Encapsulation of poorly soluble substances via self-assembling of amphiphilic copolymers and polyelectrolytes

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Introduction

Poorly water soluble drugs (PSD) represent a large group of therapeutically active agents. The improvement of bioavailability of PSD is one of the actual tasks of pharmaceutical technology. Colloidal delivery systems were proved efficient for both increasing PSD apparent solubility in water and protecting from degradation in the case of labile drugs [Torchilin V.P., 2005; Agnihotri S.A., 2004]. Of particular interest are pH-sensitive microsystems based on polyelectrolytes, we recently elaborated the major principles for their constructing and behavior [Balabushevich N.G., 2004]. The aim of this study was to encapsulate PSD via self-assembling of amphiphilic copolymers and polyelectrolytes. Here we used as PSD phenolic antioxidant – quercetin (Q) and tamoxifen (Tam)– a well known antiestrogen, used in breast cancer therapy. For self-assembling we applied recently synthesized amphiphilic graft copolymer dextran-poly-ε-caprolactone (Dex-PCL) [Gref R., 2003], as well as water-soluble conjugates of chitosan (Ch) with Q being polycations and polyanion of chitosan sulfate (ChS).

Materials and Methods

Materials. Ch enzymatically hydrolysed (Ch_{30}) M_W 30,000, DDAC 85 % (Biochit, Russia); ChS M_W 150,000, 70 % DS of $–CH_2OH$ (Moscow State Texile University, Russia); Tam, Q, sodium lauryl sulfate (SLS) (Sigma); Nile Red (Molecular Probes). Block copolymer Dex_{10}-PCL with 10 w. % of Dex was synthesized as described in [Gref R., 2003].

Encapsulation of Tam and Nile Red. 5 mg Dex_{10}-PCL was dissolved under stirring in 1 ml of ethyl acetate, containing different amounts of Tam (or 0.3 mg Nile Red). 4 ml of distilled water was added with intensive mixing and then the emulsion (with Tam) was sonicated for 1 minute, as described above. The organic solvent was then evaporated. The non-solubilized tamoxifen was removed by the filtration of the nanoparticles suspension through a 0.4 μm filter Millipore or by centrifugation at 1 000 rpm. The filtrate containing colloidal suspension of nanoparticles was stored away from light at room temperature. Tam encapsulation efficiency was calculated as the ratio of the Tam content in nanoparticles assayed spectrophotometrically [Abaev V.M., 2001] to the initial amount of Tam used for nanoparticle preparation. Loading was determined as the ratio of the mass of Tam in the nanoparticles to the mass of the lyophilized suspension.

Synthesis of soluble Ch_{30}-Q conjugate. The conjugates of Ch_{30} with Q (Ch_{30}-Q) were synthesized using 1-(3-dimetylaminopropyl)-3-ethylcarbodiimide hydrochloride after preliminary modification of Q by succinic anhydride. The Ch_{30}-Q conjugate was isolated from the reaction mixture by precipitation into acetone (followed by reprecipitation from water into acetone) and washed with acetone (to remove the unreacted agents), then dried under vacuum. Not varying the molar ratio between Q and Ch_{30}, we derived Ch_{30}-Q conjugates, where the Q content was 1-8 w %.

Fabrication of insoluble polyelectrolyte microcomplexes between Ch_{30}-Q and ChS. Polyelectrolyte complexes were prepared by incubating 0.1 % Ch_{30}-Q and 0.1 % ChS at pH 3.0. The mixture was shaken for 20 min and centrifuged. The procedures of resuspension, centrifugation and

supernatant elimination were repeated twice. Microcomplexes were ultrasonicated for 2 min. The complexing efficiency was calculated as $E=((A_{mix}-A_{super})/A_{mix})\cdot 100$ %, where A_{mix} is the sum of absorbances of the initial solution of Ch_{30}-Q and ChS, A_{super} is the absorbance of the first supernatant at 340 nm.

In vitro Tam release kinetics. 10 mg of the freeze-dried Tam-loaded nanoparticles were incubated in 10 ml of PBS, pH 7.4, containing SLS 0.5% (w/v) (to enhance the solubility of Tam) during 120 h at 37 °C. At predetermined time intervals the tubes were centrifuged, and 4.0 ml samples of the release medium were withdrawn. Then 4.0 ml of fresh SLS-PBS were added to the test tubes to maintain sink conditions. The supernatant release medium was filtered through a 0.12-μm membrane filter to ensure that the filtrate was free of most nanoparticles. The concentration of Tam in the filtrates was determined spectrophotometrically.

In vitro Ch_{30}-Q release studies. To study the dissolution/release, we sequentially agitated the polyelectrolyte microcomplexes in HCl solution (pH 1.7) for 2 h, in 0.05 M phosphate buffer (pH 7.0) for 3 h, and in 0.05 M phosphate buffer (pH 8.0) for 24 h at 100 rpm and 37°C. At appropriate time intervals the suspension was centrifuged for 2 min and the supernatant was separated to measure its absorption at 340 nm. Then the supernatant was mixed with the precipitate to continue the study of the release. Ch_{30}-Q release from the microparticles was characterized by the ratio of the Ch_{30}-Q content in the supernatant to the initial Ch_{30}-Q content in the microparticle suspension.

Results and Discussion

The self-assembling characteristics of amphiphilic polymers in aqueous solution has been explosively attended for development of effective targetable drug carriers. An o/w emulsion evaporation technique proved to be very effective for the preparation of micro- and nanoparticles from Dex_{10}-PCL copolymer. Dex_{10}-PCL microparticles with sizes 1-3 μm (Fig.1) and nanoparticles with sizes ranging from 100 up to 260 nm and low polydispersities could be produced with good reproducibility.

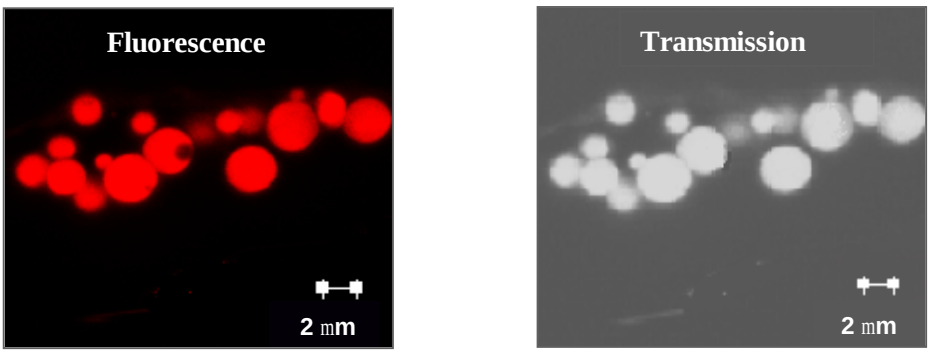

Fig. 1. CLSM-images of Dex_{10}-PCL microspheres loaded with Nile Red.

Our studies indicate that Tam can be successfully entrapped in degradable Dex-PCL nanoparticles. As shown in Table 1, the encapsulation efficiency was higher than 85%, when initial Tam concentrations were ≥ 100 μg/ml, regardless the Dex_{10}-PCL concentration. Meanwhile, drug loading increased with increasing initial Tam concentration. The maximum loading corresponded to 43.5 w %. Thus, in our study we obtained a very high loading. For comparison, the maximal loading of Tam in PCL and in poly(MePEGcyanoacrylate-co-hexadecylcyanoacrylate) 1:4 nanospheres was 5.1 w % [Chawla J.S., 2002] and 0.46 w % [Brigger I., 2001] respectively. The entrapment of Tam reduced the size of the Dex_{10}-PCL nanoparticles. Monodisperse Tam-loaded nanoparticles with average sizes around 200 nm were obtained (Fig. 2). Tam was released from the nanoparticles in SLS-containing PBS at 37°C (Fig. 3).

Table 1. Encapsulation efficiency and loading of Tam in Dex_{10}-PCL nanoparticles.

№	Concentration Dex_{10}-PCL, μg/ml	Initial concentration of Tam, μg/ml	Concentration of Tam in the nanoparticles suspension, μg/ml	Encapsulation efficiency, %	Loading, w %
1	1000	200	192±15	96±7	16.1±1.0
2	1000	400	380±15	95±4	27.5±0.8
3	1000	900	770±33	85±4	43.5±1.0
4	4500	200	180±15	90±8	3.8±0.4
5	4500	900	900±35	100±4	16.6±0.8

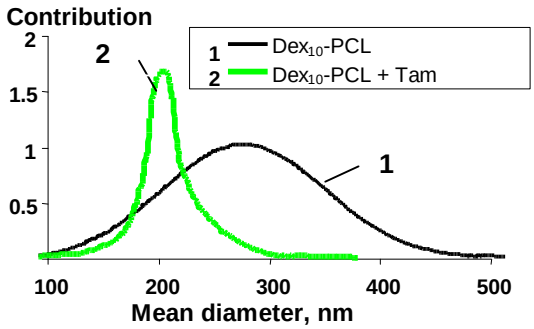

Fig. 2. Size distribution of the nanoparticles loaded with Tam (sample 3 in Table 1).

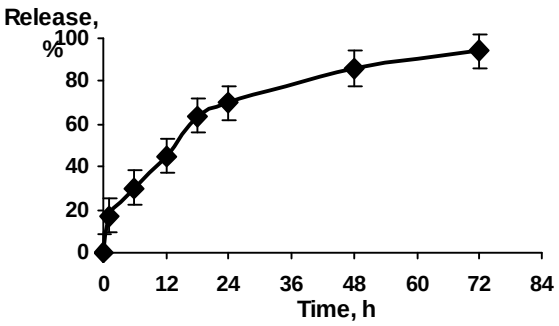

Fig. 3. Cumulative Tam release from Dex_{10}-PCL nanoparticles (pH 7.4).

Similar type of release profile was observed for Tam incorporated in poly(MePEGcyanoacrylate-co-hexadecylcyanoacrylate) 1:4 nanoparticles [Brigger I., 2001]. The release of 62% of the entrapped drug within a few minutes was observed, when the experiment was performed in cell culture medium [Brigger I., 2001]. In our case, *in vitro* release studies clearly showed a sustained release and the "burst" effect was significantly diminished compared to the above mentioned literature data. Indeed, after 72 hours incubation of in PBS-SLS, Tam release was practically completed. This result suggests that most of the active compound was located in the core of Dex_{10}-PCL nanoparticles.

The gist of the Q encapsulation process was in the electrostatic interaction of oppositely charged polyelectrolytes Ch_{30}-Q (see structural formula on Fig. 4) and ChS to form the insoluble complexes (Ch_{30}-Q)ChS at pH 3.0. Microscopic study of insoluble complexes showed that they were the closed microparticles of irregular form (Fig. 5). The ultrasonic treatment decreased the sizes of particles of (Ch_{30}-Q)ChS down to 100-500 nm. Table 2 lists some properties polyelectrolyte complexes. Fig. 6 shows the dissolution of the polyelectrolyte complexes upon their sequential interaction with the media with pH values corresponding to the basic segments regions of the gastrointestinal tract and for the transit time through relevant gastrointestinal regions.

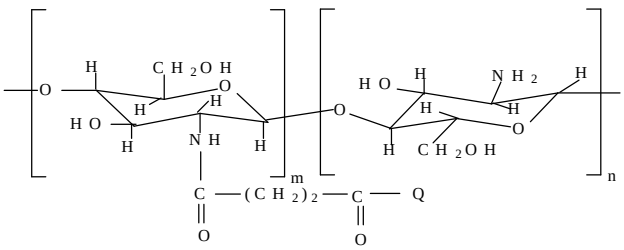

Fig. 4. Structural formula of Ch_{30}-Q conjugates.

Table 2. Characterization of insoluble polyelectrolyte complexes between the Ch_{30}-Q and ChS (pH 3.0).

Content Q, w %	Ch_{30}-Q:ChS, w/w	Size, μm
1.4	1:4	1 - 5

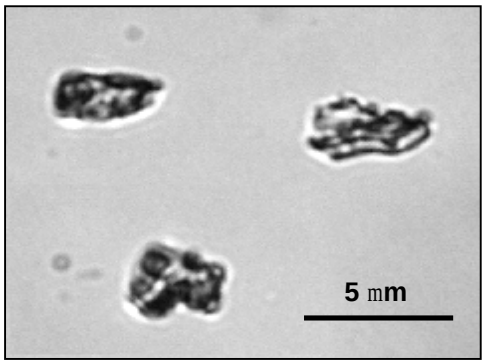

Fig. 5. Optical micrograph of polyelectrolyte complexes (Ch_{30}-Q)ChS.

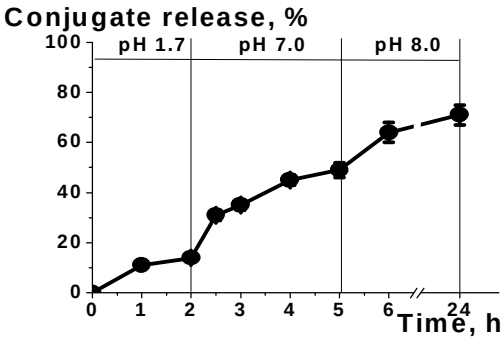

Fig. 6. Dissolution profiles of polyelectrolyte complex (Ch_{30}-Q)ChS**.**

At pH 1.7, corresponding to gastric pH, and for 2 h, the observed Ch_{30}-Q conjugate release from the insoluble complexes was rather low. At neutral intestinal pH 7.0, the complexes were gradually dissolved. Based on Andreasen et al. findings, we suppose that in vivo, the further Q release from the conjugates proceed under the action of gastrointestinal esterases from intestinal mucosa and microflora via cleavage of spacer – Q bond. These properties of the polyelectrolyte complexes with Q show the promises for their per oral administration.

Conclusions

Core-shell Dex_{10}-PCL nanoparticles may have potential to provide an alternative dosage form for Tam, one of the best and most commercially successful anticancer drugs. The microparticles of the (Ch_{30}-Q)ChS complex were proposed for pH-sensitive controlled release of the antioxidant. These microsystems protected the flavonoid against detrimental action of gastric medium.

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Acknowledgements

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