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ScienceDirect
International Journal of Pharmaceutics 338 (2007) 297–305
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Physico-chemical characterization of nanocapsule polymeric wall using fluorescent benzazole probes

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Received 12 December 2006; received in revised form 27 January 2007; accepted 29 January 2007
Available online 3 February 2007

Abstract

Fluorescent polymers were used to prepare innovative formulations with the objective of verifying the chemical composition of the particle/water interface of nanocapsules at a molecular level. The benzazole dyes distinguish between apolar and polar/protic environments. Comparing the fluorescent behavior of benzoxazole-loaded nanocapsules (entrapped dye) with that of fluorescent-polymeric nanocapsules (chemically bound dye), the results indicated that the latter was exposed to a different environment than that to which the entrapped dye was exposed. The polymer in the nanocapsule suspensions is actually at the oil/water interface, interacting with both inner and outer pseudo-phases at the same time. The polymer is restricted at the particle/water interface forming a wall in nanocapsules. The physico-chemical stability of nanocapsules was studied by fluorescence, light scattering, ζ-potential and potentiometry. After 15, 30, 45 and 60 days of preparation different fluorescent behaviors were observed for the benzimidazole physically entrapped in nanocapsules compared to the benzimidazole chemically bound to the polymer wall. This spectrum presented an isoemissive point indicating that only two species were in equilibrium in the medium. The study showed that the water is increasingly interacting with the polymer in the nanocapsule suspensions.

Keywords: Polymeric nanoparticles; Nanocapsules; Benzazole; Fluorescent polymers; ESIPT; Stability study

1. Introduction

In the past 20 years, nanocarriers for drug delivery have been extensively studied in the pharmaceutical nanotechnology field, as well as, more recently, in different nanoscience areas including chemistry, physics and biology. Different systems have been proposed comprising inorganic or organic nanodevices. In general, some promising nanocarriers are the liposomes (Fattal et al., 2001; Koynova and MacDonald, 2004), the solid lipid nanoparticles (Müller et al., 2000), the self-assembled lipid superstructures (Barauskas et al., 2005), the polymeric micelles (Kang et al., 2005) and the polymeric nanoparticles (Brannon-Peppas, 1995; Fernández-Urrusuno et al., 1997; Couvreur et al., 2002; Schaffazick et al., 2003; Schaffazick et al., 2005; Teixeira et al., 2005). The nanoencapsulation improves drug efficacy, drug bioavailability or it is proposed to decrease drug side effects (Couvreur et al., 2002; Schaffazick et al., 2003).

Among the different nanocarriers, the polymeric nanoparticles are sub-micrometric drug carriers prepared by *in situ* polymerization or by precipitation of pre-formed polymers. The general term, nanoparticles, includes nanospheres and nanocapsules, which are made of polymer and polymer and oil, respectively. The theoretical models for those nanoparticles are, respectively, a polymeric matrix and a vesicle (Couvreur et al., 2002). The release properties of nanospheres and nanocapsules are frequently compared to each other, as well as those nanoparticles are compared to nanoemulsions (Calvo et al., 1996; Guterres et al., 2000; Pohlmann et al., 2004; Cruz et al., 2006a) that are sub-micrometric emulsions. Overall, the physico-chemical characteristics of those nanocarriers have been identified as critical for the control of the release properties of encapsulated drugs (Leroueil-le Verger et al., 1998; Couvreur et al., 2002; Pohlmann et al., 2004).

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0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.01.051

Different raw materials are proposed to prepare nanocapsules, including polyesters or acrylic polymers, as polymers, and triglycerides, benzyl benzoate, large size alcohols or mineral oil, as oil cores (Schaffazick et al., 2003). Several surfactants are used as phospholipids, sorbitan monostearate, polysorbates, dextran, poly(ethyleneglycol) or mixtures of poly(ethyleneglycol) and poly(propyleneglycol) to stabilize the systems (Fessi et al., 1989; Couvreur et al., 2002). Due to the complexity of the systems, any model of the organization of those components at a molecular level can only be proposed for each nanocarrier system after a full physicochemical study (Mosqueira et al., 2000; Müller et al., 2001). For instance, nanocarriers prepared with poly(lactide) or poly(ε-caprolactone), as polymers, and benzyl benzoate, as oil, correspond to nanoemulsions instead of nanocapsules (Guterres et al., 2000). This conclusion was supported by the results of swelling experiments, proposed to verify the solubility of those polymers in this oil, and HPLC, which showed the drug concentration after spray-drying due to losses of benzyl benzoate from the formulation. The organization of the components at a molecular level has been proposed for nanocapsules prepared with poly(ε-caprolactone), caprylic/capric triglyceride, sorbitan monostearate and polysorbate 80 (Müller et al., 2001). DSC curves showed the melting peak of the polymer, the absence of the sorbitan monostearate melting and the decrease of the melting temperature of the triglyceride indicating that, in those nanocapsules, the sorbitan monostearate is dissolved in the oil phase and that the polymer is probably surrounding the core. This model was corroborated by SAXS analyses carried out more recently (Cruz et al., 2006a,b). In another work (Pohlmann et al., 2002), a comparative study has been carried out by dynamic light scattering using nanocapsules prepared with poly(ε-caprolactone), mineral oil, sorbitan monostearate and polysorbate 80 and the respective nanospheres (omitting the oil), nanoemulsion (omitting the polymer) and nanodispersion of surfactants (omitting both the polymer and the oil). In this case, after determining the relative virial diffusion coefficients (k_D) for each formulation, those nanocapsules presented low interaction with water, due to the presence of sorbitan monostearate dispersed in the polymer. This model was corroborated by SAXS analyses (Cruz et al., 2006a,b).

The use of fluorescent dyes to label structures is a versatile method to probe a system (Campo et al., 2000; Rodembusch et al., 2005). The benzazole dyes are able to differentiate chemical environments due to dual emissions (normal and ESIPT). The ESIPT is resulted from an intramolecular proton-transfer mechanism in the excited state (Rodembusch et al., 2005). In the ESIPT mechanism (Fig. 1), the UV light absorption by the enol-*cis* (E_I) produces the excited enol-*cis* (E_I*), which is quickly converted to an excited keto-tautomer (K*) by an intramolecular proton transfer. The tautomerism occurs because the hydrogen in E_I* becomes more acid and the nitrogen more basic than in the E_I conformer. In the K*, an intramolecular hydrogen bond is also formed by the interaction between N–H and C=O (Rodembusch et al., 2005). The K* decays to a keto-tautomer (K) emitting fluorescence, and the initial E_I is regenerated after tautomerism without any photochemical change. Depending on

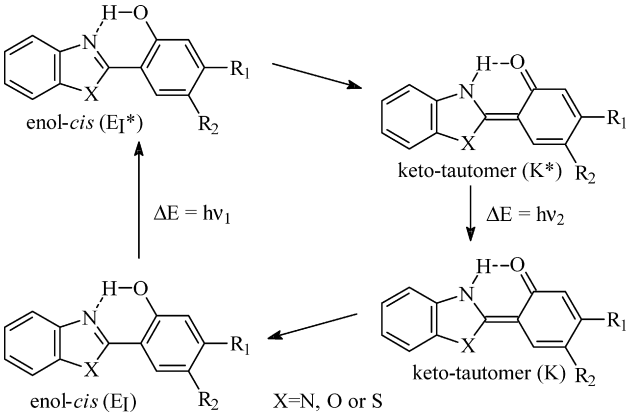

Fig. 1. Excited state intramolecular proton-transfer mechanism (ESIPT).

the chemical environment, E_I can equilibrate with other conformers, like enol-*cis* open conformer (E_{II}) (Fig. 2), which can be stabilized by intermolecular hydrogen bond with the solvent. E_{II} is originated from the E_I intramolecular hydrogen bond rupture between the hydrogen of the OH group and the nitrogen at the position 3, followed by rotation of the 2-hydroxyphenyl group. In non-polar solvents additional enol-*trans* conformers (E_{III}) in benzoxazoles and benzothiazoles (X = O and S, respectively) and enol-*trans* open (E_{IV}) in benzimidazoles (X = NH) could also exist. The conformers (E_{II} to E_{IV}) present normal relaxation and can compete with E_I, which produces E_I* and consequently the excited keto-tautomer (K*), responsible for the ESIPT mechanism (Rodembusch et al., 2005). In this way, the benzazoles are able to differentiate the chemical environments of either polar and non-polar or protic and non-protic solvents.

Fluorescent polymers prepared by radicalar polymerization of methyl methacrylate and benzazoles have been reported (Campo et al., 2000; Rodembusch et al., 2005). Then, we have chosen two of those benzazole-labeled polymers to prepare innovative formulations containing fluorescent-labeled polymeric nanocapsules with the objective of verifying the physicochemical interactions at a molecular level in the particle/water interface of those systems. Our specific goal was to determine the chemical composition of the interface of nanocapsules prepared

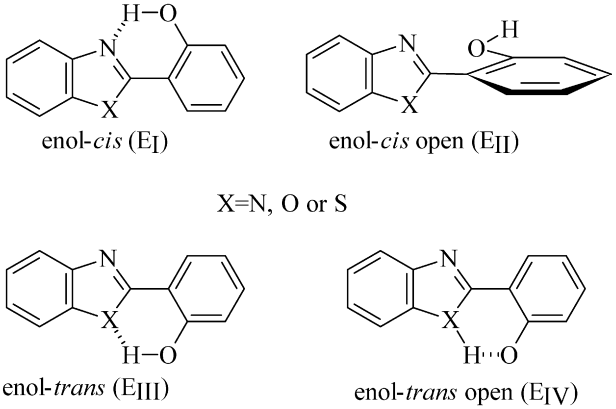

Fig. 2. Structures of enol-*cis* (E_I), enol-*cis* open conformer (E_{II}), enol-*trans* conformers (E_{III}) and enol-*trans* open (E_{IV}).