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Note

Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan

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Abstract

Oil-in-water nanoemulsions were prepared using a series of synthetic graft copolymers with a backbone of dextran (DEX) and a number of side chains of poly-ε-caprolactone (PCL). In this paper, we focus on the o/w emulsion stabilizing abilities of these novel PCL-DEX copolymers, using a recently developed optical analyzer (Turbiscan). The main advantage of Turbiscan is to detect the destabilization phenomena in non-diluted emulsion, much earlier than the naked eye's operator, especially in the case of an opaque and concentrated system. This study shows that PCL-DEX copolymers successfully stabilized ethyl acetate-in-water emulsions, even in the absence of additional surfactants, whereas they were not efficient in stabilizing methylene chloride-in-water emulsions which coalesced fast and irreversibly. The ethyl acetate-in-water emulsion stabilizing ability of PCL-DEX seemed to be related to the localization of their blocks with regard to the oil–water interface.

Keywords: Nanoemulsion; Amphiphilic copolymers; Stabilization; Turbiscan

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In the last decades, an intensive research has been focused on the development of biodegradable and biocompatible materials presenting emulsion stabilizing abilities for pharmaceutical application. These stabilization properties appear crucial particularly in the case of the formulation of colloidal carriers.

Macromolecules, such as amphiphilic copolymers consisting of polyester blocks (poly(lactic) acid) coupled to a hydrophilic polymer (polyethylene glycol) revealed excellent emulsion stabilizing abilities and could advantageously replace the commonly employed surfactants such as poly(vinyl alcohol) (Gref et al., 1998).

More recently, a novel family of amphiphilic PCL-grafted dextran (PCL-DEX) copolymers was synthesized for medical application (Gref et al., in press). The aim of this work was to investigate the o/w emulsion stabilizing abilities of a series of PCL-DEX, using a recently developed optical analyzer (Turbiscan).

This apparatus was successfully used to study the stability of various emulsions and concentrated colloidal dispersions (Mengual et al., 1999). Indeed, in these cases, destabilization can occur following irreversible processes such as coalescence or flocculation, or following reversible processes such as creaming or sedimentation. The main advantage of Turbiscan is to

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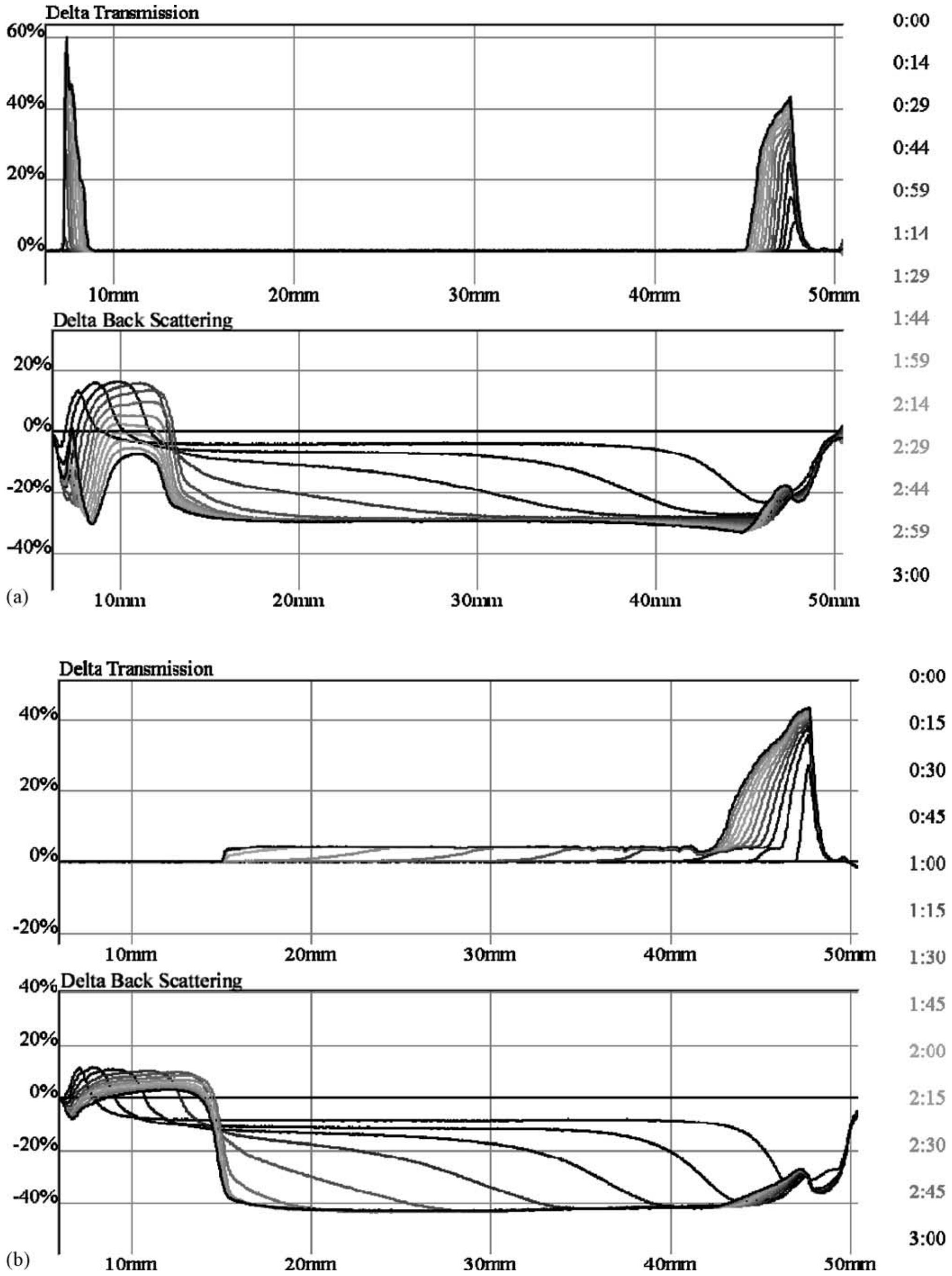

Fig. 1. Transmission and backscattering data of methylene chloride-in-water emulsions stabilized with PCL-DEX copolymers bearing 3 PCL side chains, in the absence (a) or in the presence (b) of sodium cholate. These data are represented as a function of time (0:00 to 3:00 hours) and of sample height (6 to 50 mm).

detect these phenomena much earlier than the naked eye's operator, especially in the case of opaque and concentrated systems.

PCL-DEX copolymers were obtained by grafting preformed PCL chains to DEX by means of ester bridges (Gref et al., in press). Amphiphilic copolymers with 3–7 grafted PCL chains and with hydrophilic–lipophilic balances (HLB) ranging from 2 to 7 were thus obtained. Graft copolymers are known to be among the most effective stabilizers (Napper, 1977). PCL-DEX were insoluble in water and in most of the commonly used organic solvents for nanoparticle preparation. However, a method was developed: PCL-DEX copolymers (5 mg) were added in a glass vial with 1 ml of organic solvent (methylene chloride or ethyl acetate) and 5 ml of water or surfactant solution (sodium cholate 0.1% (w/v)). An o/w rough emulsion was formed under magnetic stirring. The size of the droplets was reduced by sonication (Microprobe, 60 s, pulses of 1 s each, Vibra Cell^{TM} VC750, Sonics and Materials, Newtown, USA) thus forming a nanoemulsion.

The stability of the o/w emulsions obtained using PCL-DEX copolymers bearing 3–7 grafted PCL side chains was analyzed using a Turbiscan MA 2000 (Formulaction, L'Union, France). In this study, the molecular weights of DEX backbone and PCL side chains were, respectively, 5000 and 2000 g/mol. Five ml of emulsion samples prepared as previously described were placed in a cylindrical glass cell. The detection head was composed of a pulsed near-infrared light source ($\lambda = 850$ nm) and two synchronous transmission (T) and back scattering (BS) detectors. The T detector received the light which goes across the sample (at 180° from the incident beam), while the BS detector received the light scattered backward by the sample (at 45° from the incident beam). The detection head scanned the entire height of the sample (about 50 mm), acquiring T and BS data each 40 μm and each minute during at least 3 h. The principle of the measurement was based on the variation of the droplet volume fraction (migration) or diameter (coalescence), resulting in a variation of BS and T signals.

As expected, in the absence of PCL-DEX copolymers, ethyl acetate or methylene chloride-in-water emulsions prepared in the same conditions split very rapidly in two phases due to their unfavorable thermodynamic state (data not shown). The fast apparition of a clear aqueous phase was reflected by an increase in the T signals. The addition of the anionic water soluble surfactant sodium cholate allowed to delay phase separation, but could not avoid it. However, the presence of amphiphilic PCL-DEX copolymers in the system could greatly influence the stability of these o/w emulsions, depending on the nature of the organic phase. Two cases will be further analyzed: stable emulsions obtained with ethyl acetate and unstable ones obtained with methylene chloride.

Fig. 1 shows a typical transmission (T) and back scattering (BS) profiles (14 selected scans, one every 15 min) obtained in the case of methylene chloride-in-water emulsions stabilized with a PCL-DEX copolymer bearing in average 5.5 grafted PCL side chains, as function of the height of the sample. It should be pointed out that BS signal can only be analyzed if T signal is nil. Otherwise, the partial reflection of the light crossing the sample by the walls of the measurement cell would interfere with the BS signal. The methylene chloride-in-water emulsions were milky and opaque all over the experiment. At $t = 0$ the T signal was nil and the BS signal was around 50%. To better visualize the signal modifications as a function of time, T and BS profiles at $t = 0$ were subtracted from all the others. The BS profiles clearly indicate a destabilization process occurring in three steps schematically represented in Fig. 2A.

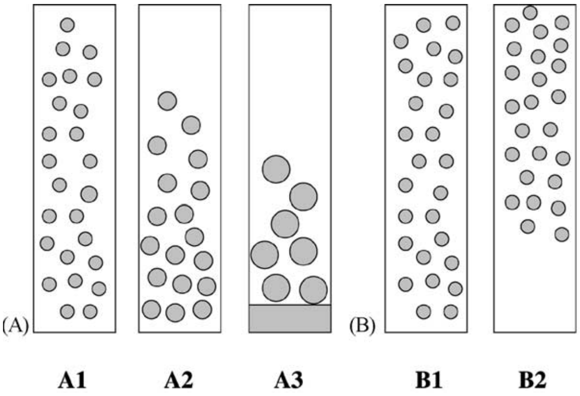

Fig. 2. Schematic representation of the evolution in time of methylene chloride-in-water (A) and ethyl acetate-in-water (B) emulsions stabilized with PCL-DEX copolymers. A1 and B1: freshly prepared emulsions; A2: coalescence and sedimentation; A3: phase separation; B2: reversible creaming.

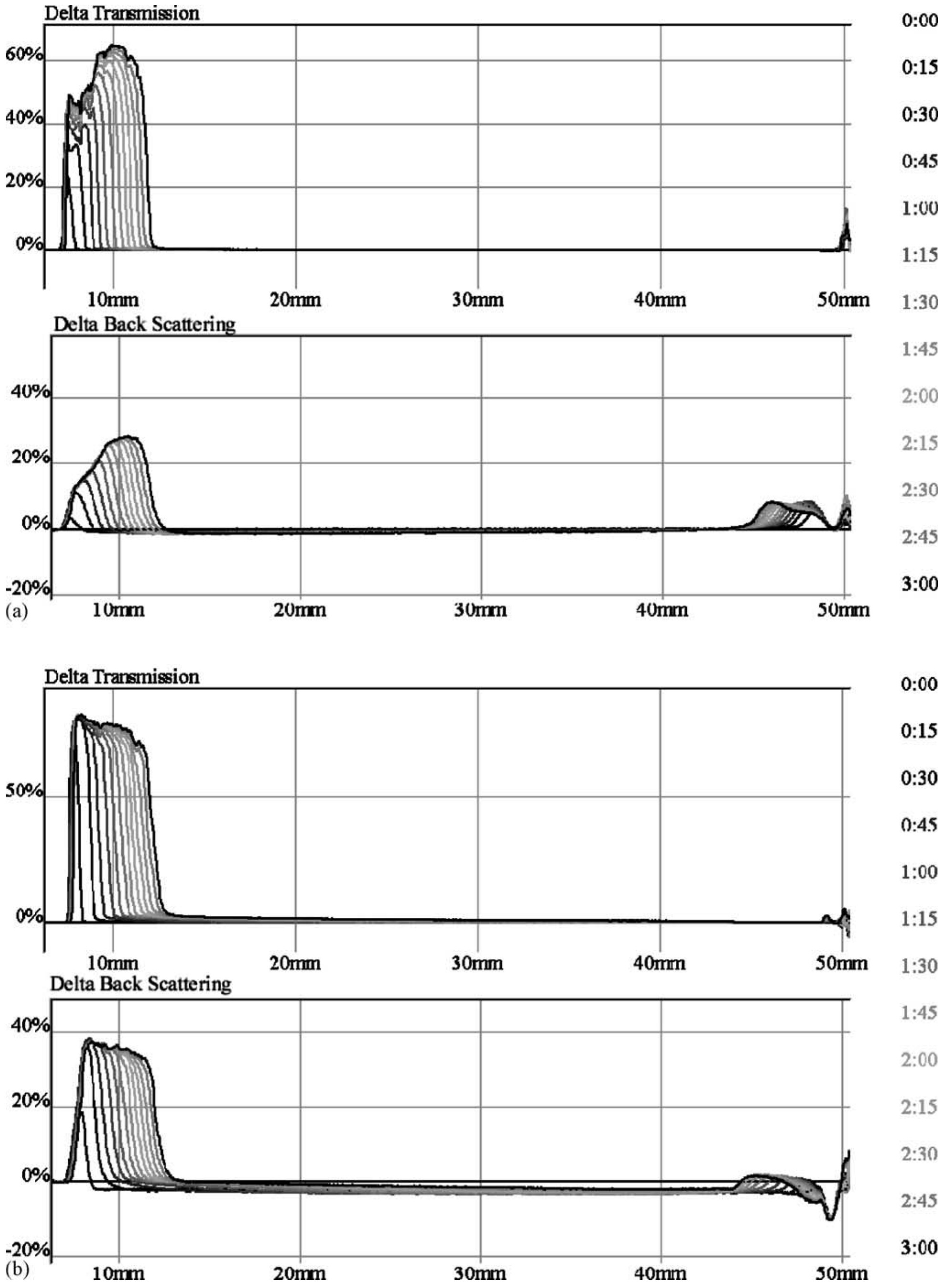

Fig. 3. Transmission and backscattering data of ethyl acetate-in-water emulsions stabilized with PCL-DEX copolymers bearing 5.5 PCL side chains, in the absence (a) or in the presence (b) of sodium cholate. These data are represented as a function of time (0:00 to 3:00 hours) and of sample height (6 to 50 mm).

(a) Firstly, BS signal decreased between 12 and 50 mm and increased in the bottom of the sample (8–12 mm), indicating that the methylene chloride nanodroplets migrated to the bottom of the sample due to their higher density (1.36 at 20 °C).
(b) Secondly, BS signal decreased in the bottom of the sample (6–8 mm), indicating that the droplets started to coalesce. Possibly, the increase in nanodroplet density due to sedimentation promoted their contact and therefore their coalescence.
(c) Finally, after about 1 h, an increase of the T signal in the bottom of the sample was observed, indicating that this zone was starting to clarify. This was attributed to an irreversible phase separation occurring at this late stage of coalescence, when the interfacial polymeric film was broken, leading to methylene chloride expulsion (Fig. 2A3). When a hydrophilic surfactant (sodium cholate) was added in the aqueous phase, no T peak appeared in the bottom of the sample (Fig. 1b), indicating that phase separation was delayed.

In conclusion, DEX-PCL copolymers failed to stabilize methylene chloride-in-water emulsions, due to the inadequacy of their HLB (<7) for this type of emulsions (Briant, 1989). On the contrary, when methylene chloride was replaced by ethyl acetate, all DEX-PCL copolymers successfully stabilized the o/w emulsions, whatever their HLB (Fig. 3). During the three hours of scanning, no coalescence occurred. Indeed, both T and BS remained close to zero in the middle of the samples. The only phenomenon observed was droplet migration to the top of the sample due to the lower density of ethyl acetate (0.9 at 20 °C). The clarification of the sample bottom (7–15 mm) correlated with the apparition of positive T peaks. Simultaneously, the droplet concentration (creaming) at the sample top (44–50 mm) correlated with the increase in the BS signals. The addition of sodium cholate in the aqueous phase contributed to slow down the process of creaming (Fig. 3b), probably because of the reduction in the initial droplet mean diameter.

Taking into account that droplet migration is a reversible process, and that the nanodroplets' mean diameters were constant during the 3-h experiments, we could conclude that PCL-DEX copolymers were effective in stabilizing ethyl acetate-in-water emulsions, despite of their hydrophobic character ($2 < \text{HLB} < 7$). To explain this interesting property, we took into consideration the possible conformations of the hydrophobic PCL blocks and of the hydrophilic DEX backbone at the o/w interface.

At room temperature, PCL is insoluble in water, highly soluble in methylene chloride, but poorly soluble in ethyl acetate, a more polar solvent. DEX is soluble only in water. When PCL-DEX copolymers are put in contact with the organic and water phases, DEX migrates to the water interface. In methylene chloride, PCL would adopt a coil conformation, whereas in ethyl acetate it would more probably adapt a flattened conformation, at the interface of the two phases. This location would increase the exposure of the DEX backbone in the water phase and therefore maximize the thickness of the steric protective layer.

PCL-DEX copolymers successfully stabilized ethyl acetate-in-water emulsions, even in the absence of additional surfactants. Their emulsion stabilizing ability are supposed to be related to the localization of their blocks with regard to the oil–water interface. Turbiscan revealed to be a helpful tool to study the destabilization of methylene chloride-in-water emulsions and help choosing the most adapted solvent for emulsion stabilization.

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