

Microencapsulation of dehydroepiandrosterone (DHEA) with poly(ortho ester) polymers by interfacial polycondensation

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An original encapsulation process of DHEA was developed, based on the formation of poly(ortho ester) membrane from interfacial polycondensation in an oil-in-oil emulsion. First, the formation of poly(ortho ester) (POE) in solution under anhydrous conditions between a polyol, a lactide diol and a diketene acetal (3,9-diethylidene-2,4,8,10-tetraoxaspiro-[5.5]-undecane) was studied in order to determine the structural and thermal characteristics of the POE polymer. The optimization of the formation of a fine and stable emulsion with the required size distribution was performed in relation with the type of the internal and external phases, the type and the concentration of the surfactant and the stirring rate and duration. The diffusion of monomers and DHEA was evaluated by GC-MS analysis in order to determine the mechanisms of the membrane formation. Finally the synthesis of poly(ortho ester) DHEA-loaded microcapsules was performed under anhydrous conditions required by the particular synthesis of POE. Stable poly(ortho ester) microcapsules containing DHEA were obtained with particle sizes $\sim 1 \mu\text{m}$.

Keywords: Interfacial polycondensation, poly(ortho ester), dehydroepiandrosterone (DHEA), Microencapsulation, emulsion.

Introduction

Microcapsules consisting of a liquid or solid core and a permeable or non-permeable membrane may be produced by carrying out an interfacial polymerization reaction at the surface of droplets containing the material to be encapsulated (Arshady 1989). In this way, polymers such as poly(amides) (Chang *et al.* 1966, Muramatsu *et al.* 1994), poly(ureas) (Pensé *et al.* 1994, Yadav *et al.* 1997) and poly(esters) (Suzuki *et al.* 1968) were synthesized. Such microcapsules are usually obtained from a water-in-oil or oil-in-water emulsion, each phase containing a different monomer, hydrophobic and hydrophilic, which are particularly reactive together, inducing the formation of the polymeric membrane at the emulsion interface.

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Poly(ortho esters) are a versatile family of hydrophobic biodegradable polymers. Since the 1970s, three families of poly(ortho esters) have been synthesized to provide bioerodible carriers for drug delivery (Shi *et al.* 1991, Merkli *et al.* 1993, 1996). A more recent family (Ng *et al.* 1997), corresponding to auto-catalysed poly(ortho esters), can be considered as an evolution of the second family (Shi *et al.* 1991). The preparation of the auto-catalysed poly(ortho esters) has been developed, in homogenous solution of tetrahydrofuran or p-dioxane as reaction solvent (Heller *et al.* 1981), by a condensation reaction between di- or tri-functional monomers, such as ketene acetals and hydroxylic compounds. Unlike most conventional condensation reactions, the reaction between ketene acetal and alcohol proceeds without the formation of small molecule by-products, which must be removed in order to achieve high molecular weight. Furthermore, this new process of auto-catalysed poly(ortho ester) synthesis produces the polymer in short reaction times, at essentially room temperature and under atmospheric pressure. These bioerodible polymers are useful in the fabrication of devices and coating for delivering beneficial agents (Lin and Vasavada 2000).

The aim of this work was to study the encapsulation of DHEA by poly(ortho esters) obtained by interfacial polycondensation. Microcapsules, composed of an oily core containing DHEA, were obtained from an original formulation system based on a stable and fine oil-in-oil emulsion. First, the formation of poly(ortho ester) in solution under anhydrous conditions between a polyol, a lactide diol and a diketene acetal (3,9-diethylidene-2,4,8,10-tetraoxaspiro-[5.5]-undecane) was studied in order to determine the structural and thermal characteristics of the POE polymer. The formation of a fine and stable emulsion with the required size distribution was optimized in relation with the type of the internal and external phases, the type and the concentration of the surfactant and the stirring rate and duration. The diffusion of monomers and DHEA was evaluated by GC-MS analysis in order to determine the mechanisms of the membrane formation. Finally, the synthesis of poly(ortho ester) DHEA-loaded microcapsules was performed under anhydrous conditions required by the particular synthesis of POE. Size distribution and DHEA content of the microcapsules were then determined.

Experimental

Materials

Polydimethylsiloxane (PDMS) with a viscosity of $300 \text{ mm}^2\text{s}^{-1}$ at 20°C was supplied by Rhodia (France).

Dehydroepiandrosterone (DHEA), DL-lactide, 1,6-hexanediol, 3,9-divinyl-2,4,8,10-tetraoxaspiro-[5.5]-undecane (DVTOSU) and tetrahydrofuran (THF) (99.9%) were purchased from Sigma Aldrich (France).

The surfactants (Span[®] 83, Span[®] 20, Tween[®] 21, Tween[®] 61, Tween[®] 65) were kindly supplied by SEPPIC (France).

Monomer and polymer preparation

Monomer synthesis. The diketene acetal 3,9-diethylidene-2,4,8,10-tetraoxaspiro-[5.5]-undecane DETOSU (b) was prepared from a base-catalysed rearrangement of the commercially available precursor 3,9-divinyl-2,4,8,10-tetraoxaspiro-[5.5]-undecane (DVTOSU) (a), as described in Ng *et al.* (1985) (figure 1).

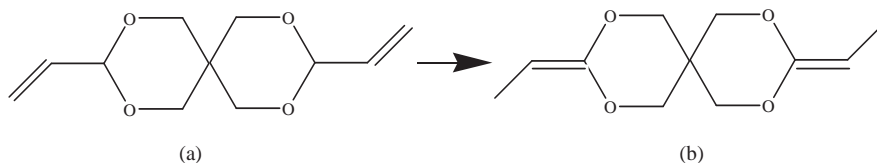


Figure 1. Synthesis of (3,9-diethylidene-2,4,8,20-tetraoxaspiro-[5.5]-undecane) ((b) DETOSU) by a base catalyzed rearrangement of the commercially available precursor 3,9-divinyl-2,4,8,10-tetraoxaspiro-[5.5]-undecane ((a)DVTOSU).

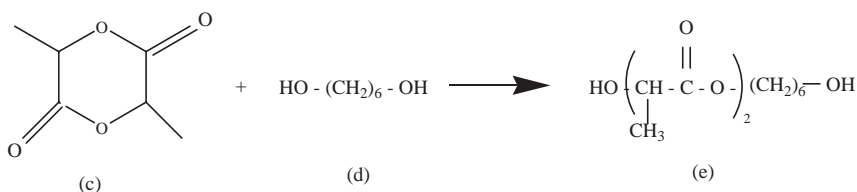


Figure 2. Synthesis of lactide-1,6-hexanediol (e) by addition reaction between DL-lactide (c) and 1,6-hexanediol (d) at 160°C.

Lactide-1,6-hexanediol (e) was prepared by an addition reaction between 25 mmol DL-lactide (c) and 50 mmol 1,6-hexanediol (d) at 160°C in a sealed 100 mL round-bottom flask for 48 h at 160°C (Sintzel *et al.* 1998) (figure 2).

The resulting lactide-diol (e) was then used without further purification. Chemical structures of (b) and (e) were confirmed by FTIR and ^1H nuclear magnetic resonance spectroscopy.

Polymer synthesis. Under anhydrous conditions, 38 mmol of 1,6-hexanediol (d) and 2 mmol of lactide-1,6-diol (e) dissolved in 50 ml of dried tetrahydrofuran (THF) were poured into a 250 mL round bottom flask; 40 mmol of DETOSU (b) were then added. p-toluenesulphonic acid (p-TSA) (1% w/w) was used as a catalyst. After the exothermic reaction (figure 3) subsided during 1 h at room

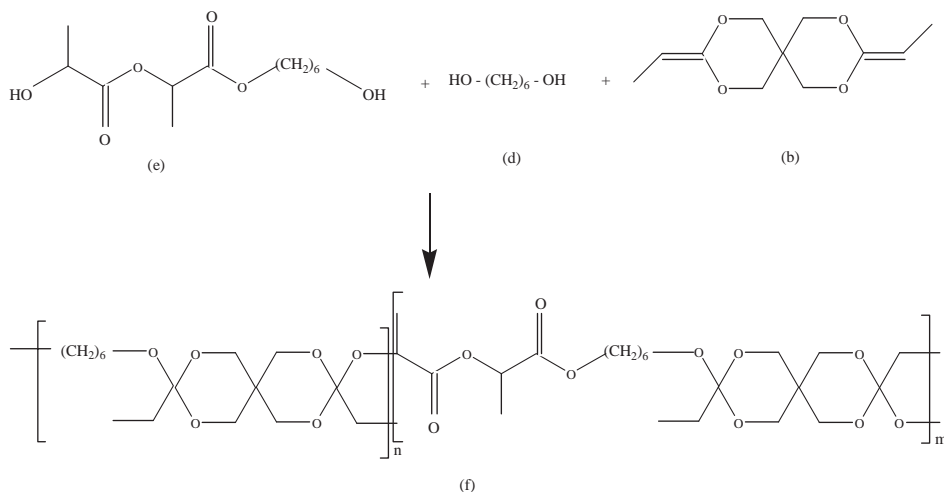


Figure 3. Synthesis of self-catalyzed poly(ortho esters) (f) by condensation reaction between lactide-1,6-diol (e) and 1,6-hexanediol (d) and of (3,9-diethylidene-2,4,8,10-tetraoxaspiro-[5.5]-undecane) (b) DETOSU.

temperature, the polymer (f) was isolated by precipitation into 200 mL methanol containing a small amount of triethylamine (TEA) and filtrated (Heller *et al.* 1981).

Monomer and polymer characterization. The chemical structure of the monomers was determined by $^1\text{H-NMR}$. 1,6-hexanediol $\delta(^1\text{H})$ in CDCl_3 : 3.63 ppm (triplet, 4H); 1.58 ppm (triplet, 4H); 1.39 ppm (multiplet, 4H) DL-lactide $\delta(^1\text{H})$ in CDCl_3 : 5.05 ppm (quadruplet, 2H); 1.65 ppm (doublet, 6H) lactide-1,6-hexanediol $\delta(^1\text{H})$ in CDCl_3 : 5.1 ppm (multiplet, 1H); 4.2 ppm (multiplet, 3H); 3.6 pm (triplet, 2H); 1.5 ppm (multiplet, 14H).

The polymer structure was confirmed by using Fourier Transform Infra-Red spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR). FTIR spectra were obtained with a Perkin-Elmer 1600 series FT-IR spectrometer. $^1\text{H-NMR}$ spectra were recorded in CDCl_3 on a Bruker AM 300 (300 MHz for ^1H) at 298K. UV-visible spectroscopy experiments were conducted on a KONTRON instrument.

GC-MS: Gas Chromatography—mass spectrometry experiments were performed on the HP 6890/5973 chromatograph equipped with a capillary column HPMS (30 m \times 250 μm \times 0.25 μm) with a heating rate of 10°C/min from 120–210°C.

Determination of the diffusion rate

As the polymerization reaction occurs at the interface of the emulsion, some of the monomers dissolved in each phase diffuse at the site of the reaction. Thus, the formation of the membrane by interfacial polycondensation requires the study of the distribution of monomers which depends on their partition coefficients (Wittbecker and Morgan 1959, Koishi *et al.* 1969).

The diffusion rate of the monomers was determined in the same way as in the emulsion preparation.

DETOSU partition coefficient. 10 mL of benzyl benzoate were added to 100 mL of the mixed organic solvent (THF/PDMS 1/1 vol) containing Span[®] 83 (2% w). After 15 min of mechanical stirring at 500 rpm, 50 mL of 0.2 M of DETOSU solution in THF/PDMS (1:1 vol) was added to the emulsion and the mixed solution was emulsified at 500 rpm for 15 min. Immediately after the stirring stop, the emulsion was centrifuged at 2700 g for 30 min and then at 6200 g for 15 min. DETOSU concentration in the benzyl benzoate solution was determined by UV-visible spectroscopy from a calibration curve at $\lambda = 246$ nm.

The partition coefficient of DETOSU was then calculated from its initial and final concentration in the mixed organic solvent (THF/PDMS 1:1 vol).

1,6-hexanediol and lactide-1,6-hexanediol diffusion. 10 mL of 0.1 M of 1,6-hexanediol (or lactide-1,6-hexanediol) benzyl benzoate solution were added to 150 mL of the mixed organic solvent (THF/PDMS 1/1 vol) containing Span 83 (2% w). After 15 min of stirring, the two phases were separated by centrifugation in the same conditions as DETOSU. The benzyl benzoate solution was analysed by GC/MS for the determination of 1,6-hexanediol concentration and by UV-visible spectroscopy for the determination of lactide-1,6-hexanediol concentration. The

lactide-1,6-hexanediol concentration was then determined spectrophotometrically from a calibration curve at $\lambda = 260$ nm.

The diffusion rate of the lactide and 1,6-hexanediol was determined from their initial and final concentrations in benzyl benzoate solution.

Diffusion of THF. The miscibility of THF and benzyl benzoate could favour the diffusion of THF toward the internal phase and then the diffusion of DETOSU, suggesting that the polymerization occurs in the internal phase. The THF diffusion toward the benzyl benzoate droplets was evaluated by measuring the THF concentration after 10, 15, 20 and 25 min of mechanical stirring of the emulsion, at 500 rpm, prepared as before but without monomers. After the emulsification step, the benzyl benzoate solution collected was analysed by GC/MS and the THF concentration was deduced from a calibration curve.

Diffusion of DHEA. One of the most important factors influencing the encapsulation yield of an active agent is its affinity toward the external phase (Zydowicz and Nzimba-Ganyanad 2002). To reach high encapsulation yields, a low solubility of the encapsulated agent in the external phase is required. In order to estimate the efficiency of the encapsulation system, the final DHEA concentration in benzyl benzoate solution after various times of emulsification step was determined by GC-MS from a calibration curve. The emulsification step was followed with the same manner as before.

Determination of the encapsulation yield

The encapsulation yield (Y) was calculated from the total DHEA concentration (T_{DHEA}) and loaded DHEA concentration (L_{DHEA}) as follows:

$$Y = \frac{L_{\text{DHEA}}}{T_{\text{DHEA}}} \times 100$$

Total DHEA concentration (T_{DHEA}). The reactional mixture was poured into acetone and sonified for 30 min, in order to dissolve the polymeric microcapsules. Free DHEA was then separated by ultra-centrifugation from the polymer that has precipitated in methanol. Finally, the total DHEA concentration was determined by GC-MS.

Loaded DHEA concentration (L_{DHEA}). Five grams of the microcapsules suspension were ultra-centrifuged at 10 000 rpm. The supernatant containing the DHEA-loaded microcapsules was isolated. Ten milligrams of these microcapsules were dissolved in 20 mL of acetone and sonified for 30 min. Loaded DHEA was then separated by ultra-centrifugation from the polymer that has precipitated in methanol. Finally, DHEA content of the microcapsules was determined by GC-MS.

Optimization of the emulsification process

All the experiments concerning the optimization of the emulsion formation process were performed at 37°C.

Choice of surfactants. In order to determine the HLB required by the emulsion, the internal phase containing 1% (w/w) of different kinds of surfactant was introduced in the organic external phase under mechanical stirring at 500 rpm.

After 5, 10, 15 and 20 min of stirring, the mean size of the emulsion was observed by optical microscopy. The emulsion was also analysed by static laser light scattering after 20 min of stirring.

Surfactant concentration. Once the HLB value was determined, the size and the stability of the emulsion prepared with various surfactant proportions (0.5, 1, 2 and 2.5% w) were studied. After 5, 10, 15 and 20 min of mechanical stirring at 500 rpm, the mean size of the emulsion was observed under optical microscopy. The emulsion was analysed by static laser light scattering after 20 min of stirring.

Stirring rate. The influence of the stirring speed, varying in a range of 500–700 rpm, was studied, in the presence of surfactant (2% w), by optical microscopy.

Stirring time. The influence of the stirring time was determined by optical microscopy, in the presence of surfactant (2% w) at 500 rpm. The emulsion observations were done after 5, 10, 15 and 20 min of stirring.

Particle formation

To 100 mL of THF/PDMS (1:1 vol) mixture were added 10 mL of benzyl benzoate solution containing 0.14 mmol of lactide-1,6-hexanediol, 1.27 mmol of 1,6-hexanediol and 100 mg of DHEA under anhydrous conditions. The temperature was maintained at 37°C and the mechanical stirring at 500 rpm.

After 15 min of stirring, 50 mL of THF/PDMS (1:1 vol) solution containing 8.46 mmol of DETOSU and some drops of p-toluene sulphonic acid (p-TSA) (1% w/w in THF) were added. The colour of the emulsion immediately turned to brown and the temperature of the preparation increased abruptly up to 40°C. The polymerization reaction was kept for 1 h.

After the polymerization step, the mechanical stirring was stopped and 300 mL of methanol were poured into the preparation. A white cloud of particles appeared and precipitated at the bottom of the vessel.

Emulsion and particle characterization

The size distribution of the emulsions and the microcapsules was determined by static laser light scattering on a LS 230 COULTER granulometer.

The stability of the emulsion and the microcapsules were observed by using a Leica DMLM optical microscope in the transmission mode and photographs were obtained by instantaneous digitalization by means of a Sony hyper HAD video.

Thermal properties of the polymer were also studied by thermal gravimetric analysis (TGA). Sample weighing between 5–10 mg were heated at 10°C min⁻¹ from 25–500°C in a stream of helium in the microbalance 2950 Du pont Instruments thermal gravimetric analyser. Glass transition temperature (T_g) of the polymer was determined by Differential Scanning Calorimetry (DSC). Samples weighing between 5–10 mg were heated at 10°C min⁻¹ from -100 to +250°C in a stream of helium in the oven of a DSC 2920 Modulated DSC TA Instruments.

Results and discussion

Polymer characterization

The polymer was firstly synthesized in solution (THF), in order to verify the reactivity of the diol monomers toward DETOSU. The characterization of the chemical structure and some physical-chemical properties of the poly(ortho ester) polymer are informative for the performance of the encapsulation experiments.

Chemical structure. The chemical structure of the monomers was verified by FTIR and $^1\text{H NMR}$, especially the chemical structure of DETOSU which was synthesized according to Ng *et al.* (1985). The analysis of the $^1\text{H NMR}$ spectrum of DETOSU revealed the presence of residual vinyl protons ($\delta = 6\text{--}4.5$ ppm) from DVOSU (4% mol). The monomers were used for polymer synthesis without any distillation.

The linear self-catalysed poly(ortho ester) was then synthesized in THF, according to the method proposed by Heller *et al.* (1981).

The chemical structure of the polymer was then studied after precipitation in methanol (Sintzel *et al.* 1998) by FTIR and $^1\text{H NMR}$ spectroscopy.

The absence of a vibration band at 3500 cm^{-1} indicated that the hydroxyl groups of 1,6-hexanediol and lactide 1,6-hexanediol have reacted. Furthermore, the disappearance of the vibration band at 3059 cm^{-1} corresponding to the vinyl protons of DETOSU indicated that the polymerization reaction had occurred. The presence of the vibration band at 1740 cm^{-1} , corresponding to the stretching vibration of ester groups, also confirmed the chemical structure of the polymer chemical structure. Moreover, no unknown vibration band was observed, suggesting that no side reaction had occurred (table 1).

The $^1\text{H NMR}$ spectrum of the polymer clearly showed several differences compared to the $^1\text{H NMR}$ spectra of DETOSU and the diol monomers, suggesting that the reaction had occurred and confirming the FTIR results. More precise assignments and quantitative information could not be investigated, because of the overlay of the resonances corresponding to the monomer units.

Thermal properties. DSC thermogram confirmed the semi-crystalline character of the polymer (Sintzel *et al.* 1998), with an endothermic peak at 212°C corresponding to the fusion transition and a glass temperature (T_g) at 7°C . The relatively low value of T_g is coherent with the soft-solid behaviour of the POE

Table 1. Infra red vibrations of functional groups of auto-catalyzed poly(ortho esters).

Vibration band (cm^{-1})	Vibrations	Functional group
2980–2855	ν (C–H) sy, as	CH, CH_2 , CH_3
1747	ν (C=O)	R–COOR (aliphatic ester)
1466–1455	δ (C–H) as	CH_2 , CH_3
1390–1360	δ (C–H) sy	C– CH_3
1245–1196	ν (C–O) as	C–O–C (cycle)
1175–1141	δ (C–H)	CH_3
1042	ν (C–O) sy	C–O–C (cycle)
943	δ (C–O)	C–O–C (cycle)
722	δ (C–C)	C– $(\text{CH}_2)_n$ –C, pour $n \geq 4$

at room temperature, contrarily to the semi-solid like previous family of POE (Merkli *et al.* 1994). The T_g value suggests that the molecular weight of the POE is relatively low and contributes to the mechanical properties of the polymer studies (Heller *et al.* 1995). The fusion enthalpy was 152 J/g.

Thermal gravimetric analysis is a much-used technique for characterizing polymer thermal stability and is often informative about polymer characteristics (Mathiowitz and Cohen 1989, Zydowicz *et al.* 2001, Zydowicz and Nzimba-Ganyanad 2002). The TGA thermogram showed a thermal degradation temperature of the polymer around 250°C, which is a relatively low value for a polymer. This information is interesting, as no data about the POE degradation temperature is reported in the literature, but no polymeric characteristic can be deduced.

Optimization of the formulation

The aim of the formulation study was to determine the optimal formulation allowing the formation of a stable emulsion, characterized by droplets with mean diameter $\sim 1 \mu\text{m}$, and a narrow size distribution, which is required for a successful encapsulation process.

The emulsion formulation study represents a major step in the microcapsules synthesis by interfacial polycondensation (Zydowicz *et al.* 2001). The difficulty of this encapsulation process relies on the heterogeneous polymerization in dispersed medium. Thus, a theoretical study of the monomers and the DHEA solubility parameters and diffusion rates in the solvents on one hand, and the optimization of the experimental conditions of the emulsion formation, such as the type and the proportion of the external and internal phases, the type and the concentration of the surfactant, the stirring time and rate, on another hand, are required before performing the encapsulation experiments.

Choice of solvents. As the preparation of polycondensate microcapsules by interfacial polycondensation requires the precipitation of the initially-formed oligomer molecules during the polycondensation process and then the growth of the primary membrane by diffusion of one of the monomers through the membrane, the role of the type of the organic phase is very important. The process of polycondensate precipitation and the formation of the primary membrane around the droplets is largely controlled by the solvency of the medium (Morgan and Kwolek 1959). It is well known that the solvent should favour the precipitation of the polymer, but also should allow the diffusion of the monomer through the polymeric membrane providing the growth of the membrane (Janssen and Nijenhuis 1992). The polymerization rate is controlled by the mass transfer of the monomer towards the polymerization locus. In fact, the diffusion of the monomer will be improved by the swelling of the membrane by the organic phase (Wittbecker and Morgan 1959, Zydowicz *et al.* 2001). The strength of the polymer-solvent interactions will influence not only the morphology and the thickness of the polymeric wall (Danicher *et al.* 1999), but also the inherent viscosity of the polymer which depends on its average molecular weight (Zydowicz *et al.* 2001).

As microcapsules contain DHEA, the internal phase must be a solvent of DHEA and, furthermore, it must be suitable for pharmaceutical application that means not toxic. Moreover, as it has been specified before, the solvents should dissolve the monomers, but not the polymer. In the literature, poly(ortho esters)

were prepared in organic solvents such as tetrahydrofuran (Sintzel *et al.* 1998) or p-dioxane (Shi 1995), that are also solvents of DETOSU and polyols. It must also be taken into account that poly(ortho ester) synthesis requires anhydrous experimental conditions, inducing that the use of water, which is a perfectly non-toxic solvent, is impossible. Benzyl benzoate and cyclohexanol are known to be acceptable (European Pharmacopoeia 1997). In order to optimize the choice of the solvents, the solubility parameters of DETOSU, lactide-1,6-hexanediol and 1,6-hexanediol were calculated (table 2), according to Hoftyzer and Van Krevelen (1990). The solubility parameters of various solvents such as THF and benzylbenzoate were also calculated (table 2).

It appeared that the monomers, DHEA and benzyl benzoate present relatively close solubility parameters, suggesting that this solvent could be the internal phase containing DHEA and one or two monomers. These results were then confirmed by solubility tests of the monomers and DHEA in THF and benzyl benzoate, at 37°C (table 3).

These tests confirmed that 1,6-hexanediol and lactide-1,6-hexanediol would be soluble in the internal phase and DETOSU in the external phase, i.e. THF. Nevertheless, THF is miscible with benzyl benzoate, hindering the formation of the emulsion. It was then necessary to find a non-toxic co-solvent of THF, miscible with it, but not miscible with benzyl benzoate and not reactive toward DETOSU. Poly(dimethylsiloxane), PDMS, with a solubility parameter of $15 \text{ J}^{1/2} \text{ cm}^{-3/2}$ (Brandrup 1989) and a viscosity of $300 \text{ mm}^2 \text{ s}$ appeared an appropriate candidate. Furthermore, the relatively high viscosity of PDMS should improve the

Table 2. Calculated solubility parameters (δ) at 25°C of DHEA, DETOSU, lactide-1,6-hexanediol, 1,6-hexanediol, tetrahydrofuran (THF), benzylbenzoate and poly(dimethylsiloxane) (PDMS), according to Hoftyzer and Van Krevelen (1990).

Substance	δ_d	δ_p	δ_h	$\delta \text{ (J}^{-1/2} \text{ cm}^{-3/2}\text{)}$	$\delta \text{ (J}^{-1/2} \text{ cm}^{-3/2}\text{)}^*$
THF	16.3	4.75	6	18	18.6
Benzyl benzoate	20.5	2.85	6.4	21.7	
PDMS					15.04
DETOSU	22.1	5.6	9.1	24.5	
DHEA	21.7	5	11	24.8	
1,6-hexanediol	17.5	6.1	18.5	26.2	
Lactide-1,6-hexanediol	17.6	4.5	15.7	24	

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2; \delta_d^2: \text{Dispersion index}; \delta_p: \text{Polarity index}; \delta_h: \text{Hydrogen index.}$$

*Brandrup and Immergut (1989).

Table 3. DETOSU, 1,6-hexanediol, lactide-1,6-hexanediol and DHEA solubility tests (mg/mL) in various solvents at 37°C.

	THF	Benzyl benzoate	THF/PDMS 1 : 1 vol
DETOSU	> 80		> 50
1,6 Hexanediol		20	
Lactide 1,6-hexanediol		> 50	
DHEA		> 50	

emulsion stability (Rosen 1978, Meyers 1988). The adequate amount of PDMS must be such to allow the emulsion formation without causing the precipitation of DETOSU. Therefore, attempts of DETOSU solubility were performed in the mixed organic phase at 37°C, by varying the proportion of THF and PDMS in the continuous phase (3 : 1, 2 : 1, 1 : 1 and 1 : 2 vol). Finally, the THF/PDMS 1 : 1 (vol) mixture allowed the formation of a fine and stable emulsion with benzyl benzoate. This formulation was retained for the microcapsules synthesis.

Then, for the microcapsules synthesis, DHEA and monomers were used in their respective solvents at their solubility.

Choice of the surfactants. The choice of the appropriate surfactant followed the optimization of the organic external phase composition. The choice of the surfactant for a given application must take into consideration the type of the emulsion desired and the nature of the oil phase. In the present emulsion, the surfactant was selected according to its solubility in the organic external phase (Bancroft 1913). According to Griffin's (1954) method, the hydrophilic lipophilic balance (HLB) of the external phase was determined by the performance of a number of experiments involving various surfactants, with a range of HLB numbers between 4.3–13. The stability of the resulting emulsions after 5, 10, 15 and 20 min of stirring was evaluated by optical microscopy and static laser light scattering. The emulsion prepared with Span 83 (HLB = 4.3) appeared as the most stable, with a single narrow distribution (figure 4).

The stability of the emulsions, stored at room temperature, was then followed by optical microscopy during a few days. Emulsions prepared with Tween 21 (HLB = 10), Tween 65 (HLB = 9.6) and Tween 61 (HLB = 13) as surfactants were completely unstable, even after some hours. Emulsions prepared with Span 20 (HLB = 6) and Span 83 (HLB = 4.3) appeared stable after 24 h, but after 3 days a phase separation was observed.

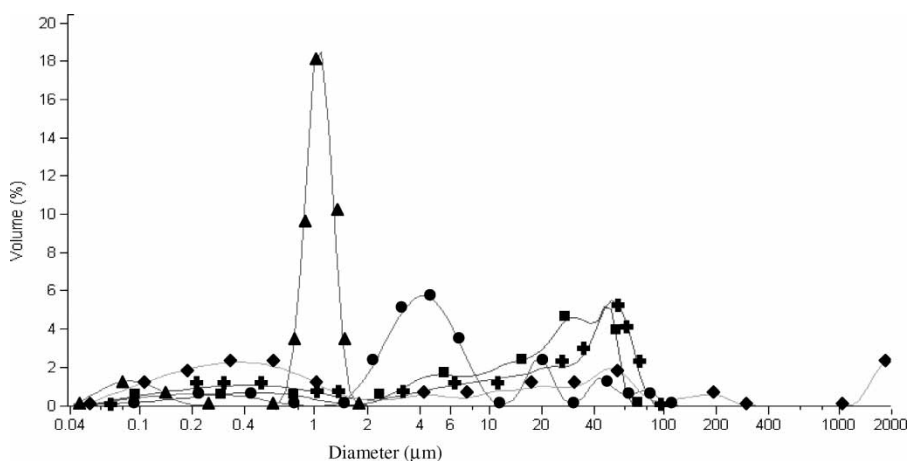


Figure 4. Influence of the HLB value on the size distribution of benzyl benzoate/(THF-PDMS, 1 : 1 v) emulsion HLB = 4.3 ▲; HLB = 6 ●; HLB = 13 +; HLB = 10 ◆; HLB = 9.6 ■.

The stability and size distribution results of the different emulsions suggested that the HLB value of the emulsion was equal to 4.3. Thus, Span 83 was retained as a surfactant for the emulsion stabilization.

The concentration of the surfactant, the stirring rate and time were then optimised, in order to produce the more stable emulsion. It was observed, as described in literature (Bru *et al.* 1998), that an increase of the surfactant concentration induced the decrease of the emulsion droplets diameter and improved the stability of the emulsion. Above a surfactant concentration of 2% (w/w), the mean diameter of emulsion droplets reached a plateau, thus the concentration was taken equal to 2% for the encapsulation experiments.

Although the improvement of the emulsion stability is essentially due to thermodynamic factors, kinetics also plays an important role (Meyers 1988). The lowering of the interfacial tension between phases induced by the adsorption of the surfactant molecules at the interface of the two immiscible phases requires a minimal time corresponding to the reach of equilibrium. It was then observed that a minimal stirring time of 15 min was required to produce a stable emulsion with a single narrow size distribution (figure 5).

A longer stirring time induced the formation of a second particles population with smaller diameter. This behaviour could be due to the Ostwald ripening effect because of the partial solubility of the internal phase (benzyl benzoate) in the external one (THF) (Meyers 1988a). Furthermore, too long a stirring time could improve the diffusion of THF toward the internal phase, providing the disruption of the emulsion. Thus, mechanical stirring was limited to 15 min for encapsulation experiments.

The influence of the stirring rate on the emulsion size is known to be lowered when surfactant is added in the external phase (Koishi *et al.* 1969). A slight decrease was observed when the stirring rate was increased from 500–600 rpm, whereas a stirring speed of 700 rpm induced a marked decrease of the particle size of 50% and the presence of a second population (table 4) at lower diameter.

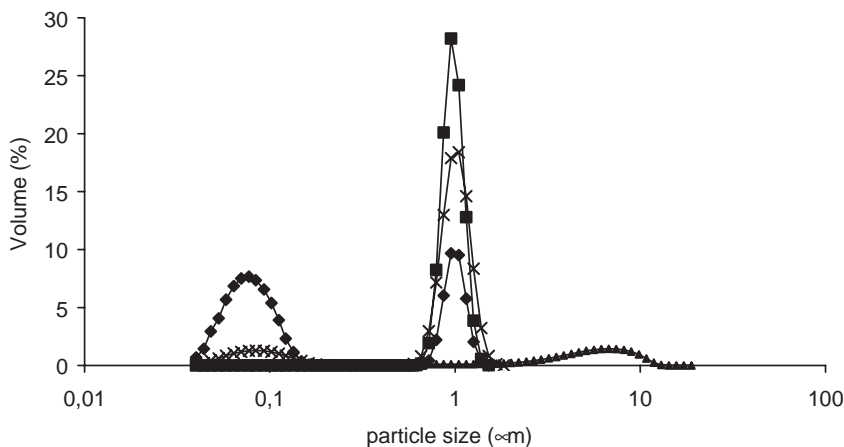


Figure 5. Size distributions of benzyl benzoate/(THF-PDMS, 1:1 vol) emulsions prepared with Span 83 (2% w) obtained after various stirring durations. 5 min ▲; 10 min ×; 15 min ■; 20 min ◆.

Table 4. Influence of the stirring speed on the size of emulsions obtained with 2% (weight) of *Span 83* after 15 min of stirring. Mean droplet diameters are determined by static laser light scattering.

Stirring speed (rpm)	Mean droplet diameter (μm)
500	1.0
600	1.0
700	0.5

These results could probably be explained by the physical nature of the adsorbed film and the interfacial rheology of the system, particularly the elasticity of the interfacial film (Meyers 1988b, Lee *et al.* 1997), inducing the droplets breaking at high shear rates. Furthermore, a too high speed could induce undesirable thermal effects on the emulsion stability. Thus, a stirring rate of 500 rpm was chosen for the encapsulation experiments, in order to limit the thermal effects of a high stirring rate, which could also disturb the stability of the system.

Determination of the diffusion rates. The diffusion of the monomers through the interface is a necessary step to the polymeric membrane formation and, thus, the determination of the diffusion rate of the monomers is required, in order to estimate the whole polymerization rate (Zydowicz *et al.* 2001). Contrarily to other systems (Koishi *et al.* 1969, Pensé *et al.* 1994), the locus of the polymerization is not known in the present oil-in-oil emulsion system, as these organic solvents have never been used in these conditions. In order to understand the reactional mechanism of the POE membrane formation, it is necessary to determine the diffusion rate of each monomer.

It was then found that 27% (w) of DETOSU diffused toward the internal phase. Almost the entire lactide-1,6-hexanediol was retained in the internal phase, whereas 43% (w) of the 1,6-hexanediol diffused toward the external phase. The relatively high diffusion rates of DETOSU and 1,6-hexanediol, compared to the lactide-1,6-hexanediol, could suggest that the POE membrane of the microcapsules should result more probably from the reaction between DETOSU and 1,6-hexanediol than from the reaction between DETOSU and lactide 1,6-hexanediol. The determination of the chemical structure of the POE membrane by ^{13}C NMR should be informative on the reactivity of the monomers and on the polymerization locus. Contrarily to another more classical polycondensation reaction, where only one type of monomer diffuses toward the organic phase, it is difficult to deduce, in this case, the locus of the reaction and the direction of the membrane growth.

The THF partition toward the internal phase, determined by GC-MS after 10, 15, 20 and 25 min of stirring of the system, was found equal to 11.7, 6.1, 11.8 and 11.4% (w), respectively. These results show that the diffusion rate does not increase with the increase of the stirring time, suggesting that the THF diffusion toward the benzyl benzoate phase occurs rapidly at the beginning of stirring and then is relatively stable. The THF diffusion should favour the DETOSU diffusion toward the internal phase.

Finally, the DHEA diffusion toward the external phase was also investigated. The diffusion rate of DHEA toward the external phase should influence the DHEA loading rate inside the microcapsules. A high loading rate requires a low diffusion rate of DHEA. It was found that $\sim 63\%$ (w) of DHEA has diffused. This rate was particularly high, suggesting that the DHEA loading of the microcapsules would not be high.

Microencapsulation experiments

Based on these preliminary studies, the DHEA microencapsulation experiments could be performed.

After 1 h of stirring at 37°C , in a closed reactor and under anhydrous conditions, the microcapsules were recovered by precipitation induced by the addition of 300 mL methanol. Microcapsules were washed several times in methanol, in order to eliminate PDMS, and then analysed by optical microscopy and static laser light scattering. Three populations of microcapsules are observed between $0.2\text{--}3\ \mu\text{m}$ (figure 6).

Optical micrographs confirmed the static laser light scattering results, showing three kinds of microcapsules with the higher diameters $\sim 2\ \mu\text{m}$.

The total (T_{DHEA}) and loaded (L_{DHEA}) DHEA concentrations were found equal to 0.601 and 0.174 g/L, respectively. The yield of encapsulation was then found equal to 29% (w). This value is in accordance with the relatively high DHEA diffusion rate (63%) toward the external phase.

Conclusion and perspectives

This paper describes the synthesis of DHEA-loaded poly(ortho ester) (POE) microcapsules obtained by interfacial polycondensation. This polymerization route used for POE microcapsules formation has never been reported and the

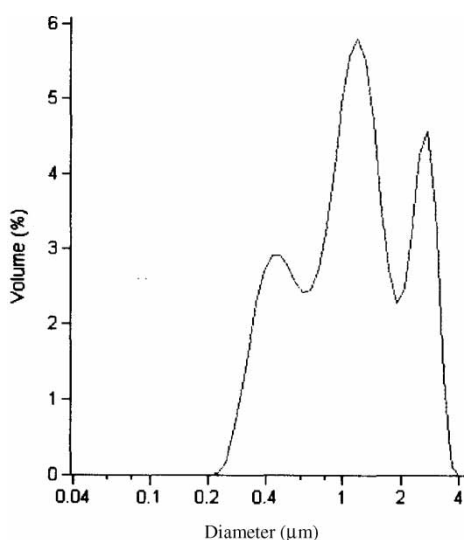


Figure 6. Size distribution of DHEA-loaded poly(ortho ester) microcapsules.

encapsulation studies and experiments described here are particularly original, based on an oil-in-oil emulsion. Furthermore, the formation of poly(ortho ester) microcapsules by interfacial polycondensation compared to other microencapsulation processes such as solvent evaporation should allow the control of the thickness and the porosity of the polymeric membrane and, thus, the drug release kinetics.

The encapsulation of DHEA was performed after the theoretical study of the solubility parameters and diffusion rate of each monomer and the optimization of the emulsion formulation. POE microcapsules with three size distributions centred around 0.4, 1 and 3 μm were obtained. The encapsulation yield was equal to $\sim 30\%$ (w), according to the high diffusion rate of DHEA toward the external phase.

The results should be completed by the study of the chemical structure of the POE membranes of the microcapsules by ^{13}C NMR and MALDI-TOF mass spectrometry in order to verify the reactivity of the monomers and to determine the locus of polymerization.

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