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SHORT COMMUNICATION

Preparation of poly(butylcyanoacrylate) drug carriers by nanoprecipitation using a pre-synthesized polymer and different colloidal stabilizers

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Abstract Classically, drug-loaded poly(alkylcyanoacrylate) colloidal carriers are prepared by the drug entrapment during emulsion polymerization. However, a number of chemically sensitive drugs are unstable in the conditions of polymerization or can be irreversibly inactivated by the highly reactive monomer. Furthermore, the particle size distribution and the molecular weight of formed polymer depend strongly on the polymerization conditions. Here, we investigate the nanoprecipitation approach for the preparation of pure and drug-load poly(butylcyanoacrylate) nanoparticles. This method allows the successful entrapment of lipophilic and chemically labile drugs by avoiding the contact with highly reactive monomers. The anticancer agent chlorambucil is chosen as the model drug for the incorporation and release studies. Pure and drug-loaded nanoparticles are successfully prepared using various stabilizers (Polysorbate 80, Pluronic F68, Dextran 40). The nanoparticles coated with Polysorbate 80 are of highest interest since they could overcome the blood–brain barrier and the multidrug resistance in cancer cells. Such nanoparticles can be easily prepared by the nanoprecipitation approach reported here.

Keywords Poly(butylcyanoacrylate) · Nanoparticles · Nanoprecipitation · Chlorambucil · Pluronic F68 · Polysorbate 80 · Dextran 40

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Introduction

The poly(alkylcyanoacrylate) (PACA) polymers are biocompatible, biodegradable, and low-toxic materials, approved for human use (the respective monomers are used as surgical glues) [1–4]. The PACA nanoparticles are among the most perspective nanosized drug carriers attracting a great interest for application in targeted delivery of different biologically active substances, such as anticancer agents [5–9], antibiotics [10–15], peptides [16, 17], nucleic acids [18], and antiviral drugs [19, 20]. The PACA nanoparticles have been prepared for the first time in 1979 by emulsion polymerization of alkylcyanoacrylate monomer in acidic aqueous solution [21]. Since then, the emulsion polymerization has been intensively applied for the entrapment of various drugs in PACA nanoparticles under different conditions (for detailed reviews, see Ref. [1–3]). The utilization of this method resulted in a significant advancement of the development of novel nano-formulations of classical drugs; however, a number of serious limitations still exist. For example, the molecular weight of the formed polymer and the nanoparticle size depend strongly on the polymerization conditions, being sometimes difficult to control [22–28]. Also, certain chemically sensitive drugs could be unstable in the conditions of the polymerization reaction or could initiate the polymerization, being irreversibly inactivated by the highly reactive monomer [29–31].

On the other hand, the nanoprecipitation is a classical approach for the preparation of polymer colloids [32, 33]; however, it is not popular for the preparation of PACA homopolymer nanoparticles. This method is based on a phase separation induced by the addition of a rather diluted polymer solution to a non-solvent for the polymer. The polymer particles form spontaneously, and the polymer solvent is further removed from the obtained colloidal

dispersion by evaporation. In order to facilitate the formation of colloidal particles, the phase separation is usually performed with a water-miscible solvent (such as acetone, tetrahydrofuran, etc.). Similar technique has been used in the PACA chemistry for the preparation of nanoparticles composed of diblock amphiphilic polyethyleneglycol (PEG)–PACA copolymers, such as poly[methoxypoly(ethylene glycol)cyanoacrylate-*co*-hexadecylcyanoacrylate] [34–36]. However, the emulsion polymerization is still more popular and widely used method for the preparation of PACA nanoparticles. Despite of the advantages of the nanoprecipitation approach, little is known about its application for the preparation of PACA homopolymer nanoparticles.

Here, we report the preparation of poly(butylcyanoacrylate) (PBCA) homopolymer nanoparticles by the nanoprecipitation method, using a pre-synthesized polymer and different colloidal stabilizers. We are particularly concerned with the PBCA, which is among the most suitable materials for drug-delivery applications. This is so because it has been previously shown that such nanoparticles are non-toxic [37], biodegradable (form non-toxic water-soluble products upon biodegradation) [38–40], could overcome the multidrug resistance in cancer cells [41–43], and could target drugs to the brain [44–46]. We found that the key to the successful formation of PBCA nanoparticles by nanoprecipitation is the synthesis of initial polymer, whose molecular weight is low enough (ca. 1,500 Da) to be suitable for biological use, as well as soluble in organic solvents (such as acetone or tetrahydrofuran). Since the PBCA is not commercially available, the initial polymer in our experiments is synthesized using emulsion polymerization of pure alkylcyanoacrylate monomer in aqueous medium. The obtained polymer is soluble in acetone, forming a stable clear solution. This solution is then added dropwise to a water solution of a colloidal stabilizer upon vigorous stirring, thus resulting in a precipitation of the polymer in the nanocolloids and formation of PBCA nanoparticles. This strategy is especially important for the nano-encapsulation of chemically unstable lipophilic drugs, which can be readily dissolved in the acetone phase prior to the nanoparticle formation. We demonstrate such a possibility using the anticancer drug chlorambucil, which has been successfully entrapped in PBCA nanoparticles, coated with three different stabilizers (Polysorbate 80, Pluronic F68, and Dextran 40). The nanoparticles coated with Polysorbate 80 are of highest interest since they could overcome the blood–brain barrier [44] and the multidrug resistance in cancer cells [41]. Such nanoparticles are classically prepared by polymerization-based methods in the presence of dextrans as colloidal stabilizers, followed by surface exchange of the dextran with Polysorbate 80 [46, 47]. Our attempts to prepare directly polysorbate-coated PBCA nanoparticles by emulsion polymerization in the presence of Polysorbate 80 were unsuccessful, since this surfactant could not stabilize the initially formed emulsion. However, the Polysorbate 80-coated PBCA nanoparticles (pure and drug-loaded) can be easily prepared by the nanoprecipitation approach reported here.

Experimental procedures

Materials and reagents

Butylcyanoacrylate monomer was from Special Polymers Ltd. (Bulgaria). Phosphate-buffered saline (PBS, tablets; pH 7.4), citric acid (anhydrous), sodium hydroxide (>99%), acetone, Pluronic F68, Dextran 40 (M_r~40,000), chlorambucil, and Polysorbate 80 (Tween 80) were from Sigma (Germany). Glucose (10%, *w*/*w*) was from Actavis (Bulgaria). Distilled water was used for all preparations.

Synthesis of PBCA polymer

The PBCA polymer was prepared by emulsion polymerization in the following way. The polymerization medium was prepared by dissolving Pluronic F68 (500 mg) and citric acid (400 mg) in distilled water (200 ml). Then, the monomer (2.0 ml) was added dropwise to the polymerization medium upon vigorous mechanical stirring (~600 rpm). The emulsion became milky white within the first 10 min and was left to polymerize for 6 hours. The pH of the obtained dispersion was adjusted at 5.6 by the addition of 1 M NaOH (4.0 ml). The polymer dispersion was centrifuged (14,500 rpm, 15 min) and washed twice with distilled water by centrifugation. The white sediment was then dried under vacuum to obtain fine white powder, which was further utilized for the preparation of nanoparticles by the nanoprecipitation method (see below).

Polymer characterization

The as-obtained polymer was analyzed by nuclear magnetic resonance (^{1}H NMR) with Bruker Avance II+600 spectrometer (600.13 MHz) in acetone-d_6. Fourier transform infrared (FTIR) spectra were taken with Bruker Tensor 27 spectrometer using the KBr-tablet technique. The molecular weight of polymer was determined by gel-permeation chromatography (GPC). The chromatograms were measured using a GPC system with refractive index (RI Waters M410) and ultra-violet (UV Waters M484) detectors, operating at a wavelength of 254 nm. Styragel columns with nominal pore sizes of 100 and 500 Å were utilized. Tetrahydrofuran (THF) was used as the eluent, with a flow rate of 1 ml/min, at 45 °C. The samples were prepared as solutions in THF (3–4 mg/ml). The calibration was carried out with PEG standards.

Preparation of pure nanoparticles

The precipitation medium was prepared by dissolving a colloidal stabilizer (Pluronic F68, Polysorbate 80, or Dextran 40) (20 mg) and citric acid (20 mg) in glucose solution (5%, 10 ml). The pre-synthesized PBCA (50–100 mg) was dissolved in acetone (5 ml) and then added dropwise to the nanoprecipitation medium upon vigorous magnetic stirring (~600 rpm). The acetone was evaporated during stirring of the obtained suspension in a fume hood for 5 h. The residual acetone was removed by vacuum evaporation, and the final volume of dispersion was adjusted to 10 ml by the addition of distilled water. *Note*: The preparation of nanoparticles by this method works also without the citric acid; however, we use it here in order to keep similar conditions like these for the preparation of drug-loaded nanoparticles (see below), where acidic medium is required to avoid the drug hydrolysis.

Preparation of drug-loaded nanoparticles

Drug-loaded nanoparticles were prepared by a procedure, similar to the one described for pure particles above. The difference was that chlorambucil (10 mg) was dissolved in the acetone solution of PBCA beforehand in order to be entrapped in the nanoparticles during their formation.

Characterization of the nanoparticles

The obtained pure and chlorambucil-loaded nanoparticles were imaged by a scanning electron microscope (SEM) JSM-5510 (JEOL). The particle size distribution was measured by dynamic light scattering (DLS) system Zetasizer Nano ZS (Malvern Instruments, UK) (each value was obtained as average of five measurements). The drug content (DC) is the weight fraction (given in %) of chlorambucil in drug-loaded nanoparticles. It was determined by the following way. An aliquot of the as-prepared dispersion was centrifuged (14,500 rpm, 60 min) in a pre-weighted tube, washed with water, and dried in vacuum. The dried nanoparticles were weighted and dissolved in dichloromethane (CH_2Cl_2), followed by spectrophotometric measurement of the drug concentration in the obtained solution at 260 nm (using a double-beam UV–vis spectrophotometer Evolution 300, Thermo Scientific). The drug stability upon entrapment in nanoparticles was monitored by thin-layer chromatography (TLC). The TLC analysis was carried out using aluminum sheets pre-coated with silica gel (ALUGRAM® SIL G/UV_{254}, Germany). The mobile phase consisted of chloroform/methanol (6/1, *v*/*v*).

Drug release

The drug release was studied by dialysis of the colloidal dispersions in 0.01 M PBS (pH 7.4, 37 °C) serving as the release medium. The dialysis tubing (cellulose membranes of molecular weight cut-off size 12,000 Da) was from Sigma (Germany). Before use, a dialysis membrane was washed out with hot distilled water (60 °C) for 10 min. An aliquot (1 ml) of the as-prepared dispersions was centrifuged, washed with distilled water, redispersed in 1 ml of PBS, and transferred into the dialysis tube. The dialysis was carried out upon magnetic stirring (300 rpm) against 100 ml of PBS in a closed vessel to avoid evaporation of the release medium. The amount of released drug was determined by spectrophotometric measurements of aliquots, taken at given time intervals (the aliquots were returned back to the release vessel after measurement to keep constant the volume of release medium). Each experiment was carried out thrice.

Results and discussion

The PACA polymers are not soluble in water and can form different kinds of nanoparticles [1–5]. Such nanoparticles are suitable for carriers of hydrophobic drugs, since such drugs could be entrapped in the hydrophobic interior of the particles. However, the nanoparticle surface could be made hydrophilic by modification with suitable colloidal stabilizers or by using PEG–PACA amphiphilic copolymers [34–36]. The coating reduces the adsorption of plasma proteins, thus making the nanoparticles biocompatible and stable in biological fluids. Such nanoparticles can penetrate through the hyper-permeable vasculature, which is typical for the most solid tumors, reaching the tumor interstitium and degrade there, releasing the drug, and creating its high local concentration. Furthermore, most of the solid tumors have disrupted lymphatic drainage, which causes retention of the nanoparticles in the tumor. This is the so-called enhanced permeability and retention effect, which is used for the passive targeting of the nanoparticle drug carriers to solid tumors [1, 5]. Also, the nanoparticle carriers are widely used for lymph node targeting of drugs, which could be beneficial for the treatment of lymph node metastases [48–51].

The PBCA, used in our preparations, is soluble in acetone; water is a non-solvent for the polymer. To stabilize the obtained nanoparticles, the water phase contains water-

Table 1 Results from the GPC analysis of pre-synthesized PBCA polymer used in the preparation of nanoparticles by nanoprecipitation

Detection	Max RT, min	Mp	Mw	Mz	Mn	PD
UV-254	22.95	1,510	1,890	2,430	1,440	1.31
RI	22.95	1,270	2,200	2,890	1,670	1.32

Mw weight-average molecular weight, *Mn* number-average molecular weight, *Mz* *Z*-average molecular weight, *Mp* peak molecular weight, *PD* polydispersity, *PD* Mw/Mn

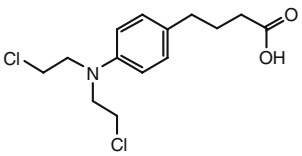

Fig. 1 Chemical structure of chlorambucil

soluble colloidal stabilizers, such as Dextran 40, Pluronic F68, or Polysorbate 80. Such stabilizers could adsorb on the nanoparticle surface, making it more hydrophilic and preventing the particle aggregation (it makes the obtained colloid stable and suitable for biomedical applications). Since these are all non-ionic substances, the pH does not affect their properties. The value of pH in our system is acidic (~2.7, adjusted with citric acid) in order to prevent hydrolysis of the entrapped drug (chlorambucil). The classical emulsion polymerization could provide for control of the molecular weight of the PACA polymers [24–28]. It usually leads to the formation of oligomers (usually decamers), which are suitable for biological use. For that reason, for the preparation of PBCA, we utilize the classical emulsion polymerization. The obtained polymer is isolated, purified, and used further for the nanoparticle preparation by nanoprecipitation. The identity and purity of the obtained PBCA is confirmed by ^{1}H NMR and FTIR spectroscopy. The molecular weight of the polymers, as determined by GPC, is found to be 1,000–1,500 Da (Table 1).

The chemical structure of chlorambucil, which is used in our experiments as a model drug, is given in Fig. 1. It is a hydrophobic anticancer drug used for the treatment of different proliferative diseases [52]. Chlorambucil is a nitrogen mustard derivative, which is relatively unstable and easily hydrolyses in aqueous medium [53, 54]. It is believed that its incorporation into nanosized drug carrier systems could decrease its side effects and increase its efficiency. Indeed, previous investigations using chlorambucil loaded in lipid nanoparticles revealed increased concentration of the drug in tumors [55].

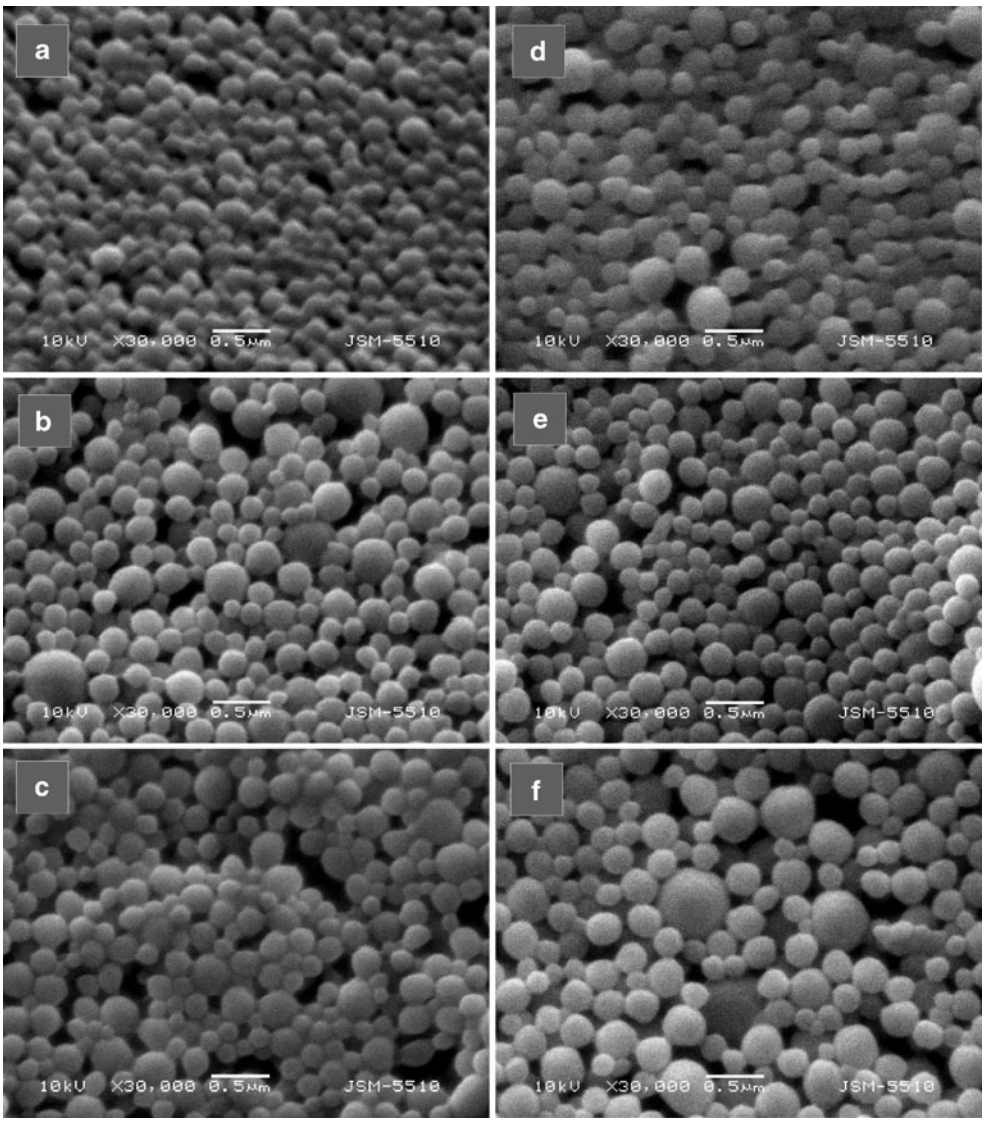

Fig. 2 Representative SEM images of PBCA drug carriers prepared by nanoprecipitation: **a** pure, coated with Polysorbate 80 (U-P80); **b** pure, coated with Dextran 40 (U-D40); **c** pure, coated with Pluronic F68 (U-F68); **d** chlorambucil-loaded, coated with Polysorbate 80 (C-P80); **e** chlorambucil-loaded, coated with Dextran 40 (C-D40); **f** chlorambucil-loaded, coated with Pluronic F68 (C-F68). All types of particles are prepared by using 10 mg/ml PBCA. The size bars represent 500 nm (magnification, ×30,000)

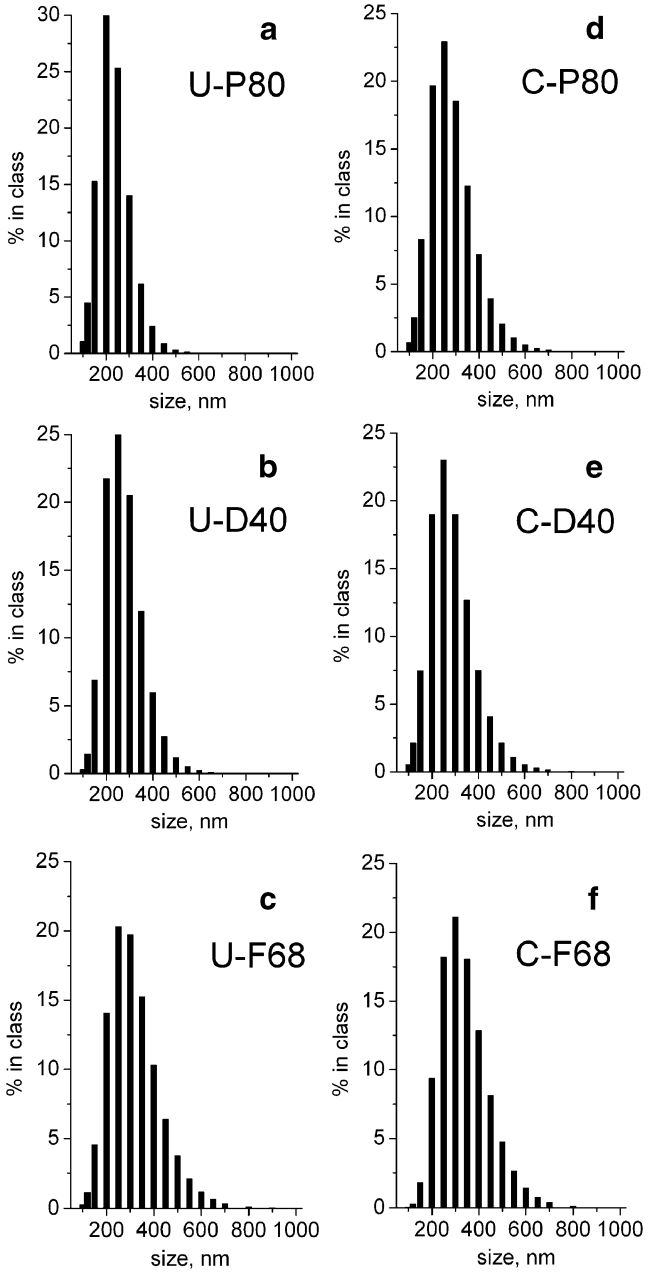

Fig. 3 Representative size distributions of PBCA nanoparticles determined by DLS (analysis by intensity). The histograms from **a** to **f** and the respective particle abbreviations correspond to those depicted in Fig. 2

The effect of colloidal stabilizers is well studied in the classical emulsion polymerization of alkylcyanoacrylates [23, 28]. However, the preparation of PACA nanoparticles by nanoprecipitation with different stabilizers has not been studied so far. For the preparation of PBCA drug carriers by nanoprecipitation, we use three types of stabilizers, approved for biomedical applications: Polysorbate 80 (monomer surfactant, PEG-modified sorbitane-monooleate), Pluronic F68 (polymer surfactant, PEG–PPO–PEG amphiphilic triblock copolymer), and Dextran 40 (polysaccharide, colloidal stabilizer). The most interesting is the Polysorbate 80 because it allows the transportation of nanoparticles through the blood–brain barrier. The mechanism of this process probably involves adsorption of apolipoprotein E from the blood plasma, followed by receptor-mediated transcytosis through the endothelial cells of the brain capillaries [45]. This makes such nanoparticles especially important for the treatment of very aggressive brain tumors, such as glioblastomas [47]. The Polysorbate 80-coated nanoparticles are classically prepared by polymerization-based methods in the presence of dextrans, followed by exchange of the dextran with Polysorbate 80 [46]. We found that the nanoprecipitation approach can be used to prepare directly Polysorbate 80-coated nanoparticles as demonstrated below.

The representative SEM images shown in Fig. 2 demonstrate the spherical shape of the obtained polymer particles and their submicron size. The size distributions (obtained by DLS) are monomodal, as seen from the histograms given in Fig. 3. The utilization of Polysorbate 80 leads to the formation of relatively small PBCA nanoparticles (~210 nm). Larger particles are obtained in the cases of Dextran 40 and Pluronic F68 (~240 and ~270 nm, respectively). The size distribution in the case of Pluronic-coated particles is slightly wider in comparison with the Polysorbate- and Dextran-coated particles. The drug-loaded nanoparticles are obtained with size distributions similar to those of the respective pure particles. The results from light scattering analysis of nanoparticles with different stabilizers are summarized in Table 2 and illustrated in Fig. 4a (data are based on DLS analysis of the distribution by intensity).

It is important to note that our attempts to produce chlorambucil-loaded PBCA nanoparticles by polymerization-based method in the presence of Dextran 40 as a colloidal stabilizer resulted in the formation of much larger particles of average size ~400 nm with bimodal size distribution (data not shown here). However, the nanoprecipitation approach

Table 2 Characteristics of the PBCA particles, prepared by nanoprecipitation using different stabilizers

Type of particles	Stabilizer	Average size, nm (±SD)	Polydispersity index (±SD)	Diffusion coefficient, μ^2/s (±SD)
Pure	Polysorbate 80	210±5	0.068±0.017	2.35±0.06
	Dextran 40	238±3	0.059±0.03	2.07±0.02
	Pluronic F68	269±4	0.158±0.014	1.83±0.03
Drug-loaded	Polysorbate 80	242±6	0.117±0.022	2.04±0.05
	Dextran 40	245±5	0.094±0.016	2.02±0.02
	Pluronic F68	295±5	0.158±0.023	1.67±0.03

The particles are prepared using 10 mg/ml of PBCA

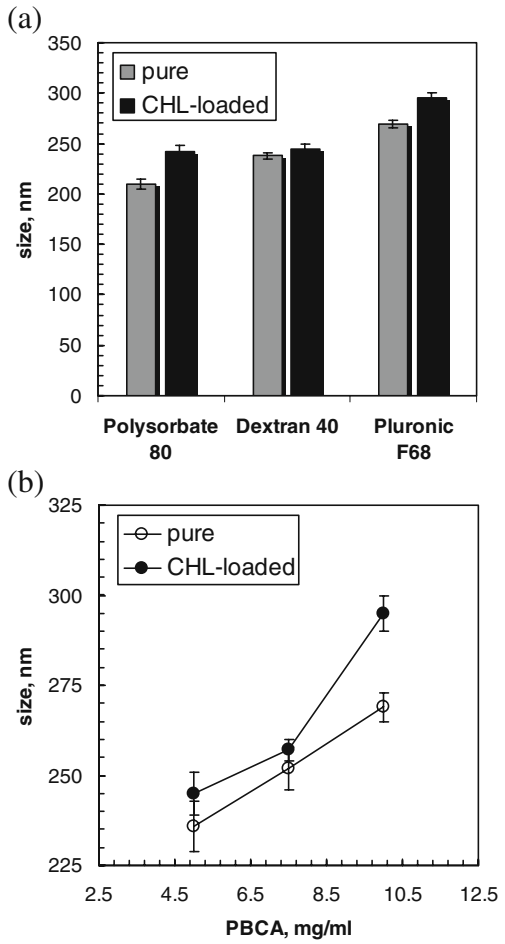

Fig. 4 Schematic representation of the average size of pure and chlorambucil-loaded PBCA nanoparticles as a function of **a** the type of stabilizer (using 10 mg/ml of PBCA) and **b** the amount of PBCA used for their preparation (for nanoparticles, coated with Pluronic F68)

reported here resulted in the formation of smaller (~240 nm in size) dextran-coated nanoparticles with monomodal size distribution, which are expected to be more suitable for future biomedical tests.

Table 3 Characteristics of PBCA particles prepared by nanoprecipitation using different amount of polymer

Type of particles	Amount of PBCA, mg/ml	Average size, nm (±SD)	Polydispersity index (±SD)	Diffusion coefficient, μ^2/s (±SD)
Pure	5.0	236±7	0.138±0.053	2.08±0.06
	7.5	252±6	0.121±0.024	1.95±0.04
	10.0	269±4	0.158±0.014	1.83±0.03
Drug-loaded	5.0	245±6	0.103±0.015	2.01±0.05
	7.5	257±3	0.138±0.024	1.92±0.02
	10.0	295±5	0.158±0.023	1.67±0.03

The amount of polymer is given in milligrams of PBCA per milliliter of nanoprecipitation medium. The particles are prepared using Pluronic F68 as a stabilizer

Table 4 Drug content in chlorambucil-loaded PBCA nanoparticles prepared by nanoprecipitation using various amounts of polymer and different stabilizers

Type of stabilizer	Amount of PBCA, mg/ml	Drug content, %
Polysorbate 80	10.0	6.6
Dextran 40	10.0	7.9
Pluronic F68	10.0	7.5
Pluronic F68	7.5	8.6
Pluronic F68	5.0	11.2

We found that by varying the amount of polymer, one can finely control the size of pure and drug-loaded PBCA particles prepared by nanoprecipitation; increasing the amount of polymer results in the formation of larger nanoparticles. The results from the investigation of this effect in the case of Pluronic-coated particles are summarized in Table 3 and Fig. 4b. Similar tendencies are observed for both pure and drug-loaded nanoparticles.

The results from determination of the drug content are summarized in Table 4. As seen, a relatively large amount of the drug is incorporated in the nanoparticles due to its lipophilic character. Increasing the amount of polymer leads to lower drug content. Experiments with thin-layer chromatography revealed that the chlorambucil remains unchanged after incorporation in nanoparticles (R_f=0.54). Insignificant amount of a hydrolysis product is detected (R_f=0.22) in the aqueous phase.

The drug release studies indicate that most of the entrapped drug is released relatively fast during the dialysis experiments (Fig. 5). No significant difference in the release rates is observed in the cases of using different colloidal stabilizers. This is probably a result of the

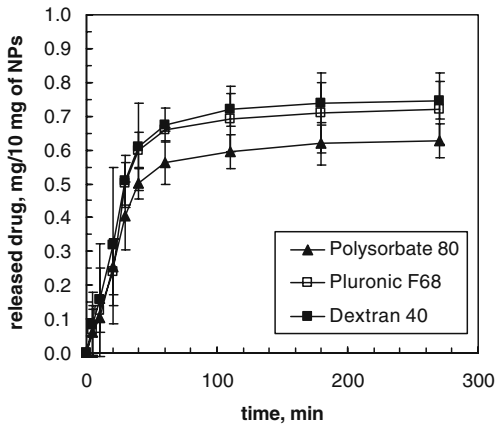

Fig. 5 Release of chlorambucil from drug-loaded PBCA nanoparticles as studied by dialysis. The release medium is phosphate-buffered saline at 37 °C (pH 7.4). The particles are prepared by nanoprecipitation in the presence of different stabilizers (given in the legend); the amount of PBCA is 10 mg per milliliter of nanoprecipitation medium

relatively fast release of the drug from the nanoparticles. It is rather possible that the release is rate-limited by the drug diffusion through the solution inside the dialysis tube and through the dialysis membrane.

Preliminary tests of the formulation stability indicate that the drug-loaded nanoparticles should be stored at a low temperature (4 °C for few days or −20 °C for longer periods) in order to avoid the drug hydrolysis. The storage of nanoparticles at −20 °C resulted in no significant hydrolysis of the drug. The nanoparticles can sediment during storage as a liquid dispersion, but can be redispersed upon shaking. The nanoparticle dispersions are stable in the frozen state at −20 °C, except those prepared with Dextran 40; in this latter case, the nanoparticles coagulate after unfreezing.

Conclusions

In this paper, we report the preparation and characterization of pure and drug-loaded poly(butylcyanoacrylate) nanoparticles by nanoprecipitation approach in the presence of different colloidal stabilizers (Polysorbate 80, Pluronic F68, and Dextran 40). The size of the obtained nanoparticles (200–300 nm) falls within the size ranges suitable for drug-delivery applications and can be controlled by varying the amount of polymer. In comparison with the polymerization-based methods, the nanoprecipitation has the advantage that the used polymer is well characterized, and its characteristics do not depend on the conditions of the nanoparticle preparation. The method could be utilized for the entrapment of drugs, which otherwise could be inactivated upon contact with the highly reactive alkylcyanoacrylate monomers. Also, it is difficult to obtain such particles by direct emulsion polymerization in the presence of Polysorbate 80 because this surfactant cannot stabilize well the initially formed emulsion. However, we found that these limitations could be overcome by using the nanoprecipitation approach, which resulted in the formation of stable Polysorbate 80-coated nanoparticles, suitable for future biomedical tests. We expect that the preparation of Polysorbate 80-coated drug-loaded poly(alkylcyanoacrylate) nanoparticles by nanoprecipitation will reveal new opportunities to improve the treatment of resistant cancers and drug targeting to the brain.

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