Cent. Eur. J. Chem. • 10(2) • 2012 • 305-312
DOI: 10.2478/s11532-011-0143-2

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Central European Journal of **Chemistry**

Development of cephalexin-loaded poly(ethyl cyanoacrylate) colloidal nanospheres

Research Article

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Received 12 August 2011; Accepted 31 October 2011

Abstract: This article considers the preparation and physicochemical characterization of a novel colloidal formulation of the β-lactam antibiotic cephalexin, loaded in poly(ethyl cyanoacrylate) colloidal nanospheres. The drug was loaded by means of drug incorporation in the interior of poly(ethyl cyanoacrylate) particles during the polymerization of the respective monomer in aqueous medium. The obtained colloids were characterized by scanning electron microscopy, dynamic and electrophoretic light scattering, Fourier transform infrared and nuclear magnetic resonance spectroscopy. It was found that the drug loading efficiency depends on the initial concentration of monomer and cephalexin in the polymerization medium. The average size of cephalexin-loaded particles was around 400 nm and did not depend significantly on the concentrations of drug and monomer. Drug-loaded particles with drug content as high as 21% (*w/w*) were prepared. The drug release kinetics was studied in physiological phosphate-buffered saline. It was found that a biexponential model could describe well the experimental release kinetics.

Keywords: *Cephalexin • Poly(ethyl cyanoacrylate) • Colloidal particles • Nanospheres • Drug release*

1. Introduction

Cephalexin (CPX) is a cephalosporin antibiotic (Fig. 1), which is used to treat urinary tract infections, respiratory tract infections, skin and soft tissue infections [1]. It is predominantly active against Gram-positive bacteria. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. Such antibiotics diffuse into but do not accumulate in phagocytes, probably because of their acidic character [2]. The recent advances in pharmaceutical nanotechnology resulted in the development of various colloidal nanoparticles for targeted delivery of antibiotics to infected phagocytes [3-8]. Poly(alkyl cyanoacrylate) (PACA) nanoparticles have been previously shown as suitable for the preparation of colloidal carriers for antibiotics [9-21]. For example, the entrapment of ampicillin in poly(hexyl cyanoacrylate) colloids has been found to increase by 120-fold the efficacy of the antibiotic in experimental murine salmonellosis [13,14]. These promising results motivate the need for further development of novel PACA-based colloidal formulations of antibiotics.

The colloidal nanospheres of poly(ethyl cyanoacrylate), PECA, a kind of PACA nanoparticles, are relatively non-toxic (at concentrations up to 25 μg mL^{-1}, [12]) and have been used recently as a drug carrier system for the anticancer drug epirubicin [22]. PECA-based nanoparticles of core/shell type with a magnetic core intended for targeted drug delivery are also known [23,24]. It has been found that drugs could be loaded in such nanospheres by entrapment in the polymer network (absorption) and/or surface adsorption, and the type of drug incorporation could affect the drug release profile [24]. Colloidal formulations of the antibiotics cefaclor and cefsulodin, using PECA nanospheres coated with various surfactants, have also been prepared and characterized [21]. The drug release rate from such systems usually involves diffusion of drug molecules from the particle interior toward the particle surface and desorption from the surface [21,24], however the drug release could be facilitated by degradation/erosion of the polymer [25], which is likely to occur in the presence of enzymes in biological systems (and especially inside the phagolysosomes).

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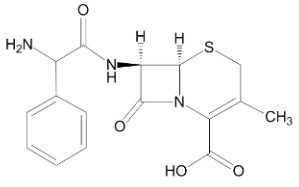

Figure 1. Chemical structure of cephalexin.

This article describes the development of a novel colloidal formulation of the β-lactam antibiotic cephalexin, loaded in PECA nanospheres. The drug-loaded particles were prepared by a polymerization-based method in aqueous medium. Two different methods of drug loading were tested – by means of drug adsorption on the surface of preformed colloidal particles, and by incorporation of the drug molecules in the polymer matrix of the particles during their formation. In particular, we studied the effects of the drug concentration and concentration of ethyl cyanoacrylate in the polymerization medium on the particle size distribution and the drug loading efficiency. The particles of pure PECA, as well as the cephalexin-loaded (CPX-PECA) ones, were characterized by various methods, such as scanning electron microscopy, dynamic and electrophoretic light scattering, infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. The drug release in phosphate-buffered saline was studied at pH 7.4 and 37°C and evaluated by using different models.

2. Experimental procedure

2.1. Materials and reagents

Ethyl cyanoacrylate (ECA) was purchased from Special Polymers Ltd (Bulgaria). Cephalexin (CPX; water content ~6.38% according to the technical specification), dextran 40, phosphate-buffered saline (PBS, tablets), Pluronic F68 (PEO-PPO-PEO triblock copolymer), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were from Sigma. The water used in all studies was distilled and filtered through membrane filter (pore size 0.2 μm) before use.

2.2. Preparation of pure PECA colloidal nanospheres

The preparation of pure PECA nanospheres was carried out similarly to previously described emulsion polymerization [26]. Pluronic F68 (20 mg) was dissolved in distilled water (8.7 mL) and acidified with HCl (1 M, 0.1 mL). ECA (60-100 μL) was added dropwise to the polymerization medium upon magnetic stirring (600 rpm). The obtained emulsion became milky-white within the first 10 minutes. The colloidal dispersion was neutralized after 30 minutes by addition of 1 M NaOH (0.1 mL) and buffered at pH 7.4 by addition of 1.0 mL phosphate-buffered saline (10×PBS). The neutralized dispersion was stirred for 3 hours for complete polymerization.

2.3. Preparation of cephalexin-loaded PECA colloidal nanospheres

The cephalexin-loaded PECA (CPX-PECA) nanospheres were prepared similarly to the pure ones. Two methods for drug loading were tested: i) inclusion of the antibiotic in the polymer matrix during the polymerization and particle formation; and ii) adsorption of the drug on the surface of preformed particles. CPX was loaded by adsorption on the surface of presynthesized PECA nanospheres (100 mg) by addition of CPX (60 mg) to the PECA particles in phosphate buffer (pH 7.4) and stirring for 4 hours at room temperature. The preliminary tests indicated that only few percents of the drug were loaded in the nanospheres by adsorption and therefore, only loading by inclusion of the antibiotic in the polymer matrix during the polymerization was studied further. For that purpose, two sets of experiments were carried out. In the first set, CPX (various amounts from 20 to 100 mg) and Pluronic F68 (20 mg) were dissolved in distilled water (8.7 mL). The polymerization medium was acidified with HCl (1 M, 0.1 mL) and the ECA monomer was added dropwise upon magnetic stirring (600 rpm). The amount of ECA was kept constant (100 μL) in each experiment. The particles were formed within the first few minutes from the addition of monomer and the dispersion became milky-white. The dispersion was stirred for 30 min (this time was found enough for the particles to form and longer periods were avoided to prevent drug hydrolysis in the acidic environment) and then NaOH (1 M, 0.1 mL) was added for neutralization. The pH of the resulting dispersion was buffered at 7.4 with 10×PBS (1 mL). The dispersion was stirred additionally for 3 hours for complete polymerization. In the second set of experiments, various amounts of ECA (40-200 μL) were utilized at constant CPX concentration (2 mg mL^{-1}). All concentrations of ECA and CPX were calculated with respect to the final volume of the dispersion (10 mL). All other procedures were as described in section 2.2. All experiments were performed in triplicate in order to evaluate the reproducibility of the preparation procedure.

2.4. Characterization of the nanospheres

The obtained nanospheres were observed by a scanning electron microscope (SEM) JSM-5510 (JEOL), operated at 10 kV of acceleration voltage. The samples for

SEM were prepared by evaporation of dilute aqueous dispersions on glass substrates, followed by coating with gold thin film by JFC-1200 fine coater (JEOL). The size of at least 500 particles was determined from the SEM images in order to evaluate the particle size distributions. Dynamic light scattering (DLS) system Malvern 4700C (Malvern Instruments, UK) was used to measure the particle size and size distribution in water at 25°C and light-scattering angle of 90°. Electrophoretic light scattering (ELS) system ZETASIZER IIC (Malvern Instruments, UK) was used to measure the ζ-potential of the colloids in physiological phosphate-buffered saline (PBS, 0.01 M with respect to phosphates) at pH 7.4. Each value was the average of five measurements. Fourier transform infrared (FTIR) spectra were taken with Bruker Tensor 27 spectrometer in the interval 400-4400 cm^{-1} (the samples were prepared for FTIR study using the KBr-tablet technique). Nuclear magnetic resonance (^{1}H-NMR) spectra were taken with Bruker Avance II+ 600 spectrometer (spectrometer frequency 600.13 MHz). The samples for NMR analysis were prepared in the following way: aliquots of the as-prepared particle dispersions were centrifuged and washed with distilled water to remove salts, Pluronic F68 and unloaded drug, then dried under vacuum and dissolved in dimethyl sulfoxide (DMSO-d6). The spectra were taken at room temperature in the interval 0-15 ppm.

2.5. Drug loading

The amount of CPX loaded in PECA nanospheres was determined by centrifugation of the as-prepared CPX-PECA dispersions at 14000 rpm for 60 minutes. The drug concentration (C_s) in the obtained optically clear supernatant was measured spectrophotometrically by a double-beam UV-vis spectrophotometer at a wavelength of 260 nm (Evolution 300, Thermo Scientific). The drug loading efficiency (L, %) is defined by Eq. 1.

$$L = 100 \times \frac{C_i - C_s}{C_i} \quad (1)$$

Here, C_i is the total (initial) amount of drug (calculated from the amount of CPX used and the final volume of the dispersion) and C_s is the amount of drug in the dispersing medium (unloaded drug, determined spectrophotometrically) after the synthesis. The drug content (DC, %) in nanospheres is defined as the mass fraction of drug in drug-loaded particles (Eq. 2). It could be determined from the NMR spectra (see section 3.3, Eqs. 3 and 4).

$$\mathrm{DC} = \frac{\text{mass of loaded drug}}{\text{mass of drug-loaded particles}} \times 100 \quad (2)$$

2.6. *In vitro* studies of drug release

The CPX-PECA nanospheres for the drug release studies were prepared by using 60 mg of CPX and 100 μL of monomer (loading efficiency ~25%). A volume of 10 mL of the as-prepared particle dispersion (phosphate-buffered at pH 7.4) was centrifuged and washed with water to remove the unloaded drug. Then, the particles were dispersed in PBS (0.01 M, 10 mL, pH 7.4, containing 0.2% dextran 40 as a colloidal stabilizer) in a closed vessel (to prevent evaporation of the release medium) and gently stirred at 37±1°C for 16 hours. Aliquots (0.5 mL) were taken from the dispersion at regular time intervals and centrifuged (14000 rpm, 20 min). The amount of released drug in the supernatant was determined by spectrophotometric measurement at 260 nm (taking an aliquot of 0.100 mL of the supernatant and diluting with PBS buffer to a total volume of 4.00 mL). The experiment was carried out in triplicate. The obtained release profile was analyzed by using different models (see section 3.6).

3. Results and discussion

3.1. Mechanisms of particle formation and drug loading

The polymerization of alkyl cyanoacrylates in aqueous solutions is initiated by the hydroxide ions (OH^-) from water [27]. The anionic polymerization is favored because the two strong electron acceptor groups (-CN and -COOR) bound to the same carbon atom stabilize the respective anions during the polymerization. Interestingly, it has been found that once terminated the process can be re-initiated, which has an important effect on the process of nanoparticle formation [27]. Actually, the process of nanoparticle formation seems to be rather complex and still not fully understood. Drugs can be loaded in PACA nanoparticles by means of adsorption on the surface of preformed particles or by drug entrapment inside the polymer matrix of nanospheres during their formation [10,11]. Various drug-polymer non-covalent interactions, such as H-bonding and electrostatic attraction, may favor drug association with nanoparticles. Surface adsorbed drugs are usually weakly bound to the particles and could be easily desorbed. Equilibrium exists between the adsorbed drug and the drug dissolved in the dispersing medium, and usually loading efficiencies are low. Drug loading by surface adsorption is usually used for drugs, which are sensitive at the conditions of nanoparticle formation and/or could react with the highly reactive monomers. Drug loading by incorporation in the interior of nanoparticles during their formation usually

results in higher loading efficiencies and the loaded drug is released slower. Drug molecules could become physically entrapped inside nanoparticles by growing polymer chains and aggregation of polymer molecules during the formation of nanospheres. It is expected that drugs, which interact with the polymer *via* various non-covalent interactions could be more easily entrapped in the nanospheres. Our attempts to load CPX on preformed PECA nanospheres resulted in loading efficiencies of only 1-2%. Therefore, we considered this method of drug loading as inefficient. Further studies were performed using only CPX loading inside PECA nanospheres (drug loading during polymerization). This method resulted in drug content as high as 21% (see section 3.4).

3.2. Size distribution of PECA and CPX-PECA nanospheres

The SEM images (Fig. 2) show that both PECA and CPX-PECA particles are submicron in size, of spherical shape and exhibiting monomodal size distributions (Fig. 3). The size distributions, measured by light scattering (DLS) well correspond to those obtained from the SEM observation (Fig. 3). The CPX-PECA particles were relatively smaller (~430 nm) than the pure PECA particles (~550 nm). The exact reason for differences in the size of pure and drug-loaded particles is currently unclear for us. The CPX somehow affects the polymerization process, which probably results in particles of different size. The effect of CPX on the particle formation can be clearly observed during the synthesis: the dispersions containing CPX become milky-white faster after monomer addition than those of pure polymer. However, further studies are required in order to reveal the intimate mechanisms of the effect of CPX on the processes of polymerization and particle formation. Previously reported preparations of PECA nanospheres have resulted in the formation of slightly smaller in size (380±120 nm) particles [23]. Surprisingly, previous preparations of pure and drug-loaded PECA colloids by a similar procedure and using the same stabilizer (Pluronic F68) have resulted in the formation of very large particles of average size ~790 nm. In our experiments, the preparations of CPX-PECA nanospheres at various monomer concentrations (in the interval of 4-12 μL mL^{-1}; at constant CPX concentration of 2 mg mL^{-1}) resulted in the formation of almost the same in size particles. The change of CPX concentration (at constant ECA concentration of 10 μL mL^{-1}) also resulted in similar in size nanospheres. The relatively large size (>200 nm) of the obtained drug-loaded particles might be important for their targeting to phagocytic cells in future biomedical tests. It should be noted that we observed variable particle size using

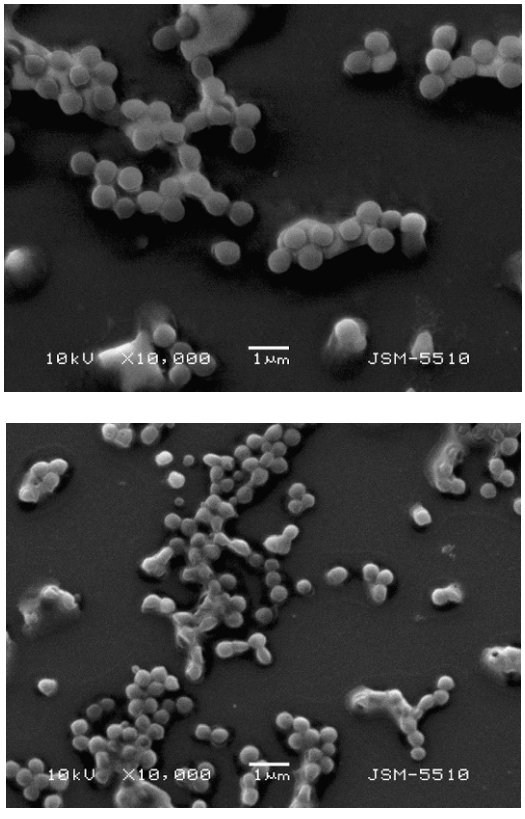

Figure 2. Representative SEM images of (a) pure PECA, and (b) CPX-PECA nanospheres. The samples are prepared with concentrations of ECA and CPX, both 10 mg mL^{-1}.

different batches of monomer. Such batch-to-batch variations are well known for the preparation of PACA colloids by polymerization-based methods [27]. We obtained drug-loaded particles of average size from 380 to 600 nm using different batches of monomer. For that reason, in order to obtain reproducible results and study the effects of drug and monomer concentrations, we performed the above-described experiments with the same batch of monomer.

3.3. Structural characterization

The ^{1}H-NMR spectrum of pure PECA is shown in Fig. 4a. The peak of solvent (DMSO-d6) at 2.49 ppm was used as a chemical shift reference. The signals at 4.2 ppm and 2.6 ppm correspond to the methylene (CH_2) protons (2H) from both: the ethyl group and the polymer backbone, respectively. The signal at 1.3 ppm corresponds to the protons (3H) from the methyl group. The spectrum of CPX-PECA (Fig. 4b) shows signals for both components: PECA and CPX. The signal at 9.17 ppm could be attributed to the proton (1H) from the amide CONH group of CPX. The signals for the aromatic protons (5H) of CPX appear at 7.2-7.6 ppm. The signals at 2.8-3.1 ppm (2H) and 1.99 ppm (3H)

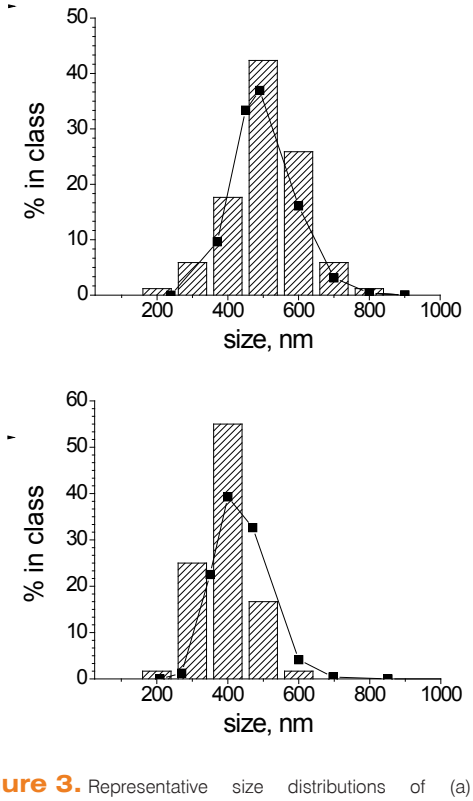

Figure 3. Representative size distributions of (a) PECA, and (b) CPX-PECA colloidal nanospheres. The size distributions are determined by DLS (solid line) and SEM (bars).

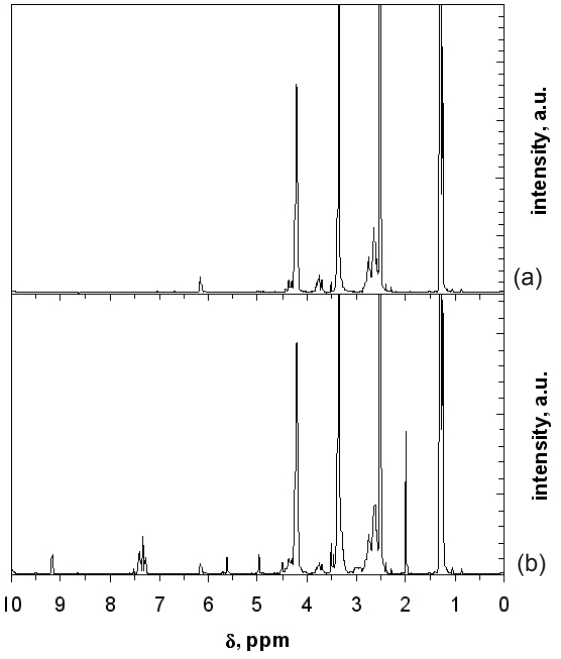

Figure 4. ^{1}H NMR spectra of: (a) PECA, and (b) CPX-PECA (with drug content of 21%, w/w). The CPX-PECA nanoparticles were prepared by using 10 μL mL^{-1} of ECA and 10 mg mL^{-1} of CPX.

correspond to the methylene (CH_2) and the methyl group (CH_3) of CPX. The signals at 5.62 ppm (1H), 4.97 ppm (1H) and 4.49 ppm (1H) are attributed to the other protons from CH-groups in the CPX molecule.

The data from NMR spectrum can be used to estimate the drug content in CPX-PECA as previously described for chlorambucil-loaded poly(butyl cyanoacrylate) nanoparticles [29]. For that purpose, one could use the integral intensity of signals for aromatic protons from CPX (5H, 7.2-7.6 ppm; I_{Ar}) and the integral intensity of methyl protons (3H per monomer unit; $I_{Me/PECA}$) from the polymer (1.0-1.5 ppm). Then, a formula for calculation of the mass fraction of loaded drug (DC, %) in the particles can be derived, similarly to that reported in [29] (Eq. 3).

$$DC = \frac{3M_{CPX}I_{Ar}}{3M_{CPX}I_{Ar} + 5M_{ECA}I_{Me/PECA}} \times 100 \quad (3)$$

Here, M_{CPX} is the molecular mass of CPX (347.4 g mol^{-1}), and M_{ECA} is the molecular mass of the ECA monomer (125.1 g mL^{-1}). The drug content (DC) in CPX-PECA particles (prepared from 100 µL of ECA and 100 mg of CPX) according to Eq. 3 was found to be 21% (w/w). Because the signals for methylene protons from CPX ($I_{Me/CPX}$) and PECA ($I_{Me/PECA}$) do not overlap, one can use these signals in a similar formula for calculation of the drug content (Eq. 4):

$$DC = \frac{M_{CPX}I_{Me/CPX}}{M_{CPX}I_{Me/CPX} + M_{ECA}I_{Me/PECA}} \times 100 \quad (4)$$

The experimental ratio of the integral intensity of signals $I_{Me/PECA}$: $I_{Me/CPX}$: I_{Ar} was 1.00:0.095:0.165. The drug content calculated by using Eq. 4 for the same sample was found to be also 21% (w/w).

The FTIR spectrum of pure CPX shows the characteristic absorption bands of the β-lactam C=O (1758 cm^{-1}), the amide carbonyl CONH (1690 cm^{-1}) and C=C bonds (1595 cm^{-1}). There is a broad band at 2610 cm^{-1}, which was attributed to NH_3 stretching vibration (the amino group is protonated by the carboxylate group, which results in the formation of a zwitterion). The FTIR spectrum of pure PECA shows the characteristic absorption bands for the carbonyl C=O ester (1750 cm^{-1}), C≡N groups (2251 cm^{-1}), C-H (2880-3050 cm^{-1}), and C-O-C (absorbance at 1256 and 1015 cm^{-1} correspond to the asymmetric and symmetric stretches, respectively). The spectrum is similar to previously reported FTIR studies of PECA [23,24,28] and is not shown here. The FTIR spectrum of CPX-PECA shows all the characteristic absorption bands of pure PECA, which remained unchanged. The CPX characteristic absorbance bands overlapped with the strong absorbance bands of the polymer and do not provide additional information of entrapped CPX.

3.4. Drug loading efficiency

The adsorption of CPX onto preformed particles resulted in a very low loading efficiency (~2%). This was probably a result of low affinity of CPX to the particle surface or/and limited capacity of the particle surface for adsorption of drug molecules. For that reason, only the CPX-PECA particles prepared by drug incorporation during the polymerization, where loading efficiencies as high as 30% were observed, were considered as suitable for CPX-carriers. The polymerization of ECA in aqueous solution of CPX allows the formation of CPX-PECA colloids of various loading efficiency by varying the concentrations of CPX (Fig. 5a) and ECA (Fig. 5b). In the first set of experiments, the concentration of ECA is kept constant (10 μL mL^{-1}), while the concentration of CPX is varied between 0.5 and 10 mg mL^{-1}. The loading efficiency increased from 10 to 30% with increasing the concentration of CPX up to 6 mg mL^{-1} (Fig. 5b). Experiments with higher concentrations of CPX (>10 mg mL^{-1}) were not performed, because of solubility limitations (it was found that at concentrations higher than 15-20 mg mL^{-1} CPX formed suspensions in PBS at pH 7.4). In the second set of experiments, the concentration of CPX was kept constant (2 mg mL^{-1}), while the concentration of ECA was varied between 4 and 20 μL mL^{-1}. In this case the loading efficiency increased almost linearly from 10 to 30% with increasing the concentration of ECA (Fig. 5b). Formation of macroscopic polymer aggregates was observed at higher monomer concentrations, which was a limitation for further increase of the ECA concentration. Interestingly, variations in the loading efficiency (about ±7%) depending on the batch of monomer used were observed. In order to avoid confusion from such batch-to-batch variations, monomer from the same batch was used.

3.5. ζ – potential

The ζ-potential measurements showed that the PECA colloids have negative values of the ζ-potential (-2.9±0.5 mV) in physiological phosphate-buffered saline (pH 7.4). The negative value of ζ-potential in the case of pure PACA particles has been previously attributed to the existence of free carboxylic groups (in the form of anionic carboxylates) from the polymer [23]. In contrast, the CPX-PECA particles show positive values of about 2.0±0.5 mV at the same other conditions, which did not depend significantly on the concentration of CPX. These findings support the hypothesis that the adsorption of CPX molecules on the particle surface changes the ζ-potential, which changes from negative (for the pure PECA) to positive (for the CPX-PECA). The relatively low absolute value of the ζ-potential is a result of the relatively high ionic strength of the PBS buffer (which contains 0.137 M NaCl as the main electrolyte).

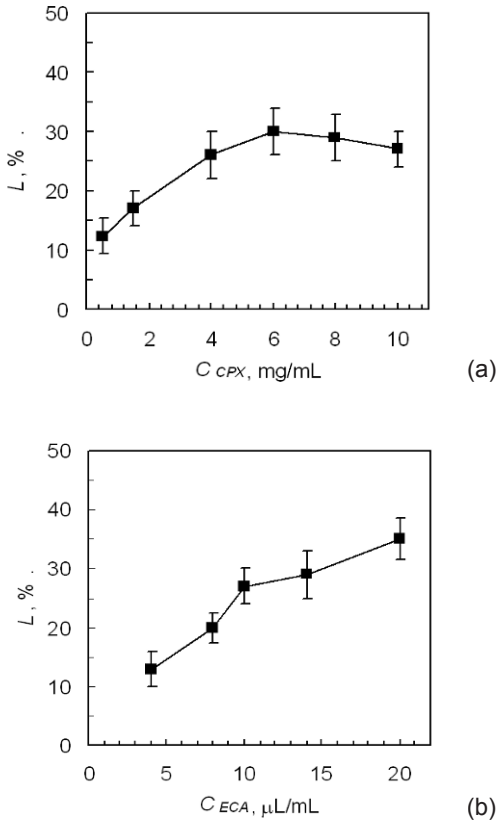

Figure 5. Dependence of the drug loading efficiency (L) on the: (a) concentration of ECA (at constant CPX concentration of 2 mg mL^{-1}); (b) concentration of CPX (at constant ECA concentration of 10 μL mL^{-1}).

3.6. In vitro drug release studies

The drug release from CPX-PECA particles was studied in 0.01 M PBS (pH 7.4) at 37°C, which was intended to provide a preliminary idea of the drug release in physiological-like systems. The particles with highest drug content (21%) were used for the release study. One milliliter of this formulation contained 10 mg CPX, 27% (2.7 mg) of which were associated with the particles. About 2.2 mg of CPX could be released from CPX-PECA particles isolated from one milliliter of dispersion during 16 hours (this time was enough to reach almost constant concentration of drug in the release medium - the release profile is shown Fig. 6). After this period the particles became unstable in the PBS medium and tended to form aggregates, which could be a reason for disturbance of the release profile. In the analysis of the release profile, M_0 was the maximum amount of releasable drug, M_t was the amount of drug released at time t, and k was the rate constant.

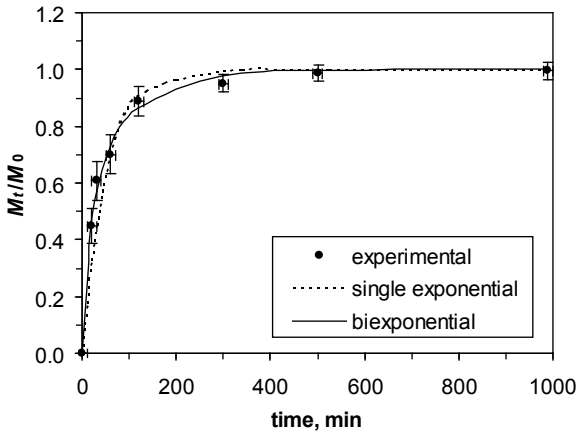

Figure 6. Experimental release kinetics of cephalexin (CPX) from CPX-PECA colloidal particles fitted with a single and biexponential functions. Experimental data are given with circles (•), while the respective fits are given with dashed and solid lines as shown in the legend.

Drug release from PACA colloidal systems may involve diffusion of drug molecules through the particle interior and desorption from the particle surface. Drugs, which are strongly associated with the polymer, could be released during erosion of the PACA materials *via* hydrolysis of the ester bonds (such erosion usually takes place in biological systems in the presence of enzymes) [10]. The drug release from drug-loaded colloids is a complex process, which depends on many different factors and its mathematical modeling should be performed carefully in order to avoid inadequate conclusions [30]. Most of the methods for *in vitro* studies of drug release involve separation of the colloidal particles from the dispersing medium and determination of the concentration of released drug. Investigations of the release of cephalosporins from PECA colloids have been previously done by the dialysis method [21]. These authors observed interesting dependence of the release rate on the type of surfactant used and explained their results by the different interaction of the particles with the dialysis membrane. However, it has been shown previously that the drug release kinetics in experiments with dialysis membranes is controlled by the partition coefficient of the drug and its diffusion through the membrane, and is independent of the release rate constant, which is of course the release rate, which is intended to be measured [30]. On the other hand, separation methods such as filtration could have disadvantages such as adsorption of drug on filter membranes. For that reason, in our experiments we separated the particles from the release medium by centrifugation, however it required at least 20 min (at 14000 rpm) per sample and resulted in the x-axis error bars shown in Fig. 6.

In the analysis of the release profile of CPX from CPX-PECA particles we tested the square-root model [31], the model of Baker-Lonsdale [32], and the model of Sinclair-Peppas [33], but these models could not fit well our data. Therefore, we tested the widely used single exponential (Eq. 5) and biexponential (Eq. 6) empirical models [30]. The experimental data and the respective fits are shown in Fig. 6.

$$\frac{M_t}{M_0} = 1 - \exp(-kt) \tag{5}$$

$$\frac{M_t}{M_0} = 1 - \left[A\exp(-k_1 t) + B\exp(-k_2 t)\right] \tag{6}$$

The fit with single exponential model (Eq. 5) resulted in a rate constant (k) 0.020 min^{-1} (R^2 = 0.984). However, the biexponential model (Eq. 6) fitted better our data (R^2 = 0.997). At equal amplitudes (A=B), the values of the rate constants k_1 and k_2 were 0.011 min^{-1} and 0.068 min^{-1}, respectively. As seen, the biexponential model consists of a rapid and a slow function, which could be assigned to "burst release" and "sustained release", respectively. However, the existence of these two different physical processes should be considered with some skepticism. Previously performed modeling of the release kinetics from colloids with lognormal size distribution has resulted in strongly curved profile [30]. Therefore, the initial "burst release" could be a result of surface release and release from small particles, while the "sustained release" could be attributed to deep release from the larger particles.

4. Conclusions

This work presents the development of cephalexin-loaded poly(ethyl cyanoacrylate) colloidal particles as a potential drug carrier system. The colloidal particles are prepared by the polymerization of ethyl cyanoacrylate monomer in the presence of cephalexin solution. The investigation by electron microscopy shows spherical in shape colloidal particles of average sizes 400-500 nm depending on the polymerization conditions. The particle size distribution is monomodal in all cases, as measured by light scattering, which well correlates with the data from electron microscopy. The ζ-potential is slightly positive for drug-loaded colloidal particles in phosphate-buffered saline, whereas it is slightly negative for the pure particles. The analysis by FTIR and NMR spectroscopy confirmed the chemical composition of the particles. The loading with cephalexin by adsorption in phosphate-buffered saline results in a very low loading efficiency (~2%), which is insufficient for future biomedical tests.

The polymerization of ethyl cyanoacrylate in solution of cephalexin allowed the preparation of formulations with a drug content as high as 21%. The studies on drug release kinetics indicate an exponential release profile, which could be well described by a biexponential function. It is expected that the novel formulation of cephalexin described here might by useful in future biomedical tests.

Acknowledgements

The author is thankful to COST Action D43 of EC. The technical assistance of Mr. Nikola Dimitrov and Dr. Mariana Boneva for the SEM and DLS measurements, respectively, is greatly acknowledged. The FTIR and NMR spectra were taken at the Institute of Organic Chemistry (Bulgarian Academy of Sciences).

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