

Simultaneous emulsification and interfacial polycondensation for the preparation of colloidal suspensions of nanocapsules

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Abstract

Sub-micrometric particles having an oil core and a polymer shell (nanocapsules) have been prepared by combining in a single stage the emulsification process and an interfacial polymerization reaction. The spontaneous emulsification produced very fast a dispersion of oil droplets of 100–400 nm mean diameter at the surface of which the subsequent polycondensation reaction took place. The process has been optimized with respect to the choice of α -tocopherol as the oil and made robust regarding the presence of monomers in the aqueous and oil phases and their conversion into polymers. The major cause of troubles was the large concentration of diol or diamine monomers in the aqueous phase that made the oil droplets unstable with respect to aggregation immediately after their formation. Once the emulsifier has adsorbed and the polymerization has completed, the final suspensions of nanocapsules were quite stable over long periods. A secondary population of micrometric particles that coexisted with the nanocapsules was present in several cases, which was unfavourable regarding their application as a drug delivery system for cosmetic applications.

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1. Introduction

Drug delivery systems that consist in the encapsulation of drugs inside a carrier are manifold [1]. They include molecular encapsulation by means of complexation to a molecular or macromolecular carrier such as cyclodextrins or water-soluble functional polymers. Liposomes and oil-in-water emulsions provide a true encapsulation into supramolecular assemblies that are easily disrupted in the living organism. Drug carriers made of polymer particles have the advantages of a slower delivery rate and a protection of the encapsulated drug towards its enzymatic degradation in the living tissues [1,2].

Microcapsules are an attractive way to encapsulate a large variety of drugs, hydrophilic or hydrophobic inside their aqueous or oily core [3]. They consist in structured particles having a liquid core surrounded by a polymer shell that controls the leaching and protects the encapsulated drug from the external agents. The formation of the polymer coating in

situ at the surface of the emulsion droplets by interfacial polycondensation is an interesting route [4].

Most preparation processes of particulate drug carriers lead to micrometric particles: microspheres or microcapsules. A submicrometric size would be more suitable for several applications such as intravenous administration and skin penetration in cosmetic applications. The most popular preparation process of nanoparticles is nanoprecipitation where a solution of the polymer in a polar organic solvent is precipitated as an aqueous suspension of particles when it is mixed with a large amount of water [5]. Nanometric sizes can be obtained in favourable cases, the range of particle diameters being 100–500 nm.

Interfacial polycondensation is the in situ synthesis of the polymer at the surface of an oil droplet [4]. The polymer might not stay at the interface where it has been formed, however. The most frequent monomers are the water-soluble diol or diamine and the oil-soluble di(acid chloride) or diisocyanate, leading to polyesters, polyamides, polyurethanes or polyureas [4]. Interestingly, alkylcyanoacrylate anionic polymerization is easily performed at an oil–water interface because polymeri-

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zation of the oil-soluble monomer is initiated by hydroxide ions in the aqueous phase [6–8].

Conventional emulsification processes using laminar or turbulent flows allow the formation of micrometric droplets, either oil-in-water or water-in-oil according to the emulsifier type. The typical size range is 10 μm . The emulsification processes allowing the preparation of sub-micrometric size require very high shear rates or abrupt pressure drops realized in a high-pressure homogenizer or using high power ultrasound. Alternative emulsification processes referred to as “spontaneous emulsification” are derived from the methods used for the production of inorganic nanocolloids [9] or to the “nanoprecipitation” technique used for the preparation of polymer nanoparticles [5]. Generally speaking, starting from a supersaturated solution, the nucleation and growth of the particles is controlled by a suitable formulation and the process parameters. Thus, an oil-in-water emulsion is formed when a solution of oil in a water-soluble organic solvent is mixed with water. The polar organic solvent dissolves into water and leaves a supersaturated solution of oil in water. The subsequent liquid–liquid phase separation results in the formation of oil droplets into an aqueous continuous phase. This process has been described earlier as the “diffusion and stranding” mechanism by Davies and Haydon [10,11]. Miller [12,13], Vitale and Katz [14] have recently proposed the classical “nucleation and growth” mechanism as the possible route for oil droplet formation. This last mechanism looks likely because it explains the main features of the process [15], in particular the failure of emulsification at high oil contents.

The present work deals with attempts to combine into a single process, two steps that are usually performed separately: the emulsification and the interfacial polycondensation. The oil droplets have to be present for interfacial polycondensation, which means that the two steps have to take place subsequently. The emulsification process has to be fast with respect to the polycondensation reaction. Therefore, conventional emulsification methods using strong mechanical means are not suitable because the time required for breaking large droplets into nanometric sized droplets is necessary long (i.e. several minutes). The similar combination of the spontaneous emulsification and interfacial anionic polymerization of alkylcyanoacrylate led to the successful formation of nanocapsules having a thin polymer membrane [16,17]. It seemed however that the polymerization of the cyanoacrylate monomer was too fast with respect to the emulsification process since nanoparticles of pure polymer have left the nanocapsules in formation as a result of the emulsification process [18]. Another constraint that has to be managed with is the presence of the monomers in both the oil and aqueous phases. Investigations and optimization of the formulation pertaining to the several steps of the process are reported as follows: the emulsification step of the monomer-free recipe has been optimized in a first stage and a robust formulation has been selected where the monomer could be added. The polycondensation is subsequently reported, followed by a discussion on the alteration brought about by the presence of the monomers and polymerization with respect to the monomer-free system.

2. Materials and methods

2.1. Materials

The solvents were 99.5%+ ACS reagent grade from Sigma-Aldrich. Cosmetic grade oils α -tocopherol, hexyl laurate, Miglyol[®] 812 and Myritol[®] 318 were from Coletica. The emulsifiers Tween[®] 20 and Span[®] 85 were supplied by Seppic (France).

2.2. Methods

Droplet size distributions were analyzed by means of small-angle light scattering with a Coulter LS230 granulometer equipped with supplementary large-angle detectors working under the polarisation intensity differential scattering mode (PIDS). Such equipment allowed the measurement of broad distribution of sizes. The PIDS enlarged the measurable size domain towards smaller sizes, giving the size distribution from 50 nm up to some millimeters. The dilutions of the emulsions required for a right measurement were carried out so as to reach 10% obscuration as recommended. The Lorenz–Mie theory of scattering was used for converting the size distribution of scattered intensities into the size distributions of droplet volumes or number. This required the refractive indexes of both the dispersed and dispersing media to be correctly known. Size distributions of droplet volumes are given in following.

Transmission electron microscopy pictures were taken by a TOPCON 002B microscope operating at 200 kV acceleration. The sample preparation was performed according to similar previous studies [19]. The emulsions were diluted by a factor of 10, deposited on the copper grid and dried before observation. Negative staining with a 2% sodium phosphotungstate solution was made directly on the deposit.

Infrared spectra were recorded in transmission mode on KBr pellets with a Perkin-Elmer 1600 FT-IR spectrometer.

Interfacial tension measurements have been carried out by means of the pendent drop method with a Krüss DSA10 apparatus. Pictures of a pendent drop of the aqueous phase formed inside the oil phase were analyzed for their shape so as to obtain the interfacial tension with the help of the standard data analysis routine of the apparatus.

The encapsulation yield of α -tocopherol was determined according to the following particular sampling process [20]: The aqueous suspension of nanocapsules was centrifuged at 45,000 rpm with a Beckman-Coulter Optima MAX-E ultracentrifuge for 20 min; the supernatant was recovered and the pellet was washed twice with an aqueous solution of 0.3% Tween[®] 20 followed by ultracentrifuge separation. The washed nanocapsules were subsequently dissolved in isopropanol and analyzed for the α -tocopherol by HPLC. The free α -tocopherol content was determined by the HPLC analysis of the aqueous phase recovered after centrifugation and the total content of α -tocopherol was determined from the analysis of the full sample dissolved in isopropanol.

3. Results and discussion

3.1. The monomer-free spontaneous emulsification process

In the present spontaneous emulsification process, the oil droplets were formed when a solution of the oil in a water-soluble organic solvent was mixed with water. A typical experiment that led to the formation of an emulsion of α -tocopherol as an oil in water continuous phase was as follows. The following organic and aqueous solutions were prepared. A solution in acetone containing α -tocopherol (10 g/L) and the Span[®] 85 emulsifier (2 g/L) and an aqueous solution containing the Tween[®] 20 emulsifier (1.7 g/L). 100 mL of the organic solution were poured into 200 mL of the aqueous solution under smooth magnetic stirring. The mixture immediately turned cloudy, showing the fast formation of oil droplets. The mean droplet size was 163 nm. The emulsification was called spontaneous because it did not require a large mechanical energy input as in a conventional emulsification process. The magnetic stirring was necessary for the mixing process to be fast enough. But stirring did not break the droplets into smaller ones. The yield of emulsification and droplet size did not depend on the rotation rate of the magnetic stir bar. The acetone was subsequently evaporated under vacuum, leaving a stable emulsion of 0.5% α -tocopherol in water.

The formulation of the emulsion has been optimized with respect to the oil type and content, nature of the polar organic solvent and choice of the emulsifiers. This section is a more comprehensive report of an optimization study that has been reported in part [21]. The results are discussed in the framework of the “nucleation and growth” mechanism of droplet formation [14,15].

The concentration of oil phase was quite low but could not be increased. Larger amounts of oil resulted in an incomplete emulsification; the larger the oil content, the lower the emulsification yield which manifested by the creaming of an oily layer on top of the emulsion. Increasing the oil content above 0.5% was possible in some cases but limited to few percents. The low emulsification yield was in agreement with observations reported in the literature and with the mechanism proposed by Vitale and Katz [14,15]. Emulsification failure corresponded to a droplet formation by a mechanism of spinodal decomposition. It was difficult to measure with accuracy the low emulsification yields that resulted from oil concentrations slightly in excess because creaming was slow, so that the determination of the maximum oil content that gave full emulsification was also quite inaccurate. The oil concentration of 0.5% was safe in most instances and was retained throughout the whole investigation.

The choice of the emulsifiers was made with the help of the HLB numbers and was not crucial [21]. The mixture Span[®] 85/Tween[®] 20 40/60 of HLB=11 ensured a satisfactory stability, so did several other types of emulsifying systems. The long-time stability was never a problem because the emulsions were quite dilute. It has been retained for the whole investigation. The final size of the droplets depended on the choice of the emulsifier however. In particular, emulsifying

systems containing the Pluronic[®] F68 yielded oil droplets of very small size, as already reported in the different instance of nanoprecipitation of polymer particles [8,22,23].

The type of oil influenced the emulsification process at a microscopic scale, as revealed by the size distribution of the oil droplets. Thus, emulsification of α -tocopherol produced small droplets that had a monomodal size distribution; the mean diameter was 163 nm. On the contrary, the emulsification of various fatty esters such as hexyl laurate or the caprylic/capric triglycerides Miglyol[®] 812 and Myritol[®] 318 yielded a bimodal distribution of droplets. The small size population of 200 to 400 nm diameter was the most abundant and a secondary population of larger droplets of 2 μ m diameter was always present (Fig. 1). It was presumed that the small size population corresponded to the primary oil droplets that were formed from the supersaturated solution according to the nucleation and growth mechanism. But such a colloidal suspension of oil droplets was not stable unless the emulsifier has adsorbed to a large enough extent. Therefore, limited coagulation took place during the emulsifier adsorption period until the droplets were stabilized, giving rise to a small amount of larger droplets appearing as a secondary population in the size distribution. The size distribution changed very slowly over 6 months storage, showing that the population of large particles was not the result of the beginning of a fast aggregation process; the particles of 2 μ m diameter have been formed during the emulsification stage.

The amount of large droplets of 2 μ m diameter reflected the stability of the bare droplets immediately after their formation. It was correlated with the size of the primary droplets: the smaller the primary droplets, the lower the amount of large droplets (Table 1 and Fig. 1). Thus, the emulsification of α -tocopherol produced the smallest droplets. They were also the most stable since no significant amount of large droplets could be detected. Miglyol[®] 812 and Myritol[®] 318 that phase-separated as the largest droplets, gave the largest amount of secondary population, while the hexyl laurate oil gave

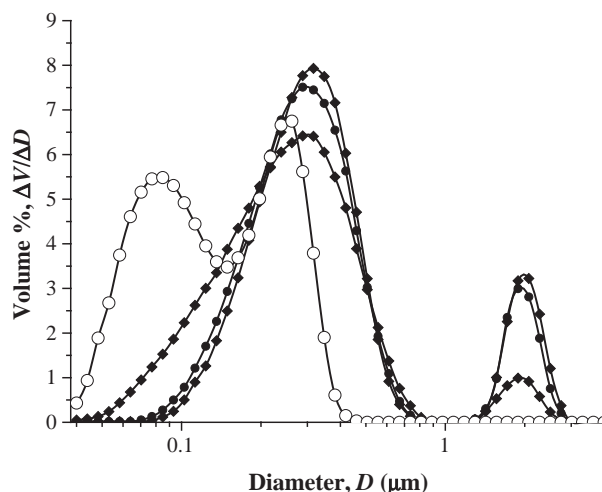


Fig. 1. Size distributions for different oils emulsified with acetone as a polar organic solvent: ○: α -tocopherol; ◇: hexyl laurate; ●: Miglyol[®] 812; ■: Myritol[®] 318.

Table 1
Granulometric analysis of the emulsions prepared with different oils as measured by small-angle light scattering

Oil	Mean diameter of the two populations (nm)		Volume fraction of the large-size population
	Small-size population	Large-size population	
α -tocopherol	163	None	0
Hexyl laurate	262	1900	25%
Miglyol [®] 812	310	2000	48%
Myritol [®] 318	320	2000	52%

intermediate results. It is noticeable that α -tocopherol has a surfactant-like chemical structure with a hydroxyl polar group attached to a long phytyl chain of 16 carbon atoms. It resembles an emulsifier of low HLB number. The bare emulsion droplets of α -tocopherol were quite stable with respect to coagulation because they were covered with the polar groups of α -tocopherol at their surface that made the oil–water interfacial tension low. Thus, the measured interfacial tension of α -tocopherol against water was $\gamma = 17 \pm 1$ mN/m, much the lower than that pertaining to the other oils. This value was at the upper bound of the values for normal fatty alcohols [24]. The value $\gamma = 25.0 \pm 0.5$ mN/m was found for hexyl laurate. It was not possible to measure the oil–water interfacial tensions of the triglycerides Miglyol[®] 812 and Myritol[®] 318 with accuracy because of the surface contamination with residual fatty acids. An estimate of such an interfacial tension is 30 mN/m. The low interfacial tension of α -tocopherol was also possibly the origin of a facilitated nucleation of the droplets from the supersaturated solution. Let us recall that the free energy of formation of a stable nucleus is proportional to

the third power of the interfacial tension in the classical theory of homogeneous nucleation. A fast nucleation rate gave rise to more numerous droplets, and therefore, to the smaller sizes that were observed.

The long-time colloidal stability of the emulsions was very good. The size distribution of the emulsions of α -tocopherol did not vary upon storage during 6 months at different temperatures up to 45 °C. But the emulsions made with the three other oils that showed a secondary population of larger droplets of 2 μ m diameter slowly varied over the 6 months storage. Storage at 4 °C kept the size distribution identical to that at observed the end of the preparation. Accelerated ageing by storage at 45 °C only resulted in a slight increase of the population of large size (Fig. 2). The visual aspect of the emulsions did not change, no creaming nor sedimentation could be observed. The instantaneous stability of the bare particles and the long-time stability of the emulsions covered with the emulsifiers looked related. The slow variation of size that mainly manifested by the growth of the larger population at the expense of the smaller, strongly suggesting Ostwald ripening as a mechanism.

The type of polar organic solvent also contributed to the efficiency of the spontaneous emulsification process. This has been investigated for the emulsification of α -tocopherol as an oil. Thus, the fully water-soluble ethanol and acetone allowed the successful emulsification. Acetone was preferred because ethanol was reacting with the monomers used for the polycondensation. Starting from this basic recipe, solvent mixtures have been investigated by replacing part of the acetone by various polar organic solvents. The substitution of 15% of the acetone by either tetrahydrofuran (THF) or methyl acetate (MeOAc) impaired the stability of the emulsion.

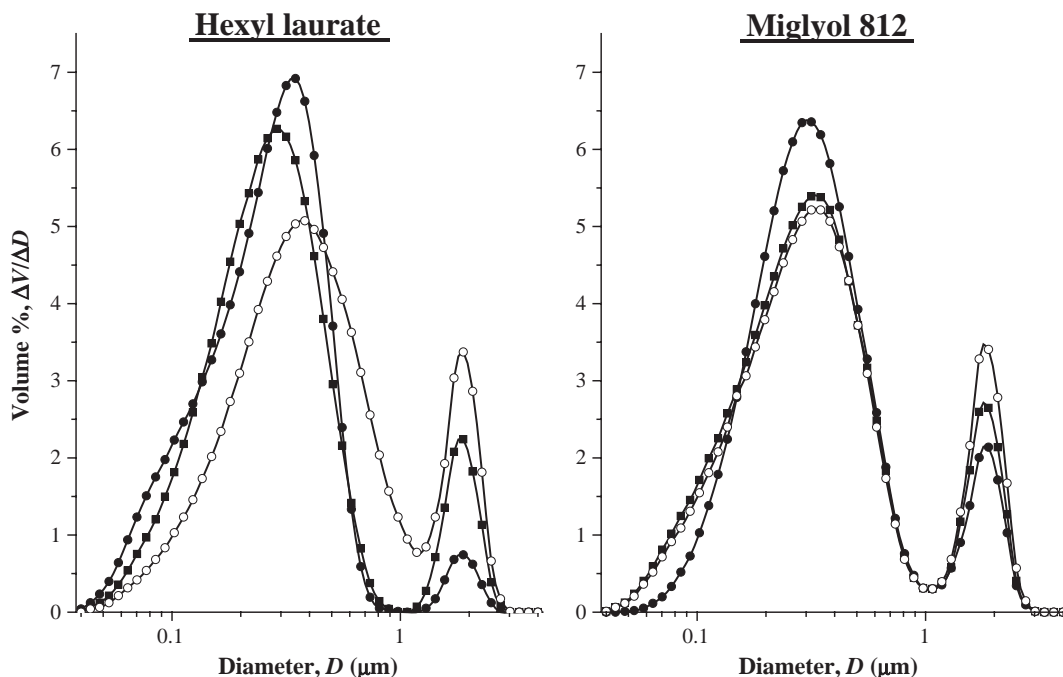


Fig. 2. Size distributions after storage during 6 months at different temperatures for different oils emulsified with acetone as a polar organic solvent. The examples of hexyl laurate (left) and Miglyol[®] 812 (right) are shown; Myritol[®] 318 was similar to Miglyol[®] 812. ●: 4 °C; ■: 25 °C; ○: 45 °C.

Conversely, substitution by either methyl ethyl ketone (MEK) up to 30% or ethyl acetate (EtOAc) up to 20% allowed the preparation of stable emulsions. Larger substitution rates gave unstable emulsions. In the case of MEK which had a large solubility in water (26.8%), the size distribution was monomodal and the average diameter was 400 nm. This indicated that the bare emulsions were stable enough with respect to coagulation, as in the similar case of pure acetone. But the mean size was larger however, which reflected a slower nucleation rate of oil droplets. Ethyl acetate was much less soluble in water; the maximum substitution rate of acetone by EtOAc was related to the solubility of EtOAc in water (8.7%). The origin of the failure observed above 20% EtOAc substitution was different of the that of the unsuccessful water-soluble solvents THF and MeOAc; it simply corresponded to cases where EtOAc could not dissolve to completion in water because of its limited solubility. For the successful substitution rates of EtOAc, the size distribution of the oil droplets was bimodal: a population of small primary particles (200 to 500 nm) coexisted with a population of secondary droplets of 2 μm diameter. The larger the amount of EtOAc, the most abundant the population of secondary droplets (Fig. 3).

In the framework of the “nucleation and growth” mechanism of droplet formation, such solvent effects might be caused by either a lower supersaturation or a higher interfacial tension. The rate of diffusion of the polar organic solvent out from the nucleation site and the partition of the polar organic solvent between the oil droplets and the aqueous phase are related parameters of relevance. Thus, the partition of the organic solvent between the aqueous phase and the oil droplets retains some of the solvent at the site where the emulsification takes place. The partial solubilization of the organic solvent inside oil concerns both the emulsion droplets and the transient nuclei that possibly lead to droplet formation when they are large enough. The consequence is a slower solvent diffusion from the droplet formation site and a slower nucleation rate because the local supersaturation of oil is low. The less polar the solvent

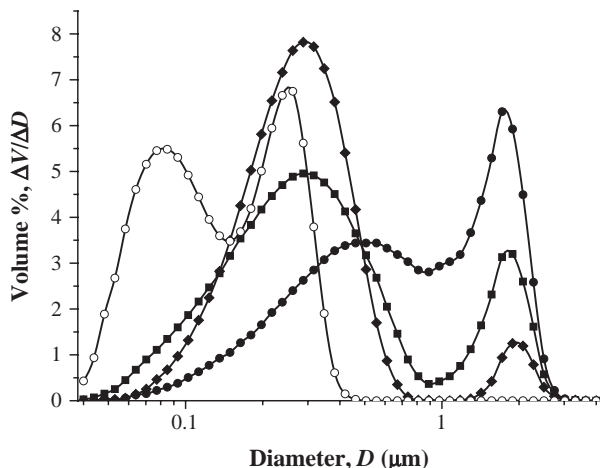


Fig. 3. Size distributions for α -tocopherol emulsified with mixtures of acetone and ethyl acetate of various compositions as a polar organic solvent: acetone/ethyl acetate = 100/0: \circ ; 90/10: \blacklozenge ; 85/15: \blacksquare ; 80/20: \bullet .

the stronger this effect. This tentative mechanism explains the larger sizes of primary droplets that were observed upon substitution of acetone by the more hydrophobic solvents MEK and EtOAc. The poor colloidal stability of the bare primary droplets immediately after their formation might also be related to the swelling by the organic solvent. This has especially a striking effect for the most hydrophobic EtOAc solvent. The presence of the solvent inside the oil droplets also influences the interfacial tension. It should be acknowledged that such mechanistic interpretations are largely speculative but checking against their validity by means of experiments would be quite a difficult task because fast transient phenomena are involved. The troubles encountered with the most hydrophilic solvents THF and MeOAc were different: the emulsification was successful but the emulsion was not stable with respect to coagulation.

3.2. Description of the optimized process with interfacial polycondensation

Throughout this work, the oil was α -tocopherol and different polymers have been formed by means of interfacial polycondensation at the surface of the emulsion droplets. A general recipe has been designed, which could work for the different polycondensates. Since several examples of polycondensation reactions have been investigated, this optimized process is thought to be quite general. Thus, several polyurethanes, polyureas and polyamides have been investigated where the oil-soluble monomer was either a diisocyanate or a di(acid chloride) and the water-soluble monomer was either a diol or a diamine. The list of the monomer pairs is given in Table 2.

A general process has been designed, which could be successful for the different polycondensates of the present investigation. The difference with respect to the emulsification process reported above was the presence of large amounts of monomers in the initial formulation and the progressive formation of polymers. The polymers might not stay at the interface during the course of the reaction. The requirement regarding the recipe and emulsification process was robustness against the presence of large amounts of reagents that partition between the two phases and the interface and the evolution of the chemical composition of the system. The demand was the long-time stability of the emulsion, but also the successful application of the spontaneous emulsification process.

Thus, the concentration of oil phase (α -tocopherol) in the final emulsion was restricted to 0.5%. Pure acetone was selected as the polar organic solvent with the mixing ratio acetone/water of 1/2. The emulsifier system was the Span[®] 85/Tween[®] 20 (40/60) mixture reported in Section 3.1. The low-HLB emulsifier Span[®] 85 was introduced in the organic phase and the hydrophilic emulsifier Tween[®] 20 was dissolved in the aqueous phase. In preliminary attempts, stoichiometric amounts of the two monomers were used and the monomer load was equal to the expected amount of polymer at the interfacial membrane. Such a recipe did not lead to the formation of capsules having a thick enough polymer

Table 2

Monomer pairs, corresponding polycondensates, the spectroscopic characteristics used for their chemical analysis and the encapsulation yield of α -tocopherol

Abbreviation	Water-soluble monomer	Oil-soluble monomer	IR spectroscopy characteristics of the polymer	Encapsulation yield (%)
<i>Polyurethane</i>				
IPDI-EG	Ethylene glycol	Isophorone diisocyanate	Carbamate and urea bands at	86
IPDI-BD	Butane diol	(IPDI)	1742 and 1634 cm^{-1}	88
IPDI-HD	Hexane diol			90
IPDI-PEG200	Polyoxyethylene glycol (PEG)		No residual $-\text{N}=\text{C}=\text{O}$	91
IPDI-PEG300	$M_w=200, 300, 400, 600$ g/mol		band at 2263 cm^{-1}	91
IPDI-PEG400				91
IPDI-PEG600				92
<i>Polyurea</i>				
IPDI-DETA	Diethylene triamine, DETA	Isophorone diisocyanate	Urea band at 1641 cm^{-1}	67
IPDI-DPTA	Dipropylene triamine, DPTA	(IPDI)	No residual $-\text{N}=\text{C}=\text{O}$ band at 2263 cm^{-1}	71
<i>Polyamide</i>				
SC-DETA	Diethylene triamine, DETA	Sebacoyl chloride	Amide bands at 1647 and 1558 cm^{-1}	76
SC-DPTA	Dipropylene triamine, DPTA		No residual $-\text{COCl}$ band at 1815 cm^{-1} nor $-\text{CO}_2\text{H}$ band at 1710 cm^{-1}	75

membrane at their surface that could influence the encapsulation and drug delivery properties [25]. The origin of such a failure was the low concentration of the water-soluble reagent (diol or diamine) because the emulsion was dilute. The consequence was a slow polycondensation rate and a relative importance of the competitive hydrolysis of the IPDI or sebacoyl chloride. Thus, the concentrations of diol or diamine were set high, so that these reagents were added in large excess with respect to the stoichiometry. According to a monomer concentration of 0.025 mol/L in the oil phase and a 10-fold excess of water-soluble monomer, the concentration of monomers in the aqueous phase was 0.125 mol/L in the optimized process. This large concentration of monomer corresponded to weight fractions ranging from 1% for EG to as high as 10% for PEG600. This large load of polar organic molecules that could act as co-solvents in water, was the most important difference with respect to the monomer-free emulsification process.

3.3. The interfacial polycondensation

The polycondensation reaction led to the formation of new chemical linkages that could be observed by means of their infrared absorption bands. Full conversion of the monomers could be monitored by the disappearance of their IR bands. Lastly, the isocyanate and acid chloride groups were prone to hydrolysis; their hydrolysis products, if any, could also be detected by IR spectroscopy (Table 2). Thus, the formation of the polyurethane led to the appearance of IR absorption bands characteristic of the carbamate linkage at 1742 cm^{-1} and a secondary band at 1634 cm^{-1} showing the formation of urea linkages; the band pertaining to the isocyanate group at 2263 cm^{-1} has vanished. The formation of urea linkages was caused by the hydrolysis of the isocyanate into amine groups followed by the polycondensation of the amine groups into urea. The hydrolysis side-reaction did not impair the polymerization course since the hydrolysis products were reactive and could be incorporated into the polymer chains; a poly(urethane-co-urea)

copolymer resulted. The same types of observations reported in Table 2 have been made for polyurea and polyamide. In this later case, the formation of carboxylic acid by hydrolysis of the acid chloride could be ruled out because no characteristic IR band at 1710 cm^{-1} could be detected. The IR characterization of the dried emulsions allowed the analysis of the polymers. It showed the formation of the expected polymers and full conversion of the isocyanate or acid chloride. The hydrolysis of the monomer was not significant, at least in the case of the polyamide capsules. The characterization of the polymers would deserve much more attention however. A NMR analysis and the measurement of the molar masses by means of light scattering or size exclusion chromatography would be informative, but it would require the polymer to be separated from the other components of the formulation (oil, emulsifiers).

The formation of a polymer membrane was assessed from transmission electron microscopy. Direct observations allowed an analysis of the particle size distribution which is discussed next. Since the contrast between the internal oil and the polymer membrane was low, and it was very difficult to detect the presence of the membrane at the surface of the droplets. In some favourable cases, negative coloration by sodium phosphotungstate allowed the observation of the polymer membrane as a line surrounding the circular droplets (Fig. 4). It was concluded that the membrane was indeed present but very thin. A rough estimate of the thickness was 2 nm. The membrane was much thinner than expected regarding the amount of polymer. This suggested that a large part of the polymer was dissolved inside the oil core of the capsules. The polycondensation taking place at the surface of the emulsion droplets was quite different of the same reaction run at the interface separating macroscopic oil and water phases. Thus, in this later case, a thick polymer layer appeared at the interface, which could be recovered with a spatula. This membrane was 10 μm thick and had enough tensile strength. The types of polymers were clearly different in both cases. It was speculated that the interfacial polycondensation taking place in an

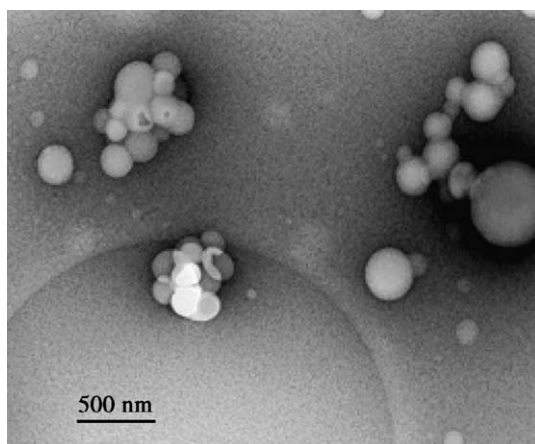


Fig. 4. Transmission electron microscopy pictures of IPDI-EG nanocapsules. The negative staining observation made the capsules appear as white surrounded by a vanishingly thin black membrane.

emulsion yielded low molar mass polymers that could easily leave the interface during the polymerization reaction. On the contrary, polymerization at a macroscopic interface yielded high molar mass polymers that were entangled into a self-supported material.

The encapsulation yield of α -tocopherol was a proof of the presence of a polymer membrane and an indirect measurement of its permeability. This was measured by an HPLC analysis of α -tocopherol after having separated the nanocapsules by ultracentrifugation and washed them with a solution of detergent. Thus, the suspension of capsules was centrifuged at 45,000 rpm for 20 min; the supernatant where the nanocapsules have creamed up was recovered and washed twice with an aqueous solution of 0.3% Tween[®] 20. The washed nanocapsules were subsequently dissolved in isopropanol and analyzed for the α -tocopherol by HPLC. The lost α -tocopherol molecules corresponded to those which were removed at the washing step. The encapsulation yield was an estimate of the ability of the polymer membrane to protect the internal content of the capsules against washing. This type of measurement was of course very dependent on several experimental parameters such as the number and duration of washing cycles or the concentration of the detergent, so that only relative values of the encapsulation yields made sense. From the encapsulation yields reported in Table 2, the membranes made of polyurethane were significantly superior to those made of polyamide and polyurea.

3.4. The emulsification step

The spontaneous emulsification process was strongly affected by the presence of the monomers. This was especially obvious when the particle size distribution was compared to that of the monomer-free system. The spontaneous emulsification of α -tocopherol was a clean process where a single population of nanometric droplets was formed. The particle size distribution as measured at the end of the polycondensation was shifted towards larger sizes and might appear bimodal in some cases. Thus, the mean diameter increased with respect

Table 3

Granulometric analysis of the nanocapsules prepared from different diols as measured by small-angle light scattering

Diol	Mean diameter of the two populations (nm)	
	Small-size population	Large-size population
Ethylene glycol, EG	230	None
Butane diol, BD	260	None
Hexane diol, HD	310	None
PEG 200	220	None
PEG 300	370	1900
PEG 400	320	1900
PEG 600	370	1800

to 170 nm for the monomer-free emulsion in the order $EG < BD < HD < PEG200$ and a population of aggregated primary droplets was present for the higher PEGs (Table 3). The relative abundance of large size population of 2 μ m diameter grew in the order of the PEGs' molar masses (Fig. 5). Because of the dependence on the nature of the diol, it was inferred that the presence of the diol was the origin of the troubles in the emulsification step in the same way as the polar organic co-solvents THF, methyl acetate or ethyl acetate. The diols were indeed quite concentrated in the aqueous phase and the manifestations on the size distribution were the same. The early aggregation of the bare emulsions was most pronounced with the PEG systems because they were larger molecules: for the same concentration as expressed in moles per liter, the volume fraction of organic materials in the aqueous phase was larger. The organic co-solvents discussed above were introduced in the organic phase and were thought to diffuse slowly into the aqueous phase because of their partition into the phase separating oil droplets. On the contrary, the PEGs were introduced in the aqueous phase. The origin of the perturbed emulsification might be the poor adsorption of the water-soluble Tween[®] 20 emulsifier because of several effects. The emulsifier was retained in the aqueous phase of hydro-organic nature because the hydrophobic effect that determined the tensioactive properties drops off when the water structure has been disrupted [26,27]. Owing to the tensioactive properties of

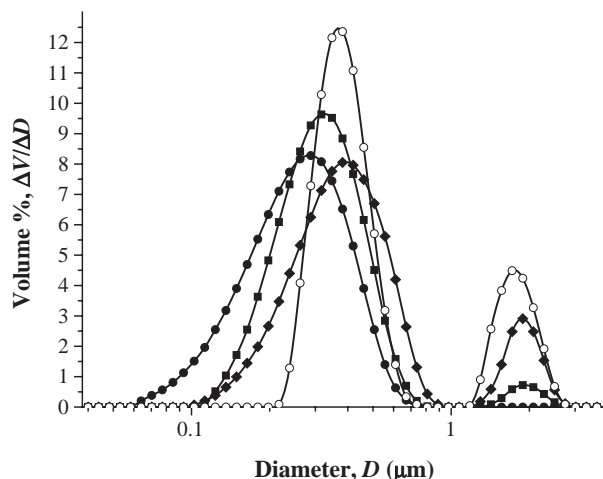


Fig. 5. Size distributions of α -tocopherol nanocapsules with polyurethane polycondensates made from PEG200 (●), PEG300 (◆), PEG400 (■) and PEG600 (○).

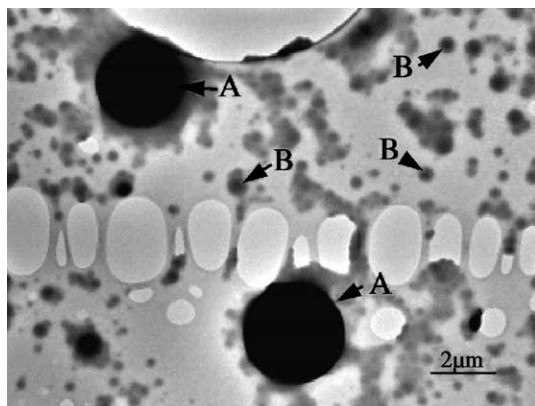


Fig. 6. Transmission electron microscopy picture of IPDI-PEG400 nanocapsules. The capsules appear as black in the direct observation mode. The picture provides evidence of the bimodal size distribution with a population of large particles of 2 μm diameter (A) supplementing the main population of 200 nm mean diameter (B), as was observed by means of small-angle light scattering analyses.

PEG, adsorption of PEG at the surface of the oil droplets impeded that of the emulsifier.

Direct observations by means of transmission electron microscopy allowed an analysis of the particle size distribution that confirmed the presence of two distinct populations: the predominance of nanometric particles (diameter of the order 200 nm) and a supplementary population of larger particles having a diameter of 1–2 μm (Fig. 6). The limited aggregation of small particles that led to larger particles of some micrometers diameter was confirmed by means of such direct observations. Indeed, there are no larger particles that the light scattering technique would have missed.

4. Conclusions

The combination of the spontaneous emulsification and interfacial polycondensation could be merged into a single process. This was possible because the time scale of the two processes were quite different. The emulsification was almost instantaneous while the time required for the polycondensation reaction to reach full conversion of the diisocyanate or di(acid chloride) amounted in minutes to hours [28–30].

The main advantage of the spontaneous emulsification process was the easy preparation of nanometric droplets. Nanometric emulsions could be prepared by means of conventional mechanical means such as high-pressure homogenizer or high-power ultrasounds. The later mechanical methods require a rather long emulsification time which prohibits the interfacial polycondensation to be carried out in the same operation. A two-steps process has to be designed. Apart from the time and energy saving, the spontaneous emulsification process is a mild process that might be suited for fragile materials to be encapsulated such as proteins or nucleic acids. Mechanical emulsification device might break large biomolecules because of the high shear, but also because such lengthy operations produce much heat that might denature biological materials. One sound criticism against the spontaneous emulsification process is the use of organic solvents

(acetone) that are able to denature proteins. The solvents that have been investigated in the present study were taken from the Class III list of safest solvents of the European Pharmacopeia.

Such benefits of the spontaneous emulsification process have their counterparts. The organic solvent has to be eliminated after the emulsification. It is easy to evaporate acetone at low temperature under reduced pressure, but this represents a supplementary stage in the preparation process. As another drawback, this process does not allow the preparation of concentrated emulsions because the phase separation at large supersaturation takes place by means of a spinodal decomposition mechanism [14,15].

The presence of monomers and the polycondensation reaction influenced the emulsification process. Since the hydrophobic monomer, which was dissolved in the organic phase, was most often prone to hydrolysis, the polycondensation had to be accelerated with respect to hydrolysis. This was ensured by a large concentration of hydrophilic monomer in the aqueous phase. The solvent properties of such monomers made the emulsion unstable, especially during the droplet formation period before the emulsifier had adsorbed. The stability of the bare droplets was a crucial parameter of the process. Since the amounts of monomer were far from stoichiometry, the large amounts of residual hydrophilic monomer would also have influenced the long-time stability. This was not the case, suggesting that the formation of the polymer membrane at the surface of the oil droplets improved the colloidal stability of the suspension.

Quite a satisfactory system regarding the nanometric size and the stability requirements was the IPDI-PEG200 system containing the polyurethane membrane formed from IPDI and PEG200. Some aspects still remain largely unclear and would require further work. A more precise characterization of the polymers, in particular their molar masses, would help much in understanding the large differences between the interfacial polycondensation carried out at the surface of emulsion droplets and at a macroscopic interface. Off-stoichiometric conditions of monomers are known to be disastrous regarding the molar masses in polycondensation reaction, but they were useful in the present case because of the heterogeneous nature of the polymerization medium for the reaction rate to be fast enough. Therefore, the molar masses are expected to be low. The presence of a membrane was made clear from the measurements of its influence on the encapsulation yield. But the structural characterization of the membrane was quite poor and this is a difficult experimental task to investigate an organic polymer membrane, possibly swollen by solvents, at the surface of an oil droplet. For geometrical reasons, the polymer membrane cannot be identical with that of micrometric capsules because the later membranes are thicker than the full diameter of the nanoemulsion oil droplets. The membranes of micrometric capsules have a high mechanical strength and low permeability. It can be speculated that the polymer membrane at the surface of sub-micrometric capsules resembles that of the micrometric capsules at low conversion of the polycondensation reaction. Therefore, nanocapsules should not be considered as microcapsules of smaller size. Because the overall size

has been reduced by a factor of 100 to 1000, the properties of the polymer membrane (thickness, permeability, mechanical strength) are completely different. Nanocapsules are new drug carriers designed for different applications than microcapsules.

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