The effect of hydrophile–lipophile balance of water-soluble poly(ethylene glycol)–poly(lactic acid) diblock copolymers on the stability of microscopic emulsion films and nanoemulsions

Valery G. Babak,*[a] Ruxandra Gref[b] and Edith Dellacherie[b]

[a] *A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 117813 Moscow, Russian Federation. Fax: +7 095 135 5085; e-mail: babak@ineos.ac.ru*

[b] *Laboratoire de Chimie Physique Macromoléculaire, ENSIC, BP 451, 54001 Nancy Cedex, France*

The effect of the PEG and PLA block length of amphiphilic water-soluble poly(ethylene glycol)–poly(lactic acid) diblock copolymers on the stability to rupture of microscopic liquid films and nanoemulsions is investigated.

The encapsulation of drugs into colloidal injectable carriers (liposomes, micelles, nanoparticles, nanoemulsions, *etc.*) represents a very promising way to preserve the drug activity, control its release directly in the vascular compartment and/or achieve targeting to specific tissues. It has been shown that an appropriate hydrophilic and neutral coating, and particularly the one formed by a poly(ethylene glycol) (PEG) corona, sterically protects the carriers, avoids their interaction with plasma proteins and therefore makes these carriers ‘invisible’ for the phagocytic cells.[1–3] This significantly increases the circulatory blood half-lives of these carriers.

Among the various ‘stealth’ systems presented in the literature, the biodegradable nanospheres composed of water-insoluble diblock copolymers PEG–poly(lactic acid) (PEG–PLA) or PEG–poly(lactic-co-glycolic acid) (PEG–PLGA) have a good mechanical resistance due to their compact cores and offer the possibility to achieve a controlled release of various drugs (hydrophobic molecules, proteins, peptides),[4] depending on the physico-chemical properties of the cores. These nanospheres are obtained by the solvent evaporation method from oil-in-water nanoemulsions, the oil phase being a solution of the polymer in an organic solvent.

Our interest in this work was focused on decane-in-water nanoemulsions (droplet size of the order 300 nm) and microscopic decane–water–decane emulsion films (radius of the order of 50 μm) sterically stabilised by the adsorption layers of water-soluble PEG–PLA diblock copolymers. The steric repulsion between the extended (in the aqueous phase) PEG chains coating the decane droplets is explained by the osmotic pressure and the elastic restoring forces which compete with the van der Waals molecular attraction forces in the case of relatively short polymeric chains.[5–7] It is expected that the thickness of these adsorption layers, and consequently, the steric stabilisation effect, should increase as the area per one PEG chain decreases. The PEG conformation at the surface passes from a ‘mushroom’ in the dilute, unhindered state at low surface concentration, to an extended ‘brush’ at a high surface concentration.

To investigate the effect of the PEG corona on the interfacial and emulsion stabilisation properties, we studied a family of PEG–PLA copolymers with different hydrophile–lipophile balance (HLB), obtained by varying successively both PEG and PLA chain lengths.

Monomethoxy poly(ethylene glycol) (MPEG) with weight average molecular masses (MW) 5 and 10 kg mol^{-1} were purchased from Sigma, France. A family of diblock copolymers MPEG–PLA was synthesised as previously described,[8] by ring opening polymerisation of lactide (Lancaster, UK), initiated by the hydroxyl terminal group of MPEG and catalysed by tin(II)ethyl hexanoate (Alfa, Germany). These copolymers were further used as emulsifying and stabilising agents. PLA is hydrophobic and biodegradable, whereas PEG is hydrophilic and bioeliminable (MW < 20 kg mol^{-1}). The MW of the PLA block was 0.5, 1 and 2 kg mol^{-1}, as determined by ^{1}H NMR and vapour pressure osmometry. These diblock copolymers will be further named PEG$_{5K}$–PLA$_{0.5K}$, PEG$_{5K}$–PLA$_{1K}$, PEG$_{5K}$–PLA$_{2K}$ and PEG$_{10K}$–PLA$_{2K}$.

The surface tension (σ) was measured at 25 °C using the Wilhelmy plate method with a K-10ST tensiometer (Krüss, Germany) with an accuracy of 0.3 mJ m^{-2}. The polymer solutions were prepared using twice-distilled and deionized water.

The measurements of the stability (the lifetime τ before rupture) of microscopic (about 100 μm in diameter) emulsion films (MEF) were carried out using the method of contact

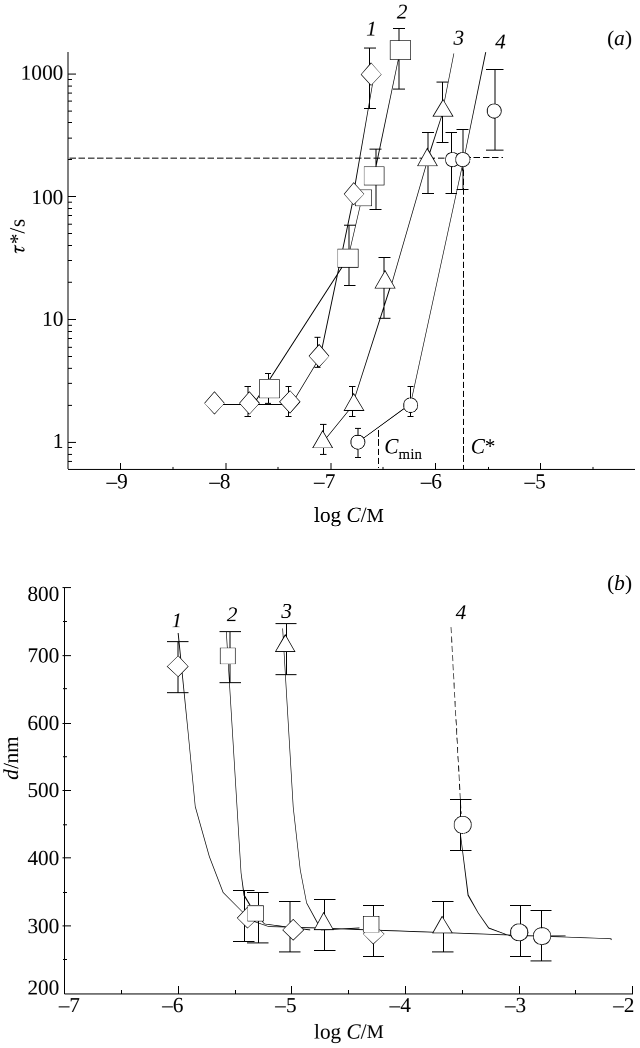

Figure 1 Lifetime before rupture τ^* of microscopic emulsion (decane–water–decane) films (*a*) and diameters of decane droplets (*b*) as a function of the molar concentration of the PEG–PLA diblock copolymers: PEG$_{10K}$–PLA$_{2K}$ (*1*), PEG$_{5K}$–PLA$_{2K}$ (*2*), PEG$_{5K}$–PLA$_{1K}$ (*3*) and PEG$_{5K}$–PLA$_{0.5K}$ (*4*).

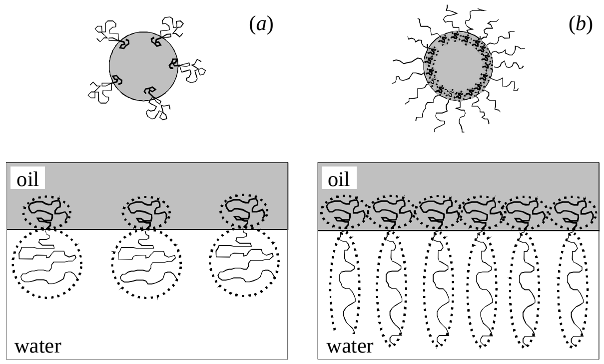

Figure 2 Scheme illustrating the transformation of the adsorption layer structure from two dimensional ‘gas-like’ distribution with a ‘mushroom’ structure of hydrophilic PEG blocks (*a*) to sterically-interacting surfactant molecules with a ‘brush’ like structure of PEG blocks (*b*) layers in the course of increasing the adsorption amount of the surfactant molecules at the oil/water interface.

interaction described in refs. 9 and 10. The characteristic lifetime of the MEF, τ^*, corresponding to the probability of destruction of 63% of these films, was determined from the experimental histograms of the MEF distribution over their lifetime, which were plotted on the basis of 10 to 20 independent measurements of τ.

PEG–PLA freshly-prepared solutions were tested with regard to their ability to stabilize decane-in-water emulsions. The o/w emulsions were prepared by sonicating (15 W, 30 s, pulses of 1 s each, followed by 1 s pause) 8 μl of decane and 4 ml of an aqueous solution containing the surfactant. The droplet size distribution was determined at 30 °C by photon correlation spectroscopy (PCS) using a Malvern 4600 (Malvern Instruments Ltd., UK). The emulsion droplet mean diameter *d* was measured by PCS during several hours. In all the systems studied, the size distribution of the decane-in-water emulsion droplets was monomodal.

Figure 1(*a*) represents the mean lifetime τ^* of microscopic emulsion films formed between two decane droplets in aqueous solutions of surfactants as a function of their molar concentration *C*. The characteristic feature of all the isotherms $\tau^*(C)$ is their very pronounced transfer from non stable films (with a lifetime of the order of 1 s) to very stable films living 10^3 s and more in the relatively narrow range of surfactant concentration. To each surfactant may be attributed a certain value of the minimum concentration C_{min} corresponding to this transfer and characterising its stabilising ability. More unambiguously and with a higher precision, the stabilising ability of a surfactant may be characterised by the concentration C^* corresponding to the standard lifetime of the film (usually chosen as equal to 200 s for experimental convenience reasons, *i.e.* the necessity to proceed to a measure of 10 to 20 microscopic liquid film ruptures).

For example, the C^* value corresponding to the surfactant $\mathrm{PEG_{10K}}$–$\mathrm{PLA_{2K}}$ is *ca.* 2×10^{-7} M, whereas the value of C^* corresponding to the sample $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{0.5K}}$ is *ca.* 2×10^{-6} M. This means that the stabilising ability of the second sample is ten times smaller compared to the first one. Therefore, in order to achieve the conventionally chosen standard lifetime of 200 s, the concentration of $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{0.5K}}$ should be ten times higher than for the $\mathrm{PEG_{10K}}$–$\mathrm{PLA_{2K}}$ surfactant. Consequently, the stabilising ability of the copolymers studied, determined on the basis of their C^*, decreases in the series: $\mathrm{PEG_{10K}}$–$\mathrm{PLA_{2K}}$ > > $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{2K}}$ > $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{1K}}$ > $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{0.5K}}$.

As regards the stability of nanoemulsions, Figure 1(*b*) shows that the diameters *d* of the emulsion droplets, measured 1 h after emulsion ripening, undergo a sharp increase below a given minimum surfactant concentration C_{min} whose value increases with decreasing PLA block length of the surfactants. Thus, the ability of the surfactants studied to stabilise the o/w emulsion varies in the series: $\mathrm{PEG_{10K}}$–$\mathrm{PLA_{2K}}$ ≅ $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{2K}}$ > > $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{1K}}$ > $\mathrm{PEG_{5K}}$–$\mathrm{PLA_{0.5K}}$. These results clearly show the main role played by the PLA chain length in emulsion stabilisation.

This feature may be explained by the fact that the PLA blocks, being insoluble both in decane and in water, must adopt the most energetically favourable conformation at the interface which is presumably the globule-like formation schematically represented in Figure 2. The hydrophilic PEG blocks extend in the water phase with either a ‘mushroom’ [Figure 2(*a*)] or a ‘brush’ [Figure 2(*b*)] conformation.

At low surfactant concentrations the degree of filling of the adsorption layer is low, and molecules behave as a two dimensional ‘gas-like’ distribution [Figure 2(*a*)] characterized by high lateral mobility. The area *q* per surfactant molecule in the adsorption layer, estimated from the surface tension isotherms,[11] is of the order of 50 nm^2 in the concentration range corresponding to emulsions and microscopic emulsion film (MEF) instability. This value of *q* is much greater than the hydrodynamic radius $R_{\mathrm{g}} \sim 1$–2 nm of the nonperturbed statistical coil of the hydrophilic PEG chain in the good solvent (water).[12] The PEG blocks acquire a ‘mushroom’ conformation characterized by a relatively low extention of these blocks in the aqueous phase and a low protection of microscopic emulsion films (MEF) against rupture and of emulsion droplets against coalescence.

Beginning with some critical concentration C^* corresponding to the sharp increase of the MEFs and emulsion stability, a saturated adsorption layer is formed at the decane–water interface [Figure 2(*b*)]. The PEG blocks adopt a ‘brush’ conformation in the adsorption layer. The lateral mobility of the adsorbed molecules decreases because of their more tight filling and mutual steric repulsion which contributes to the stability of emulsion films to the rupture. The area *q* per surfactant molecule in the adsorption layer is of the order of 1 nm^2 which corresponds to the size of the globule-like PLA blocks. The longer the PLA blocks, the higher their effective geometrical area and the less their density of filling in the saturated adsorption layer and their critical concentration C^* corresponding to the sharp increase of the stability of MEFs (Figure 1).

The results presented in this paper suggest that the microscopic emulsion films and the emulsion droplets bearing adsorption layers of surface active water soluble PEG–PLA diblock copolymers become stable to coalescence when the surfactant macromolecules form a dense coating layer and have a low lateral mobility. Therefore, the minimum area q_{min} per adsorbed surfactant molecule at the interface is determined by the size of the hydrophobic PLA block (in the range studied 0.5–2K). As a consequence, the critical concentration C^* increases with decreasing PLA block length. Accordingly, the minimum surfactant concentration C_{min} necessary to stabilize the decane-in-water emulsions varies in a similar way with the PLA chain length. However, at identical PLA MW, the length of the PEG block, extending in the aqueous medium and ensuring steric stabilization, also has an effect on the emulsion stability. The longer the PEG blocks, the thicker the hydrophilic coating layer, and the better the stabilisation.

These results show the possibility of stabilising o/w emulsions using biocompatible PEG–PLA surfactants, and open a new field of applications for these copolymers in the manufacture of nanoparticles with a hydrophilic PEG coating.

References

1 S. Stolnick, L. Illum and S. S. Davis, *Adv. Drug Deliv. Rev.*, 1995, **16**, 195.
2 R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin and R. Langer, *Science*, 1994, **263**, 1600.
3 D. Bazile, C. Prud’homme, M. Bassoullet, M. Marlard, G. Spenlehauer and M. Veillard, *J. Pharm. Sci.*, 1995, **84**, 493.
4 M. T. Peracchia, R. Gref, Y. Minamitake, A. Domb, N. Lotan and R. Langer, *J. Control. Release*, 1997, **46**, 223.
5 P. de Gennes, in *Model Polymers at Interfaces*, ed. P. Bongrand, CRC Press, Florida, 1988, p. 91.
6 E. B. Zhulina and T. A. Vilgis, *Macromolecules*, 1995, **28**, 1008.

7 V. G. Babak, *Usp. Khim.*, 1994, **63**, 228 (*Russ. Chem. Rev.*, 1994, **63**, 217).

8 P. Bouillot, R. Gref, A. Petit and E. Dellacherie, *J. Appl. Pol. Sci.*, 1998, **68**, 1695.

9 V. G. Babak, *Fizikokhimiya mikroskopicheskikh zhidkikh plenok, stabilizirovannykh polimerami. 2. Modelirovanie kontaktnykh vzaimodeistvii v polimersoderzhashchikh dispersnykh sistemakh* (*Physicochemistry of Microscopic Liquid Films Stabilized by Polymers. 2. Modelling of Contact Interactions in Polymer-containing Disperse Systems*), Urals University Press, Sverdlovsk, 1988 (in Russian).

10 V. G. Babak, *J. Chem. Soc., Faraday Trans.*, 1993, **89**, 4059.

11 R. Gref, V. G. Babak, P. Bouillot, I. G. Lukina, M. M. Bodorev and E. Dellacherie, *Colloids Surfaces. A. Physico-chemical and Engineering Aspects*, 1998 (in press).

12 S. I. Jeon, J. H. Lee, J. D. Andrade and P. G. de Gennes, *J. Colloid Interface Sci.*, 1991, **142**, 149.

Received: Moscow, 31st March 1998
Cambridge, 1st May 1998; Com. 8/02789G