Colloid Polym Sci (2010) 288:893–899
DOI 10.1007/s00396-010-2219-5

SHORT COMMUNICATION

Preparation and physicochemical characterization of novel chlorambucil-loaded nanoparticles of poly(butylcyanoacrylate)

Georgi G. Yordanov · Zorka A. Bedzhova · Ceco D. Dushkin

Received: 14 October 2009 / Revised: 1 April 2010 / Accepted: 1 April 2010 / Published online: 30 April 2010

Abstract The aim of the present study is the preparation and physicochemical characterization of chlorambucil-loaded poly(butylcyanoacrylate) nanoparticles. Chlorambucil is a lipophilic drug, which is used clinically against chronic lymphocytic leukemia, lymphomas, and other types of malignant diseases. However, the chlorambucil use is limited by its chemical instability and toxic side effects. A promising approach to circumvent these drawbacks is the entrapment of chlorambucil in a suitable nanosized carrier. Toward this goal, poly(butylcyanoacrylate) nanoparticles meet the requirements for a drug carrier system due to their biocompatibility, biodegradability, low toxicity, and ability to overcome the multidrug resistance in cancer cells. We prepared chlorambucil-loaded poly(butylcyanoacrylate) nanoparticles, which are characterized for chemical composition, particle size, drug content, and drug release. It is expected that the utilization of poly(butylcyanoacrylate) nanoparticles as a drug carrier system will pave the way toward more effective use of chlorambucil in the treatment of cancer.

Keywords Poly(butylcyanoacrylate) · Nanoparticles · Chlorambucil · Emulsion polymerization

Introduction

The nanoparticles can prove to be very useful in cancer therapy by allowing for the effective and targeted drug delivery by overcoming many biological, biophysical, and biomedical barriers that the human body possesses against a standard intervention such as the administration of drugs or contrast agents [1–8]. The nanoparticle drug carrier systems for cancer treatment can alter the unfavorable properties of "free" anticancer agents, such as a poor specificity and high toxicity. Toward this goal, the poly(alkylcyanoacrylate) (PACA) nanoparticles meet the requirements for a drug carrier system due to their biocompatibility, biodegradability, low toxicity, and ability to overcome the multidrug resistance in cancer cells. These nanoparticles can provide a controlled and targeted delivery of the drug with a better efficacy and less side effects [9–12]. The lipophilic drugs are more readily incorporated in a hydrophobic polymer like PACA than the hydrophilic compounds, although the latter may be adsorbed onto the particle surface [10–12].

Chlorambucil (CHL) is a lipophilic anticancer drug, which is used clinically against chronic lymphocytic leukemia, lymphomas, and some other types of advanced cancer [13, 14]. However, the use of CHL is limited by its poor solubility in water, chemical instability, and severe toxic side effects [15–20]. CHL is a classical alkylating agent, which is believed to interact with DNA, thus causing cytotoxic effect. Recent efforts to decrease the CHL toxicity have been devoted to the development of new CHL derivatives [21] and liposomal formulations of prodrug lipids [22]. A promising approach to target tumors and circumvent toxic effects is the entrapment of CHL in suitable nanosized carrier systems, such as parenteral emulsions [23] and lipid nanoparticles [24]. A prolonged and significantly higher drug concentration in tumor tissue has been observed in the case of CHL encapsulated in lipid nanoparticles in comparison with the free drug, which indicates a possible enhanced therapeutic activity [24].

Here, we present a new alternative approach for the nanoencapsulation of chlorambucil in polymer nanoparticles of

G. G. Yordanov · Z. A. Bedzhova · C. D. Dushkin (✉)
Laboratory of Nanoparticle Science and Technology,
Department of General and Inorganic Chemistry,
Faculty of Chemistry, University of Sofia,
1 "James Bourchier" Blvd.,
1164 Sofia, Bulgaria
e-mail: nhtd@wmail.chem.uni-sofia.bg

poly(butylcyanoacrylate) (PBCA). The preparation and physicochemical characterization of this formulation is described. The obtained CHL-loaded PBCA nanoparticles are characterized by scanning electron microscopy (SEM), nuclear magnetic resonance (^{1}H NMR), and dynamic light scattering (DLS). Various colloidal stabilizers are used to prepare nanoparticles by emulsion and dispersion polymerization. Drug entrapment efficiency and release kinetics are also studied. The chemical composition and formulation stability upon various storage temperatures is investigated by using thin-layer chromatography (TLC).

Experimental procedures

Chemicals Butylcyanoacrylate (BCA) monomer was purchased from Special Polymers Ltd (Bulgaria). CHL, phosphate-buffered saline (PBS), Pluronic F68, citric acid (anhydrous), acetone (>99.5%), and sodium hydroxide (>98%) were from Sigma. Dextran 40 (M_w 40,000 Da; isolated from *Leuconostoc* ssp.) and Polysorbate 80 (Tween 80) were from Fluka. Glucose (water solution; 5%, *w*/*w*) was from Actavis (Bulgaria). Distilled water was used for all preparations. All other chemicals were of analytical grade.

Preparation of nanoparticles Chlorambucil-loaded PBCA (CHL-PBCA) nanoparticles were prepared by the following manner. A polymerization medium was prepared by dissolving the colloidal stabilizer (Pluronic F68; Dextran 40, or Polysorbate 80) (20 mg) and citric acid (20 mg) in glucose solution (5%, *w*/*w*, 10 ml). A mixed solution, consisting of butylcyanoacrylate monomer (100–200 μl), CHL (10 mg), and acetone (5 ml), was added dropwise to the polymerization medium upon vigorous magnetic stirring (~600 rpm). The emulsion became milky white and was left to polymerize on open air for 4 h. The residual acetone was removed by vacuum evaporation, and the final volume of the dispersion was adjusted to 10 ml by addition of distilled water. The obtained colloidal dispersion was kept at 4 °C for short-term storage (few days) or at −20 °C for long-term storage (months).

Characterization of nanoparticles The obtained nanoparticles were imaged by a SEM JSM-5510 (JEOL). The samples for SEM were prepared by evaporation of dilute nanoparticle dispersion on a glass substrate and coating with gold thin film by JFC-1200 fine coater (JEOL). DLS system Malvern 4700C (Malvern Instruments, UK) was used to measure the nanoparticle size in water dispersion at 25 °C and scattering angle 90° (each value was obtained as the average of five measurements). The nuclear magnetic resonance (^{1}H NMR) spectra were taken with Bruker Avance II+ 600 spectrometer (spectrometer frequency 600.13 MHz). For that purpose, aliquots (ca. 1 ml) of the as-prepared unloaded and CHL-loaded nanoparticle dispersions were centrifuged, washed with distilled water, and dried under vacuum to obtain white powdered material. The samples of dried nanoparticles (ca. 5 mg) were dissolved in 0.6 ml of deuterated acetone (acetone-d_6). The spectra were taken at room temperature in the interval 0–15 ppm.

Determination of the drug content in nanoparticles The drug content (DC, %) is defined as the weight fraction of intact drug, which is contained in drug-loaded nanoparticles:

$$\mathrm{DC} = \frac{m_{\mathrm{CHL}}}{m_{\mathrm{CHL-PBCA}}} \times 100 \quad (1)$$

Here, m_{CHL} is the mass of loaded CHL, and $m_{\mathrm{CHL-PBCA}}$ is the mass of CHL-PBCA nanoparticles. An aliquot (1 ml) of the as-prepared nanoparticle dispersion is centrifuged in a pre-weighted Eppendorf tube at 14,500 rpm for 60 min, washed with distilled water, and centrifuged again. The aqueous supernatant was then removed, and the nanoparticles were dried in vacuum. The obtained nanoparticles were weighted and dissolved in CH_2Cl_2 (10.0 ml). The obtained clear solution was then diluted 10-fold, and the concentration of CHL was measured by UV–vis absorbance spectroscopy at 260 nm using a double-beam UV–vis spectrophotometer (Evolution 300, Thermo Scientific). Pure PBCA dissolved in CH_2Cl_2 was used as a baseline reference (although PBCA does not absorb light at 260 nm). The mass of the CHL in the initial sample could be then calculated for determination of the drug content. Closed quartz cuvettes were used to prevent evaporation of the volatile solvent. The extinction coefficient of CHL in CH_2Cl_2 at 260 nm was found to be 53±5 ml/mg cm.

In vitro drug release The drug release was studied by dialysis of the nanoparticle dispersions. The dialysis tubing cellulose membranes (with molecular weight cut-off size of 12,400 Da) were from Sigma. Before use, the dialysis membranes were washed with hot distilled water (60 °C) for 20 min. For the dialysis experiments, an aliquot (1.0 ml) of the as-prepared nanoparticle dispersion was centrifuged, washed with distilled water, and redispersed in PBS buffer (1 ml; pH 7.4). The dispersion was placed within a dialysis bag, which was put in a vessel containing 100 ml of phosphate buffer (0.01 M PBS, pH 7.4). The dialysis was carried out at 37±1 °C upon magnetic stirring (300 rpm). The receiver vessel was closed to prevent evaporation of the receiver medium. The amount of released drug was determined by spectrophotometric measurements at 256 nm. The extinction coefficient of CHL at 256 nm in PBS buffer (pH 7.4) was found to be 38±3 ml/mg cm. Each aliquot was returned back to the receiver vessel immediately

after the spectrophotometric measurement. Each experiment was carried out at least triplicate in order to evaluate the reproducibility.

Formulation stability The formulation stability upon storage at various temperatures was studied by the evaluation of the chemical stability of CHL against hydrolysis using TLC. The CHL-loaded nanoparticles were stored at two different temperatures: −20 °C (deep refrigeration) and 20 °C (room temperature). The TLC experiments were carried out on aluminum sheets pre-coated with silica gel (ALUGRAM® SIL G/UV_{254}, Germany). The mobile phase was a mixture of chloroform/methanol (6:1, *v*/*v*). Samples of nanoparticle dispersions (stored at different conditions) were centrifuged; the aqueous supernatant was decanted and analyzed by TLC; the sediment of nanoparticles was washed with distilled water, centrifuged again, and dried in vacuum. The dried nanoparticles were dissolved in acetone and analyzed by TLC. Pure CHL (dissolved in acetone) was used as a reference.

Results and discussion

The PBCA nanoparticles can be prepared by different polymerization-based procedures [25, 26]: (1) emulsion polymerization and (2) dispersion polymerization. Both procedures involve dropwise addition of monomer (or solution of monomer in water-miscible organic solvent) to acidic polymerization medium. In the case of emulsion polymerization, surfactants (Polysorbate 80 and Pluronic F68 in our experiments) are used as components of the polymerization medium, which can solubilize the monomer to form swollen micelles. Colloidal stabilizers (such as Dextran 40) are used instead of surfactants in the case of dispersion polymerization. The colloidal stabilizer cannot form micelles but prevents the aggregation of growing particles by adsorption on the particle surface.

In this report, different preparations of CHL-PBCA nanoparticles are carried out depending on the type of emulsifier/colloidal stabilizer used. We found that the utilization of Polysorbate 80 (monomer surfactant, PEG-modified sorbitane-monooleate) usually results in the formation of unstable emulsion in the beginning of the process. In most cases, bulk polymer forms instead of nanoparticles. Only in few cases could nanoparticles with broad and usually bimodal size distribution be obtained (data not shown here). Therefore, this surfactant is considered as unsuitable for the preparation of CHL-PBCA nanoparticles by emulsion polymerization. The preparation of CHL-PBCA nanoparticles by dispersion polymerization (using Dextran 40) results in the formation of relatively unstable dispersion of nanoparticles. In contrast, the emulsion polymerization in the presence of Pluronic F68 (polymer surfactant, PEG-PPO-PEG amphiphilic triblock copolymer) results in the formation of stable CHL-PBCA nanoparticles, whose characteristics are described below.

In the preparation of PBCA-based nanoparticles, the polymerization of butylcyanoacrylate is initiated by the hydroxide ions (OH^-) of water in aqueous medium [27–29]. Therefore, the elongation of polymer chains occurs according to an anionic polymerization mechanism. The CHL molecules do not contain any functional groups, which could initialize polymerization reactions. The polymerization is carried out in acidic water solution (the respective medium contains citric acid, pH 2.7) in order to achieve controllable polymerization rate. Since CHL is a lipophilic drug, we found it soluble in butylcyanoacrylate monomers and readily incorporating in the hydrophobic polymer matrix of PBCA nanoparticles. For the CHL entrapment in PBCA nanoparticles, we utilize the emulsion polymerization. CHL is well soluble in butylcyanoacrylate; thus, CHL-PBCA nanoparticles can be prepared by the dropwise addition of CHL dissolved in the monomer to the polymerization medium. Typically, this procedure leads to the formation of monodisperse CHL-PBCA nanoparticles of average size ~170 nm. However, a large portion of the monomer could not be well emulsified in this procedure and forms micron-sized polymer beads instead. For that reason, we utilize acetone as a suitable water-soluble solvent, which dissolves both CHL and monomer. The dropwise addition of CHL and monomer, dissolved in acetone, to the polymerization medium results in a much better emulsification and formation of CHL-PBCA nanoparticles of high quality. The same procedure is utilized for all nanoparticle preparations described in this report.

The SEM shows that the CHL-PBCA nanoparticles, obtained by emulsion polymerization (with Pluronic F68 as a surfactant), are of spherical shape and monomodal size distribution (Fig. 1a). The nanoparticle size, measured by light scattering (DLS), well corresponds to the data from electron microscopy (the size distribution given in Fig. 1a is determined by DLS). All nanoparticles obtained are of average size, 220–250 nm, which does not depend significantly on the monomer concentration as seen from the data in Table 1. This size falls within the size ranges (below 400 nm) suitable for passive tumor targeting by nanoparticle penetrating across the fenestrations in tumor vasculature [10–12].

In comparison, the CHL-PBCA nanoparticles prepared by dispersion polymerization are much bigger, with relatively broad and bimodal size distribution (Fig. 1b). The particle dispersion is rather unstable, which does not allow accurate determination of the size distribution by DLS analysis (the size distribution given in Fig. 1b is determined from SEM images by measuring the size of at least 500 particles). Due to their large size and poor

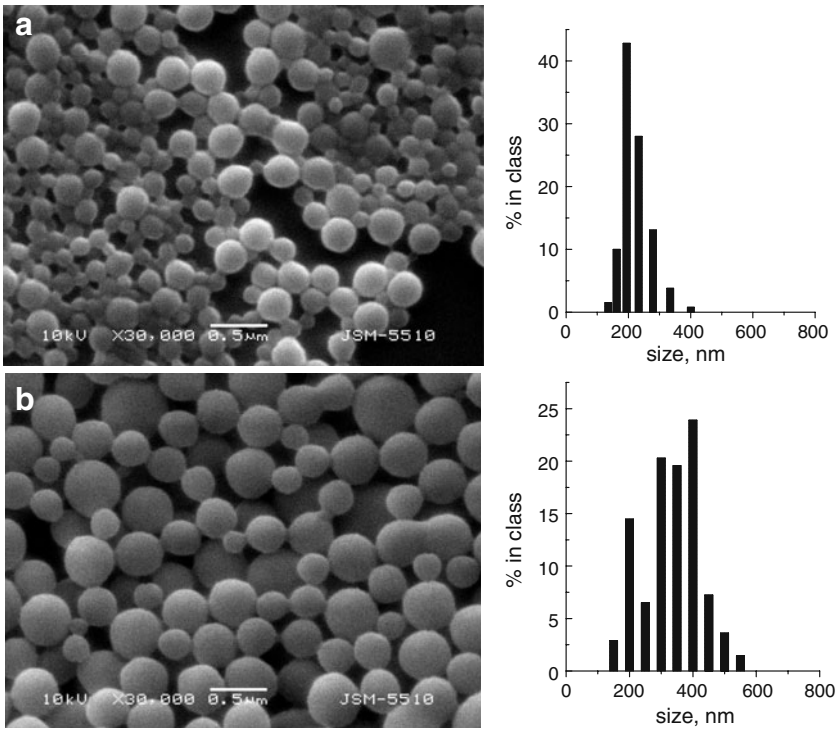

Fig. 1 Representative SEM images (*left*) and respective histograms of the size distribution (*right*) of CHL-PBCA nanoparticles prepared by **a** emulsion polymerization (using Pluronic F68) and **b** dispersion polymerization (using Dextran 40). The nanoparticles are prepared by using 10 mg of CHL and 100 μl of BCA monomer

colloidal stability, these nanoparticles are considered as less suitable for biomedical applications. Also, our experience shows that the dispersion polymerization (using Dextran 40 as a colloidal stabilizer) is less reproducible than the emulsion polymerization (using Pluronic F68 as a surfactant). For these reasons, we are focused on the characterization of the CHL-PBCA nanoparticles prepared by using Pluronic F68.

The content of CHL in PBCA nanoparticles is determined by two independent methods: ^{1}H NMR spectroscopy and UV–vis absorbance spectroscopy. As an example, parts of the ^{1}H NMR spectra of pure and CHL-PBCA nanoparticles are shown in Fig. 2. The signals at 1.5 ppm (2H), 1.7 ppm (2H), 2.9 ppm (2H), and 4.3 ppm (2H) in the spectra of pure PBCA correspond all to methylene protons. The signal at 1.0 ppm (3H) corresponds to protons from the methyl group. Additional signals from the entrapped CHL molecules appear in the spectra of CHL-PBCA nanoparticles, of which those from aromatic protons are well distinguishable at 7.11 and 6.75 ppm. The drug content (DC, %) in nanoparticles can be determined by measuring the ratio between the integral intensity of signals from aromatic protons I_{Ar} (which correspond to the 4H atoms from a CHL molecule) and the integral intensity of signals from methyl protons I_{Me} (which corresponds to the 3H atoms from an alkylcyanoacrylate monomer):

$$\mathrm{DC} = \frac{3M_{\mathrm{CHL}}I_{\mathrm{Ar}}}{3M_{\mathrm{CHL}}I_{\mathrm{Ar}} + 4M_{\mathrm{BCA}}I_{\mathrm{Me}}} \times 100 \qquad (1)$$

Table 1 Characteristics of various CHL-PBCA nanoparticles

Nanoparticles[a]	Average size (nm)	Polydispersity index	CHL-PBCA nanoparticles (mg) per ml of dispersion	Drug content (DC) in CHL-PBCA nanoparticles (%)
CHL-PBCA (100)	217±9	0.177	8.5±0.1	7.2±0.7
CHL-PBCA (150)	242±7	0.184	10.4±0.2	6.4±0.8
CHL-PBCA (200)	248±3	0.161	14.3±0.2	5.6±0.5
CHL-PBCA (100)[b]	~400	–	8.2±0.1	10.6±2.4

[a] The values given in brackets represent the volume of initial monomer (μl) used per 10 ml of polymerization medium. In all syntheses, the initial CHL is 10 mg per 10 ml of polymerization medium

[b] The nanoparticles are prepared by dispersion polymerization (using Dextran 40). In all other cases, the nanoparticles are prepared by emulsion polymerization (using Pluronic F68)

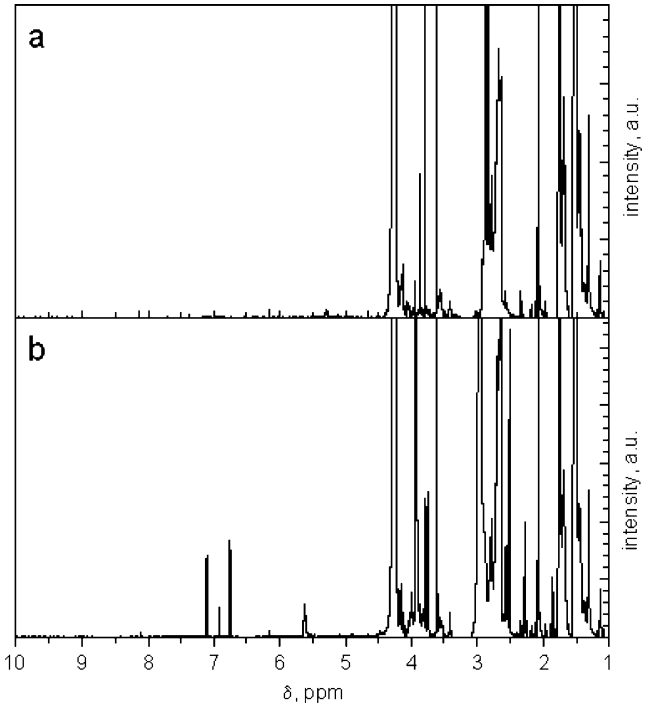

Fig. 2 ^{1}H NMR spectra of **a** pure PBCA nanoparticles and **b** CHL-PBCA nanoparticles. The signals for aromatic protons of CHL are clearly observed in the case of CHL-PBCA nanoparticles. The ^{1}H NMR spectrum can be used for determination of the drug content in nanoparticles (see the text for details)

Here, M_{CHL} is the molecular weight of CHL (304.2 g/mol), and M_{BCA} is the molecular weight of the monomer (153.2 g/ml).

The drug content in CHL-PBCA nanoparticles is determined more conveniently by UV–vis absorbance spectroscopy (see the "Experimental procedures" section for details). The results from spectrophotometric determination of the drug content are summarized in Table 1. The CHL content in CHL-PBCA nanoparticles is about 5–8%. As seen, the drug content in nanoparticles slightly decreases with increasing the amount of monomer (at constant amount of initial drug). Drug content is highest for nanoparticles prepared by dispersion polymerization (~10%). It is important to note that not the entire amount of initial butylcyanoacrylate is transformed into nanoparticles. For example, the weight of CHL-PBCA nanoparticles prepared from 200 µl (193 mg) of monomer is 143 mg (Table 1). About 5.6% of this mass (8.0 mg) is CHL, which means that about 30% of the initial monomer is not included in the obtained CHL-PBCA nanoparticles. Some of the initial monomer is transformed into polymer, which is found deposited on the Teflon-coated stirring bar after the nanoparticle preparation. One can estimate also that not the entire amount of initial CHL is loaded in the obtained nanoparticles. For example, the CHL-PBCA (200) nanoparticles (see Table 1) contain 5.6% of drug (8.0 mg CHL in 143 mg CHL-PBCA). Taking into account that the initial amount of drug is 10 mg, it appears that about 20% of the CHL is not loaded in nanoparticles. This drug is probably entrapped in the small amount of bulk polymer (deposited on the stirring bar). Also, the TLC experiments (see below) indicate that the aqueous phase contains small amounts of CHL derivatives (products of hydrolysis).

The CHL release from drug-loaded nanoparticles is studied in 0.01 M PBS (pH 7.4) at 37 °C, which provides a basic idea of the drug release in physiological systems. One should take into account that CHL is released in the PBS buffer in its anionic form, which is soluble in water. The released amount of CHL per 10 mg of CHL-PBCA nanoparticles is plotted as a function of dialysis time (Fig. 3). Most of the loaded CHL is released within the first 1–2 h of dialysis. The CHL-PBCA (200) nanoparticles release relatively more amount of CHL, which is related to the higher drug content in this case (see Table 1). The release profiles from CHL-PBCA nanoparticles prepared with various concentrations of monomer do not differ significantly. Probably, the release of CHL from the nanoparticles is a rather fast process, and the rate-limiting step is the diffusion of the drug through the solution and out of the dialysis tube. This is likely the reason for the similarity between the different release rates. The results from the drug release experiments indicate that the CHL could be released rather fast from the nanoparticles, which could decrease the efficiency of the drug carrier system if applied intravenously (the drug could be released before the nanoparticles reach the target site). Therefore, we suppose that the CHL-PBCA nanoparticle system could be more suitable for local application (such as intratumoral injection). Drug delivery

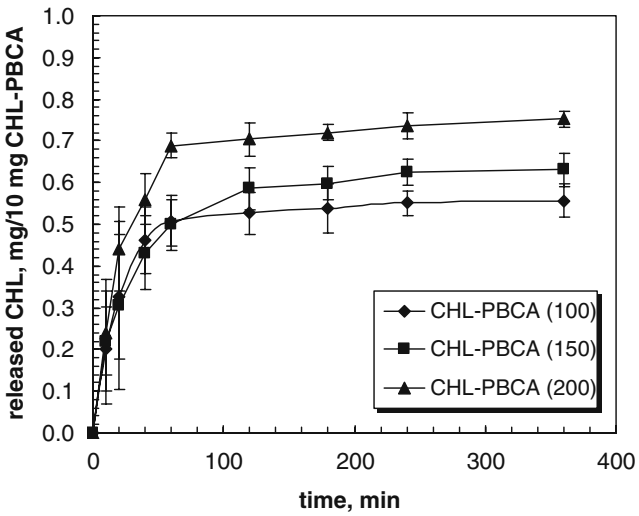

Fig. 3 Release kinetics of CHL from CHL-PBCA nanoparticles studied by dialysis in phosphate-buffered saline at 37 °C. The types of nanoparticles, given in the legend, correspond to those depicted in Table 1

to lymph node metastases is another possible application, as previously demonstrated for various types of nanosized drug carriers [30–33]. However, this should be evaluated in future biomedical tests.

In the CHL-PBCA nanoparticle preparations, reported here, the acidic polymerization medium (pH 2.7) is not neutralized after completion of the polymerization reaction in order to avoid the hydrolysis of CHL. At the same time, the PBCA polymer remains stable in the presence of citric acid if stored at refrigeration. The previous studies of CHL hydrolysis have led to the acceptance of a mechanism involving a reaction proceeding via aziridinium ion intermediate [15–18]. This reaction results in the formation of respective mono- and dihydroxyl derivatives of CHL. The rate of CHL hydrolysis can be significantly decreased in acidic medium (pH<3) [15, 18]. TLC is used to evaluate the stability of CHL-PBCA nanoparticles during storage at different temperatures. The fluorescent silica plates are visualized under UV (254 nm) light illumination. At these conditions, only compounds containing UV-chromophore groups are visible, such as CHL (marked as "compound 1" in our experiments) and its hydrolysis products (marked as "compound 2" and "compound 3"). All other components of the polymerization medium, including PBCA, were invisible on the fluorescent chromatographic plates. The experiments (Fig. 4) indicate that the CHL-PBCA nanoparticle dispersion stored for 3 months at deep refrigeration (−20 °C) contains two CHL derivatives in small quantities (R_f=0.22 and 0.14), most probably products of hydrolysis (Figs. 4 and 5). These by-products (compounds 2 and 3) are detected only in the aqueous phase (chromatogram "c"), while the nanoparticles contained only intact CHL (chromatogram "b"). The identity of CHL loaded in nanoparticles is confirmed by using pure CHL as a reference (R_f=0.54, chromatogram "a"). It is interesting to observe that the PBCA nanoparticles contain only CHL and no other by-products. On the other hand, the aqueous phase contains only the by-products and no detectable CHL. This is an important observation because it affords an opportunity for easy purification of the CHL-PBCA nanoparticles by

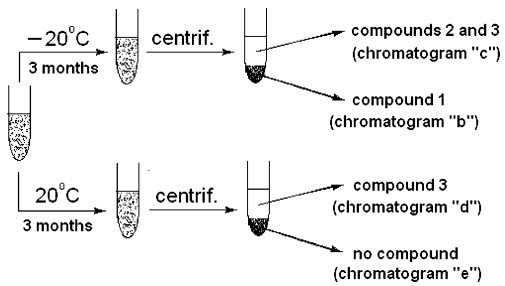

Fig. 4 Scheme of the experiments on the chemical stability of CHL-PBCA nanoparticle dispersions. The compounds 1, 2, and 3 (CHL and its derivatives) are detected by TLC (see respective chromatograms)

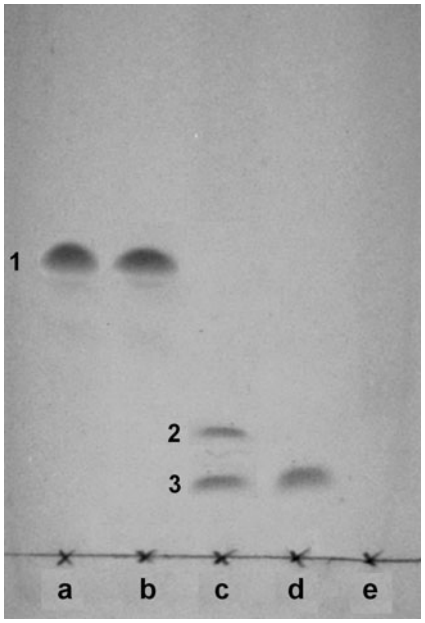

Fig. 5 A photograph of a fluorescent TLC plate (*a*) pure CHL; (*b*) CHL-PBCA nanoparticles stored 3 months at −20 °C; (*c*) aqueous supernatant, obtained after centrifugation of CHL-PBCA nanoparticle dispersion stored 3 months at −20 °C; (*d*) aqueous supernatant, obtained after centrifugation of CHL-PBCA nanoparticle dispersion stored 3 months at 20 °C; (*e*) CHL-PBCA nanoparticles stored 3 months at 20 °C. *The numbers on the chromatogram* indicate the respective compounds 1, 2, and 3 (see the text for details)

centrifugation. Actually, the formation of compounds 2 and 3 is detected by TLC experiments, performed immediately after the CHL-PBCA nanoparticle preparation (data not shown). It means that these by-products are formed during the CHL-PBCA nanoparticle preparation, but not during their storage at −20 °C. During the storage of the nanoparticles in the frozen state, the loaded CHL remains unchanged, indicating that the as-obtained formulation is stable at these conditions. Furthermore, the nanoparticle colloidal stability is not affected by the storage in the frozen state due to the presence of glucose as a cryoprotector. The parallel experiments on the storage of nanoparticles at −20 °C without glucose show lack of colloidal stability. However, the CHL-PBCA nanoparticles are found to be unstable during the storage at room temperature (20 °C). The TLC experiments indicate that the CHL is finally transformed into compound 3, which is water soluble and is detected only in the aqueous phase (chromatogram "d"). The nanoparticles contain no CHL or its hydrolysis products after storage for 3 months at room temperature (chromatogram "e").

It is well known that the CHL easily undergoes hydrolysis in aqueous medium resulting in the formation of two products: the respective mono- and dihydroxyl derivatives [15–18]. Therefore, taking into account the results from the TLC experiments, it can be assumed that the compounds 2

and 3 are actually the products of CHL hydrolysis. In order to verify this suggestion, we performed an experiment on the hydrolysis of pure CHL in alkaline aqueous solution and monitored the process by TLC (data not shown). This experiment indicated that the CHL indeed forms two products of hydrolysis (with the same R_f as those for the compounds 2 and 3) and is finally transformed into one of them—a compound with the R_f value being the same as that for the compound 3. These experiments show that compounds 2 and 3 are the mono- and dihydroxyl derivatives of CHL, respectively.

Conclusions

The anticancer agent chlorambucil is loaded in poly (butylcyanoacrylate) nanoparticles by emulsion polymerization in the presence of Pluronic F68 as a surfactant. The obtained chlorambucil-loaded nanoparticles are of average size 220–250 nm and rather monodisperse in size. The preparation of chlorambucil-loaded nanoparticles by dispersion polymerization, using Dextran 40 as a colloidal stabilizer, results in the formation of relatively unstable colloid with relatively larger particle size (~400 nm in diameter). The drug content in the obtained nanoparticles is found to be 6–10%. The as-obtained formulation can be stored at deep refrigeration (−20 °C), thus avoiding the drug hydrolysis as revealed by thin layer chromatography. The entrapment of chlorambucil in poly(butylcyanoacrylate) nanoparticles results in a stable formulation, which has the potential to be applied in future biomedical tests for targeted cancer treatment.

Acknowledgment The authors are thankful to COST Action D43 of the European Community.

References

1. Vasir J, Reddy M, Labhasetwar V (2005) Curr Nanosci 1:47–64
2. Briesen H, Ramge P, Kreuter J (2000) AIDS Rev 2:31–38
3. Briones E, Colino CI, Lanao J (2008) J Control Release 125:210–227
4. Salata OV (2004) J Nanobiotechnology 2:3
5. Brigger I, Dubernet C, Couvreur P (2002) Adv Drug Deliv Rev 54:631–651
6. Praetorius NP, Mandal TK (2007) Recent Pat Drug Deliv Formul 1:37–51
7. Brannon-Peppas L, Blanchette JO (2004) Adv Drug Deliv Rev 56:1649–1659
8. Barratt G (2003) Cell Mol Life Sci 60:21–37
9. Pinto-Alphandary H, Andremont A, Couvreur P (2000) Int J Antimicrob Agents 13:155–168
10. Murthy R, Reddy L (2006) Poly(alkyl cyanoacrylate) colloidal particles for delivery of anti-cancer drugs. In: Amiji MM (ed) Nanotechnology for cancer therapy. CRC, Boca Raton, pp 251–288
11. Ringe K, Walz C, Sabel B (2004) Nanoparticle drug delivery to the brain. In: Nalwa HS (ed) Encyclopedia of nanoscience and nanotechnology, vol 7. American Scientific, Valencia, pp 91–104
12. Begley D, Bradbury M, Kreuter J (2000) The blood–brain barrier and drug delivery to the CNS. Marcel Dekker, New York
13. Galton D, Israels L, Nabarro J, Till M (1965) BMJ II:1172
14. Hall A, Tilby M (1992) Blood Rev 6:163–173
15. Owen W, Stewart P (1979) J Pharm Sci 68:992–996
16. Ehrsson H, Eksborg S, Wallin I, Nilsson S (1980) J Pharm Sci 69:1091–1094
17. Bosanquet A, Clarke H (1986) Cancer Chemother Pharmacol 18:176–179
18. Chatterji D, Yeager R, Gallelli J (1982) J Pharm Sci 71:50–54
19. Cullis P, Green R, Malone M (1995) J Chem Soc Perkin Trans 2:1503–1511
20. Salmaso S, Bersani S, Semenzato A, Caliceti P (2007) J Drug Target 15:379–390
21. Reux B, Weber V, Galmier M, Borel M, Madesclaire M, Madelmont J, Debiton E, Coudert P (2008) Bioorganic Med Chem 16:5004–5020
22. Pedersen P, Christensen M, Ruysschaert T, Linderoth L, Andresen T, Melander F, Mouritsen O, Madsen R, Clausen M (2009) J Med Chem 52:3408–3415
23. Ganta S, Paxton J, Baguley B, Garg S (2008) Int J Pharm 360:115–121
24. Sharma P, Ganta S, Denny W, Garg S (2009) Int J Pharm 367:187–194
25. Vauthier C, Dubernet C, Fattal E, Pinto-Alphandary H, Couvreur P (2003) Adv Drug Deliv Rev 55:519–548
26. Nicolas J, Couvreur P (2009) Wiley Interdiscipl Rev Nanomed Nanobiotechnol 1:111–127
27. Donnelly E, Johnston D, Pepper D, Dunn D (1977) J Polym Sci Polym Lett Ed 15:399–405
28. Behan N, Birkinshaw C (2000) Macromol Rapid Commun 21:884–886
29. Behan N, Birkinshaw C, Clarke N (2001) Biomaterials 22:1335–1344
30. Hawley A, Davis S, Illum L (1995) Adv Drug Deliv Rev 17:129–148
31. Moghimi S, Bonnemain B (1999) Adv Drug Deliv Rev 37:295–312
32. Nishioka Y, Yoshino H (2001) Adv Drug Deliv Rev 47:55–64
33. Oussoren C, Storm G (2001) Adv Drug Deliv Rev 50:143–156