Effect of the type of hydrophobic polymers on the size of nanoparticles obtained by emulsification–solvent evaporation

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The effect of the molecular structure of hydrophobic polymers on their interfacial activity at the methylene chloride–water interface, as well as on the emulsifying ability and the size of nanoparticles obtained by emulsification–solvent evaporation, has been studied.

Biocompatible and biodegradable hydrophobic polymers such as poly(ε-caprolactone) (PCL), poly(D,L-lactide-co-glycolides) (PLGA) and ammonio poly(methyl methacrylates) (Eudragits RL and RS) are widely used in medicine for the preparation of micro- and nanoparticles: the vectors of drugs, vaccines, enzymes, genetic materials, *etc*. The emulsification of organic solutions of these polymers in an aqueous phase followed by the evaporation of organic solvents is a common method for the synthesis of vectors.[1–3] The crucial step of this method is the emulsification of the organic solutions of hydrophobic polymers containing additional bioactive substances as colloidal dispersions in an aqueous phase with the subsequent solidification of emulsion droplets by the evaporation of the organic solvent in a vacuum.

The interfacial activity of hydrophobic polymers at a water/oil boundary and the viscosity of organic solutions of these polymers significantly affect the size of emulsion droplets and, consequently, the size of resulting micro- and nanoparticles after solvent evaporation. The nanoparticle size is a very important parameter responsible for drug distribution, absorption and biological activity after administration.[4–5]

The interfacial behaviour of a series of hydrophobic polymers in connection with their emulsifying ability was studied in detail.[4] The aim of this work was to study the effect of the molecular structure of hydrophobic polymers on the size of nanoparticles prepared by emulsification–solvent evaporation.

Poly(D,L-lactide-co-glycolide) 50/50 (PLGA) (MW 40000 Da) (Medisorb Technologies), poly(ε-caprolactone) (PCL) (MW 42000 Da) (Aldrich), Eudragit® RS100 and RL100 (MW 150000 Da) (Röhm Pharma) (Figure 1), poly(vinyl alcohol) (PVA) (MW 30000 Da, 88% hydrolysed) (Sigma), and methylene chloride (Sigma) were used. Water was purified using a Milli-Q plus 185 system (Millipore).

Nanoemulsions (a droplet size of ~10–100 nm) were prepared according to a standard procedure.[2,3] A 150 ml portion of a stock polymer solution in methylene chloride (5%, w/v) was dispersed in water saturated with methylene chloride (2.85 ml) using an ultrasound probe at 15 W for 30 s (1 s pulses at 1 s intervals).

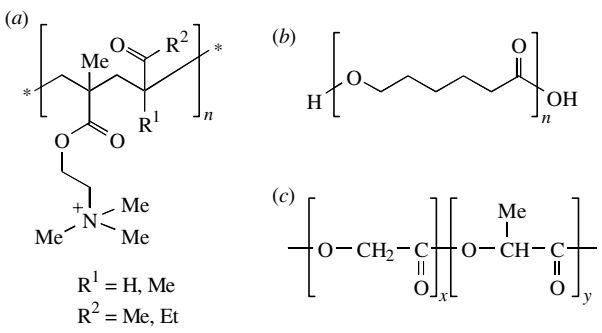

Figure 1 Structural formulae of (*a*) Eudragit RL and RS, (*b*) poly(ε-caprolactone) and (*c*) poly(lactid-co-glycolide).

Nanoparticles were prepared by oil-in-water emulsification and solvent evaporation. A 200 ml portion of a stock polymer solution (5%, w/v) was dispersed in either water (3.8 ml) or an aqueous PVA solution (0.1%, w/v) saturated with methylene chloride with an ultrasound probe for 15 s. The organic solvent was removed under a reduced pressure at 40 °C for 5 min.

The sizes of methylene chloride-in-water emulsion droplets and nanoparticles were determined by laser light scattering using a Zetamaster instrument (Malvern Instruments). The results were normalised using a polystyrene standard suspension (Malvern Instruments).

The interfacial tension at the methylene chloride–water interface was measured by the dynamic pendent drop method with a drop tensiometer (Traker).[7]

The viscosity of polymer solutions was determined using an Ubhellode viscosimeter with a glass capillary (i.d. 0.56 mm) at 20 °C.

It is well known that the type of polymers considerably affects the size and, consequently, physico-chemical parameters such as drug stability, loading and release.[3] The emulsions under consideration are widely used for the one step preparation of nanoparticles.

Figure 2 demonstrates the time dependence of the mean radius of emulsion droplets for various polymers. The emulsions were prepared under the same conditions (sonication time and power). Table 1 summarises the sizes of nanoparticles obtained from these emulsions by methylene chloride evaporation.

The emulsifying properties of polymers can be characterised by an initial droplet size at zero time. The initial droplet sizes obtained immediately after sonication increased in the order Eudragit RL ≅ Eudragit RS < PLGA < PCL, demonstrating that the emulsifying properties of polymers increase from PCL

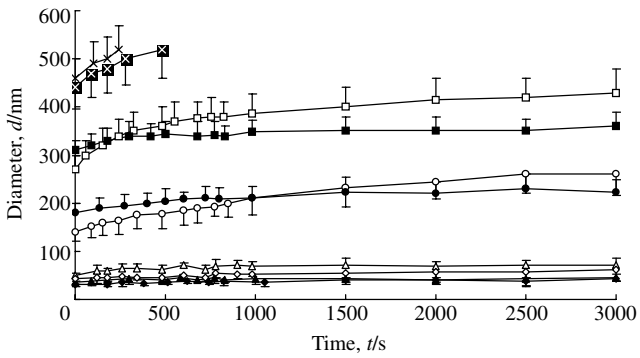

Figure 2 Mean droplet diameters of methylene chloride-in-water emulsion as a function of time in the presence (filled symbols) or absence of PVA (open symbols): Eudragit RL (◇), Eudragit RS (△), PLGA (○), PCL (□) and without polymers (×).

Table 1 Mean diameters and zeta potentials of nanoparticles prepared by oil-in-water emulsification and solvent evaporation with PCL, PLGA and Eudragit RS and RL.

Polymer	Diameter/nm		Zeta potential/mV	
PVA (%)	0	0.1	0	0.1
PCL	430±20	370±10	–20±1	–17±1
PLGA	340±3	320±5	–22±2	–17±1
Eudragit RS	290±5	240±2	+61±1	+42±3
Eudragit RL	277±4	266±10	+66±1	+53±1

to Eudragits. As assumed, the polycationic Eudragit polymers led to positively charged nanoparticles, whereas the other uncharged polymers led to slightly negative zeta potentials.

The addition of PVA to the aqueous phase of an emulsion led to a detectable but insignificant increase in the emulsifying properties of Eudragit polymers; this effect was not found for PLGA and PCL. The slopes of the kinetic curves characterised the overall stability of the emulsions: the lower the slope, the smaller the rate of droplet coalescence and the more stable the emulsion. Note that the order of polymers was the same as for the emulsifying properties. As expected, PVA played its role of a surfactant since the stabilising ability of PLGA and PCL (as found from the slopes of kinetic curves) was more pronounced than without PVA.

Figure 3 demonstrates the isotherms of the reduced viscosity η_{red} of hydrophobic polymer solutions in methylene chloride as a function of concentration. In the test concentration range, all the $\eta_{red}(C)$ isotherms are described by the Huggins equation

$$\eta_{red} = [\eta] + k_H[\eta]^2 C,$$

where $[\eta]$ is the intrinsic viscosity, and k_H is the Huggins constant.[8] The critical overlap concentrations for the polymers, which were estimated using the relationship $C^* = 1/[\eta]$ (Table 2), show that, for the polymer PCL, the semi-dilution concentration threshold (where the macromolecular coils sterically interact) is ~2%, whereas it is equal to ~3 % for PLGA. For the Eudragit polymers, the studied concentration range 0–5% corresponds to a dilute regime because the corresponding values of C^* for these polymers are equal to ~10%.

The intrinsic viscosity $[\eta]$ decreases in the order PCL > PLGA > Eudragit RS > Eudragit RL (Table 2). Therefore, the size of the macromolecular coils of these polymers in methylene

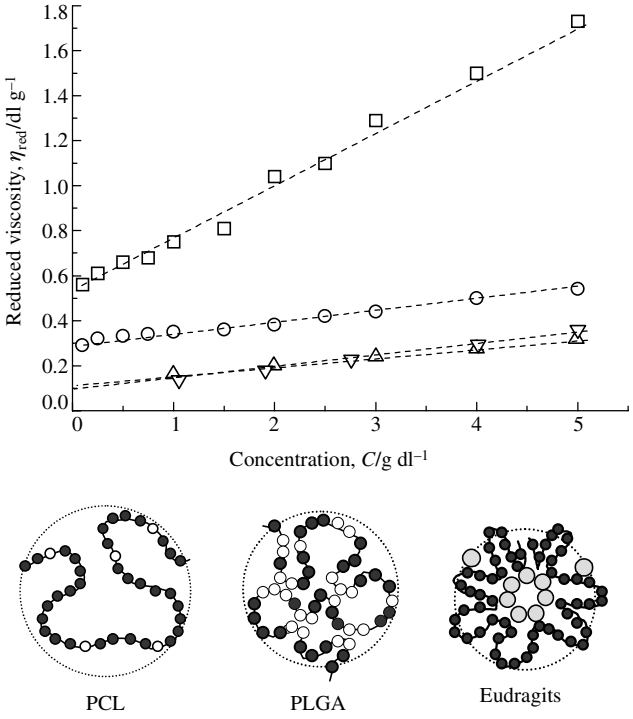

Figure 3 Reduced viscosity versus polymer concentration in methylene chloride solutions: PCL (□), PLGA (○), Eudragit RL (▽) and Eudragit RS (△).

Table 2 Main physico-chemical characteristics of Eudragit (RS and RL), PLGA and PCL solutions in methylene chloride emulsified in water without PVA.

Polymer	d_0/nm	$[\eta]$/dl g^{-1}	$\sigma_{5\%}$/mN m^{-1}	M/kDa
Eudragit RL	42±5	0.1±0.05	3±1	150
Eudragit RS	50±10	0.1±0.05	4±1	150
PLGA	140±20	0.3±0.05	24±1	40
PCL	270±20	0.55±0.05	28±1	42

chloride solutions (their hydrodynamic radius R_h) also increases in the same order; *i.e.*, it is the greatest for PCL and the smallest for Eudragit. This suggests that the thermodynamic quality of methylene chloride as a solvent is the highest for PCL, and it was lower for PLGA and Eudragits. In general, the better the thermodynamic quality of a solvent for a given hydrophobic polymer, the higher the size of a macromolecular coil and the higher the characteristic viscosity $[\eta]$. This is true, even though the molecular mass of PCL (42 kDa) is much lower than that of Eudragits (~150 kDa) (Table 1). In methylene chloride solutions, PCL behaves as a rather extended (swelled) statistical coil, whereas Eudragits takes a very compacted (collapsed) conformation (Figure 3). The macromolecule of PLGA can be represented as a rather compact coil similar to that of the Eudragit polymers.

At the molecular level, the difference in the thermodynamic quality of methylene chloride for these polymers is explained by difference in the polarity of the constitutive or pendent segments of the macromolecular backbone. The grafted polar trimethylammonium groups of the Eudragit polymers are shielded from the solvent molecules in compact micelle-like aggregates. Similarly, more polar glycolide segments of PLGA are shielded from the solvent by less polar lactide segments.

Figure 4 illustrates the behaviours of polymers at the methylene chloride/water interface. The low interfacial tension obtained with Eudragit polymers may be explained by the ionisation of hydrophilic quaternary ammonium groups, which therefore are hydrated. The desorption of these hydrophilic groups in the methylene chloride solution involves a decrease in enthalpy and the polymers are adsorbed irreversibly at the oil/water interface: there is an adsorption layer at the interface. On the other hand, PLGA and PCL polymers, which have no ionisable hydrophilic groups, do not display any significant adsorption at the methylene chloride/water interface, explaining the non-significant decrease in the interfacial tension.

However, the difference between PCL and PLGA can be explained by the more hydrophilic properties of PLGA with regards to PCL.[9] Indeed, PLGA can be slightly adsorbed at the interface, as follows from a decrease in the interfacial tension by approximately 5 mN m^{-1}, whereas no decrease was observed for PCL (Figure 4). The equilibrium interfacial tension $\sigma_{5\%}$ (Table 2) corresponds to the order PCL > PLGA > Eudragit RS > Eudragit RL. The interfacial tension of Eudragit RL lower than that of Eudragit RS may be explained by the higher density of quaternary ammonium groups in Eudragit RL.

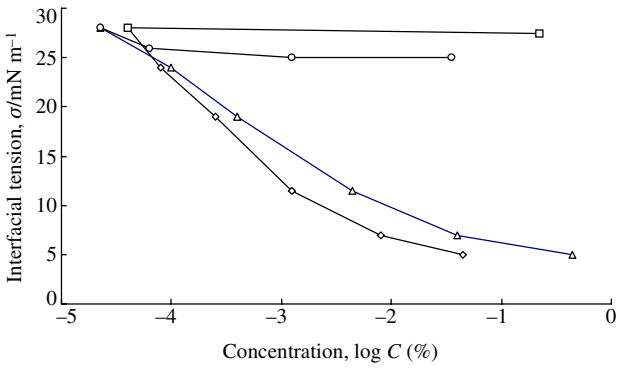

Figure 4 Interfacial tension isotherms for the methylene chloride solutions of polymers in contact with water: PCL (□), PLGA (○), Eudragit RS (△) and Eudragit RL (◇).

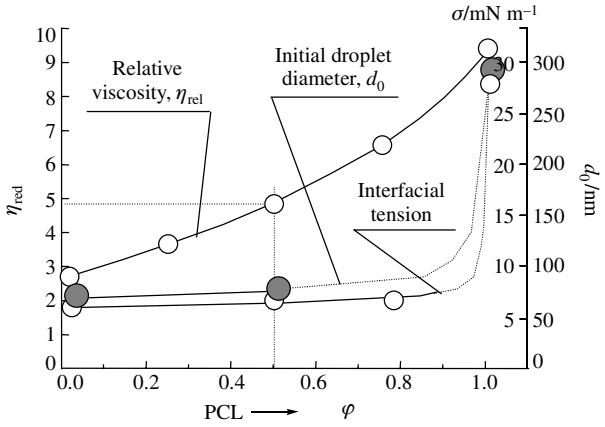

Figure 5 Relative viscosity, interfacial tension and initial droplet size of the blends of Eudragit RL and PCL dissolved in methylene chloride (5% w/v) *versus* φ.

Both the viscosity and the interfacial tension of organic polymer solutions affect the droplet size of emulsions and consequently the mean nanoparticle diameter. However, it is important to know the relative contributions of these two parameters to the emulsion droplet size. We experimentally studied the emulsion droplet size as a function of time with a blend of PCL and Eudragit RL at different concentration ratios for a total concentration of 5% (w/v).[6]

We found that the droplet diameter d_o for a mixture of 2.5% PCL + 2.5% Eudragit RL is similar to that observed in a 5% Eudragit RL solution in methylene chloride (Figure 5). The interfacial tension of this polymer mixture was consistent with that of a 5% organic solution of individual Eudragit RL. On the other hand, the relative viscosity $\eta_{rel} = \eta/\eta_o$ (η_o is the viscosity of methylene chloride) of the organic solutions increased linearly for φ up to 0.8 [$\varphi = C_{PCL}/(C_{Eud} + C_{PCL})$ is the parameter which characterizes the composition of mixed PCL/Eudragit RL solutions in methylene chloride at $C_{Eud} + C_{PCL} = 5\%$] (Figure 5). For the above concentrations (2.5% PCL + 2.5% Eudragit RL, w/v), the relative viscosity is equal to 4.7. This value is slightly greater than a half of the relative viscosity of a 5% (w/v) organic solution of PCL. Thus, we found that interfacial tension plays a much more important role in emulsification than the viscosity of a dispersed phase.

The higher emulsifying and stabilising ability of the polycationic polymers compared to PCL and PLGA may be explained by the formation of charged adsorption layers and the electric double layers of ions. According to the DLVO theory, the electrostatic repulsion of double layers is a well-known stabilising factor. In addition, the steric repulsion between the hydrophilic charged groups of Eudragits might also contribute to the overall droplet stability. Because PCL does almost not form adsorption layers, its stabilising properties are very poor. The stabilising ability of PLGA is relatively low; nevertheless, it is higher than that of PCL due to its higher adsorption ability.

By using the same preparation technique (o/w emulsification) with various types of polymers, we found that the sizes of emulsion droplets and the resulting nanoparticles were dramatically different. The physico-chemical parameters affecting the particle diameter are the viscosity and the interfacial tension of organic polymer solutions in contact with water. Moreover, the interfacial tension is of prime importance with regards to viscosity. Indeed, Eudragit polymers, which exhibit the lowest interfacial tension, afforded the smallest particles. Finally, it can be postulated that Eudragit polymers have good surface-active properties, which allow one to prepare nanoparticles without surfactants.

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