International Journal of Pharmaceutics 383 (2010) 236–243

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Determination of poly(ε-caprolactone) solubility parameters: Application to solvent substitution in a microencapsulation process

C. Bordes[a,*], V. Fréville[a], E. Ruffin[b], P. Marote[a], J.Y. Gauvrit[a], S. Briançon[b], P. Lantéri[a]

[a] Université de Lyon, F-69622, Lyon, France; Université Lyon 1, Villeurbanne; LSA, UMR 5180, CNRS, CPE, 43 bd du 11 novembre, 69100 Villeurbanne, France
[b] Université de Lyon, F-69622, Lyon, France; Université Lyon 1, Villeurbanne; LAGEP, UMR 5007, CNRS, CPE, 43 bd du 11 novembre, 69100 Villeurbanne, France

ARTICLE INFO

Article history:
Received 7 July 2009
Received in revised form 4 September 2009
Accepted 11 September 2009
Available online 23 September 2009

Keywords:
Hansen solubility parameters
Solvent substitution
Poly(ε-caprolactone)
Microencapsulation
Anisole

ABSTRACT

The evolution of regulation on chemical substances (i.e. REACH regulation) calls for the progressive substitution of toxic chemicals in formulations when suitable alternatives have been identified. In this context, the method of Hansen solubility parameters was applied to identify an alternative solvent less toxic than methylene chloride used in a microencapsulation process. During the process based on a multiple emulsion (W/O/W) with solvent evaporation/extraction method, the solvent has to dissolve a polymer, poly(ε-caprolactone) (PCL), which forms a polymeric matrix encapsulating or entrapping a therapeutic protein as the solvent is extracted. Therefore the three partial solubility parameters of PCL have been determined by a group contribution method, swelling experiments and turbidimetric titration. The results obtained allowed us to find a solvent, anisole, able to solubilize PCL and to form a multiple emulsion with aqueous solutions. A feasibility test was conducted under standard operating conditions and allowed the production of PCL microspheres.

1. Introduction

Solvents are essential products in many sectors of industry and everyday life. They are found in various fields such as detergents, agrochemicals, cosmetics, pharmaceuticals, paints, varnishes and inks. In recent years, regulations on solvents are more stringent because of their impact on the environment and health. The REACH directive (Registration, Evaluation and Authorization of Chemicals) requires manufacturers to prove the safety of the substances they use. Indeed, the impact of very few of the 100,000 chemicals used in everyday life have actually been evaluated on human health and environment. Accordingly, many formulations must be modified by replacing certain solvents by less toxic and more environmentally friendly ones.

The substitution of one or more solvents in a formulation remains a complex problem. There are more or less empirical tools allowing the prediction of the solubility of a compound in a solvent (Modarresi et al., 2008). The most common method used by formulators and applied in this study is based on the Hansen solubility parameters (Hansen, 2007). In chemical engineering developments, thermodynamic models such as Universal Quasichemical Activity Coefficient (UNIQUAC) or Non-Random Two Liquid (NRTL) models (Chen and Crafts, 2006) based on the concept of local composition or the Universal Functional Activity Coefficient (UNIFAC) predictive model (Gracin et al., 2002) using group contributions are more commonly used (Manifar and Rohani, 2005). Another approach consists in collecting experimental and theoretical molecular descriptors which are analyzed by using statistical techniques in order to obtain Quantitative Structure–Property Relationship (QSPR) models (Code et al., 2008; Tantishaiyakul et al., 2006; Yu et al., 2006) and/or solvent classifications (Chastrette et al., 1985; Gramatica et al., 1999; Katritzky et al., 2005; Xu and Redman-Furey, 2007).

In this context, we are interested in the substitution of a solvent, methylene chloride (MC), used in a microencapsulation process for therapeutic proteins (Al Haushey et al., 2007). Indeed, MC belongs to the class of solvents whose use is subject to limitation by the European Pharmacopoeia, because of their inherent toxicity (Class 2) (European Pharmacopoeia, 2009). During the process, MC makes soluble a polymer (poly(ε-caprolactone) (PCL)) which forms a polymeric matrix encapsulating the protein as the organic solvent is extracted. PCL is a biodegradable polymer (an aliphatic polyester) obtained by ring opening polymerization of caprolactone (Fig. 1).

PCL has a semi-crystalline structure and a glass transition temperature (T_g) of −60 °C. PCL easy crystallization explains its limited solubility in many solvents which are able to dissolve other amorphous polyester structures. The PCL degradation kinetics is very slow, making it suitable for slow release delivery systems with long term kinetics extending over periods exceeding 1 year (Sinha et al., 2004).

* Corresponding author. Tel.: +33 4 72 44 85 61; fax: +33 4 72 44 83 19.
E-mail address: bordes@univ-lyon1.fr (C. Bordes).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.09.023

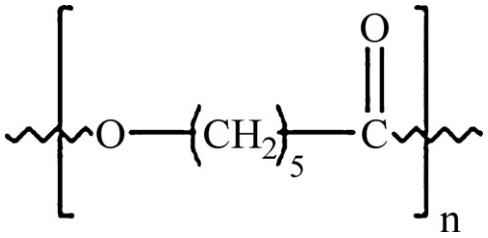

Fig. 1. Structure of poly(ε-caprolactone) (PCL).

In this study, we chose to determine the PCL Hansen solubility parameters through different techniques in order to obtain clear indications for substituting MC and to select a suitable alternative solvent belonging to the Class 3 of solvents with low toxic potential whose use is recommended by the European Pharmacopoeia.

2. Solubility parameters: theory

Hildebrand introduced the concept of solubility parameter for non-polar compounds by noting that the vaporization enthalpy (ΔH_v) reflects the amplitude of cohesion intermolecular forces in liquids (Hildebrand and Scott, 1950). The solubility parameter of a substance was defined as the square root of the cohesion energy per unit volume, with V the molar volume:

$$\delta = \left(\frac{\Delta H_v - RT}{V}\right)^{1/2} \quad (1)$$

Hildebrand has shown that the solubility of two substances 1 and 2 will occur for a minimal mixing free energy i.e. when their solubility parameters will be identical or tend toward equality $\delta_1 = \delta_2$:

$$\Delta H_M - V_M \varphi_1 \varphi_2 (\delta_1 - \delta_2)^2 \quad (2)$$

where ΔH_M is the heat of mixing, φ_1 and φ_2 are the volume fractions of substances 1 and 2 respectively and V_M is the volume of the mixture.

In 1967, Hansen suggested the splitting of the "global" Hildebrand solubility parameter into three parts derived from different types of cohesive forces (a disperse part, a polar part and a hydrogen part) according to (Hansen, 2007):

$$\delta = (\delta_d^2 + \delta_p^2 + \delta_h^2)^{1/2} \quad (3)$$

where δ_d corresponds to the so-called London interaction resulting from the existence of induced dipoles as two molecules approach one another (disperse part), δ_p corresponds to Keesom forces occurring when two permanent dipoles are present (polar part) and δ_h represents hydrogen bonding forces (hydrogen part). The unit of solubility parameters in the SI unit system is $(MPa)^{1/2}$.

Hansen solubility parameters define a three dimension "solubility space" in which all liquid or solid substances may be localized. In the "Hansen space", solvents in which a given molecule is soluble form a cloud of points corresponding in most cases to a sphere whose center point is the solute coordinates. All solvents and mixtures found in this volume are good solvents for the studied solute and the solvents outside are non-solvents. The more a solvent is close to the solute in the "Hansen space", the better its affinity for this solute.

Another approach to understand the solubility of a polymer was developed in the early 50s by Flory and Huggins (Flory, 1953). Their theory may explain the non-ideal character of polymer solutions. The Flory–Huggins parameter χ_{12} was included in the definition of the mixing enthalpy and can be related to the solubility parameters of two substances by the relation:

$$\chi_{12} = \frac{V_M}{RT}(\delta_1 - \delta_2)^2 \quad (4)$$

where δ_1 and δ_2 are the solubility parameters of the solvent and the polymer respectively. When χ_{12} is less than 0.5, the solvent is generally considered as a good solvent for the polymer, while a value higher than 0.5 corresponds to a poor solvent.

3. Materials and methods

3.1. Materials

Poly(ε-caprolactone) (M_w = 14,000 and 65,000) was purchased from Aldrich Chemical Company. Poly(vinyl alcohol) (PVA) from Fluka was used as a stabilizer in the external phase in the microencapsulation process. All other chemicals and solvents used were of analytical grade.

3.2. Determination of solubility parameters

3.2.1. Group contribution methods

Polymer solubility parameters can be calculated by several methods involving group contributions as the methods of Van Krevelen (Hoy, 1970; Van Krevelen and Hoftyzer, 1976) or more recently the method proposed by Stefanis and Panayiotou (2008). Van Krevelen approach is one of the most common methods in which each parameter can be estimated using the following equations (Van Krevelen and Hoftyzer, 1976):

$$\delta_d = \frac{\sum_i F_{di}}{V}, \qquad \delta_p = \frac{\sqrt{\sum_i F_{pi}^2}}{V}, \qquad \delta_h = \sqrt{\frac{\sum_i E_{hi}}{V}} \quad (5)$$

where F_d is the dispersion component, F_p the polar component and E_h the contribution of hydrogen bonding forces. The total solubility parameter is then calculated by Eq. (3). Tables giving group contributions are available in the literature (Barton, 1991).

3.2.2. Experimental methods (Barton, 1991; Hansen, 2007)

The experimental estimation of the solubility parameters of slightly volatile compounds can be done by several techniques: by swelling tests (Schenderlein et al., 2004), by turbidimetric titration (Schenderlein et al., 2004; Wang, 2003), by viscosity measurements (Wang, 2003) and by inverse gas chromatography (IGC) (Tian and Munk, 1994; Adamska et al., 2008; Sreekanth and Reddy, 2008). In this study, swelling tests and turbidimetric titration were performed. These methods are presented below.

3.2.2.1. Swelling tests. The experimental determination of solubility parameters generally requires the choice of reference solvents whose solubility parameters are known and well distributed in the "Hansen space". Barton and Hansen recommend the selection of about 40 solvents belonging to different compound families (Barton, 1991; Hansen, 2007). According to Hansen (2007), water has to be excluded from a standard set of test liquids because of its particular behavior in relation with its low molecular volume, its very high δ_h parameter and its tendency to self-associate or associate with other substances forming special structures. Swelling tests of the polymer have to be performed at well-defined temperature and concentration. The determination of the solubility volume (or solubility sphere) is derived from visual observations.

The effectiveness of a non-tested solvent with well-known solubility parameters can be predicted by positioning it within or outside the solubility sphere. It is then possible to compare the solubilizing power of solvents for a given solute by classifying according to their distance from the solute (the center of the sphere). Solvents which are closest to the center are those that are thermodynamically more likely to give a stable solution.

Table 1
PCL solubility parameters obtained by different methods.

	Method	δ_d	δ_p	δ_h	R_s
PCL	Group contribution	17.0	4.8	8.3	–
PCL14000 (0.5 g/5 mL)	Swelling tests	17.8	6.1	7.8	7.1
	Heptane/butanol titration	16.2	3.3	9.1	$4.5 < R < 7.0$
	Hexane/butanol titration	16.1		8.8	$4.5 < R < 7.4$
PCL14000 (2.5 g/5 mL)	Swelling tests	17.6	6.2	8.0	7.1
PCL65000 (0.5 g/5 mL)	Swelling tests	17.8	6.2	7.7	5.5
	Heptane/butanol titration	16.1	3.3	8.7	$5.3 < R < 6.8$
	Hexane/butanol titration	16.1	3.4	8.9	$5.3 < R < 7.0$
PCL65000 (2.5 g/5 mL)	Swelling tests	17.0	7.7	8.3	5.0

The distance D between a solvent (S) and the solute (P) in the "solubility space" is calculated by the following equation:

$$D = (4(\delta_{dS} - \delta_{dP})^2 + (\delta_{pS} - \delta_{pP})^2 + (\delta_{hS} - \delta_{hP})^2)^{1/2} \quad (6)$$

Hansen has suggested on the basis of empirical tests the doubling of the dispersion parameter in Eq. (6) in comparison with the two other parameters. Indeed, this weighting converts the elliptic shape of the solubility volume to an almost spherical one.

In the ideal case, the solubility sphere includes all the solvents and excludes all the non-solvents. It is characterized by the three coordinates of its center δ_{dP}, δ_{pP}, δ_{hP} (solubility parameters of the compound to solubilize) and by its radius R_s.

$$\delta_{dP} = \frac{\sum_{S=1}^{N} \delta_{dS}}{N}, \qquad \delta_{pP} = \frac{\sum_{S=1}^{N} \delta_{pS}}{N}, \qquad \delta_{hP} = \frac{\sum_{S=1}^{N} \delta_{hS}}{N} \quad (7)$$

where N is the number of solvents able to solubilize the molecule.

The radius R_s can be determined by different methods: by determining the maximum distance between the good solvent the furthest from the center and the sphere center (Eq. (8)) or by calculation with the minimization of the outlier number (the numbers of good solvent outside the sphere and non-solvents inside).

$$R_S = Max(4(\delta_{dS} - \delta_{dP})^2 + (\delta_{pS} - \delta_{pP})^2 + (\delta_{hS} - \delta_{hP})^2)^{1/2} \quad (8)$$

3.2.2.2. Turbidimetric titration. This experimental method based on the Flory–Huggins theory allows the clarification of the solubility volume limits by studying several mixtures of solvents and non-solvents (Suh and Clarke, 1967). Indeed, the addition of a certain amount of non-solvent to a polymer solution causes the polymer precipitation. Then, for each chosen mixture, the measurement principle consists in varying the proportions of the two types of solvent until reaching the "solubility boundary". The mass fraction of the liquids provides information on the interactions between polymer molecules.

For classical turbidimetric titration, two non-solvents have to be chosen so that one (1) has a solubility parameter lower than the solvent (2) solubility parameter and the second (3) has a higher one. Each non-solvent is mixed with the solvent and the molar volume V_m of these mixtures are obtained by the following equation:

$$V_{m,low} = \frac{V_1 V_2}{\varphi_1 V_2 + \varphi_2 V_1} \quad \text{and} \quad V_{m,high} = \frac{V_2 V_3}{\varphi_2 V_3 + \varphi_3 V_2} \quad (9)$$

with V_i the molar volume, φ_i the volume fraction.

One of the two non-solvents is added to the polymer solution until reaching turbidity. At this moment, the polymer solubility parameter $\delta_{app,p}$ is about the apparent Flory–Huggins parameter and is defined by the following equation:

$$\delta_{app,p} = \frac{\delta_{m,low}\sqrt{V_{m,low}} + \delta_{m,high}\sqrt{V_{m,high}}}{\sqrt{V_{m,low}} + \sqrt{V_{m,high}}} \quad (10)$$

$$\text{with } \delta_m = \varphi_1\delta_1 + \varphi_2\delta_2 \text{ or } \varphi_2\delta_2 + \varphi_3\delta_3 \quad (11)$$

Experiments have to be carried out in different solvents to determine the partial solubility parameters of the polymer. Then, each partial solubility parameter is graphically obtained and corresponds to the intersection between the plotted regression line obtained from Eq. (10) and the line $\delta_{app} = \delta_{solv}$.

3.3. PCL microsphere preparation

PCL microspheres were prepared by a multiple emulsion process followed by a solvent extraction/evaporation method previously described (Al Haushey et al., 2007). The microspheres were dedicated to the encapsulation of therapeutic proteins. Within this study, no protein has been introduced in the formulations since the main objective was to verify the feasibility of microsphere preparation by substituting MC in the microencapsulation process. Briefly, an internal aqueous phase was emulsified in an organic phase, a solution of MC and PCL (2 g of PCL in 5 mL of MC). A multiple emulsion $W_1/O/W_2$ was then obtained by mixing the first emulsion with an external aqueous phase containing poly(vinyl alcohol) (PVA) as a stabilizer and isopropanol to extract MC. The extraction of MC and evaporation of both solvents led to the formation of PCL microparticles which were then filtered, washed and dried.

4. Results and discussion

4.1. Determination of the PCL solubility parameters

4.1.1. Group contribution method

The most common method based on the contributions of functional groups was proposed by Van Krevelen and Hoftyzer (1976). On its base, the results obtained for PCL partial solubility parameters were $\delta_d = 17$, $\delta_p = 4.8$, $\delta_h = 8.3$ and are reported in Table 1. However, this calculation takes into account neither the molecular weight nor polymer concentration.

4.1.2. Swelling tests

0.5 g of PCL were introduced into sealed test tubes containing 5 mL of solvent. The tubes were put under magnetic stirring for 1 h, and immersed in a bath thermostated at 25 °C for 24 h. A visual observation was conducted and the results are classified into three categories: soluble (a), partially soluble (b) and non-soluble (c) (Fig. 2).

The concentration 0.5 g of polymer in 5 mL of solvent corresponds to the polymer concentration classically used in the literature (Barton, 1991; Hansen, 2007). Swelling tests were also performed at the maximum concentration 2.5 g of PCL in 5 mL used for the microencapsulation process and for two types of PCL with different molecular weight 14,000 (PCL14000) and 65,000 g/mol (PCL65000).

Table 2
Swelling test results: soluble (a), partially soluble (b) and non-soluble (c).

CAS number	Solvent name	PCL14000[a]	PCL14000[b]	PCL65000[a]	PCL65000[b]	CAS number	Solvent name	PCL14000[a]	PCL14000[b]	PCL65000[a]	PCL65000[b]
56235	Carbon tetrachloride	b	b	c	c	75854	2-Methyl-2-butanol	c	c	c	c
56815	Gycerol	c	c	c	c	75898	2,2,2-Trifluoroethanol	a	a	a	a
57556	1,2-Propanediol	c	c	c	c	76051	Trifluoroacetic acid	a	a	a	a
60297	Diethyl ether	c	c	c	c	78922	2-Butanol	c	c	c	c
62533	Aniline	a	b	a	b	78933	2-Butanone	b	b	c	c
64175	Ethanol	c	c	c	c	84662	Diethyl phthalate	c	c	c	c
64197	Acetic acid	a	b	a	b	93890	Ethyl benzoate	b	b	c	c
67561	Methanol	c	c	c	c	95476	*o*-Xylene	a	b	b	b
67630	Isopropanol	c	c	c	c	95501	1,2-Dichlorobenzene	a	a	a	c
67641	Acetone	b	b	c	c	96220	3-Pentanone	b	b	c	c
67663	Chloroform	a	a	a	a	98862	Acetophenone	a	b	a	c
67685	Dimethyl sulfoxide	c	c	c	c	98953	Nitrobenzene	a	b	a	c
68122	N,N-dimethylformamide	b	b	c	c	100414	Ethylbenzene	b	b	c	c
71238	1-Propanol	c	c	c	c	100516	Benzyl alcohol	a	a	a	c
71363	1-Butanol	c	c	c	c	100527	Benzaldehyde	a	a	a	b
71410	Pentanol	c	c	c	c	100663	Anisole	a	a	a	b
71432	Benzene	a	a	a	b	102716	Tiethanolamine	c	c	c	c
74964	Bromoethane	b	b	c	c	105588	Diethyl carbonate	c	c	c	c
75036	Iodoethane	b	b	c	c	106423	*p*-Xylene	b	b	c	c
75058	Acetonitrile	b	b	c	c	107062	1,2-Dichloroethane	a	a	a	a
75092	Methylene chloride	a	a	a	a	107073	2-Chloroethanol	a	a	a	c
75127	Formamide	c	c	c	c	107108	Propylamine	b	b	c	c
75183	Dimethyl sulfide	a	a	a	b	107211	Ethylene glycol	c	c	c	c
75310	Isopropylamine	b	b	c	c	107313	Methyl formate	a	a	a	b
75365	Acetyl chloride	a	a	a	a	107879	2-Pentanone	b	b	c	c
108101	4-Methyl-2-pentanone	c	c	c	c	112403	*n*-Dodecane	c	c	c	c
108203	Isopropyl ether	c	c	c	c	112607	Tetraethylene glycol	c	c	c	c
108247	Acetic anhydride	c	c	c	c	119368	Methyl salicylate	a	b	b	b
108327	Propylene carbonate	c	c	c	c	121448	Triethylamine	c	c	c	c
108883	Toluene	a	b	a	c	123422	4-Hydroxy-4-methyl-2-pentanone	c	c	c	c
108941	Cyclohexanone	b	b	c	c	123513	3-Methyl-1-butanol	c	c	c	c
109433	Dibutyl sebacate	c	c	c	c	123864	*n*-Butyl acetate	c	c	c	c
109660	Pentane	c	c	c	c	123911	1,4-Dioxane	a	a	a	c
109693	Chlorobutane	b	b	c	c	124185	Decane	c	c	c	c
109739	*n*-Butylamine	b	b	c	c	127195	N,N-dimethylacetamide	b	b	c	c
109897	Diethylamine	c	c	c	c	131113	Dimethyl phthalate	c	c	c	c
109999	Tetrahydrofuran	a	a	a	b	141435	Ethanolamine	c	c	c	c
110009	Furan	a	a	a	b	141786	Ethyl acetate	b	b	c	c
110543	Hexane	c	c	c	c	142825	Heptane	c	c	c	c
110634	1,4-Butanediol	c	c	c	c	544763	Hexadecane	c	c	c	c
110827	Cyclohexane	c	c	c	c	554121	Methyl propionate	b	b	c	c
110861	Pyridine	a	a	a	b	563804	3-Methyl-2-butanone	b	b	c	c
110918	Morpholine	a	a	a	c	629141	1,2-Diethoxyethane	c	c	c	c
111400	Diethylenetriamine	c	c	c	c	872504	N-methylpyrrolidone	b	b	c	c
111557	Ethylene glycol diacetate	c	c	c	c	1330207	Xylenes	b	b	c	c
111659	*n*-Octane	c	c	c	c	1330785	Tritolyl phosphate	c	c	c	c
111842	*n*-Nonane	c	c	c	c	1634044	tert-Butyl methyl ether	c	c	c	c
111875	1-Octanol	c	c	c	c	5989275	Limonene	c	c	c	c
111900	2(2-Ethoxyethoxy) ethanol	c	c	c	c	7732185	Water	c	c	c	c
112276	Triethylene glycol	c	c	c	c						

[a] 0.5 g PCL in 5 mL solvent.
[b] 2.5 g PCL in 5 mL solvent.

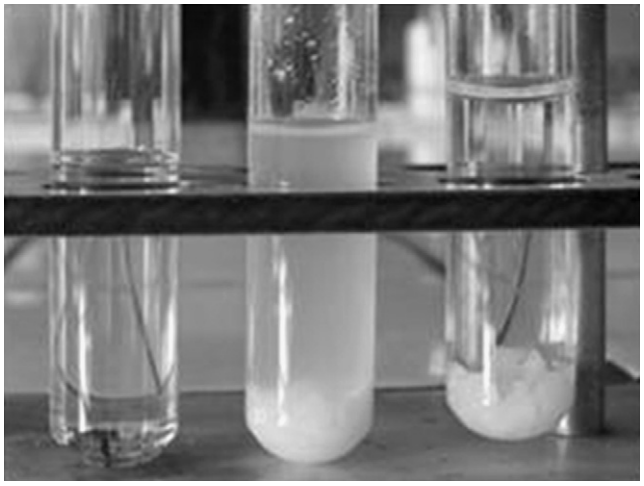

Fig. 2. Pictures of PCL solubility states. From left to right: soluble (a), partially soluble (b) and non-soluble (c).

Test results are given in Table 2. Experiments have been conducted in 99 available solvents of analytical grade and have identified 26 good solvents for PCL14000 (0.5 g in 5 mL), 23 partial solvents and 50 non-solvents. The solubility parameters of PCL are then calculated using Eq. (7) and reported for each case in Table 1. As recommended by Hansen (2007), water was excluded from all calculations.

At the lowest polymer concentration, molecular weight had a slightly influence on the solubility parameters. At 2.5 g/5 mL, an increase in molecular weight decreased the disperse fraction of solubility parameter δ_d and increased δ_p and δ_h. The same evolution was observed for a given molecular weight by increasing the polymer concentration. As expected, increasing the molecular weight or the quantity of PCL to dissolve implied a decrease in polymer solubility and therefore the number of solvents and partial solvents in contrast to non-solvents (see Table 3).

Among the 26 solvents of PCL14000, the solvent the furthest from the PCL in the solubility parameter space is the 2-chloroethanol. It determined the value of the solubility sphere radius by using relation (8): $R_s = 9.8$. For such a diameter, the number of solvents outside the solubility sphere and of non-solvents within is 50 (or 50% of the studied solvents).

Table 3
Results of swelling tests: numbers of PCL solvents, non-solvents and partial solvents with percentage of outliers.

Swelling tests	Solvents	Non-solvents	Partial solvents	% outliers
PCL14000[a]	26	50	23	24.2
PCL65000[a]	24	73	2	30.3
PCL14000[b]	19	73	7	24.2
PCL65000[b]	6	83	10	22.2

[a] 0.5 g PCL in 5 mL solvent.
[b] 2.5 g PCL in 5 mL solvent.

Another more common way to estimate the sphere radius is to minimize the number of outliers i.e. to include as many solvents in the solubility sphere and exclude as many non-solvents as possible. The results are given in Table 3 with the percentage of outliers. Most of solvents dissolving PCL are included in the spherical region: 20–30% are outliers. As expected, the sphere radius decreased significantly as the molecular weight of PCL increased (Table 1). Furthermore, in the case of PCL65000, increasing polymer concentration significantly decreased the sphere radius.

Fig. 3(a) and (b) shows the 98 solvents (water excluded) in the solubility parameter space and the solubility sphere for the PCL14000 (0.5 g in 5 mL) and PCL65000 (0.5 g in 5 mL) respectively. Hansen approach is only suitable for amorphous polymers (Terada and Marchessault, 1999; Hansen, 2007) but gave very interesting results in the case of PCL, a semi-crystalline polymer, since 76% of solvents are well-predicted.

4.1.3. Turbidimetric titration

Five solvents (methylene chloride, 1,4-dioxane, tetrahydrofuran, furan and 1,2-dichloroethane) and two non-solvent combinations (Heptane/butanol and Hexane/butanol) were used to perform turbidimetric titration experiments. Briefly, 0.2 g PCL was dissolved in 2 mL in closed test tubes using magnetic stirrer for one hour. The mass of non-solvent inducing a persistent turbidity in the tube was measured. The apparent solubility parameters δ_{app} were calculated for each solvent according to Eqs. (10) and (11) and graphically represented as a function of the Hansen solubility parameters.

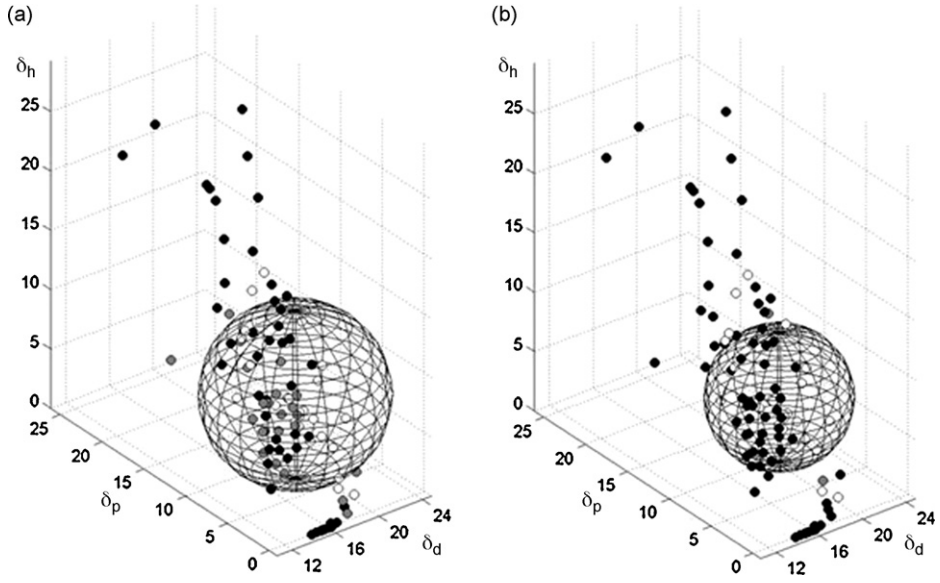

Fig. 3. Three-dimensional plot of Hansen solubility parameters for 98 solvents and solubility sphere for PCL14000 (a) and PCL65000 (b) at a concentration of 0.5 g in 5 mL solvent.

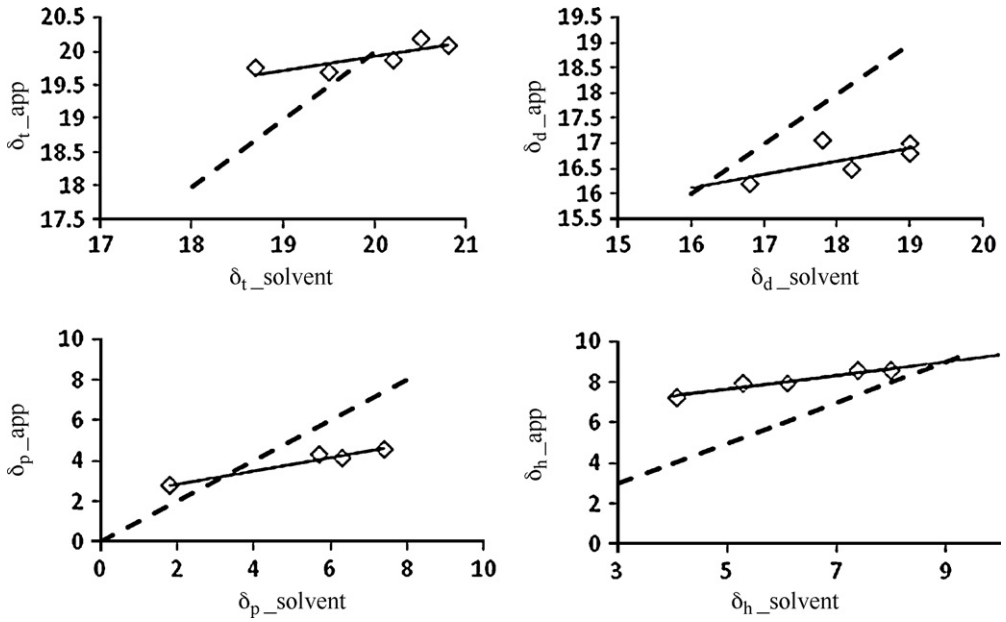

Fig. 4. Graphical determination of PCL solubility parameters by turbidimetric titration with Heptane/butanol combination. Experiments (◊) were carried out in five different solvents and plotted with the corresponding regression line obtained from Eq. (10). The dotted line is the first bisector corresponding to the line $\delta_{app} = \delta_{solv}$.

PCL solubility parameters were found at the intersection between the plotted regression line obtained from Eq. (10) and the line $\delta_{app} = \delta_{solv}$. Fig. 4 shows the results obtained for PCL14000 by using the heptane/butanol non-solvent combination. Fig. 4 (a)–(d) correspond to δ_t, δ_d, δ_p and δ_h respectively.

Moreover, the titration method allows the determination of the solubility volume limits. The radius of the solubility sphere corresponds to the solubility boundary i.e. the distance between PCL and the mixture allowing turbidity in the "Hansen parameter space" (Eq. (6)). The results are reported in Table 1. The values obtained for the solubility parameters of the 2 PCL were very close but a relatively wide range of values (between 4.5 and 7.5) was obtained for the sphere radius.

The range of each partial solubility parameter was similar to those determined by the other methods (see Table 1). However, the values of δ_p were very low and seem unrealistic as the results obtained by Schenderlein et al. (2004) by turbidimetric titration for δ_p partial solubility parameter of poly(D,L-lactide-co-glycolide).

Three methods have been employed to determine the partial solubility parameters of PCL: a theoretical one (group contribution) and two experimental techniques (swelling tests and turbidimetric titration). The results obtained with the method of group contributions and the swelling tests were very similar; however, titration experiments showed relatively different values particularly in the case of the polar solubility parameter δ_p which seemed very low. The determination of the solubility sphere dimensions remained difficult and the results were quite different from one method to another. In the case of swelling tests, the use of an optimization method for determining the diameter limited the number of outliers at 25%. This limited result can be partly explained by the fact that the concept of solubility parameters is only applicable for amorphous polymers while PCL exhibits a semi-crystalline structure.

The retained solubility parameters of PCL measured at 25 °C are $\delta_d = 17.7$, $\delta_p = 6.2$, $\delta_h = 7.8$. These results are in good agreement with the values calculated by Huang et al. (2006) from experimental results obtained at 70, 80, 90, 100 and 110 °C with inverse gas chromatography by Tian and Munk (1994). Indeed, according to Hansen and Beerbower (1971), the partial solubility parameters decrease as temperature increases. Table 4 reports the values of the PCL Hansen parameters as a function of temperature and shows the same evolution.

4.1.4. Identification of the substitution solvent

Table 5 lists the solvents belonging to the Class 3 as defined by the European Pharmacopoeia with their partial solubility parameters and their distance to PCL in the "Hansen space". Nineteen solvents from this list have been used to conduct swelling tests. According to Hansen theory, the solvents whose distance from PCL is greater than about 7.5 (see Table 1) should not solubilize PCL. However, the results of swelling tests showed that many theoretical PCL solvents did not dissolve the polymer at the concentrations studied. Experiments allowed the identification of only 2 solvents with low toxical potential: acetic acid and anisole (methoxybenzene). Anisole was one of the closest solvent to PCL in the solubility parameter space ($d = 2.4$) and acetic acid was one of the most distant ($d = 8.7$).

From a theoretical point of view, according to the distances from PCL in the "Hansen space", methyl acetate and ethyl formate could be also suitable for the PCL solubilization (see Table 5).

An additional criterion, water solubility, has been taken into account for the determination of the substitution solvent since the microencapsulation process was based on a $W_1/O/W_2$ emulsion. We have considered solvents whose water solubility was limited and close to the MC one, about 10 g/L at 20 °C. Anisole was the only solvent very slightly soluble in water (about 1.5 g/L at 20 °C) following by ethyl formate with a water solubility of about 100 g/L. Therefore anisole was chosen to perform a feasibility test conducted to obtain PCL microspheres, although it becomes a par-

Table 4
Evolution of PCL solubility parameters as a function of temperature.

	25 °C	70 °C[a]	80 °C[a]	90 °C[a]	100 °C[a]	110 °C[a]
δ	19.7	17.39	16.78	16.43	16.10	15.79
δ_d	17.7	15.53	14.9	14.52	14.15	13.83
δ_p	6.2	2.42	2.57	2.28	2.24	2.21
δ_h	7.8	7.44	7.28	7.35	7.34	7.29

[a] The values correspond to the results obtained by Huang et al. (2006) by using a linear method as the three-dimensional model.

Table 5
Partial solubility parameters of solvents belonging to the Class 3 as defined by the European Pharmacopeia and their distance to PCL in the "Hansen space". The corresponding results of swelling tests for PCL14000 and PCL65000 are reported (NT = non tested).

CAS number	Solvent	δ_d	δ_P	δ_h	PCL14000[a]	PCL14000[b]	PCL65000[a]	PCL65000[b]	Distance to PCL
100663	Anisole	17.8	4.1	6.7	a	a	a	b	2.4
141786	Ethyl acetate	15.8	5.3	7.2	b	b	c	c	4.0
79209	Methyl acetate	15.5	7.2	7.6	NT	NT	NT	NT	4.5
123864	*n*-Butyl acetate	15.8	3.7	6.3	c	c	c	c	4.8
109944	Ethyl formate	15.5	8.4	8.4	NT	NT	NT	NT	5.0
78933	Methyl ethyl ketone	16	9	5.1	b	b	c	c	5.2
67641	Acetone	15.7	5.3	11.7	b	b	c	c	5.7
108101	Methyl isobutyl ketone	15.3	6.1	4.1	c	c	c	c	6.1
1634044	Methyl tert-butyl ether	14.8	4.3	5	c	c	c	c	6.7
123513	3-Methylbutane-1-ol	15.8	5.2	13.3	c	c	c	c	6.8
71410	Pentanol	15.9	4.5	13.9	c	c	c	c	7.3
60297	Diethyl ether	14.5	2.9	5.1	c	c	c	c	7.7
78922	Butanol-2	15.8	5.7	14.5	c	c	c	c	7.7
108214	Isopropyl acetate	14.9	1.4	4.1	NT	NT	NT	NT	8.3
98828	Isopropylbenzene	16.2	7.0	0	NT	NT	NT	NT	8.4
71363	Butanol-1	16	5.7	15.8	c	c	c	c	8.7
64197	Acetic acid	14.5	8.0	13.5	a	b	a	b	8.7
67630	Isopropyl alcohol	15.8	6.1	16.4	c	c	c	c	9.4
78831	Isobutanol	15.1	5.7	15.9	NT	NT	NT	NT	9.6
71238	Propanol-1	16	6.8	17.4	c	c	c	c	10.2
67685	Dimethyl sulfoxide	18.4	16.4	10.2	c	c	c	c	10.6
142825	Heptane	15.3	0	0	c	c	c	c	11.1
109660	Pentane	14.5	0	0	c	c	c	c	11.8
64175	Ethanol	15.8	8.8	19.4	c	c	c	c	12.5
64186	Formic acid	14.3	11.9	16.6	NT	NT	NT	NT	12.5

[a] 0.5 g PCL in 5 mL.
[b] 2.5 g PCL in 5 mL.

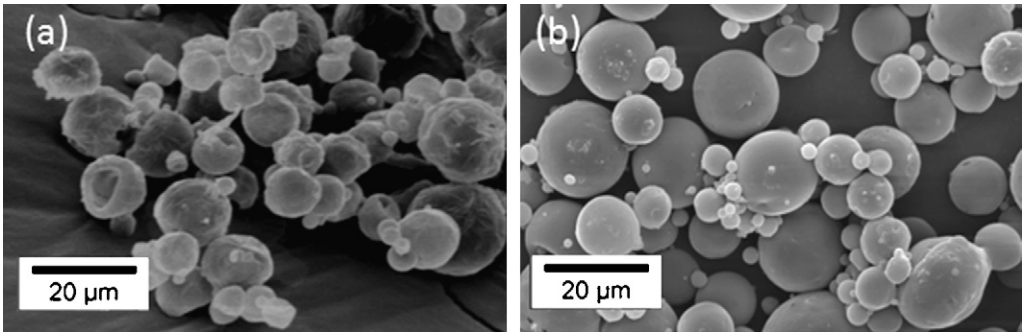

Fig. 5. SEM images of PCL microparticles obtained with anisole (a) or MC (b) as polymer solvent.

tial solvent for the PCL65000 at 2.5 g/5 mL as shown by swelling tests.

4.2. Microsphere feasibility test

PCL microsphere production was conducted by using the method of multiple emulsification with solvent extraction/evaporation described in Section 3.3. Methylene chloride was replaced by anisole as PCL14000 solvent and isopropanol by 1-pentanol (2%, v/v) in the extracting aqueous phase to remove anisole. After 24 h of extraction/evaporation, PCL particles were washed, filtered and dried. Fig. 5 (a) shows SEM images of the PCL microparticles obtained which were relatively spherical with rough and heterogeneous surface and an average diameter about 20 μm. In the case of MC, the microparticles were spherical, with smooth and homogeneous surface and an average diameter of 15 μm (Fig. 5 b). Fig. 6 shows the particle size distribution of both the formulations reflecting quite similar granulometry.

Unlike MC (relative density = 1.4 and boiling temperature = 40 °C), the boiling temperature of anisole (154 °C) is greater than that of water and its relative density is approximately 1. These properties induced difficulties for the solvent extraction/evaporation step. Therefore, the extraction time was set for the feasibility test at 24 h instead of 3 h as in the case of the MC process. Moreover the nature of the alcohol used to extract anisole was changed: 1-pentanol was chosen to replace isopropanol since pentanol-1 is the heaviest of the alcohol substances belonging to

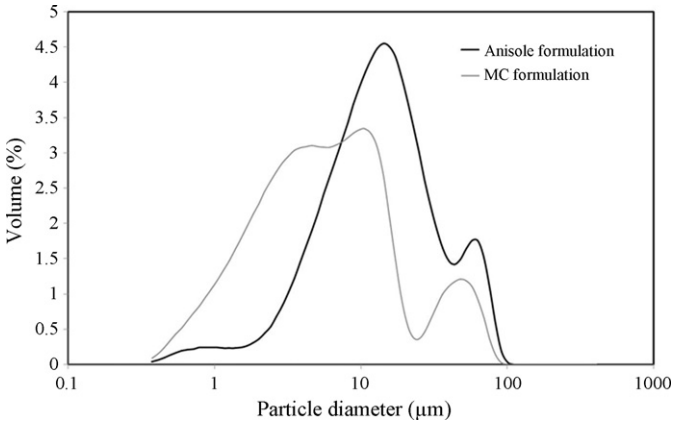

Fig. 6. Particle size distribution of microspheres obtained with anisole or MC as PCL solvent.

Class 3 as defined by the European Pharmacopoeia and does not dissolve PCL (see Table 2). The aim of the study being only to assess the feasibility of MC substitution by anisole for the production of PCL microspheres, this extraction step was not optimized. In future work, we will focus on this particular step to improve the surface state of the microparticles and to evaluate the effectiveness of protein encapsulation.

5. Conclusion

The aim of the study was to replace MC used as a solvent of PCL in a microencapsulation process for therapeutic protein by a nontoxic solvent belonging to Class 3 as defined by the European Pharmacopoeia. Therefore, the solubility parameters of PCL were determined by several methods: group contribution, swelling tests and turbidimetric titrations. The results are relatively close with values slightly lower in the case of the titration method in particular for the polar fraction. The accuracy obtained for the solubility sphere dimensions shows that the results are only suitable for qualitative assessments. Nevertheless, the methodology of Hansen parameters highlighted a nontoxic solvent, anisole, whose solubility parameters are close to PCL and distant from water. A feasibility test was conducted with anisole which allowed the obtaining of PCL microparticles in spite of physico-chemical properties very different from those of MC (density, boiling point, etc.).

References

Adamska, K., Bellinghausen, R., Voelkel, A., 2008. New procedure for the determination of Hansen solubility parameters by means of inverse gas chromatography. J. Chromatogr. A 1195, 146–149.

Al Haushey, L., Bolzinger, M.-A., Bordes, C., Gauvrit, J.-Y., Briançon, S., 2007. Improvement of a bovine serum albumin microencapsulation process by screening design. Int. J. Pharm. 344, 16–25.

Barton, A.F.M., 1991. Handbook of Solubility Parameters and other Cohesion Parameters, 2nd ed. CRC Press, Boca Raton.

Chastrette, M., Rajzmann, M., Chanon, M., Purcell, K.F., 1985. Approach to a general classification of solvents using a multivariate statistical treatment of quantitative solvent parameters. J. Am. Chem. Soc. 107, 1–11.

Chen, C.C., Crafts, P.A., 2006. Correlation and prediction of drug molecule solubility with the NRTL-SAC Model. Ind. Eng. Chem. Res. 45, 4816–4824.

Code, J.E., Holder, A.J., Eick, J.D., 2008. Direct and indirect quantum mechanical QSPR Hildebrand solubility parameter models. QSAR Comb. Sci. 27, 841–849.

European Pharmacopoeia, 2009, PhEur 6th ed. (6.5), General Texts, Residual solvents. Council of Europe, Strasbourg, pp. 603–610.

Flory, P.J., 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca, NY.

Gracin, S., Brinck, T., Rasmuson, A.C., 2002. Prediction of solubility of solid organic compounds in solvents by UNIFAC. Ind. Eng. Chem. Res. 41, 5114–5124.

Gramatica, P., Navas, N., Todeschini, R., 1999. Classification of organic solvents and modelling of their physico-chemical properties by chemometric methods using different sets of molecular descriptors. Trends Anal. Chem. 18, 461–471.

Hansen, C., Beerbower, A., 1971. Solubility parameters. In: Standen, A. (Ed.), Kirk–Othmer Encyclopedia of Chemical Technology, Suppl. vol. 2, 2nd ed. Interscience, New York, pp. 889–910.

Hansen, C.M., 2007. Hansen Solubility Parameters: A User's Handbook, 2nd ed. CRC Press, Boca Raton.

Hildebrand, J., Scott, R.L., 1950. Solubility of Nonelectrolytes, 3rd ed. Reinhold, New York.

Hoy, K.L., 1970. New values of solubility parameters from vapor pressure data. J. Paint Technol. 42, 76.

Huang, J.-C., Lin, K.-T., Deanin, R.D., 2006. Three-dimensional solubility parameters of poly(ε-caprolactone). J. Appl. Polym. Sci. 100, 2002–2009.

Katritzky, A.R., Fara, D.C., Kuanar, M., Hur, E., Karelson, M., 2005. The classification of solvents by combining classical QSPR methodology with principal component analysis. J. Phys. Chem. A 109, 10323–10341.

Manifar, T., Rohani, S., 2005. Measurement and prediction of solubility of four arylamine molecules in benzene, hexane and methanol. J. Chem. Eng. Data 50, 1794–1800.

Modarresi, H., Conte, E., Abildskov, J., Gani, R., Crafts, P., 2008. Model-based calculation of solid solubility for solvent selection—a review. Ind. Eng. Chem. Res. 47, 5234–5242.

Schenderlein, S., Lück, M., Müller, B.W., 2004. Partial solubility parameters of poly(D,L-lactide-co-glycolide). Int. J. Pharm. 286, 19–26.

Sinha, V.R., Bansal, K., Kaushik, R., Kumria, R., Trehan, A., 2004. Poly-ε-caprolactone microspheres and nanospheres: an overview. Int. J. Pharm. 278, 1–23.

Sreekanth, T.V.M., Reddy, K.S., 2008. Evaluation of solubilility parameters for nonvolatile branched hydrocarbons by inverse gas chromatography. J. Appl. Polym. Sci. 108, 1761–1769.

Stefanis, E., Panayiotou, C., 2008. Prediction of Hansen solubility parameters with a new group-contribution method. Int. J. Thermophys. 29, 568–585.

Suh, K.W., Clarke, D.H., 1967. Cohesive energy densities of polymers from turbidimetric titrations. J. Polym. Sci. Part A-1: Polym. Chem. 5, 1671–1681.

Tantishaiyakul, V., Worakul, N., Wongpoowarak, W., 2006. Prediction of solubility parameters using partial least square regression. Int. J. Pharm. 325, 8–14.

Terada, M., Marchessault, R., 1999. Determination of solubility parameters for poly(3-hydroxyalkanoates). Int. J. Biol. Macromol. 25, 207–215.

Tian, M., Munk, P., 1994. Characterization of polymer–solvent interactions and their temperature dependence using inverse gas chromatography. J. Chem. Eng. Data 39, 742–755.

Van Krevelen, D.W., Hoftyzer, P.J., 1976. Properties of Polymers, 2nd ed. Elsevier, New York.

Wang, Y.-Z., 2003. Solubility parameters of poly(sulfonyldiphenylenephenylphosphonate) and its miscibility with poly(ethyleneterephtalate). J. Polym. Sci. Part B: Polym. Phys. 41, 2296–2301.

Xu, D., Redman-Furey, N., 2007. Statistical cluster analysis of pharmaceutical solvents. Int. J. Pharm. 339, 175–188.

Yu, X., Wang, X., Wang, H., Li, X., Gao, J., 2006. Prediction of solubility parameters for polymers by a QSPR model. QSAR Comb. Sci. 25, 156–161.