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Monoglyceride-based self-assembling copolymers as carriers for poorly water-soluble drugs

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ABSTRACT

To develop self-assembling polymers forming polymeric micelles and increasing the solubility of poorly soluble drugs, amphiphilic polymers containing a hydrophilic PEG moiety and a hydrophobic moiety derived from monoglycerides and polyethers were designed. The biodegradable copolymers were obtained via a polycondensation reaction of polyethylene glycol (PEG), monooleylglyceride (MOG) and succinic anhydride (SA). Polymers with molecular weight below 10,000 g/mol containing a minimum of 40 mol% PEG and a maximum of 10 mol% MOG self-assembled spontaneously in aqueous media upon gentle mixing. They formed particles with a diameter of 10 nm although some aggregation was evident. The critical micellar concentration varied between 3×10^{-4} and 4×10^{-3} g/ml, depending on the polymer. The cloud point ($\geq$66 °C) and flocculation point ($\geq$0.89 M) increased with the PEG chain length. At a 1% concentration, the polymers increased the solubility of poorly water-soluble drug candidates up to 500-fold. Drug solubility increased as a function of the polymer concentration. HPMC capsules filled with these polymers disintegrated and released model drugs rapidly. Polymer with long PEG chains had a lower cytotoxicity (MTT test) on Caco-2 cells. All of these data suggest that the object polymers, in particular PEG1000/MOG/SA (45/5/50) might be potential candidates for improving the oral biopharmaceutical performance of poorly soluble drugs.

1. Introduction

Among the different approaches which are well-known as potential modalities to increase the solubility of poorly water-soluble drugs, lipid-based delivery systems have encountered great interest. S(M)EDDS (Self-(micro)emulsifying drug delivery systems) consists of blends of mono-, di- and tri-glycerides, hydrophobic surfactants and/or water-soluble cosolvents. These compositions, while complex with regard to development, improved bioavailability and reduced intra- and inter-individual variability (Shah et al., 1994; Lawrence and Rees, 2000; Strickley, 2004; Porter et al., 2008; Jannin et al., 2008). An example of the beneficial properties of such systems is the reformulation of cyclosporin A as the marketed dosage form Neoral® in which the drug is compounded with medium-chain-length partial glycerides, a medium-chain-length triglyceride oil and surfactants. This formulation evolved from the first commercially available form, Sandimmun® which could be characterized as a coarse emulsifying system. Systems containing polyglycolyzed glycerides with varying glycerides and polyethyleneglycol (PEG) chain lengths have also been reported as high loading capacity systems (Shah et al., 1994; Chambin and Jannin, 2005).

The use of lipid moieties as hydrophobic blocks coupled to hydrophilic PEG chains can provide additional advantages for system stability when compared with conventional amphiphilic polymeric micelles due to the existence of acyl groups which may contribute to increased hydrophobic interactions between the polymeric chains in the micellar core. Different lipid-capped polymers, including diacyllipid-PEG, PEG-phosphatidylethanolamines and fatty acid-PEG conjugates have been shown to form micelles and to efficiently incorporate poorly soluble drugs resulting in improved apparent solubility (Torchilin, 2001; Torchilin, 2002; Lee et al., 2003; Lukyanov and Torchilin, 2004; Torchilin, 2007). These amphiphilic polymers of a polymer-lipid type form very stable micelles with low critical micellar concentration (CMC) values in aqueous media which remain stable in biological fluids and

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demonstrate prolonged circulation times *in vivo*. They can be effectively loaded with a number of poorly soluble pharmaceuticals and hydrophobic or amphiphilic diagnostic agents. The grafting of ligands onto the micelle surface was shown to preserve their specific activity and may target micelles to specific areas in the body (Torchilin, 2007).

However, the loading of these micelles usually requires the dissolution of the actives in organic solvents which can be toxic or provoke undesirable side effects and need to be eliminated (Lee et al., 2003; Lukyanov and Torchilin, 2004; Torchilin, 2007). As most of the polymeric micelles require organic solvent and/or long manufacturing procedures, there is a need to develop new polymers with self-assembling properties.

In our laboratory, diblock copolymers made of caprolactone (CL) and trimethylene carbonate (TMC) initiated by monomethoxylated PEG, mmePEG750-p(CL-co-TMC)(50/50), have been developed as self-assembling systems to solubilize poorly water-soluble drugs (Ould-Ouali et al., 2004). Results have shown that these copolymers self-assemble in water without the need of organic solvents. The polymeric micelles significantly improve the solubility of several BCS class II compounds. Solubility enhancement depended strongly on the interactions between the drug and the polymers (Latere Dwan'Isa et al., 2007).

To improve the solubilizing properties of the self-assembling polymers for preclinical and clinical studies, new polymers containing lipids were designed. The rationale for their synthesis included: (i) for self-assembling properties, copolymers should contain a hydrophilic PEG moiety and a lipophilic moiety. As they need to be liquid at room temperature and at 37 °C, low molecular weight copolymers were used (ii) to improve solubilizing properties and compatibility with drugs, the lipophilic moiety of the polymer was formed by a mixture of lipid (monoglyceride) and diacid (e.g., succinic acid). Therefore, a novel group of amphiphilic copolymers based on C_{18} monoglycerides, succinic anhydride and polyethylene glycol was synthesized.

The aim of the present work was to evaluate the self-assembling properties of the newly synthesized copolymers and to characterize the colloidal systems formed. The evaluation of the self-assembling properties of the copolymers was based on the construction of ternary (polymer–water–poloxamer 105) and binary phase diagrams (polymer–water). The size and zeta potential of the particles were determined. The CMC as well as the cloud and flocculation points were measured. The solubility of BCS class II drugs in the polymeric solutions was assessed. The cytotoxicity of the polymers was evaluated in Caco-2 cells. The in vitro release of model compounds from the self-assembling formulations was measured as was the disintegration and dissolution behaviour of capsules filled with the polymers of interest.

2. Material and methods

2.1. Materials

Monostearoylglycerol (MSG) Myverol 1806 CH_3–$(CH_2)_{16}$–COO–CH_2–CHOH–CH_2OH and monooleylglycerol (MOG) Myverol 1899 CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$–COO–CH_2–CHOH–CH_2OH were purchased from Quest International. Other monomers were purchased from Sigma–Aldrich or Fluka. Poloxamer 105 was a gift from ICI surfactants (Cleveland, UK). All chemical reagents used for the Caco-2 culture were purchased from Invitrogen Life Technologies (Merelbeke, Belgium). All the other reagents were purchased from Sigma–Aldrich. ^{14}C-labelled and non-labelled risperidone and ketoconazole were provided by Janssen Pharmaceutica (Beerse, Belgium). Hydrocortisone, cyclosporin and indomethacin were purchased from Omega Pharma (Nazareth, Belgium).

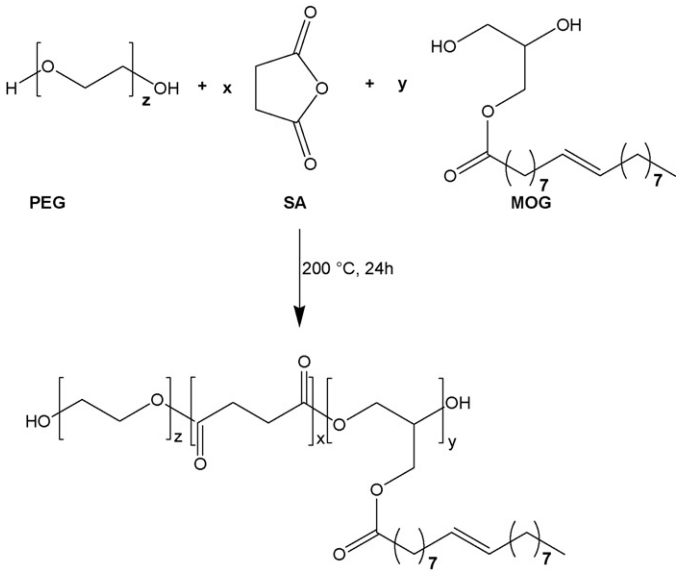

Fig. 1. Synthesis scheme of copolymers made of polyethylene glycol (PEG), monooleylglycerol (MOG) and succinic anhydride (SA) by polycondensation.

2.2. Polymer synthesis and characterization

The copolymers were synthesized by polycondensation at the Johnson & Johnson-Center of Biomaterials and Advanced Technologies (Somerville, NJ, USA) according to the synthesis pathway reported in Fig. 1. For the PEG400/MOG/SA (45/5/50 mol/mol) polymer, for instance, 13.1 g (0.037 mol) of monooleyl glycerol (Myverol 1899) and 132.14 g (0.33 mol) of polyethylene glycol (MW 400 g/mol) were placed under nitrogen and the temperature was raised to 140 °C. Once at 140 °C, the 36.67 g (0.367 mol) of succinic anhydride were added and the temperature was increased to 200 °C. The reaction was maintained at 200 °C for 24 h. The reaction flask was removed from the oil bath and allowed to cool. The polymer was stored under nitrogen. The other polymers were synthesized using a similar procedure by adapting the monomers and their quantities (Table 1).

The polymer composition and residual monomer content were analysed by proton NMR. The copolymers were dissolved in hexafluoroacetone sesquideuterate and deuterobenzene or deuterated chloroform. Spectra were taken using a Unity-Plus 400 NMR spectrometer. The ratios of the various monomers in the polymer were determined by integrating the methylene and methyl resonances in the 0 to 7.5 ppm spectral region and calculating the mole percent of each monomer in the polymer from the normalized surface area of the respective monomers (polymerized and monomer form).

Gel permeation chromatography was employed to determine the molecular weight and the polydispersity of the polymers. A Waters Alliance 2690 separation module equipped with a Wyatt Optilab DSP refractometer, a Dawn multi-angle laser photometer (Wyatt), and Waters Styragel HR 3–4 columns was used. Polystyrene standards were used for calibration. HPLC grade tetrahydrofuran or hexafluoroisopropanol were used as solvent and mobile phase.

2.3. Assembling and self-assembling properties

The feasibility to obtain emulsifying systems with the copolymers as lipophilic phase was evaluated by the construction of ternary phase diagrams. Binary systems containing various ratios of polymer and Poloxamer 105 ranging from 10/90 w/w to 90/10 w/w were prepared and equilibrated at 37 °C. Ultrapure water was added at 37 °C to the polymer-surfactant mixture aliquot by aliquot.

Table 1
Apparent weight-averaged molecular weight (M_W) and polydispersity (PD) of monoglyceride-based pegylated copolymers determined by gel permeation chromatography.

Polymer composition (mol%)			M_w[a]	PD[a]	Clear solution[b]	Maximum amount of water in clear solution[b]	Self-assembling properties[c]
Saturated monoglyceride-based polymers							
2.5% PEG 400	47.5% MSG	50% SA	28620	6.1	No		No
2.5% PEG 1000	47.5% MSG	50% SA	16000	4.2	No		No
2.5% mmePEG2000	47.5% MSG	50% SA	7758	2.2	No		No
2.5% mmePEG5000	47.5% MSG	50% SA	19884	4	No		No
5% PEG 600	45% MSG	50% SA	21307	5.2	No		No
5% mmePEG750	45% MSG	50% SA	11779	3.5	No		No
30% PEG 600	20% MSG	50% SA	41088	2.06	o/w	30%	No
30% PEG 900	20% MSG	50% SA	3813	1.95	No		No
40% PEG 400	10% MSG	50% SA	3827	1.9	o/w	90%	No
45% PEG 400	5%MSG	50% SA	3226	1.94	o/w	90%	No
45% PEG 600	5% MSG	50% SA	3270	1.97	o/w	50%	No
Unsaturated monoglyceride-based polymers							
20% PEG1000	30% MOG	50% SA	5500	1.6	ND		No
20% PEG2000	30% MOG	50% SA	5495	1.4	ND		No
25% PEG 400	25% MOG	50% SA	3370	2.11	ND		No
25% PEG 400	25% MOG	50% SA	3300	2.3	ND		No
40% PEG600	10% MOG	50% SA	4160	1.8	o/w	> 99%	Yes
40% PEG1000	10% MOG	50% SA	4600	2.2	o/w	> 99%	Yes
45% PEG 400	5% MOG	50% SA	3366	2.04	o/w	> 99%	Yes
			3784	1.6	o/w	> 99%	Yes
			3497	2.24	o/w	> 99%	Yes
			3421	1.9	o/w	> 99%	Yes
			3770	2.3	o/w	> 99%	Yes
			3348	2.3	o/w	> 99%	Yes
			3531 ± 197	2 ± 0.3			
45% PEG400	5% MOG	25% SA, 25% PGDA	3169	2.5	o/w	> 99%	Yes
45% PEG400	5% MOG	50% PGDA	4051	2.5	o/w	> 99%	Yes
45% PEG600	5% MOG	50% SA	3020	1.9	o/w	> 99%	Yes
45% PEG1000	5% MOG	50% SA	4640	2	o/w	> 99%	Yes
			7438	1.5	o/w	> 99%	Yes
45% PEG2000	5% MOG	50% SA	7050	1.5	o/w	80%	Yes
			9719	1.4	o/w	80%	Yes

ND, not determined; PEG, polyethylene glycol; mmePEG, monomethoxylated polyethylene glycol; SA, succinic anhydride; PGDA, PEG600 diacid; MSG, monostearoylglycerol; MOG, monooleylglycerol.
Emulsion formation and self-assembling properties are provided: emulsions were formed by water titration of a mixture of copolymer (lipophilic phase) and Poloxamer 105 (surfactant phase).
[a] M_w and PD (Polydispersity) were determined by GPC.
[b] Ternary diagram water/Poloxamer 105/polymer.
[c] Binary diagram water/polymer.

The systems were evaluated visually and through polarized light for the formation of a clear isotropic solution (microemulsion or micelles).

The self-assembling properties of the copolymers were assessed by adding water gradually to the polymer under gentle stirring. The formation of a clear gel or a clear isotropic solution was evaluated visually and under polarized light (Ould-Ouali et al., 2004).

2.4. Physico-chemical characterization

2.4.1. Particles size

The size of the particles was determined by photon correlation spectroscopy using a Malvern Autosizer 4700 at 25 °C. The measurements were carried out at a scattering angle of 90°.

2.4.2. Surface charge

The zeta potential of the particles formed was determined with a Zetasizer 2000 (Malvern, Worcestershire, UK). The analyses were performed on samples diluted at 20 g/l in water, phosphate buffer or carbonate buffer (pH 4.4, 6.6, 9.6, respectively). Results are the mean of at least 15 measurements.

2.4.3. Critical micellar concentration and micellisation energy

The CMC was measured by the fluorescence probe method. Three milliliters of a pyrene stock solution (10^{-6} M) in acetone were evaporated. Five milliliters of an aqueous solution of the copolymer in phosphate buffer (pH 7, 0.05 M) were then added to the pyrene. The pyrene concentration was fixed at 6×10^{-7} M for all samples. The polymer concentration varied from 10^{-8} g/ml to 0.1 g/ml. Solutions were placed in a water bath at 70 °C with stirring for 1 h and were then degassed for 5 min by bubbling with oxygen-free nitrogen before recording the spectrum at room temperature (Zhao and Winnik, 1990; Ould-Ouali et al., 2004)

Fluorescence spectra were recorded with a SLN 48000 S spectrometer (Aminco) using an excitation wavelength of 334 nm. The selected emission wavelengths were the maximum intensities of the first (I_1: 372–373 nm) and third peak (I_3: 383–384 nm) in the emission spectrum. Each spectrum was corrected for scattering caused by the aqueous polymer solution using a blank solution with an identical polymer concentration in the absence of pyrene. The I_1/I_3 values were averaged over three determinations and plotted versus the polymer concentration. As the polymer concentration in the aqueous solution increased, the I_1/I_3 ratio decreased eventually reaching a plateau level. The CMC was determined at the intersection of the 2 lines obtained by linear regression.

The micellisation energy was calculated as follow (Chen et al., 1998):

$$\Delta G_0 = RT \ln X_{CMC}$$

where R, gas constant = 8.3143 J/K mol; T, temperature in °K; X_{CMC}, concentration at CMC in molar fraction; ΔG_0, micellisation energy in kJ/mole

2.4.4. Colloidal stability

The cloud point was determined by turbidimetry. It is defined as the temperature at which the solution becomes turbid and was determined as the temperature at which the absorbance at 400 nm increased sharply (Jönsson et al., 1998).

The critical flocculation point (i.e. the ionic strength where turbidity starts to increase) was determined in the presence of increasing Na_2SO_4 concentration. One-half milliliter of the 10% (w/v) polymeric solution was added to 2.5 ml of Na_2SO_4 (0 to 0.6 M). The turbidity was measured at 400 nm after 15 min using a Unicam 8625 Spectrophotometer. The critical flocculation point was determined as the ionic strength at which turbidity started to significantly increase (Riley et al., 1999; Ould-Ouali et al., 2004).

The influence of the pH and albumin on the self-assembling properties of the polymers was analyzed by measuring the absorbance of 1% (w/v) polymer solutions at 400 nm using a Unicam 8625 Spectrophotometer. Three different pH conditions were tested (pH 3.3, 6.86 and 8.73) as well as four albumin concentrations (0.5%, 1%, 2% and 4%, w/v).

2.5. Solubilization of poorly soluble drugs

To assess whether the polymer increased drug solubility, an excess of drug was mixed for 24 h with the polymer at room temperature employing a magnetic stirrer. A phosphate buffer (pH 7, 0.05 M) was then added to reach a polymer concentration of 1%, 3.15% or 10%, w/v. The drug–polymer–buffer mixture was stirred for 24 h. The suspension containing the polymer and the drug was filtered through 0.45 μm PVDF membrane filter (Millipore SLHV025LS). The drug concentration was determined immediately by UV spectroscopy against a blank containing the same polymer concentration at room temperature using a UV–vis spectrometer HP8453 (Hewlett Packard). The absorbance was measured at 275, 230, 322, 247 and 226 nm for risperidone, ketoconazole, indomethacin, hydrocortisone and cyclosporin, respectively. The solubility of the drugs in the buffer solution (in the absence of the polymer) was measured by mixing appropriate amounts of the drugs with the phosphate buffer (pH 7.02, 0.05 M), equilibrating the mixture for 24 h and measuring the absorbance of the solution. Data are expressed as mean values of triplicate determinations.

Drug loading content (%) was calculated as

$$\frac{\text{mass of drug}}{\text{mass of drug} + \text{mass of polymer}} \times 100$$

2.6. Drug release

Drug release was evaluated by dialyzing the self-assembling solution containing the drug of interest. The release of risperidone and ketoconazole was studied. Two detection methods were compared: UV spectroscopy and liquid scintillation counting of the radioactive drug.

2.6.1. UV spectrometry

The solutions were prepared by mixing an excess of risperidone (20 mg) with 1% (w/v) PEG400/MOG/SA (45/5/50) for 24 h before adding 10 ml of phosphate buffer (pH 7, 0.05 M). After 24 h mixing, the mixture was filtered through a 0.45 μm filter. One milliliter of the solution was dialysed (Spectra/por cellulose ester, M_w cutoff 1000, Spectrum Laboratories) against 130 ml of buffer. Three ml were taken for each measurement and replaced by 3 ml of buffer. The amount of risperidone in the dialysates was quantified with a spectrometer HP8453 from Hewlett Packard at $\lambda = 275$ nm at room temperature. A standard curve was previously established. The reference was a 1% polymeric solution dialysed in the same conditions. The experiment was performed in duplicate.

2.6.2. Liquid scintillation counting

For the risperidone release study, the 1% (w/v) polymer solutions were prepared as follows: 250 μl of a stock solution of C^{14}-radiolabelled risperidone in ethanol (10 mg drug/ml) were mixed with 50 mg of the polymer PEG 400/MOG/SA(45/5/50), the solvent was evaporated and the system equilibrated for 24 h. The self-assembling solution was then made by adding 5 ml of phosphate buffer (pH 7, 0.05 M) followed by filtration. One milliliter of this solution was placed in the dialysis bag and dialyzed against 225 ml of buffer at 37 °C. Five milliliters were taken for each measurement and replaced by 5 ml of buffer. The experiment was performed in duplicate. The release of risperidone in the absence of the MSGA polymer from the dialysis bag was also studied under similar experimental conditions.

For the ketoconazole release study, the 10% (w/v) polymer solutions were prepared by evaporating the solvent of 460 μl of a C^{14}-radiolabeled ketoconazole stock solution (2.62 mg/ml in ethanol). One hundred fifty milligrams of PEG400/MOG/SA (45/5/50) were added and mixed for 24 h. The self-assembling solution was made by adding 1.5 ml of phosphate buffer (pH 7, 0.05 M) followed by filtration. One milliliter of this solution was placed in a dialysis bag and dialyzed against 250 ml of buffer. Five milliliters were taken for each measurement and replaced by 5 ml of buffer. Since the solubility of ketoconazole was under the detection limit for the analytical method, it was not possible to study its release in the absence of the MSGA polymer in an aqueous medium.

The radioactivity of the samples was determined with a Wallac 1410 liquid scintillation counter (Pharmacia) using Ultima Gold (Packard Bioscience) as the liquid scintillation analyzer. Knowing the radioactivity of the initial solution, it was possible to determine the drug concentration in the dialysate.

2.7. Disintegration and dissolution tests

Disintegration experiments were performed as described in the European pharmacopoeia (2.9.1). HPMC capsules (size 0) were filled with approximately 700 mg of PEG400/MOG/SA (45/5/50) or PEG1000/MOG/SA (45/5/50) and placed in a dissolution vessel. The medium used was ultra-pure water and the temperature was 37 °C. The disintegration time was evaluated visually. The disintegration time of an empty capsule has also been determined. Experiments were performed in duplicate.

Dissolution experiments were performed as described in the European pharmacopoeia (2.9.3) using a phosphate-citrate buffer medium pH 6.8 at 36.6 °C and with a paddle speed of 100 rpm. HPMC capsules (size 0) were filled with approximately 700 mg of a mixture of polymer PEG400/MOG/SA (45/5/50) or PEG1000/MOG/SA (45/5/50) and risperidone at a drug/polymer ratio of 10% (w/w). The capsules were placed in baskets to prevent the floating of the capsules. The amount of drug released from the formulation was quantified by UV-Spectroscopy. Three milliliters were sampled for quantification. The dissolution of a 3 mg Risperidal commercial tablet was also studied as a reference. Experiments were completed in duplicate.

2.8. In vitro cytotoxicity test

The cytotoxicity was assessed by the MTT test. The Caco-2 cell line was obtained from ATCC (American Type Culture Collection, USA). Caco-2 cells were maintained in DMEM (Dulbecco's minimal essential medium) supplemented with non-essential amino acids, 2 mM of L-glutamine, 10% of fetal bovine serum, penicillin

(100 U/ml) and streptomycin (100 U/ml). Cells with passage numbers between 40 and 50 were used.

Ninety six-well plates were seeded with 1×10^4 Caco-2 cells and maintained at 37 °C and 5% (v/v) CO_2 in a cell culture incubator. One hundred eighty microliters of the micellar solution at the desired concentration in PBS were incubated for 4 h with the cells. The micellar solution was then replaced by PBS buffer and 25 μl of MTT (1 g/l in PBS) were added after 45 min. The MTT solution was removed after 120 min of incubation at 37 °C and the cells lysed by the addition of 100 μl of dimethylsulfoxide. The optical absorbance of the solution was measured at 490 nm after the addition of 25 μl of glycine buffer. The PBS buffer was taken as the negative control and 1% Triton X100 served as the positive control. The experiments were performed in triplicate (Ould-Ouali et al., 2005).

Cell viability was expressed as a percentage compared to PBS buffer by the following equation:

$$\text{Cell viability}\,(\%) = \left(\frac{A_{\text{PBS}}}{A_{\text{p}}}\right) \times 100$$

where A_{PBS} is the absorbance of the PBS buffer at 490 nm and A_{p} the absorbance of the polymer solution at 490 nm.

The IC_{50} (i.e., the polymer concentration at which 50% of the cells died) was determined from the percentage of viability obtained at different polymer concentrations (0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 5% and 10%, w/v).

3. Results

3.1. Polymer characterization

Polymers composed of 2.5 to 45 mol% PEG with molecular weights between 400 and 2000 g/mol, 5 and 47.5 mol% monostearylglycerol (MSG) or monooleylglycerol (MOG), 50 mol% succinic anhydride (SA) or PEG diacid (PGDA) or 25 mol% SA and 25 mol% PGDA were synthesized.

The polycondensation of the different monomers, represented in Fig. 1 most probably led to random copolymers.

Table 1 summarizes the composition, M_W and polydispersity of the synthesized polymers. Copolymers with $M_W \geq 4600$ were solid at room temperature. The other copolymers were liquid or waxy. The polydispersity was in general between 1.3 and 2.5, except for a few copolymers with a low (2.5 or 5 mol%) mol fraction of hydrophilic moieties (PEG) and saturated monoglyceride (MSG) that reached polydispersities as high as 6.2. The amount of residual monomer was less than 0.1%.

The reproducibility of the polymer synthesis can be appreciated by a comparison of different batches of PEG400/MOG/SA (45/5/50). The variation in molecular weight and polydispersity was approximately 5% and 10%, respectively.

3.2. Assembling and self-assembling properties

The assembling properties of the polymers reported in Table 1 were evaluated by constructing ternary phase diagrams using a water titration method of a mixture of the polymer (lipophilic phase) and a surfactant, Poloxamer 105. The polymers were considered self-assembling when the polymer formed clear isotropic solutions in water in the absence of the surfactant.

The PEG400/monostearoylglycerol/succinic anhydride (PEG400/MSG/SA) (40/10/50) copolymers formed a clear isotropic solution when water was added to the binary mixture of the polymer containing at least 10% of the surfactant, Poloxamer 105. A large area of clear solution was observed (Fig. 2). An increase in the PEG chain length decreased the microemulsion domain: the maximum amount of water that could be added to the

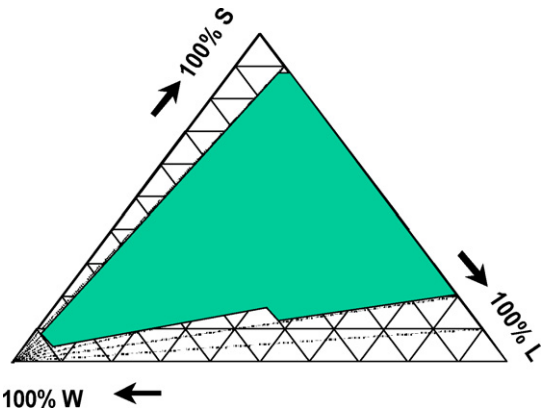

Fig. 2. Ternary phase diagram of polymer PEG400/MSG/SA (40/10/50) (L) in presence of surfactant Poloxamer 105 (S) obtained by titration with water (W).

PEG600/MSG/SA (45/5/50) copolymer was 50% while it was 90% for the PEG400/MSG/SA (45/5/50). A further increase of the PEG chain length resulted in polymers that did not form isotropic solutions. Furthermore, it was shown that by comparing the behaviour of the different PEG600/MSG/SA copolymers, that the maximal amount of water present in the microemulsion decreased from 50% to 0 when the mol% of PEG present in the polymer decreased from 45% to 5% or when the mol% of MSG present in the polymer increased from 5% to 45%. Polymers with a short PEG chain length (400 g/mol), a high proportion of PEG (45%) and a low proportion of monoglyceride (5%) formed the largest microemulsion area. However, these copolymers did not emulsify without a surfactant.

Replacement of the saturated MSG monoglyceride by an unsaturated monooleylglyceride (MOG) led to the synthesis of a self-assembling polymer, namely PEG400/MOG/SA (45/5/50). The viscosity of the solution decreased as water was added. As the PEG chain length was increased, all polymers, even the PEG/MOG/SA with long PEG chains, demonstrated self-assembling properties. The polymers containing PEG chains of 1000 and 2000 g/mol were solid at room temperature. They required mixing with a minimal amount of water to become sufficiently liquid to self-assemble and required hydration for a few hours at room temperature or 37 °C (addition of 20% to 30% of water) to self-assemble. The introduction of a polyethylene glycol diacid (PGDA) instead of succinic anhydride did not modify the self-assembling properties of the polymer.

Copolymers with 40 mol% of PEG 600 or PEG1000, 10 mol% MOG and 50 mol% SA had also self-assembling properties. On the other hand, polymers with 25% PEG and 25% MOG did not self-assemble.

In summary, copolymers containing 40 or 45 mol% of PEG varying from 400 to 2000 g/mol, 5 or 10 mol% unsaturated monoglyceride, from 25 to 50 mol% succinic anhydride and from 0 to 25 mol% PGDA, self-assembled spontaneously in water upon gentle mixing at room temperature.

3.3. Physico-chemical characterization

Physico-chemical properties of the self-assembling systems were determined. The size, critical association concentration, micellisation energy, cloud point and flocculation of the different self-assembling polymers are reported in Table 2.

3.3.1. Particles size

Whatever the polymer concentration (5×10^{-2} to 1×10^{-3} M), a multimodal size distribution was observed. One population was always detected between 10 and 20 nm and may represent micellar constructs. Another higher size population was also detected around 7.5 μm and, in some cases around 250 nm. The filtering of

Table 2
Physicochemical characterization of the PEG/MOG/SA (45/5/50) copolymers.

Polymer composition	Size (nm)	CAC (pyrene) (g/ml)	I_1/I_3	Micellisation energy (kJ/mol)	Cloud point (°C)	Flocculation point (ionic strength) (M)
PEG400/MOG/SA (45/5/50) ($n=6$)	10+ aggregates	$3.4\times10^{-4}\pm1.2\times10^{-4}$	1.3	-32 ± 2	66.5	0.89
PEG600/MOG/SA (45/5/50)	10+ aggregates	1×10^{-3}	1.3	−31	78	1.2
PEG1000/MOG/SA (45/5/50) ($n=2$)	15+ aggregates	1×10^{-3}, 4×10^{-3}	1.3	-31 ± 1	94	1.5
PEG2000/MOG/SA (45/5/50) ($n=2$)	15+ aggregates	1.5×10^{-3}, 4×10^{-3}	1.3	-30 ± 2	> 95	1.55

the solution did not change significantly these observations suggesting the formation of aggregates.

The influence of the stirring conditions was evaluated at 37 °C by mixing a 2% (w/v) PEG400/MOG/SA (45/5/50) solution at different speeds (350 and 750 rpm) and for different durations (30 min, 1, 2 and 3 h). The particle size was measured 5 min or 24 h after the end of the stirring. No significant difference in the size pattern was observed: there was always a major population at approximately 15 nm and one or more populations at larger dimensions, the size of these higher populations varying from one measurement to the other.

3.3.2. Surface charge

As expected for PEGylated nanocarriers, the zeta potential of all copolymers was neutral (-2 ± 2 mV) and was independent of the solution pH.

3.3.3. Critical micellar concentration and micellisation energy

The size measurements suggest that the amphiphilic polymers of interest would form micelles. In order to verify the hypothesis, CMC of the different self-assembling polymers were determined using a fluorimetric method. The progressive decrease of I_1/I_3 ratio of the pyrene spectrum up to a value of approximately 1.3 confirms that a CMC could be determined. The CMC of the different batches of the PEG400/MOG/SA (45/5/50) was $3.4\times10^{-4}\pm1.2\times10^{-4}$ g/ml. The CMC of the PEG600/MOG/SA, PEG1000/MOG/SA and PEG2000/MOG/SA (45/5/50) were in the range of 1×10^{-3} to 4×10^{-3} g/ml.

The I_1/I_3 ratio of the pyrene absorption spectrum gives indications about the polarity of the micelles core. Pyrene is a very apolar probe whose spectrum is sensitive to its environment. In an aqueous amphiphilic colloidal system, pyrene will tend to migrate to the more hydrophobic area. The more hydrophobic the environment of the probe, the lower the I_1/I_3 ratio. In all polymeric self-assembling solutions, the decrease of I_1/I_3 ratio of the pyrene spectrum was down to a value of 1.3.

The micellisation energy was always around −30 kJ/mol. These negative values confirm the spontaneous formation of the colloidal system.

3.3.4. Colloidal stability

The cloud point increased with the PEG chain length: varying from 66.5 °C for the PEG400/MOG/SA (45/5/50) to more than 95 °C for PEG2000/MOG/SA (45/5/50). An increase in the PEG chain length enhanced the stability of the colloidal system to temperature.

The influence of the PEG chain length of the PEG/MOG/SA (45/5/50) copolymers on the colloidal stability to ionic strength was studied by means of the critical flocculation point. The PEG chain length had a positive influence on the stability to ionic strength of the medium (Na_2SO_4): the critical flocculation point increased as a function of the PEG chain length from 0.89 to 1.55 M when the PEG chain length increased from 400 to 2000 g/mol. This phenomenon was reversible; that is when the polarity of the medium was decreased by adding water to the solution, it became clear and isotropic. The self-assembling properties did not change with pH or in presence of albumin (data not shown).

3.4. Solubility

In a first series of experiments, the solubility of five BCS class II drugs, namely risperidone, ketoconazole, indomethacin, hydrocortisone, and cyclosporin in PEG400/MOG/SA (45/5/50) was determined. These drugs have been selected for their diversity in log P (hydrophobicity) and/or pKa (acidity/basicity).

The effect of the polymer composition, more precisely of the PEG chain length and of the succinic anhydride/PEG diacid (PGDA) ratio, on the solubility of a model drug, risperidone, was then studied.

3.4.1. Solubility of drugs in PEG400/MOG/SA (45/5/50)

The solubility of ketoconazole, risperidone, indomethacin, hydrocortisone, and cyclosporin in the PEG400/MOG/SA (45/5/50) polymeric solutions at pH 7.02 was measured. The results are reported in Table 3.

The solubility in a 1% (w/v) polymer solution was 0.17, 1.54, 1.05, 0.39 and 0.14 mg/ml, respectively, for the drugs of interest. Knowing the solubility of ketoconazole, risperidone, hydrocortisone, indomethacin, and cyclosporin in PBS, which is 0.01, 0.06, 0.012, 0.002 and 0.001 mg/ml, respectively, the encapsulation of these drugs into the polymeric systems generated a 17-, 26-, 33-, 525- and 140-fold increase, respectively, in solubility. The solubility of the drugs increased linearly with the polymer concentration except for indomethacin. In the case of this latter drug, a plateau was observed.

The drug-loading efficiency depended strongly on the drug: the highest loading obtained using the 1% (w/v) PEG400/MOG/SA (45/5/50) solution was obtained for risperidone (15.4%) and indomethacin (10.5%). The indomethacin loading content decreased by a factor of 5 as the polymer concentration increased whereas the risperidone loading content was more stable as a function of the polymer concentration (11.7% at 10% polymer concentration). The ketoconazole, hydrocortisone and cyclosporin loading content of 1% PEG400/MOG/SA (45/5/50) solutions was between 2% and 4% and decreased as the polymer concentration increased.

These data clearly demonstrate that PEG400/MOG/SA (45/5/50) increased the solubility of poorly water-soluble drugs and that the solubility enhancement and drug loading depended on the physicochemical properties of the drug.

3.4.2. Effect of the polymer composition on risperidone solubility

To determine the influence of the PEG chain length and the monomer composition on the solubility of a model drug, risperidone was encapsulated in PEG400/MOG/SA (45/5/50), PEG400/MOG/PGDA/SA (45/5/25/25), PEG400/MOG/PGDA (45/5/50), PEG400/MOG/SA (45/5/50), PEG1000/MOG/SA (45/5/50) and PEG2000/MOG/SA (45/5/50) in phosphate buffer solutions.

As reported in Table 4, the PEG chain length only slightly influenced the solubilization of risperidone in the polymeric solutions. The solubility obtained by a 1% (w/v) polymer concentration was similar for the three polymers showing a slight decrease from 1.65 to 1.25 mg/ml when the PEG chain length increased from 400 to 2000 g/mol. The solubility difference increased when the polymer concentration increased such that for a 10% (w/v)

Table 3
Solubility (mg/ml) of risperidone, ketoconazole, indomethacin, hydrocortisone, cyclosporine in phosphate buffer (pH 7.02) or in PEG400/MOG/SA (45/5/50) solutions ($n = 3$) and drug loading content (%) in a 1% (w/v) PEG400/MOG/SA (45/5/50) solution.

	PBS	Solubility (mg/ml) (Polymer) (w/v, %)			Drug loading content (%) in 1% polymer
		1	3.15	10	
Ketoconazole	0.01	0.17	0.23	0.85	1.7
Risperidone	0.06	1.54	2.97	11.69	13.3
Hydrocortisone	0.012	0.39	0.59	1.22	3.8
Indomethacin	0.002	1.05	1.89	1.8	9.5
Cyclosporin	0.001	0.14	0.09	1.26	1.4

polymer solution, the solubility was three-times higher with the PEG400/MOG/SA (11.15 mg/ml) than for systems using the PEG2000/MOG/SA (4.38 mg/ml).

The solubility of risperidone also decreased as the fraction of the PGDA monomer increased (i.e., as the fraction of succinic anhydride decreased) so that the solubility decreased from 2.58 to 0.95 mg/ml when the PGDA increased from 0 to 50 mol% in a polymer concentration of 3.15% with a PEG400 chain.

3.5. Drug release

The results of two independent drug release experiments of risperidone from PEG400/MOG/SA (45/5/50) solutions are presented in Fig. 3. UV spectrometry of risperidone and scintillation analysis of the radiolabelled drug gave similar results. Fifty percent of the drug was released within 6 h. Almost all the drug was released after one to 1.5 days. The release of the drug encapsulated in the polymer is slower than the appearance of the free drug in the dialysate in which case 50% of the drug was released in 2 h and 90% after 5 h.

As liquid scintillation counting is more sensitive than UV spectrometry, this first method was applied to ketoconazole the water solubility of which being very low. Fifty percent of ketoconazole was released after 3 days from a 1% (w/v) PEG400/MOG/SA (45/5/50) solution. Almost all the drug (90%) was released after 10 days. These data highlight the importance of the physicochemical properties of the drug with regard to how they interact with the polymer.

3.6. Disintegration and dissolution tests

As these polymers may be applicable for oral administration of poorly water-soluble drugs, HPMC capsules were filled with the drug-polymer mixture and assessed as potential pharmaceutical forms. In these systems, the colloidal solution would form in the gastrointestinal fluids after disintegration/dissolution of the capsule. Hence, the dissolution rate of HPMC capsules, filled with these polymers alone or combined with a drug of interest, in a simulated intestinal medium was also evaluated.

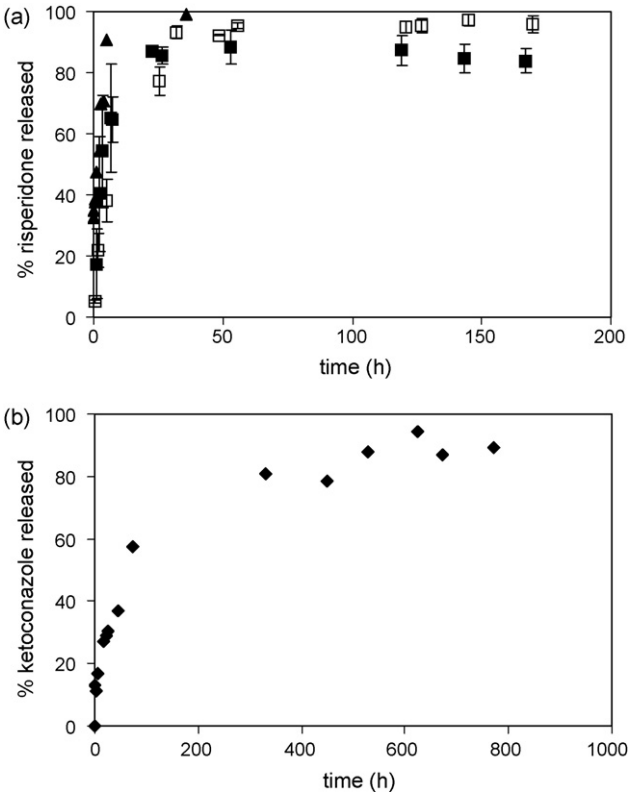

Fig. 3. (a) Release of encapsulated risperidone from a 1% (w/v) solution of PEG400/MOG/SA (45/5/50) by liquid scintillation ($n = 2$) "□" or UV spectrometry ($n = 2$) "■" and of free risperidone by liquid scintillation "▲"; (b) release of ketoconazole from a 10% (w/v) solution of PEG400/MOG/SA (45/5/50) by liquid scintillation "●".

As reported in Table 5, the empty capsule disintegrated in less than 2 min. It took slightly longer for the capsules filled with polymers to disintegrate; capsules containing the PEG1000/MOG/SA dissolved the slowest (4 min 50). The dissolution test indicated that in the case of the commercial risperidone tablet, 40% of the drug was released after 1 h and 100% after 2 h. Risperidone was released

Table 4
Solubility (mg/ml) of risperidone in polymeric solutions.

	Polymer (%, w/v)		
	1	3.15	10
Effect of PEG chain length			
PEG400/MOG/SA (45/5/50)	1.65	2.58	11.15
PEG1000/MOG/SA (45/5/50)	1.35	2.21	7.38
PEG2000/MOG/SA (45/5/50)	1.25	1.78	4.38
Effect of monomer composition			
PEG400/MOG/PGDA/SA (45/5/25/25)	1.23	2.11	ND
PEG400/MOG/PGDA (45/5/50)	0.81	0.95	5.83

ND: not determined.

Table 5
Disintegration time (s) ($n = 2$) of empty HPMC capsules or capsules filled with PEG400/MOG/SA (45/5/50) or PEG1000/MOG/SA (45/5/50), Time for the release of 50% (t_{50}) and 90% (t_{90}) of risperidone in the dissolution test of HPMC capsules containing a mixture of risperidone and PEG400/MOG/SA (45/5/50) or PEG1000/MOG/SA (45/5/50) and of a 3 mg commercial tablet (Risperidal) in a simulated intestinal fluid (pH 6.8) ($n = 2$).

	Disintegration time (s)	t_{50} (min)	t_{90} (min)
HPMC	110		
HPMC + PEG400/MOG/SA (45/5/50)	210	20	60
HPMC + PEG1000/MOG/SA (45/5/50)	290	20	60
Risperidal® tablet		60	120

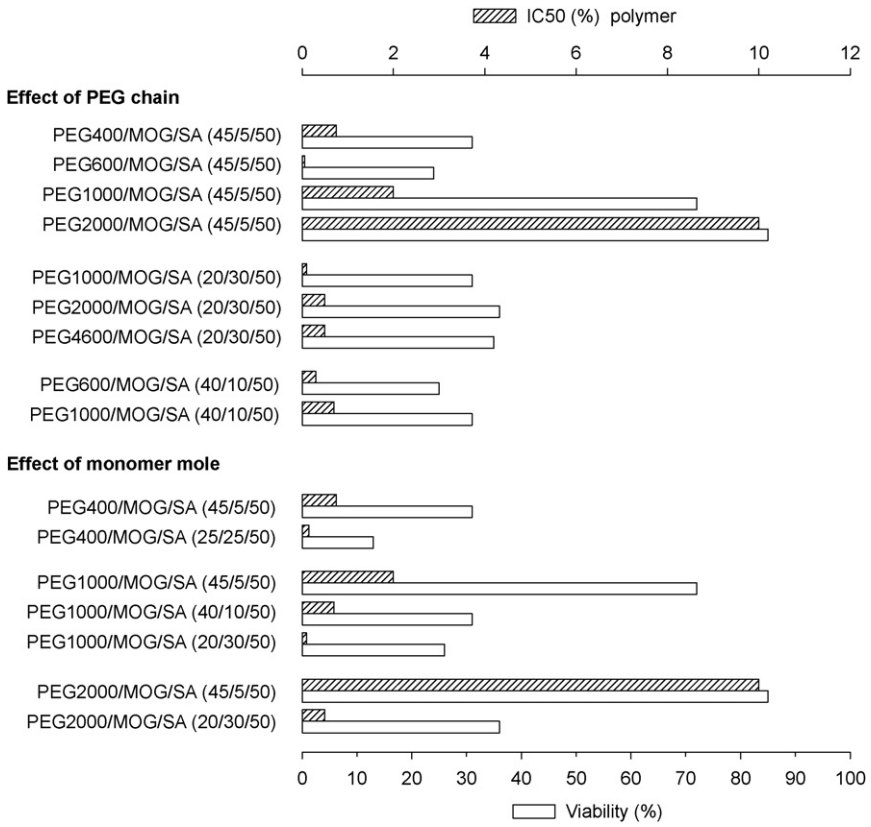

Fig. 4. Caco-2 cell viability (%) (empty column) resulting from incubation with 1% polymer solutions and IC50 (plain columns) of the polymers.

rapidly from the PEG/MOG/SA (45/5/50) polymers: after 20 min, almost 50% of the drug was dissolved in the dissolution bath and all the drug was released after 1 h. There was no significant difference between risperidone released from PEG400/MOG/SA (45/5/50) or PEG1000/MOG/SA (45/5/50).

3.7. *In vitro cytotoxicity test*

The MTT test was used to evaluate the influence of the polymer composition on its cytotoxicity on Caco-2 cells used as model enterocytes. The influence of the PEG chain length, the PEG (or MOG) ratio and the replacement of the SA by a PEG diacid on cell viability was assessed.

The IC_{50} of the polymers is reported in Fig. 4. The polymers with long PEG chains in high percentages and with a low amount of monoglyceride (e.g., PEG1000/MOG/SA (45/5/50) and PEG2000/MOG/SA (45/5/50)) were the least cytotoxic towards Caco-2 cells: the IC_{50} of PEG2000/MOG/SA was higher than 10% (w/v) while the same value for PEG1000/MOG/SA was 1.5%. The most cytotoxic polymers were the PEG600/MOG/SA (45/5/50) (IC_{50} = 0.06%, w/v), PEG1000/MOG/SA (20/30/50) (IC_{50} = 0.1%, w/v) and the PEG400/MOG/SA (25/25/50) (IC_{50} = 0.15%, w/v).

The percentage of cell viability obtained at 1% (w/v) polymer concentrations were compared for the different polymers to analyse, more precisely, the effect of the polymer composition (Fig. 4). The PEG chain length played an important role in the cytotoxicity of the polymer: the higher the amounts of PEG (i.e., 45%), the higher the cell viability. In addition, when the PEG chain length increased from 400 to 2000 g/mol in polymers containing 45% PEG, cytotoxicity decreased with cell viabilities increasing from 31% to 85%. The PEG chain length did not seem to have such a dramatic effect when the PEG was present in lower amounts (20% and 40%). For these compositions, the cell viability was always around 30% at 1% (w/v). On the other hand, for a given PEG chain length, the increase in the amount of monoglyceride (or the decrease in the PEG amount) had a strong influence on the cytotoxicity. Whatever the PEG chain length, the higher the concentration in MOG, the higher the toxicity. The number of cells surviving increased as the fraction of succinic anhydride decreased, i.e., as the fraction of PDGA increased. The presence of PGDA, instead of succinic anhydride, significantly decreased the toxicity of the polymer. For example, 73% and 65% cellular viability was observed in the presence of PEG400/MOG/PDGA(45/5/50) or PEG400/MOG/SA/PDGA (45/5/25/25) solutions and only 31% in the presence of PEG400/MOG/SA (45/5/50).

4. Discussion

Novel self-assembling amphiphilic copolymers based on polyethylene glycol, unsaturated C_{18}-monoglyceride and succinic anhydride have been synthesized using polycondensation chemistry. An initial screening revealed that copolymers containing a high molar ratio (45%) of short PEG chains (400 g/mol), a low molar ratio (5%) of monooleylglycerol and a high molar ratio (50%) of succinic anhydride, i.e., PEG400/MOG/SA (45/5/50), self-assembled spontaneously in water upon gentle mixing at room temperature. Decreasing the mol% of PEG to 40% or increasing the mol% of MOG to 10%, replacing the succinic anhydride with a PEG600 diacid (PGDA) or with a 25/25 mixture of succinic anhydride and PGDA did not significantly affect the ability of the polymers to self-assemble. The physicochemical and self-assembling properties were affected when the PEG moiety or the monoglyceride moiety were changed. Indeed, copolymers containing PEG1000 or PEG2000 were solid at room temperature and required hydration for a few hours at room temperature or 37 °C before spontaneous micelle formation was possible. Replacement of the monooleylglycerol by the corre-

sponding C_{18}-saturated monoglyceride (monostearylglycerol) was found to inhibit the self-assembling properties. This drastic change of properties is very likely related to the lipid conformation. In summary, copolymers containing 40 or 45 mol% PEG chains varying from 400 to 2000 g/mol, 5 or 10 mol% unsaturated MOG, from 25 to 50 mol% SA and from 0 to 25 mol% PGDA, self-assembled spontaneously in water upon gentle mixing at room temperature.

According to the monomer structure, the polymers formed are most probably random copolymers, with succinic anhydride units alternating with PEG or monoglyceride units. The PEG units would thus not be localized specifically at one end of the polymer but dispersed along the entire chain. The spatial configuration of these copolymers will probably be tortuous allowing for the exposure of a number of PEG units to the aqueous phase and providing potentially a stabilizing PEG layer.

Detailed physicochemical characterization of aqueous PEG/MOG/SA (45/5/50) colloidal systems indicates that micelles are formed. Indeed, it was possible to determine (i) a size (mainly 10 nm) (ii) the CMC of the copolymers (in the range of 10^{-3} to 10^{-4} g/ml), a characteristic of micellar systems (iii) a negative micellisation energy (−30 kJ/mol), indicating spontaneous micelle formation.

This self-assembling PEG/MOG/SA (45/5/50) have a number of beneficial characteristics for oral drug delivery including their high cloud point (>65 °C) and their high flocculation point (≥0.89 M). In addition, exposure of the solutions to a wide pH range (2 to 10) and to albumin solutions (0.5% to 4%) did not affect their self-assembling properties. The neutral surface charge is consistent with PEG moieties on the micelle corona. Therefore, aggregation and opsonisation should be minimized. However, some aggregation was observed. Photon correlation spectroscopy (PCS) measurements indicated that, whatever the polymer structure and concentration, high molecular weight aggregates were present. The steric protection efficacy of the PEG against aggregation depends on the PEG surface density and on the thickness of the PEG layer (Allen et al., 1999). These aggregates could prevent the use of the polymers for intravenous administration.

The I_1/I_3 ratio of the pyrene fluorescence signal indicates that the core of the micelles is relatively hydrophobic ($I_1/I_3 = 1.3$). It is slightly lower than the 1.6 values measured for other polymeric micelles such as PEO/PLA or PEO/PCL micelles (Yasugi et al., 1999; Allen et al., 2000) as well as PEG-P(CL-co-TMC) micelles (Ould-Ouali et al., 2004), suggesting that these MSGA systems are slightly more hydrophobic. These data may suggest a better incorporation for lipophilic drugs than the known polymeric systems.

A solubility study with five BCS class II drugs (risperidone, ketoconazole, hydrocortisone, indomethacin and cyclosporin) confirms this hypothesis. The increase in solubility of indomethacin was up to 100-fold greater than the increase in solubility induced by classic solubilizing systems such as cyclodextrins, surfactants (such as Brij 35, Pluronics, PEG, PVP) or cosolvent (isopropanol, propylene glycol, glycerol, ethanol) water mixtures (Krasowska et al., 1972; Elgindy, 1993; Rao et al., 1997; Etman and Nada, 1999). The increase in solubility of hydrocortisone is 2 to 10 times higher than that observed in Tween 80/water mixtures, cyclodextrin or in poloxamer nanoparticles (Hajratwala and Taylor, 1976; Monza da Silveira et al., 2000) at the same concentration and is two times higher than the increase in solubility by encapsulation in sodium taurocholate micelles or sodium taurocholate-lecithin mixed micelles (Naylor et al., 1993).

Depending on the drug and on the polymer concentration, the drug loading efficiency varied from 2% to 15%, decreasing as the polymer concentration increased. The fast solubilization screening reported here confirmed that the compatibility between drugs and micelles core is a complex phenomenon driven by several properties such as the charge, polarity, hydrophobicity (log P) and volume of the drug and the nature and size of the hydrophobic and hydrophilic domains of the polymer (Allen et al., 1999; Latere Dwan'Isa et al., 2007). None of the drug characteristics studied seemed to have a predominant influence on the drug loading capacity of the polymers.

An increase in the PEG chain length (from 400 to 2000 g/mol) and the replacement of the SA monomer by a PEG diacid monomer decreased the solubility of risperidone. The decrease in solubility can be explained by the higher hydrophobic character of the PEG400 containing polymer in comparison with the PEG2000 containing polymer and by the higher hydrophobicity of the SA monomer in comparison with the PGDA monomer resulting from its longer hydrocarbon chain. It is important to note that these experiments have been carried out in phosphate buffer pH 7 in order to avoid pH changes that might modify the drug solubility.

The polymers presented demonstrated interesting physicochemical and solubilization characteristics which prompted further investigations into dissolution and disintegration of HPMC capsules filled with these polymers, a pharmaceutical dosage form for oral drug delivery. The drug release profile from PEG400/MOG/SA (45/5/50) was dependent on the drug and probably on the interactions between the drug and the polymer: 50% of the risperidone was released after 6 h whereas 50% of ketoconazole was released after 3 days. Risperidone was released more slowly from the micelles than from the commercial drug tablet. The capsules filled with the polymer disintegrated rapidly and conformed to European Pharmacopeia criteria. The disintegration time increased with the PEG chain length of the polymer but was still rapid (less than 5 min for the PEG1000/MOG/SA (45/5/50)). The dissolution tests indicated that, whatever the PEG chain length, risperidone was released faster from the HPMC capsules containing the PEG/MOG/SA (45/5/50) polymer than from the HPMC capsules containing Risperidal® tablet.

The MTT test on Caco-2 cells was used to estimate the cytotoxicity of the copolymers. The cell viability associated with the polymers with short PEG chain length was poor, i.e., a 1% PEG400/MOG/SA (45/5/50) solution induced the death of 70% of the cells. This cytotoxicity probably results from the release of succinic acid. Cell viability increased with increasing PEG chain length. In addition, for a given PEG chain length, the increase in the amount of monoglyceride had a strong influence on the cytotoxicity such that the higher the concentration in MOG, the higher the toxicity. Comparison with other self-assembling surfactants and amphiphilic polymers is difficult because the cytotoxicity depends on the experimental conditions e.g., cell line, cytotoxicity test (MTT, LDH) and duration of incubation (1 to 72 h). PEG-P(CL-co-TMC) had a lower cytotoxicity (IC50 > 10%) whereas low molecular weight surfactant such as Cremophor EL or Polysorbate 80 or diblock polymers such has MePEG-b-PCL had similar or higher cytotoxicity (Arechabala et al., 1999; Allen et al., 2000; Ould-Ouali et al., 2005; Elamanchili et al., 2009).

The influence of the PEG chain length on the physicochemical properties and cytotoxicity of the PEG/MOG/SA polymers is summarized in Table 6. The PEG chain length had no influence on the CMC (Jönsson et al., 1998; Torchilin, 2001). This parameter had also no influence on the micellisation energy, on the polarity of the micellar core and on the dissolution of HPMC capsules filled with polymer. The disintegration time of HPMC capsules filled with polymer, the cloud point and the flocculation point did increase with increasing PEG chain length. On the other hand, the cytotoxicity decreased with increasing PEG chain length.

As the cloud point and flocculation point increase with the PEG chain length and as the cytotoxicity decreases with the PEG chain length, the PEG2000/MOG/SA (45/5/50) could be considered optimal with regard to forming the most stable and less toxic system.

Table 6
Influence of the PEG chain length on properties of PEG/MOG/SA (45/5/50).

Property	Influence
size	No
CAC	No
Polarity (I_1/I_3)	No
Micellisation energy	No
Cloud point	Yes
Flocculation point	Yes
Solubilization	Yes
Capsules dissolution	No
Capsules disintegration	Yes
Cytotoxicity	Yes

PEG400/MOG/SA (45/5/50) seem to be the best polymer composition of the series with regard to solubility enhancement since the increase in PEG chain length and the replacement of the succinic anhydride by a PEG diacid decreased the solubility of risperidone. A good compromise between self-assembling, solubilisation, stability and toxicity seems thus to be presented by the PEG1000/MOG/SA (45/5/50).

5. Conclusion

Novel amphiphilic copolymers based on polyethylene glycol, unsaturated C_{18}-monoglyceride and succinic anhydride self-assemble without the use of solvents or heat, by gentle mixing with water. They are promising vehicles for the drug delivery of poorly water-soluble drugs and may represent an improved class of solubility enhancers than other self-emulsifying polymers. They may not be suitable for intravenous administration because of aggregation of the polymeric nanosystems. They are candidates for oral administration of poorly water-soluble drugs. HPMC capsules filled with a mixture of these polymers and an active could dissolve in the stomach or intestine and the polymer would self-assemble in contact with the surrounding fluids to generate an effective delivery system. PEG1000/MOG/SA (45/5/50) seems to be particularly promising for improving biopharmaceutical performance of poorly soluble drugs.

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