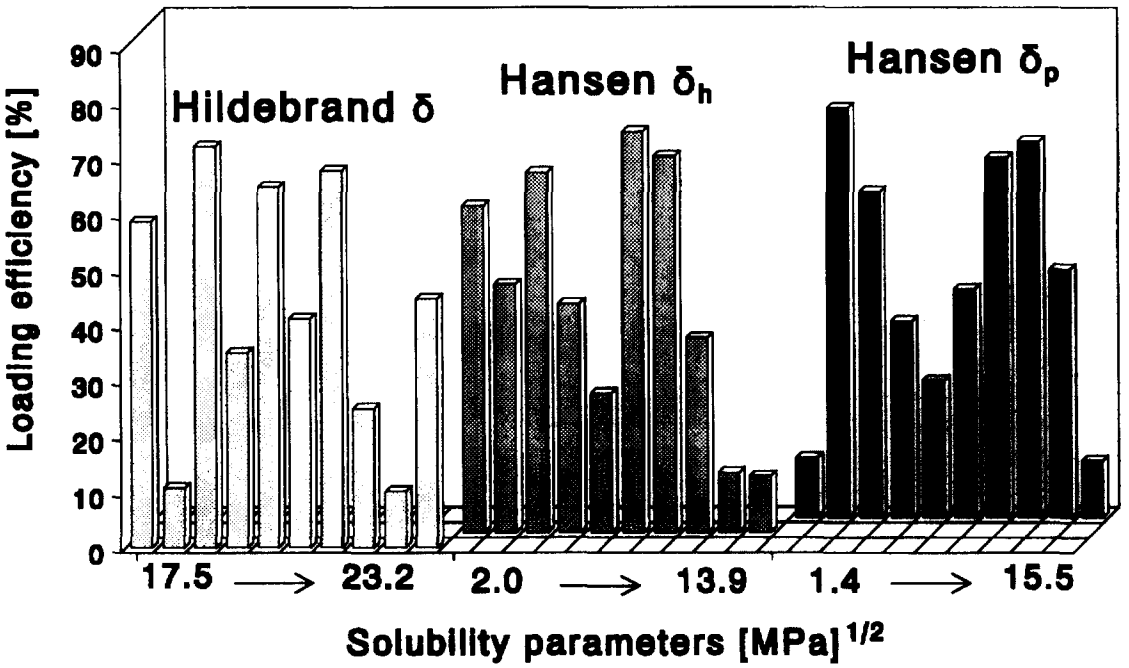

Fig. 2. Microsphere loading efficiencies versus the Hildebrand solubility parameter (δ), the Hansen hydrogen-bonding (δ_h) and polar (δ_p) cohesive parameters.

observation is further supported by the negative results obtained with the EtAc/alcohol mixtures (the hydrogen bonding parameters of isopropanol and isobutanol are 16.3 and 14.5 $[MPa]^{1/2}$, respectively). By contrast, the importance of the polar cohesion parameter in a single solvent system is ambiguous (Fig. 2). Clearly, solvents with polar cohesion parameters ranging from 1.4 to 15.5 $MPa^{1/2}$ gave suitable product quality. Therefore, the microencapsulation process appears to be much less influenced by the polarity of the solvent than by the hydrogen-bonding capacity.

Although the Hansen parameters do differentiate between dispersion, polar and hydrogen bonding forces, they still appear to lack specificity as far as H-bonding interactions are concerned. These interactions can be considered as the mutual reactions between H-donors and H-acceptors. As PLA is a Lewis base, hence H-acceptor, we can reasonably assume that this polyester interacts very differently with H-donors (DCM, alcohols) and H-acceptors (esters, ketones, acetals). Moreover, the presence of water (and also of the protein) affects this interaction. All these factors have not been taken into account so far.

A more powerful tool for estimating polymer-solvent-drug interactions is the calculation of energies of interaction using, in addition to δ_d and δ_p, the E (electrostatic) and C (covalent) parameters which account for acid-base interactions. The parameters are reported to give improved agreement with experimental data (Drago et al., 1971; Hô, 1994). The values of the E and C parameters used, taken from Drago's work (Drago et al., 1993), are summarized in Table 4. One should notice, however, that the values for PLA, toluene and nitroethane are assumptions from ethyl acetate, benzene and nitrobenzene, respectively.

Fig. 3 shows a plot of loading efficiency and burst release versus the sum of interaction energies ($\Sigma_{int}E$) from solvent-polymer and from solvent-water interactions (water was used instead of aqueous BSA solution for simplicity). By reducing the solvent-polymer and the solvent-water interactions, the water-polymer interaction is assumed to become dominant, resulting in higher protein in-

Table 4
Hansen and Drago interaction parameters used for calculating interaction energies

Substance	δ_d (MPa $^{1/2}$)	δ_p (MPa $^{1/2}$)	E^a (kJ$^{1/2}$/mol)	C^a (kJ$^{1/2}$/mol)	V (cm^3/mol
PLA	15.5	1.63	3.31[b]	2.00[b]	55.7
Water (acid)	15.6	16.0	2.68	1.60	18
Water (base)	15.6	16.0	4.66	0.20	18
DCM	18.2	6.3	1.76	0.22	63.9
Acetone	15.5	10.4	3.56	2.58	74.0
THF	16.8	5.7	3.35	4.46	81.7
EtAc	15.8	5.3	3.31	2.00	98.5
1,4-Dioxane	19.0	1.8	3.80	2.64	85.7
Toluene	18.0	1.4	1.43[c]	0.92[c]	106.8
Nitroethane	16.0	15.5	2.64[d]	0.98[d]	71.5

[a]Values taken from Drago et al. (1993).[b]Value for ethyl acetate (used as a molecular model for PLA).[c]Value of benzene.[d]Value of nitrobenzene.

corporation efficiency and lower burst release. The tendency of the results demonstrates the validity of our thermodynamic model, although 1,4-dioxane does not fit into this model. One also should keep in mind that the calculated interactions energies for toluene and nitroethane are approximations, as the E and C values for these solvents are not known. The values used here are those reported for benzene and nitrobenzene, considered as valid assumptions for toluene and nitroethane, respectively. The proposed interaction model for drug microencapsulation require confirmation by additional experiments. Clearly, taking into consideration the heat of dissolution of the

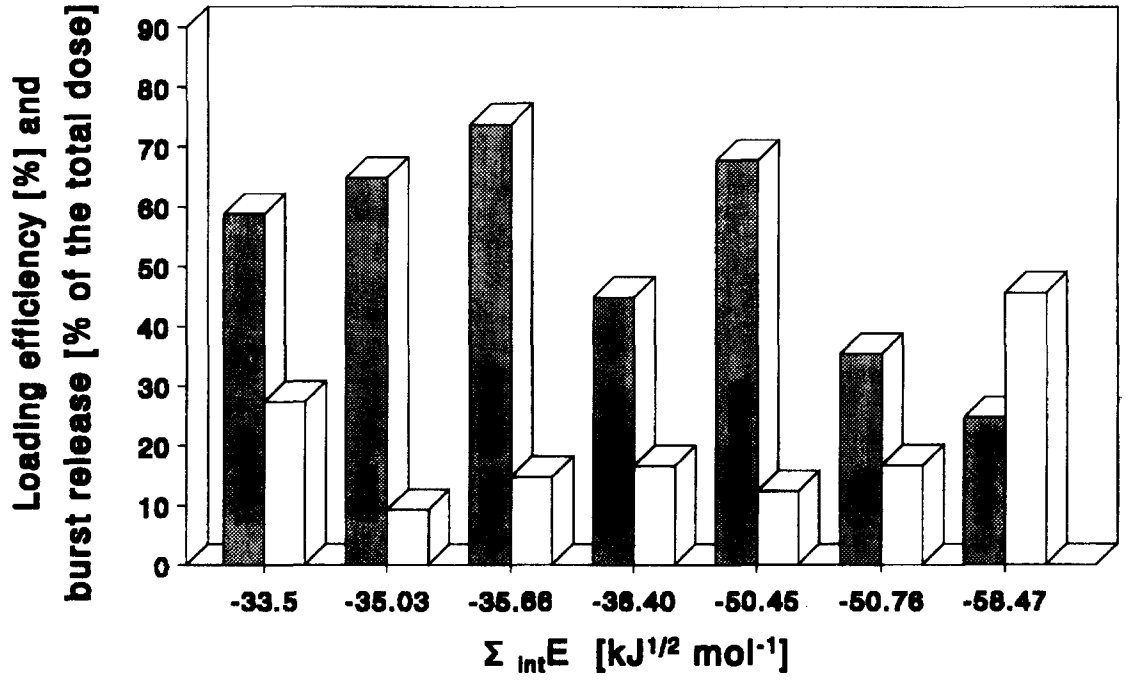

Fig. 3. Microsphere loading efficiency (hatched columns) and burst release (blank columns) versus the sum of change in interaction energy (Eqs. 1 and 2).

polymer in the different solvents, and the knowledge of the parameters δ_d, δ_p, E, C for the polymer and the solvents would definitely improve the validity of the calculated interaction energies. In this respect, a new and more developed thermodynamic model to predict protein encapsulation efficiency in PLA microspheres has been recently proposed (Gander et al., 1995a).

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References

Aftabrouchad, C. and Doelker, E., Méthodes de préparation des microparticules biodégradables chargées en principes actifs hydrosolubles. *S.T.P. Pharma Sci.*, 2 (1992) 365–380.

Almeida, A.J., Alpar, H.O. and Brown, M.R.W., Immune response to nasal delivery of antigenically intact tetanus toxoid associated with poly(L-lactic acid) microspheres in rats, rabbits and guinea pigs. *J. Pharm. Pharmacol.*, 45 (1993) 198–203.

Alonso, M.J., Cohen, S., Park, T.G., Gupta, R.K., Siber, G.R. and Langer R., Determinants of release rate of tetanus vaccine from polyester microspheres. *Pharm. Res.*, 10 (1993) 945–953.

Arshady, R., Preparation of biodegradable microspheres and microcapsules: 2. Polylactide and related polyesters. *J. Controlled Release*, 17 (1991) 1–22.

Barton, A.F.M., Cohesion parameters and liquid state thermodynamic data (Chap. 7), Particle liquid solubility scales (Chap. 8). In: *Handbook of Solubility Parameters and other Cohesion Parameters*, CRS Press, Boca Raton, FL, USA, 1983, pp. 91–200.

Bodmeier, R. and McGinity, J.W., Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by the solvent evaporation method. *Int. J. Pharm.*, 43 (1988) 179–186.

Bodmer, D., Kissel, T. and Traechslin, E., Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems. *J. Controlled Release*, 21 (1992) 129–138.

Cohen, S., Yoshioka, T., Lucarelli, M., Hwang, L.H. and Langer, R., Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. *Pharm. Res.*, 8 (1991) 713–720.

Drago, R.S., Vogel, G.C. and Needham, T.E., A four-parameter equation for predicting enthalpies of adduct formation. *J. Am. Chem. Soc.*, 93 (1971) 6014–6026.

Drago, R.S., Dadmun, A.P. and Vogel G.C., Addition of new donors to the E and C Model. *Inorg. Chem.*, 32 (1993) 2473–2479.

Esparza, I. and Kissel, T., Parameters affecting the immunogenicity of microencapsulated tetanus toxoid. *Vaccine*, 10 (1992) 714–720.

Gander, B., Merkle, H.P., Nguyên, V.P. and Hô, N.-T., A new thermodynamic model to predict protein encapsulation efficiency in poly(lactide) microspheres *J. Phys. Chem.*, 99 (1995) 16144–16148.

Gander, B., Thomasin, C., Merkle, H.P., Men, Y. and Corradin, G., Biodegradable microspheres represent a promising vaccine delivery system. *Proc. Eur. Pharm. Tech. Conf. D-Düsseldorf*, Advanstar Communications, Chester, UK, 1993, pp. 248–256.

Gander, B., Wehrli, E., Alder, R. and Merkle, H.P., Quality improvement of spray-dried protein loaded d,l-PLA microspheres by appropriate polymer solvent selection. *J. Microencapsulation*, 12 (1995) 83–97.

Hansen, C.M., The universality of the solubility parameter. *Ind. Eng. Chem. Prod. Res. Dev.*, 8 (1969) 2–11.

Hô, N.-T., A new model for evaluating interactions in liquids. *J. Phys. Chem.*, 98 (1994) 5362–5367.

Jalil, R. and Nixon, J.R., Microencapsulation using poly(D,L-lactic acid). I: Effect of preparative variables on the release kinetics. *J. Microencapsulation*, 7 (1990a) 229–244.

Jalil, R. and Nixon, J.R., Biodegradable poly(lactide acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. *J. Microencapsulation*, 7 (1990b) 297–325.

Marks, A.P. and Drago, R.S., Justification for the E and C equation. *J. Am. Chem. Soc.*, 97 (1975) 3324–3329.

Mestecky, J., Moldoveanu, Z., Novak, M., Huang, W.-Q., Gilley, R.M., Staas, J.K., Schafer, D. and Compans, R.W., Biodegradable microspheres for the delivery of oral vaccines. *J. Controlled Release*, 28 (1994) 131–141.

O'Hagan, D.T., Jeffery, H., Roberts, M.J.J., McGee J.P. and Davis, S.S., Controlled release microcapsules for vaccine development. *Vaccine*, 9 (1991) 768–771.

Okada, H., Yamamoto, M., Heya, T., Inoue, Y., Kamei, S., Ogawa, Y. and Toguchi H., Drug delivery using biodegradable microspheres. *J. Controlled Release*, 28 (1994) 121–129.

Pavanetto, F., Conti, B., Giunchedi, P., Genta, I. and Conte, U., Polylactide microspheres for the controlled release of diazepam. *Eur. J. Pharm. Biopharm.*, 40 (1994) 27–31.

Ruiz, J.M. and Benoit, J.P., In vivo peptide release from poly(D,L-lactic acid-co-glycolic acid) copolymer 50/50 microspheres. *J. Controlled Release*, 16 (1991) 177–186.

Sanders, L.M., McRae, G.I., Vitale, K.M. and Kell, B.A., Controlled delivery of an LHRH analogue from biodegradable injectable microspheres. *J. Controlled Release*, 2 (1985) 187–195.

Wu, J.C., Su, S.G., Shyu, S.S. and Chen, H.. Effect of the solvent-non-solvent pairs on the surface morphology and release behaviour of ethylcellulose microencapsules prepared by non-solvent-addition phase separation method. *J. Microencapsulation*, 11 (1994) 297–308.