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Block copolymers of the type poly(caprolactone)-*b*-poly(ethylene oxide) for the preparation and stabilization of nanoemulsions

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ABSTRACT

Block copolymers poly(caprolactone)-*block*-poly(ethylene oxide) are promising non-ionic macromolecular surfactants for the stabilization of emulsions because they display a stronger adsorption and provide an increased long-term stability. But such amphiphilic copolymers should also allow the fabrication of the suspensions according to the emulsification process used. An evaluation of such block copolymers was done regarding the nanoprecipitation and the miniemulsion polymerization processes that both afford aqueous suspensions of nanoparticles. Both the fabrication and the long-term stability were investigated. It was found that the emulsification by means of the nanoprecipitation process was successful when the amphiphilic block copolymer was added into the organic phase. The studies on the structure–activity relationships have shown that a minimum length of the poly(ethylene oxide) block was necessary in order to ensure both the long-term colloidal stability of the suspensions and the instantaneous stability during the preparation process. The length of the hydrophobic block was a parameter of less relevance, but a minimum length was required for the copolymers to be soluble in the organic phase. The miniemulsion polymerization process using block copolymer emulsifiers could be adapted to the incorporation of large loads of vitamin E acetate used as a hydrophobe stabilizer.

1. Introduction

In various application domains, the choice of emulsifiers is limited by many constraints that do not solely pertain to their emulsifying properties. This is particularly true for applications that concern living organisms, environment and humans. As example, surfactants used in detergency should be biodegradable for the correct work of water purification plants, surfactants used in body care should also have low irritancy properties; and the requirements are even more severe for cosmetic and pharmaceutical applications. Regarding the later applications, a formulation can be proposed if the safety of all excipients has been demonstrated by means of toxicology tests. This rule applies to emulsifiers as well.

As a consequence, the common practice in pharmacy consists in using compounds that are described and validated in the European Pharmacopoeia because toxicology studies have already been made. According to such restrictive scheme, the choice of emulsifiers is quite a difficult task because of the small number of described compounds. Therefore, it is frequent that a suitable emulsifier cannot be found. The utilization of a new compound requires a long and expensive toxicology study that is only decided when the optimal properties of the compound have been demonstrated. Thus, searching for new compounds having ameliorated properties and meeting the constraints of the pharmaceutical or cosmetic application is very useful.

Among emulsifiers, polymeric surfactants (Piirma, 1992; Tadros, 2001) show obvious advantages that motivate the studies that are presently reported on. In particular, the structure of block copolymers having a hydrophilic block and a hydrophobic (non polar) block is similar to that of classical emulsifiers, but the properties of each block are enhanced: the hydrophilic part is much more polar that of a classical surfactant and the same is true for the hydrophobic part. The consequence is excellent surface properties at low concentrations (Riess, 2003), which allows using lower rates of emulsifier for the stabilization of emulsions. This is an obvious economical benefit, but also the residual concentration of emulsifier in the aqueous phase is lower, limiting secondary effects such as air entrapment and foaming, adsorption at various interfaces, in particular the cell membranes.

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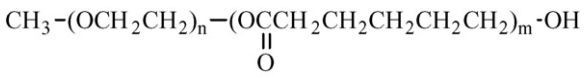
Besides the absence of toxicity of compounds and their metabolites, emulsifiers should of course allow the stabilization of emulsions or suspensions for very long periods, and be biocompatible and biodegradable. Finally for a pharmaceutical application where an active substance is encapsulated inside an emulsion, it is necessary that the dosage form reaches the therapeutic target and therefore circulates in the body for a long time. An efficient mean for preventing the detection and the elimination of particles by the immune system consists in coating the surface with a protective layer made of hydrophilic polymer (Gref et al., 1995; Holmberg et al., 1997). The hydrophilic part of a block copolymer emulsifier allows reaching such goal whereas a classical emulsifier does not, since it is a too small and mobile molecule. Polymers based on poly(ethylene oxide) are often used for that purpose.

Such type of copolymer and related copolymers are widely studied for their applications in the encapsulation and vectorisation of drugs, although no commercial application in pharmaceutical formulation has been started to date. The formulations that are generally reported for possible pharmaceutical applications mostly concern so-called "block copolymer micelles" (Allen et al., 1998, 2000; Rösler et al., 2001; Ravi Kumar et al., 2002; Soo et al., 2002; Hu et al., 2003; Ravenelle and Marchessault, 2003; Ahmed and Discher, 2004; Shuai et al., 2004; Jeong et al., 2004; Shi et al., 2005; Park et al., 2005; Mahmud and Lavasanifar, 2005; Wang et al., 2005; Meier et al., 2005). Such micelles consist of nanoparticles made of pure block copolymer but differ from micelles made of classical surfactants in several ways. In particular, block copolymer micelles cannot be prepared by means of simple dissolution of the block copolymer in water. The preparation process of block copolymer micelles is an emulsification process that has strong similarities with the nanoprecipitation process or spontaneous emulsification. Surprisingly, the utilization of such block copolymers as emulsifiers has not received so much attention, whereas the main applications of surfactants and polymeric surfactants in pharmaceutical formulation are the stabilization of emulsions and suspensions of polymer particles. The choice of the emulsifier is of chief importance for emulsions of submicronic size (nanoemulsions) because of the large specific area of such emulsions. Therefore, there is a want for technical data dealing with the use of new block copolymers as emulsifiers for the stabilization of nanoparticle suspensions.

The present study deals with the synthesis and the evaluation of the emulsifying properties of block copolymers of the type poly(caprolactone)-*b*-poly(ethylene oxide), PCL-*b*-PEO. The general chemical structure of the copolymers is shown in Scheme 1.

The hydrophobic poly(caprolactone) (PCL) block is a well-known biocompatible and biodegradable polyester (Edlund and Albertsson, 2002; Albertsson and Varma, 2003; Wang et al., 2003). PCL is indeed widely used as a matrix for the encapsulation of active substances inside polymeric microspheres or nanospheres (Benoit et al., 1996). The hydrophilic block consists in poly(ethylene oxide) (PEO), which is biocompatible but hardly biodegradable. The utilization of PEO in many non-ionic emulsifiers such as Polysorbates (Tween®) and alkyl ethers of PEO (Brij®) is however very frequent and accepted. It is indeed difficult to find out hydrophilic materials that could be substituted satisfactorily. The two parameters that control the properties of such block copolymers are the polymerization degrees of the PCL and PEO blocks. The synthesis process that has been used allows tuning the polymerization degrees of both blocks in order to allow a systematic investigation of relationships between chemical structure and properties.



Scheme 1. Chemical structure of block copolymers poly(ethylene oxide)-*b*-poly(caprolactone) (PCL-*b*-PEO).

The evaluation of the properties that is presently reported deals with two different emulsification processes of quite different nature: the "nanoprecipitation" process (Stainmesse et al., 1995; Montasser et al., 2000a,b; Moinard-Checot et al., 2006) that affords suspensions of polymer nanoparticles; and the miniemulsion polymerization of vinyl acetate that allows preparing highly loaded suspensions of nanocapsules having a poly(vinyl acetate) (PVAc) shell (Rajot et al., 2003; Bathfield et al., 2005).

The biocompatible and biodegradable polymer nanoparticles (Edlund and Albertsson, 2002; Wang et al., 2003) that are produced according to both processes can be used for the vectorisation of hydrophobic active substances that have been dissolved in the starting organic phase before the preparation of the emulsion. The overall structure of the nanoparticles is a polymer (PCL or PVAc) core surrounded by a shell made of block copolymers that ensure the colloidal stability of the suspension and prevents the recognition by the immune system owing to the PEG block. Such structure is similar to that of block copolymer micelles that have been extensively studied (Riess, 2003; Allen et al., 2000; Heald et al., 2002; Riley et al., 2003; Vangeyte et al., 2004; Letchford et al., 2004); however the particles that we report on are different because they contain a large amount of polymer material (PCL or PVAc) inside the particles and their size is much larger. For both processes, two main parameters are studied, namely the feasibility of polymer emulsions with the help of these block copolymer surfactants and the long-term stability of the emulsions.

The paper contains three main parts:

- The optimized synthesis process of the block copolymers and their characterization are presented in the first part. It is shown that the chemical structure of the block copolymers can be precisely tuned by a proper choice of the reactor feed. The control of the final product by conventional ^{1}H NMR is easy. The preparation of a series of block copolymers having various polymerization degrees of the PCL and PEO block allows investigating structure–activity relationships.
- The emulsification of poly(caprolactone) in water by means of the "nanoprecipitation" process using these block copolymers as emulsifiers is reported with emphasis on the control by the formulation parameters: the chemical compositions of the aqueous and organic phases and the choice of the block copolymer structure. This is a model study where no active substance has been incorporated. Loading with active substances that are known to enter polycaprolactone nanoparticles would be easy since the block copolymer emulsifiers adsorbed at the surface of the particles do not alter the properties of PCL the inside the particles. Such block copolymers allow the efficient and sustainable fabrication of oil-in-water emulsions with many types of common oils.
- In the third part, a block copolymer is used for the stabilization of vinyl acetate emulsion which is polymerized according to the miniemulsion polymerization process. The adaptation of this process to encapsulation of hydrophobic ingredients affords non-ionic polymer nanoparticles engulfing the hydrophobic drug (vitamin E acetate).

2. Materials and methods

2.1. Materials

The PEO block of the copolymers came from poly(ethylene glycol) mono methyl ether of different molar masses pur-

chased from Fluka and used as received. Caprolactone and vinyl acetate monomers, the initiators lauroyl peroxide, azo-bis(isobutyronitrile) (AIBN), hydrogen peroxide and ascorbic acid were reagent grade chemicals purchased from Aldrich and used as received. Triethyl aluminum was a 25 wt% solution in toluene from Aldrich. Toluene was dried on molecular sieves 3A and stored under dry argon atmosphere. Polycaprolactone (PCL) (M_W 80,000 g/mol) was from Aldrich. The active substance vitamin E acetate (DL-α-tocopherol acetate) was from Aldrich. The emulsifiers polyoxyethylene-polyoxypropylene triblock copolymer Pluronic® F-68 (PF 68) and polyoxyethylene(100) stearyl ether (Brij® 700) were from Sigma and Aldrich, respectively.

2.2. Methods

^{1}H NMR spectra were run on a Bruker AC300 spectrometer. The compounds were dissolved in $CDCl_3$ containing tetramethylsilane (TMS) as an internal standard.

Mass spectrometry measurements were performed using the MALDI-TOF method working in linear mode (Applied Biosystems Voyager System 4130). The polymers were dissolved in a dithranol–NaI matrix for laser ionization. The calibration was made with polyethylene oxide standards.

Particles sizes were measured either by dynamic or classical light scattering. These two light scattering techniques are complementary since they are sensitive to different size-ranges and they differ a lot in their basic principle. Thus small-angle light scattering is based on a measurement of the time-averaged scattered intensity as a function of the scattering angle; according to the explored angular domain, particle diameters are measured between 100 nm and 2 mm. Dynamic light scattering is a measurement of the Brownian motion of the particles that is related to their hydrodynamic diameter; the upper limit of the diameters that can be measured is 2 μm. Dynamic light scattering was performed using a Malvern® Zetasiser 3000HS operating at 633 nm wavelength and 90° scattering angle. The suspensions were diluted to approximately 10^{-4} volume fraction, so that the count rate was of the order of 200 kHz. The autocorrelation signal was analyzed by the method of cumulants, giving the *z*-average diameter of the particles. Characterization by static light scattering was performed using a Coulter® LS 230 (Beckman Coulter). The dispersion was diluted inside the instrument in such a way that the obscuration was 12% according to the instructions of the supplier. The size distribution was calculated from the Mie theory according to the suitable optical model. The real parts of the refractive indices of the optical models used were 1.33 for water, 1.45 for PCL, 1.496 for vitamin E acetate and 1.4665 for PVAc; the imaginary part of the refractive index was zero for every compound.

2.3. Synthesis of block copolymers PEO-b-PCL

The block copolymer was synthesized by anionic coordinated polymerization of ε-caprolactone with aluminum alkoxide of the convenient poly(ethylene glycol) mono methyl ether (MPEG) as the initiator. This latter was obtained by reaction of triethyl aluminum (TEA) with MPEG in excess. The reaction was performed in toluene under dry argon in a 250-mL round-bottomed flask. MPEG was firstly dissolved in dry toluene by heating and some drops of toluene were distilled in order to eliminate of the residual water contained in MPEG. After cooling at room temperature, the aluminum alkoxide was formed by adding TEA according to a ratio MPEG/TEA ≈ 10. Finally ε-caprolactone was added after 15 min and the polymerization was performed at 60 °C for 2 h. The expected degree of polymerization was controlled by the [monomer]/[MPEG] ratio. After complete conversion of the monomer, the block copolymer was precipitated in cold heptane at −18 °C. The polymer was dried at room temperature under vacuum, weighted and characterized by ^{1}H NMR. MALDI-TOF spectrometry could also be performed for copolymers of low enough molar mass.

2.4. Emulsification of PCL

Polycaprolactone emulsions have been prepared using the nanoprecipitation process. This process consisted in mixing an organic phase in acetone into water. A typical example of organic phase contained 0.2 g of PCL and 0.1 g of block copolymer in 25 mL acetone. The organic solution was rapidly injected into 50 mL water and the mixture immediately turned milky as an oil-in-water emulsion formed. Acetone was subsequently evaporated under reduced pressure. According to this process, an emulsion containing 0.6% dispersed phase was obtained. The sizes of the emulsions were measured by means of static and dynamic light scattering according to the methods described above.

2.5. Miniemulsion polymerization

Miniemulsions of the vinyl acetate monomer were prepared according to the following recipe: the organic phase was composed of the vinyl acetate monomer, vitamin E acetate and the initiator if it was of the organo-soluble type. The aqueous phase consisted in water, surfactant, and eventually the initiator if water-soluble. The two phases were weighed separately and de-oxygenated with nitrogen gas flow for 15 min. The miniemulsion was formed by dispersing the organic phase into the aqueous phase in two stages: a premix is first prepared with a magnetic stirrer; this coarse emulsion was thereafter dispersed to submicronic sizes with an ultrasonic disperser for 180 s (Sonics VibraCell apparatus equipped with a 25 mm shaft working at 750 W and 100% amplitude). During this time, the dispersion was cooled in an ice-water bath to limit heat-up.

Polymerizations were carried out under nitrogen atmosphere in a glass reactor under moderate stirring over 5 h. The polymerization temperature was 40 °C when the hydro-soluble redox initiator (hydrogen peroxide + ascorbic acid) was used. In that case, the generation of radicals is controlled by the progressive addition of the hydrogen peroxide component of the redox initiating system. A typical recipe using the hydrosoluble redox initiator was as follows: the organic phase was composed of 22 g vinyl acetate and 22 g hydrophobe; the aqueous phase was composed of 80 g water, 4 g block copolymer and 0.15 g ascorbic acid. The reactor at 40 °C was filled with the o/w emulsion and hydrogen peroxide (0.4 g H_2O_2 30%) was continuously poured into the dispersed medium at a flow rate roughly corresponding to the number of polymer particles.

The organo-soluble initiator lauroyl peroxide required elevated temperature for its thermal decomposition into radicals. The generation of radicals took place according to the decomposition kinetics of the initiator. The full initiator was dissolved in the organic phase till the beginning and the polymerization was run at 70 °C. The recipe used for the kinetics experiment is the same as above, except the initiator (1 mol%) is previously dissolved in the organic phase.

Polymerization kinetics was monitored by gas chromatography of aliquots collected at different times with ethyl acetate as internal standard. Vitamin E acetate was characterized by HPLC. Particles sizes were measured as above.

3. Results and discussion

3.1. Synthesis and characterization of block copolymers

The synthesis of PCL-*b*-PEO block copolymers was performed by coordinated anionic polymerization of ε-caprolactone from poly(ethylene glycol) methyl ether (MPEG) and $AlEt_3$. The synthesis of such copolymers is well documented and proceeds usually with stannous octanoate $Sn(Oct)_2$ as initiator and MPEG in stoichiometric amount. $AlEt_3$ was presently used in a [MPEG]/[$AlEt_3$] ratio ≈ 10, in order to synthesize the alcoholate, taking advantage of the rapid transfer reaction between alcoholate and alcohol. This reaction is a versatile one-step process for the synthesis of functional low molar mass polymers from oxygenated heterocycles (Jacquier et al., 1996; Delaite-Miola et al., 1999; Pantiru et al., 2004). It could readily be adapted to the synthesis of block copolymers using the MPEG block as transfer agent (Iojoiu et al., 2004). In addition, this reaction allowed the synthesis of polymer materials using less metal atoms than for the process using stannous octanoate. Since it is difficult to eliminate the residual metal compounds, even after precipitation of the polymer, the initiator (metal catalyst) is generally not eliminated at the end. The final material contained less metal species in the present case. The number-average polymerization degree n was easily controlled by the amount of transfer agent with respect to monomer [ε-CL]/[MPEG].

Three commercially available MPEG of number-average molar masses M_n = 350, 750, 2000 and 5000 g/mol (corresponding to number-average polymerization degrees n = 7.6, 16.7, 45.5 and 113.3, respectively) were used as transfer agents. The copolymers were characterized by means of ^{1}H NMR (Fig. 1) and MALDI-TOF mass spectrometry (Fig. 2) analyses. Usual ^{1}H NMR analysis allows controlling the conversion of the monomer and the purity of the polymer. Interestingly, ^{1}H NMR also gave the length of the PEO and PCL blocks as follows. The broad line at 3.64 ppm corresponded to the methylenes of the PEO block and the sharp singlet at 3.38 ppm corresponded to the terminal methyl group of the MPEG. The lines at 1.38, 1.64, 2.31 and 4.06 ppm were assigned to the methylenes of the PCL block. Additionally, the methylene of the PEO block that was directly linked to the PCL block was observed at 4.22 ppm; its area was 2/3 the area under the peak at 3.38 ppm corresponding to the CH_3 at the other chain end of the PEO block. This ratio of areas allowed to check against the correct linkage of the two blocks. Accordingly, the number average polymerization degrees of the PEO and PCL blocks were given by

$$n = \frac{(\text{area at } 3.64\text{ ppm})/4}{(\text{area at } 3.38\text{ ppm})/3},$$

$$m = \frac{(\text{area at } 1.38\text{ ppm})/2}{(\text{area at } 3.0\text{ ppm})/3} = \frac{(\text{area at } 1.64\text{ ppm})/4}{(\text{area at } 3.0\text{ ppm})/3}$$

The MALDI-TOF mass spectrum (Fig. 2) contains very rich information since there is one peak for each molar mass. The spectrum results from the mass distributions of the starting MPEG and PCL chains. An enlargement of a part of the mass spectrum is shown in Fig. 2b. Specific peaks of each copolymer chain were separated by m/z shifts of 114 g corresponding to one caprolactone unit and 44 g for each ethylene oxide unit. It allowed to check against the presence of by-products, particularly unreacted MPEG or PCL macrocycles, the later being hardly detected by ^{1}H NMR. ^{1}H NMR gave the number average polymerization degrees of the blocks and mass spectrometry gave the full distribution of the molar masses. The quite abundant information coming from mass spectrometry is useful for a detailed analysis of the polymerization mechanisms. ^{1}H NMR is enough for the purpose of control analysis of the synthesis. The mass spectrometry gave a confirmation of the analyses by ^{1}H NMR, the number average molar masses of the blocks calculated from the mass spectrometry data were in agreement with the ^{1}H NMR analyses.

The chemical structure of copolymers used in the following is given in Table 1. The block copolymers are abbreviated as PCL(m)-*b*-PEO(n) where, m and n are the number average polymerization degrees of the PCL and PEO blocks, respectively. The HLB numbers of the copolymer emulsifiers were estimated from the definition of Griffin using the number-average masses of the PEO block and the

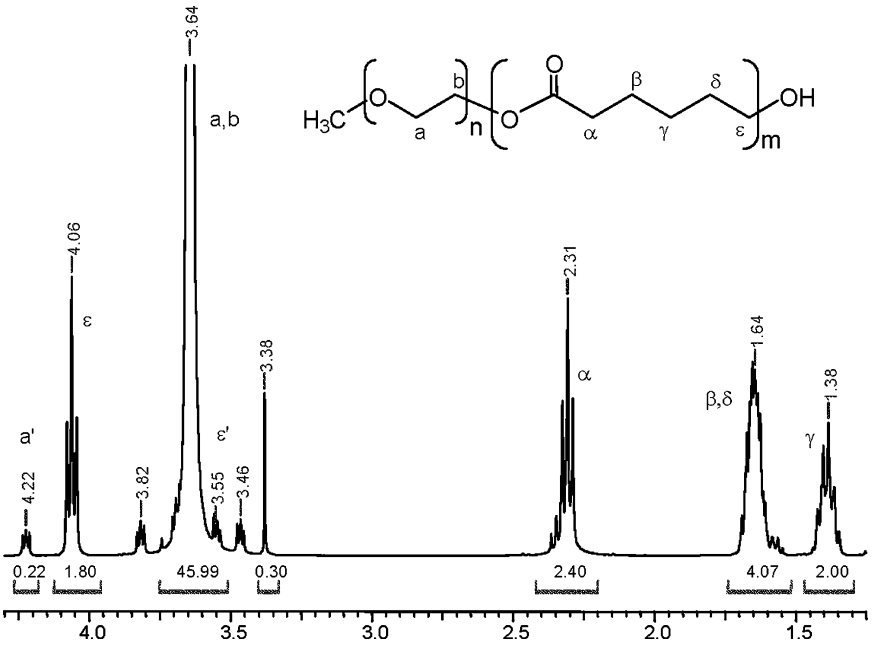


Fig. 1. ^{1}H NMR spectrum of a PCL-*b*-PEO block copolymer. The line denoted a′ corresponds to the CH_2 of the PEO block linked to the PCL block; it is 2/3 the peak terminal CH_3 at 3.38 ppm. The line denoted ε′ corresponds to the CH_2 at the PCL chain end; it is not resolved enough for looking at its area.

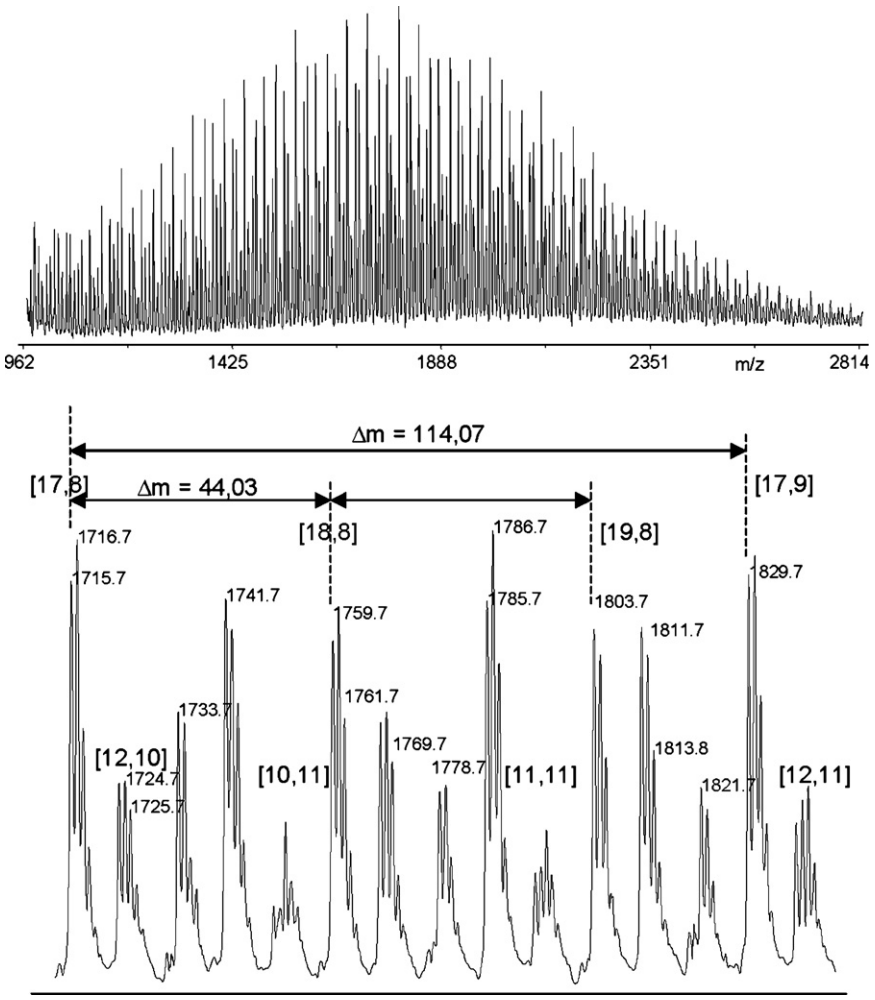


Fig. 2. (a) MALDI-TOF mass spectrum of a PCL-*b*-PEO block copolymer. (b) Enlargement of the spectrum in its central part showing the details of the different peaks. The numbers between brackets are the numbers of CL and EO units of the copolymer.

full copolymer:

$$\text{HLB} = 20 \times \frac{M_\text{n}\text{PEO}}{M_\text{n}\text{copolymer}}$$

3.2. Emulsions of polycaprolactone by nanoprecipitation

3.2.1. Fabrication of the emulsions

The block copolymer has to be compatible with the emulsification process, the nanoprecipitation process in the present case. It is known that polymers adsorb quite slowly to the surface of particles (Riess, 2003; Gohy, 2005), so that the emulsification is quite difficult and might be unsuccessful. However, some well-known polymer emulsifiers such as poly(vinyl alcohol) or (ethylene oxide)/(propylene oxide) block copolymers (Pluronic®) allow the preparation of suspensions of polyester nanoparticles. The purpose of this part of the study was to identify which PCL-*b*-PEO block copolymers were useful and how to use them properly for the nanoprecipitation of PCL.

Table 1
Chemical structure and HLB of the PCL-*b*-PEO polymeric emulsifiers

PEO(n)	PCL(m)	HLB
8	10	4.6
17	5	11.2
17	10	7.8
45	20	9.3
113	10	16.2
113	20	13.6
113	25	12.6
113	30	11.7
113	40	10.3

All copolymers listed in Table 1 were soluble in acetone. The only water-soluble copolymer was PCL(10)-*b*-PEO(113). It was indeed the compound of highest HLB number in the series.

Polycaprolactone emulsions have been prepared using the nanoprecipitation process. Nanoprecipitation was also sometimes called "spontaneous emulsification": a PCL solution in acetone was emulsified without energy supply by dispersing it into a larger volume of water. Depending on its solubility, the copolymer emulsifier was introduced either in the organic phase together with PCL, or dissolved in the aqueous phase.

The block copolymer can be incorporated in either the aqueous phase or the organic phase. It is common practice to use the emulsifier in the solvent where it is soluble, which is the continuous phase of the emulsion. Indeed, according to the Bancroft rule, water-soluble emulsifiers (high HLB) allow the stabilization of oil-in-water emulsions and the reverse holds for oil-soluble emulsifiers (low HLB). The Bancroft rule is empirical and holds for conventional processes and formulations, that is, to emulsions made by means of

Table 2
Properties of aqueous suspensions of PCL nanoparticles stabilized with various PCL-*b*-PEO block copolymers incorporated in the organic phase

Copolymer	HLB	Appearance of the suspension	Particle diameter (nm)
PCL(10)-*b*-PEO(8)	4.6	Immediate precipitation	
PCL(5)-*b*-PEO(17)	11.2	Precipitate + milky suspension	
PCL(10)-*b*-PEO(17)	7.8	Precipitate + milky suspension	
PCL(20)-*b*-PEO(45)	9.3	Milky suspension	280
PCL(10)-*b*-PEO(113)	16.2	Milky suspension	256
PCL(20)-*b*-PEO(113)	13.6	Milky suspension	204
PCL(25)-*b*-PEO(113)	12.6	Milky suspension	229
PCL(30)-*b*-PEO(113)	11.7	Milky suspension	220
PCL(40)-*b*-PEO(113)	10.3	Milky suspension	210

The recipe was: [PCL] = 0.4%, [PCL-*b*-PEO]/[PCL] = 1/2.

high shearing mechanical processes with classical non-ionic emulsifiers. Therefore, some exceptions might be found and the present case is indeed an exception to this empirical rule because the emulsifiers are not classical and the emulsification process by means of spontaneous emulsification directs the emulsion towards oil-in-water, whatever the type of emulsifier.

Since all block copolymers are soluble in acetone, all of them were incorporated in the organic phase. Stable emulsions could be prepared in the favorable cases given in Table 2. In such cases, the mixture turned milky when the acetone phase was poured into the aqueous phase; there was no solid precipitate, neither at the bottom nor on the top of the suspension. The rate of addition of the organic phase did not affect the final properties.

The PCL(10)-*b*-PEO(113) copolymer which was soluble both in water and acetone allowed to make a comparison regarding the phase solubilising the emulsifier. Thus, it was possible to prepare stable emulsions with the PCL(10)-*b*-PEO(113) block copolymer, whatever the phase it was introduced in. There was a slight difference of particle size: for the same recipe ([PCL] = 0.4%, [PCL-*b*-PEO]/[PCL] = 1/2), the particle size was 256 nm when the block copolymer emulsifier was introduced in the organic phase against 210 nm when it was introduced in the aqueous phase. Therefore, as a general rule, the block copolymer emulsifier should better be incorporated in the organic phase when the nanoprecipitation process was applied. This is a specific property of the nanoprecipitation process.

Regarding the optimum choice of the block copolymer emulsifier, Table 2 gives the observations concerning the stability of the dispersions just at the end of their preparation. Stable milky suspensions could only be prepared with copolymers having a PEO chain of molar mass between 750 and 2000 g/mol ($17 < n < 45$). Decreasing the length of the PEO block caused the partial flocculation, which most often took place immediately. The block copolymer PCL(10)-*b*-PEO(17) is close to the limit since it allows the complete emulsification of PCL; but a slow coagulation takes place after the preparation of emulsions. The limit for the PEO block length is them estimated at 1000 g/mol ($n = 22$). The length of the PCL block looked of less importance. It is obvious that a too small hydrophobic part would be disastrous with respect to the stabilization of the suspensions because such emulsifier would not adsorb at the surface of the PCL particles. But such a situation was not encountered within the series investigated. Even the PCL(10)-*b*-PEO(113) which is soluble in water allowed an efficient stabilization. Lastly, the HLB does not work satisfactorily for predicting the stability. The general trend was that a minimum HLB was required. But the limit was not well defined since PCL(5)-*b*-PEO(17) of HLB 11.2 did not ensure the stabilization whereas PCL(40)-*b*-PEO(113) of HLB 10.3 allowed an efficient stabilization; and it is likely that block copolymers having longer PCL chain would work equally well.

3.2.2. Particle sizes

In the case of stable suspensions, the sizes of the particles have been measured by means of dynamic light scattering. For a given recipe ([PCL] = 0.4%, [PCL-*b*-PEO]/[PCL] = 1/2), the particle size did not depend so much on the type of copolymer emulsifier (Table 2). The particle sizes were between 200 and 300 nm in every instance. The particle size was better controlled by the emulsification process than by the emulsifier content. The formulation parameter that allows the control of the particle size in a large domain is the PCL concentration in the organic phase (acetone) (Ganachaud and Katz, 2005).

The choice and concentration of the emulsifier allowed the control of the particle size in the narrow range available. It is worth noting that the most hydrophilic block copolymer which is soluble in water departed from the four other polymers which are not soluble in water. Therefore, the water solubility appears a relevant parameter regarding the control of the particle size. In the case of the water-soluble copolymer PCL(10)-*b*-PEO(113), increasing the polymeric surfactant content in the formulation caused the particle size to decrease (Fig. 3). This is the expected trend which is indeed observed with classical surfactants. This was not observed with the copolymers that are not soluble in water however. The comparison with classical surfactants cannot be done in that case however because classical surfactants of low HLB do not stabilize oil-in-water emulsions but water-in-oil.

As a last important observation, emulsifiers that were not soluble in water could stabilize quite well oil-in-water emulsions. This contradicted the Bancroft rule (water-soluble surfactant gives oil-in-water emulsion; oil-soluble surfactant gives water-in-oil emulsion). Thus, the nanoprecipitation process forced the emulsion type as oil-in-water, so that there was no chance for a water-in-oil emulsion to form. But the emulsions were stable and did not phase invert. The same experiment with classical surfactants of low HLB would have given an unstable oil-in-water emulsion that would rapidly phase invert into a water-in-oil emulsion, possibly leaving a non-emulsified part of pure water. The preparation of water-in-oil emulsion with insoluble amphiphilic copolymers is a difficult task that was also encountered with polysaccharide-based amphiphilic polymers (Chauvierre et al., 2004). A nice alternative to direct emulsification is *in situ* formation of the block copolymer during the polymerization process (Bertholon et al., 2006).

The ethylene oxide/propylene oxide triblock copolymer surfactant Pluronic® F68 is widely used for the fabrication and stabilization of emulsions. It is quite a satisfactory polymeric

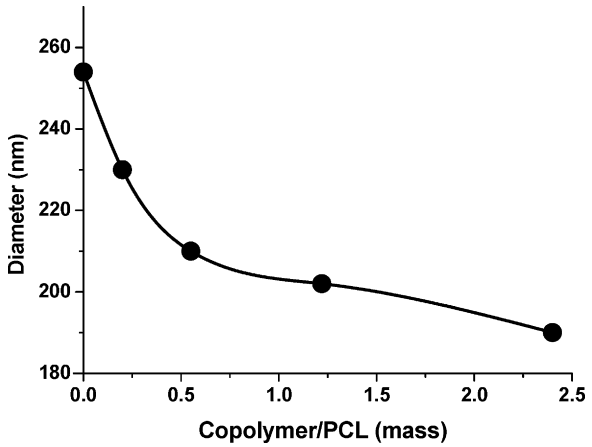


Fig. 3. Particle size as a function of the amount of PCL(10)-*b*-PEO(113) copolymer emulsifier introduced in the aqueous phase. The emulsion prepared without emulsifier was not stable but its size could be measured immediately after its preparation.

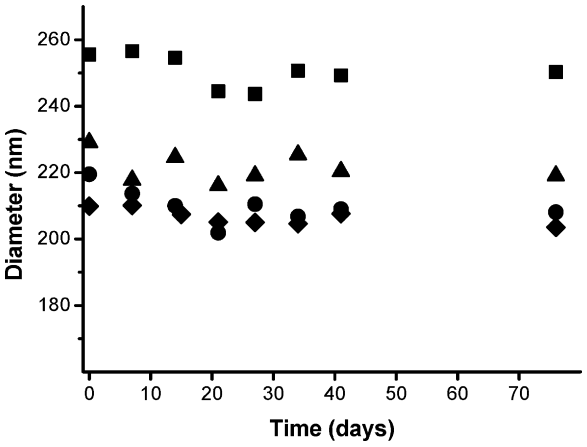


Fig. 4. Assessment of colloidal stability: particle size against storage time at 20 °C. X_n(POE) = 113; X_m(PCL) = 10 (■); 25 (▲); 30 (●); 40 (♦).

emulsifier that often allows reaching smaller particle sizes than other surfactants when conventional mechanical emulsification processes are used (Seijo et al., 1990; Frisbee and McGinity, 1994; Guinebretière et al. 2002). This is not the case in the present case since the mechanism of particle formation by means of the spontaneous emulsification process is completely different of mechanisms involved in the classical processes. The choice and the concentration of emulsifier do not influence significantly the particle size, as it has been observed in the present investigation of a series of different block copolymers. The control of the particle size comes from quite different parameters such as the supersaturation after the organic and aqueous phases have been mixed (Ganachaud and Katz, 2005). Therefore, the use of Pluronic® F68 as an emulsifier gave the same particle size (250 nm for [PF 68]/[PCL] = 1/2) as for the PCL-*b*-PEO block copolymers.

3.2.3. Stability of the emulsions

The colloidal stability of the dispersions has been evaluated in a classical way by measuring the particle size against storage time over 4 months (Fig. 4). The particle size did not significantly vary over this storage at 4, 20 and 40 °C. PF 68 also gave stable emulsions over 4 months. The emulsions that were stable immediately after their preparation showed excellent long-term stability. Coagulation took place very fast after the emulsification in cases of unstable emulsions prepared with unsuitable block copolymer emulsifiers. It was not possible to find a significant difference of emulsion stability between the block copolymers PCL(x)-*b*-PEO(113) with $10 < x < 40$.

3.3. Miniemulsion polymerization

Another interesting application for these polymer surfactants is the colloidal stabilization for the miniemulsion polymerization process with non-ionic polymer emulsifiers.

3.3.1. Description of the miniemulsion polymerization process

The miniemulsion polymerization process consists in the preparation of a submicronic emulsion of vinyl monomer droplet wherein the radical polymerization is initiated (Asua, 2002; Antonietti and Landfester, 2002; Landfester, 2003). The polymerization takes place inside each droplet as an isolated micro-reactor, whatever the choice of the initiator, either hydro-soluble or organo-soluble. Since the monomer is slightly polar, the very fine emulsion may be coarsened by the Ostwald ripening mechanism. A supplementary compound called "the hydrophobe" is added to the organic phase in order to stabilize the miniemulsion against Ostwald ripening. The presence of the hydrophobe is an absolute requirement; low rates are generally used. Hexadecane is the most popular hydrophobe used in polymer chemistry. Since a hydrophobic compound is needed to protect droplets against monomer diffusion, the idea was to use these dispersed systems for the encapsulation of hydrophobic compounds. Therefore, a hydrophobic compound of pharmaceutical or cosmetic utility was used instead of the classical hydrophobe. The challenge was to adapt the classical recipe of the miniemulsion polymerization process in order to meet the constraints of a possible biomedical or cosmetic application. This has been achieved in previous work, where Miglyol was used as hydrophobe instead of hexadecane (Rajot et al., 2003). Furthermore, since the hydrophobe has become a useful ingredient of the formulation, the amount of hydrophobe was increased with respect to the conventional recipes of miniemulsion polymerization process (Rajot et al., 2003). In the present case, vitamin E acetate was selected as the hydrophobe. Finally, since totally non-ionic particles are desired, initiators that do not give charged primary radicals have been selected. Therefore, the ammonium persulfate initiator that is widely used in emulsion polymerization processes has been replaced by a water soluble neutral system. The redox initiator system comprising hydrogen peroxide and ascorbic acid produces neutral hydroxyl radicals $OH^{\bullet}$ as hydrogen peroxide is added to the reaction medium according to the redox reaction given in Fig. 5. This initiator was preferred to the well-known Fenton salt (Fe^{2+}/H_2O_2) because the later produces ferric ions that may not be accepted regarding the pharmaceutical application. The polymerization rate can easily be controlled through the rate of hydrogen peroxide feeding the miniemulsion reactor containing ascorbic acid. The feed rate of hydrogen peroxide is adjusted such that the concentration of hydroxyl radicals is slightly lower than the number density of emulsion droplets N_p. Typically, $0.1 \times N_p < [OH^{\bullet}] < N_p$.

A large concentration of ascorbic acid allows a high initiation rate and a fast polymerization kinetic. But H^+ ions are also produced together with the primary radicals, leading to a decrease of the pH to fairly acidic (pH 1–3); this might appear incompatible with some fragile active substances unless adjusting the pH as hydrogen peroxide is fed.

3.3.2. Encapsulation of high load of vitamin E

The aim of this study was not only to check the feasibility of the colloidal stabilization of miniemulsion polymerization, but also to get dispersed systems containing high amounts of the active substance used as hydrophobe component. The amount of hydrophobe components is minimized in classical recipes of miniemulsion polymerization since it is brought only for stabilizing the miniemulsion of monomer. The development of miniemulsion polymerization systems for encapsulation purpose requires to work with dispersed systems containing high contents of drugs; this can be obtained either by increasing the concentration of the emulsion or by synthesizing highly loaded nanoparticles.

Concentrated primary emulsions of vinyl acetate monomer could be prepared using the ultrasound disperser. Such emulsions were of submicronic size using the highest power available with the equipment used. The size of the primary emulsions was below 200 nm. The same size of the emulsions was measured at the end of polymerization, showing the successful miniemulsion polymerization. The absence of shift of the droplet diameter during the polymerization process indicated that the polymerization took place independently in each monomer droplet, as was expected regarding the miniemulsion polymerization process. A typical hydrophobe content of the conventional miniemulsion polymerization is 5% with respect to the organic phase. This content was increased in the present case. The organic phase could be loaded with up to 50% vitamin E acetate, corresponding to a

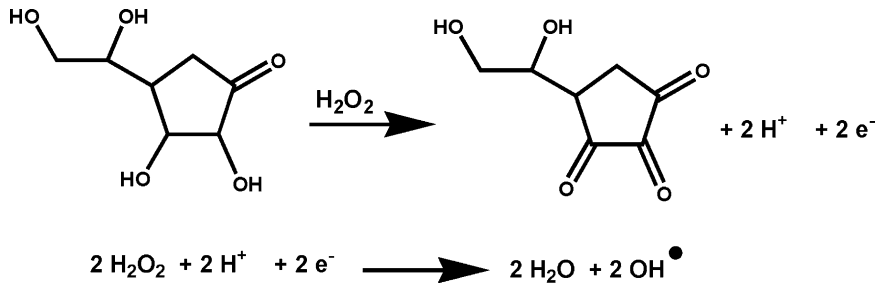


Fig. 5. Redox initiation reaction with the ascorbic acid/hydrogen peroxide system.

concentration active substance in the full emulsion of 25%. Stable aqueous suspensions of nanoparticles were produced, the size of the particle being lower than 200 nm in every instance.

3.3.3. Polymerization kinetics

The typical kinetic curves (Fig. 6) show that the miniemulsion polymerization process can be successfully used for the encapsulation of vitamin E acetate in poly(vinyl acetate) particles. Indeed, high conversions could be achieved within less than 4 h polymerization time. The polymerization started as soon as hydrogen peroxide was fed into the reactor. The absence of lag-time in the polymerization kinetics indicated that vitamin E did not delay the polymerization. Indeed, the vitamin E active substance is an antioxidant that might have trapped radicals. Obviously, vitamin E could be used safely in a radical polymerization process. The end of the polymerization is slow as in every polymerization performed in batch. The polymerization of the last monomer molecules would require a curing step at higher temperature or the addition of the hydrogen peroxide shot at the end. It is more advisable to leave the residual monomer since it can easily be cleared from the emulsion by bubbling nitrogen gas under stirring. The elimination of residual monomer was complete as checked by gas chromatography analyses.

3.3.4. Mode of initiation of the radical polymerization

Either water-soluble or oil-soluble initiators can be used. The primary radicals were generated either in the aqueous phase or inside the monomer droplets accordingly. Since the solubility of vinyl acetate in water is not very low, working with a water-soluble initiator may cause the polymerization to take place in the aqueous phase for a long part of the polymerization time. This would cause possible troubles with secondary nucleation of particles during the course of the polymerization. The formation of water-soluble oligomers can occur during the polymerization course. Such low molar mass polymers might precipitate as they grow, creating new particles. They might also be captured by emulsion particles; the entry of radicals into the particles is favorable event since the polymerization resume inside the droplets according to expectation. The secondary nucleation widens the particle size distribution characterized by the polydispersity index. The low polydispersity index measured by light scattering indicated that such secondary nucleation had a negligible effect. Hydrophobic initiators such as lauroyl peroxide (LP) or AIBN circumvent this drawback since radicals are generated inside the emulsion particles, limiting the possible secondary nucleation processes. The water-soluble system (hydrogen peroxide + ascorbic acid) is preferred because the generation of radicals takes place by means of a redox reaction that does not require heating. The polymerization can be carried out at moderate temperature (40 °C). On the contrary, LP and AIBN give rise to radicals through thermal decomposition which requires quite elevated temperature (70 °C). The highly hydrophobic initiator lauroyl peroxide gave very satisfactory results. The kinetics of polymerization initiated by the hydro-soluble redox system and the hydrophobic lauroyl peroxide were quire similar, although the polymerization temperatures were different (Fig. 6). It was therefore quite likely that the polymerization mechanisms were identical, that is, the propagation reaction took place inside the droplets with both initiators. Since $OH^•$ radicals were generated in the aqueous phase, their capture into the monomer droplets is very fast. The comparison of the hydrophilic and hydrophobic initiators showed that hydrophilic initiators that work at low temperature can be used.

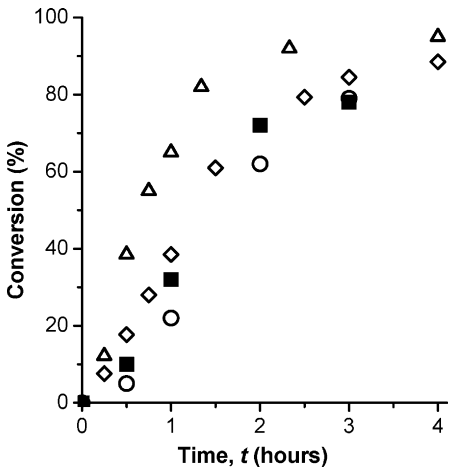


Fig. 6. Polymerization kinetics of vinyl acetate in miniemulsion using either PCL-*b*-PEO or Pluronic® F68 block copolymer emulsifiers. The recipes are those given in Section 2. (■) PCL-*b*-PEO 9%, redox system; (○) PF 68 9%, redox system; (△) PF 68 1.6%, redox system; (◊) PF 68 1.6%, LP.

3.3.5. Influence of the emulsifier type

For sake of comparison, the colloidal stabilization by a classical triblock copolymer was also investigated. The Pluronic® F68 (PF68) is a ethylene oxide/propylene oxide triblock copolymer $(EO)_n$-*b*-$(PO)_m$-*b*-$(EO)_n$ which is widely used by chemists to stabilize emulsions. The molar mass of PF68 is $M_n \approx 8400$ g/mol and it contains 80% EO; the corresponding polymerization degrees of the blocks are $n \approx 72$ and $m \approx 36$. The final particles sizes as well as the polymerization kinetics (Fig. 6) of the systems stabilized by PCL-*b*-PEO and PF68 do not differ significantly, showing that the bock copolymer PCL-*b*-PEO can be safely used instead of the Pluronic® F68. The advantage of using a PCL-based emulsifier is the biodegradability. Emulsions could also be prepared with the Brij® 700 emulsifier according to the same process.

3.3.6. Stability of the emulsions

Another important parameter which deserves investigation is the stability of the drug itself during the radical polymerization. The kinetics of polymerization indicated that vitamin E did not influ-

Table 3
Vinyl acetate miniemulsion polymerization with high load of vitamin E acetate used as hydrophobe component

Polymer surfactant	Organic phase (%)	Hydrophobe (%)	Surfactant (%)	Initiator	Particle diameter (nm)–PI
PCL-*b*-PEO	55	54.9	9	Redox	190–0.1
Pluronic® F68	55	54.9	9	Redox	170–0.1
Brij® 700	55	54.9	9	Redox	160–0.1

Comparison of the polymer surfactants (45 g water, 4.5 g surfactant (9%), 24.8 g AcV, 30.2 g vitamin E acetate).

Table 4
Assessment of stability for 1 month at 3 temperatures for 3 emulsifiers by means of measurements of the diameters of the particles, *D*, and polydispersity index, PI, as a function of storage time

Emulsifier	Time, *t*	4 °C		20 °C		40 °C	
		D (nm)	PI	*D* (nm)	PI	*D* (nm)	PI
PCL-*b*-PEO	*t* = 0	160	0.09	160	0.09	160	0.09
	1 week	172	0.09	178	0.19	178	0.16
	2 weeks	206	0.17	203	0.09	208	0.06
	3 weeks	178	0.10	191	0.06	231	0.17
Pluronic® F68	*t* = 0	170	0.13	170	0.13	170	0.13
	1 week	161	0.12	182	0.14	190	0.20
	2 weeks	177	0.18	169	0.04	210	0.12
	3 weeks	190	0.24	194	0.29	213	0.13
Brij® 700	*t* = 0	160	0.10	160	0.10	160	0.10
	1 week	179	0.12	175	0.11	187	0.12
	2 weeks	178	0.13	179	0.15	192	0.13
	3 weeks	183	0.13	206	0.05	220	0.06

t = 0 corresponds to the end of the polymerization reaction. The polydispersity index is given by the method of cumulants ($PI = \mu^2/\Gamma$). The recipe was: 45 g water, 4.5 g surfactant, 24.8 g AcV, 30.2 g vitamin E acetate, 2 g H_2O_2/2 g ascorbic acid.

ence the course of the polymerization. Titration of vitamin E at the end of the polymerization was performed in order to check against any possible reaction of vitamin E with radicals coming from the initiator or the growing polymer chains. Thus, the concentration of vitamin E acetate was measured in the miniemulsion by HPLC; the same concentration was found before and after polymerization. Vitamin E resisted the presence of radicals and the temperature of 40 °C for 5 h during polymerization.

Finally, the emulsion stability was measured at three temperatures (4, 20 and 40 °C) upon long storage time. Pluronic® F68 and Brij® 700 ($C_{18}H_{37}(OCH_2CH_2)_nOH$, $n \approx 100$) were also used as surfactant for comparison. The lengths of PEO chains are of the same order for every emulsifier, between 75 and 115 ethoxylation degree. Therefore they should provide the same steric stabilization of the colloidal suspension. The miniemulsion formulations were the same for the three experiments, namely 45 g water, 4.5 g surfactant, 24.8 g vinyl acetate, 30.2 g vitamin E acetate, 2 g H_2O_2 /2 g ascorbic acid.

As reported in Table 3, the particle sizes were in the same range whatever the polymer surfactant. The same trends were noticed for all the latexes which displayed a very good stability at 4 and 20 °C. A slight increase of the particle size was observed at 40 °C in the stability test however (Table 4). This trend may be due to accelerated ageing caused by higher temperature, the origin could lie in the temperature dependence of the POE block as well. POE is indeed known to collapse as the temperature is increased, reducing its stabilizing efficiency.

4. Conclusion

The block copolymer emulsifiers of the PCL-*b*-PEO type were found efficient for the preparation and stabilization of aqueous emulsions of PCL and as emulsifiers in the miniemulsion polymerization process. Such block copolymers are readily available since their synthesis is quite simple and could be scaled up easily. They have similar stabilizers efficiencies as the well-known ethylene oxide/propylene oxide triblock copolymers. The main benefit of using PCL-*b*-PEO block copolymers is the biodegradability of the PCL block.

The systematic study as a function of the block lengths has shown that a long enough PEO block is required, typically 1000 g/mol ($n = 22$). The copolymers that show negative results do not allow the emulsification of PCL by the nanoprecipitation process or give rise to unstable suspensions that coagulate and settle very fast. Even when the copolymer is soluble in water, it is better to introduce it in the organic phase against the Bancroft rule during the emulsification. The typical particle sizes are 200–300 nm with all copolymer emulsifiers investigated with $n = 45$ or 113. Such suspensions are stable for several month's storage and resist quite well to coagulation by electrolytes.

The successful utilization of such polymer surfactants that are not soluble in water is also of interest since all the emulsifiers macromolecules are at the surface of the particles. Therefore, the efficiency is a maximum and there is no residual surfactant left in the aqueous phase. This would not have been possible, using conventional emulsifiers that obey Bancroft rule.

The miniemulsion polymerization process gives concentrated suspensions of nanoparticles that contain high loads of active substance. The PCL-*b*-PEO block copolymers allow the stabilization of the particles during the polymerization process and on long-term storage. Their emulsifying properties are similar to emulsifiers that are well-known for their excellent efficiency. PCL-*b*-PEO block copolymers can replace ethylene oxide/propylene oxide triblock copolymers advantageously.

The present copolymers are good candidates for utilization in the formulation of emulsion made of PCL, but also of other polymers and various oils. Although the present study has been performed with the nanoprecipitation process only, it is likely that such polymeric emulsifiers be well suited for their implementation into different emulsification processes.

The strong adsorption behaviour is an obvious advantage and the absence of surfactant in the aqueous phase is a direct consequence of it. The surfactant molecules in solution in the aqueous phase cause several troubles. In particular they are bioavailable; so that they are responsible for the possible biological effects such as hemolysis, irritancy.

Non-adsorbed polymer left in the aqueous phase is also a problem in classical emulsification technologies because its presence increases the viscosity of the continuous phase of the emulsion. For example, emulsions made with large concentrations of poly(vinyl alcohol) as emulsifier are quite viscous and the consequence is a larger droplet size for a given emulsification process (Murakami et al., 1997; Guinebretière et al., 2002). Even water-soluble block copolymers would not increase too much the viscosity of the aqueous phase because they self-associate as micelles in solution.

The utilization of PCL-*b*-PEO block copolymers in pharmaceutical applications requires further more detailed evaluation of their toxicity and their behaviour *in vivo*. In particular, the evaluation

of their resistance to elimination by the immune system and their compatibility with other compounds transported in the blood circulation is of major importance.

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