Colloids and Surfaces B: Biointerfaces 108 (2013) 279–284

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Nanocolloids of indomethacin prepared using sonication and subsequent encapsulation with polysaccharide films

Viktoria Milkova, Kamelia Kamburova, Tsetska Radeva*

Institute of Physical Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

ARTICLE INFO

Article history:
Received 20 December 2012
Received in revised form 5 February 2013
Accepted 5 February 2013
Available online 15 March 2013

Keywords:
Water-insoluble drugs
Polysaccharide multilayers
Nano-particles of indomethacin
Layer-by-layer self-assembly
Electro-optics of nano-colloids

ABSTRACT

A new procedure was applied for preparation of indomethacin (IMC) nano-particles (ca. 200 nm), which includes ultrasonication of micron range IMC crystals in water, followed by short centrifugation to remove the larger drug particles. In order to stabilize the suspension against aggregation and to reduce the release time of the drug, water insoluble IMC particles were coated with chitosan/pectin (CHI/PEC) multilayer film at pH 4. Charge balance in the multilayer film and increase in the film thickness with the number of adsorbed layers was studied by means of electro-optics. The release time of IMC molecules from the encapsulated particles was measured at physiological pH 7, when the solubility of IMC in water increases several times. Addition of small amount of $CaCl_2$ after deposition of PEC layers was applied to compact the multilayer films on the IMC particles.

1. Introduction

Intravenous administration of relatively large aggregates or crystals of water-insoluble drugs may cause serious safety problems. The low solubility of such drugs, on the other hand, may not allow for achieving therapeutically effective concentrations [1]. The most popular approach to improve the solubility and dissolution rate of water-insoluble drugs is the use of micellar carriers, although micelles contain a very small percentage of the drug (usually below 5%). Wrapping drug particles with polymers is another approach for preparation of drug delivery systems with higher content of the active drug and controllable release time [1]. Recently, two strategies for preparation of stable aqueous nano-colloids of poorly soluble drugs have been demonstrated [1–3]. According to the first, micron range particles are subjected to ultrasonic treatment in order to decrease the size of the drug particles to nano level. The sonication is applied in the presence of an oppositely charged polymer, which forms highly charged layer on the particle surface and stabilize the suspension against fast particle aggregation [1,2]. The second strategy includes dissolution of the drug in organic solvent that is miscible with water, and then drug nucleation is initiated by gradual addition of an aqueous solution of polyelectrolyte during ultrasonication of the system [3]. The former procedure gives particles of size approximately 200 nm, whereas nano-colloids with diameters of less than 100 nm are obtained using the second one. After stabilization of the suspensions against aggregation, layer-by-layer encapsulation with polyelectrolyte films has been applied to assure sustained drug release of nano-particles up to several hours [1–3].

In the present study, we apply ultrasonication of water insoluble IMC with micron range particles (mostly ranging 5–10 μm) in the absence of polymer. After sonication, the obtained IMC nano-particles are encapsulated according to the layer-by-layer technique using oppositely charged polysaccharides – chitosan (CHI) and pectin (PEC) [4,5]. Our aim was to prepare stable aqueous suspension of encapsulated nano-particles, which are suitable for intravenous administration. At the same time, encapsulation of the drug particles with CHI/PEC multilayer films is applied to slow down the release rate of IMC at pH 7, which mimics the conditions expected in vivo. Prolonged sonication is applied only to the suspension of bare IMC micro-crystals in order to prevent depolymerization of the CHI chains [6,7].

Indomethacin is a non-steroidal anti-inflammatory drug, which is very appropriate for encapsulation because of its pH-dependent dissolution rate [8]. The acidic IMC particles are poorly soluble at pH 4.0 and their dissolution rate increases rapidly at higher pH-s [9]. Encapsulation of the IMC particles with polysaccharides (having pH-dependent density of charges), on the other hand, provides a way to control the drug release from such formulations. Chitosan is a polycation, which is obtained by N-deacetylation of chitin to produce (1→4)-β-D-glucosamine chains [10]. The p*K*a value of the glucosamine segments is 6.3–7 [11]. Pectin is a polyanion, consisting of linear regions of (1→4)-α-D-galacturonosyl units and their methyl esters, interrupted by (1→2)-α-L-rhamnopyranosyl units.

* Corresponding author. Tel.: +359 2 979 39 22; fax: +359 2 971 26 88.
E-mail address: radeva@ipc.bas.bg (T. Radeva).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.02.024

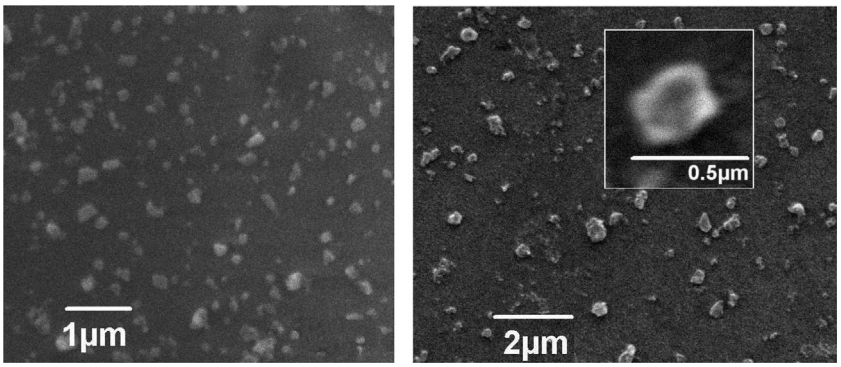

Fig. 1. Scanning electron micrographs of bare indomethacin particles (left) and indomethacin particles coated by CHI/PEC multilayer in acetic acid at pH 4 (right).

The p*K*a value of the galacturonosyl segments in PEC is 3.5 [12]. At pH 4.0, CHI is fully dissociated in a solution, whereas the PEC is dissociated ca. 75% [13]. Both polysaccharides are low cost and biocompatible and attract great scientific and industrial interest.

We employ electric light scattering and electrophoresis to follow stabilization of the IMC suspension against aggregation and to determine electrical properties and thickness of the deposited CHI/PEC layers. IMC release from the encapsulated particles was induced by exposure of the particles, coated at pH 4.0, to a buffer solution of physiological pH 7. Since polysaccharide multilayer films are characterized with great permeability for small ions and molecules, we followed the effect of Ca^{2+} (as a cross-linking agent for PEC) on the structure of the CHI/PEC coating.

2. Experimental

2.1. Materials

Indomethacin was obtained from Sigma–Aldrich. Chitosan with degree of deacetylation 85% and molecular weight 44 kDa was obtained from Sigma–Aldrich. Citrus pectin with degree of esterification 36% and molecular weight 54 kDa was obtained from CP Kelco. Both polymers were used without further purification. CHI solutions of concentration 1 g dm^{-3} were prepared by dissolution in 0.4% acetic acid solution and PEC of concentration 1 g dm^{-3} was dissolved in double-distilled water. The stock polymer solutions were filtered through a 0.45 μm filter to remove possible aggregates.

2.2. Preparation of IMC nano-particles

A certain amount (0.05 g) of IMC was dispersed in 50 ml of water and ultrasonication was applied to the suspension for 30 min at 0 °C (Sonopuls Ultrasonic Homogenizer HD 2200). After sonication, the IMC suspension was centrifugated at 4500 rpm for 20 min to remove the settled (big) particles. The supernatant, containing small IMC particles, was diluted to concentration of 0.1 g dm^{-3} and its pH was adjusted to 4.0 by adding CH_3COOH. This procedure gives particles that can be approximated with elliptical disks. Fig. 1 shows a scanning electron microscope image of the IMC particles. By electron microscopy, the average dimensions of the major and minor axes of the particles are determined to be $a = 200 \pm 70$ nm and $b = 140 \pm 50$ nm (axial ratio 1.4).

2.3. Preparation of polyelectrolyte multilayers

The layer-by-layer technique is based on an alternate adsorption of oppositely charged polyelectrolytes onto charged colloidal particles. The adsorbed polyelectrolyte layers prevent the formation of aggregates when the solution concentration of the polymers is high enough to assure overcompensation of the particle charge. The first layer was deposited by adding the IMC particles to a solution of CHI with concentration 10^{-2} g dm^{-3} (ca. 0.6×10^{-4} monoM dm^{-3}) and stirring for 20 min. The excess polymer was removed by centrifugation of the suspension at 13,500 rpm for 15 min. The settled particles were re-dispersed by short sonication (for 15 s) in a solution containing CH_3COOH to adjust to pH 4. This procedure was repeated by adding the CHI-coated particles to a solution of PEC with concentration of 2×10^{-2} g dm^{-3} (ca. 1.1×10^{-4} monoM dm^{-3}).

2.4. Release of IMC from encapsulated particles.

To study the effect of CHI/PEC coating on the release rate of IMC molecules, centrifuged bare and coated IMC nano-particles were stirred in a pH 7 phosphate buffer solution. UV absorbance at 320 nm was then measured as a function of the dipping time. All measurements were performed in a 3 mL quartz cuvette at a concentration of IMC particles of 0.33 g dm^{-3}.

2.5. Methods

The electro-optical effect studied in the present work is electric light scattering defined by $(= (I_E - I_0)/I_0$, where I_E and I_0 are the scattered light intensities in the presence and in the absence of an electric field [14,15]. The optical effect is related to the electrical moment orientation mechanism, which in turn reflects the electrical double layer features through its electrical polarizability. The kilohertz electro-optical effect, in particular, is usually related to polarization of ions in the diffuse part of the particle electrical double layers.

The transient process of Brownian particle disorientation after switching off the electric field is given by $\alpha_t = \alpha_0 \exp(6D_r t)$, where α_0 and α_t are the values of the electro-optical effect at the moment of switching off the electric field and after a time t. It permits determination of the rotational diffusion coefficient $D_r = 1/6\tau$, relative to the particle dimensions. For thin circular discs, the diameter of the particle B can be determined from the relaxation time of disorientation τ according to the Perrin equation $B^3 = (9kT\tau/2\eta)$, where η is the viscosity of the suspending medium [16]. From the change in the particle dimensions due to the polyelectrolyte adsorption, one can calculate the hydrodynamic thickness L_H of each adsorbed layer.

In this experiment, the electric light scattering from IMC suspensions is recorded at an angle of 90° with respect to the electric field, using white unpolarized light. The method and technical details are described elsewhere [14,17]. The particle electrophoretic mobilities U_e are measured using a Rank Brothers Mark II apparatus with a flat quartz cell at 25 °C.