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# Ftorafur loading and controlled release from poly(ethyl-2-cyanoacrylate) and poly(butylcyanoacrylate) nanospheres

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**Abstract**

In the present work, a method is described to prepare polymeric colloidal nanospheres, consisting of poly(ethyl-2-cyanoacrylate) (PE-2-CA) or poly(butylcyanoacrylate) (PBCA), loaded with the anticancer drug ftorafur. The method is based on the anionic polymerization procedure, often used in the synthesis of poly(alkylcyanoacrylate) nanospheres for drug delivery. A detailed investigation of the capabilities of both polymeric nanoparticles to load this drug is shown. The effect of synthesis residuals and degradation products on the absorbance of supernatants was considered in the loading and release measurement methodologies, because of their potential perturbing influence on the determination of ftorafur concentration in solution. We found the existence of two mechanisms of drug incorporation: absorption or entrapment in the polymeric network, and surface adsorption, detectable by means of zeta potential and spectrophotometric measurements. Among the factors affecting the drug incorporation to the polymer network, the type of polymer, the pH and the drug concentration are the main determining ones. Moreover, the acidity of the medium needs to be controlled in order to avoid the formation of macroaggregates of solids. The optimum loading conditions were used to perform ftorafur release evaluations from polymeric particles, and the influence of the mechanism of drug incorporation, the amount of drug loaded, and the type of polymer on the drug release were studied.


*Keywords:* Controlled release; Drug delivery systems; Drug loading; Ftorafur; Poly(alkylcyanoacrylate)

## 1. Introduction

In this paper we investigate the capabilities of two kinds of poly(alkylcyanoacrylates) as vehicles for antitumor drugs. It is well known that chemotherapeutically active concentrations of such drugs must be reached in the target tissue in order to achieve a therapeutic action. Drug delivery systems consisting of nanoparticles make this possible, and they have gained special attention due to their possibilities in increasing the sensitivity of resistant cell lines (protecting the antitumor drug against biotransformations and rapid body clearance), and in reducing adverse drug effects, as the systemic distribution of the drug is avoided (Vauthier et al., 2003a).

A very suitable group of biodegradable polymers was chosen in this work for the drug carrier design, namely, poly(ethyl-2-cyanoacrylate) or PE-2-CA, and poly(butylcyanoacrylate) or PBCA. The use of poly(alkylcyanoacrylate) (PACA) nanoparticles produces therapeutic results in the treatment of both non-resistant and resistant cancers of a wide range of cell lines, as appropriate active drug concentrations are reached in the tumor tissue. It has been suggested that such increment of the therapeutic action is due to: (i) the overpowering toxicity of the drug; (ii) the toxicity induced by high local concentrations of polymeric degradation products at the cell membrane, that leads to the inhibition of cell growth or its death; and (iii) the reversion of the multidrug resistance due to both the adsorption of loaded nanoparticles to the cell surface and to the formation, as polymeric degradation occurs, of a drug–poly(cyanoacrylic acid) ion pair, able to cross the cell membrane without being recognized by the P-glycoprotein (Lherm et al., 1992; Némati et al., 1996; Vauthier et al., 2003a,b); the interaction between the active agent and the polymeric degradation products also increases the drug penetration through the blood brain barrier (Brigger

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et al., 2004). Moreover, the arrival of nanoparticles to the target tissue occurs relatively easy, after intratumoral injection or after intraarterial injection in the arteries that feed the tumor, as nanospheres are able to extravasate across the endothelium barrier, because of the increased permeability of these vessels in the presence of solid tumors (Vauthier et al., 2003b; Brigger et al., 2004). The suitability of PACA nanoparticles as drug delivery systems rises from their mechanical properties, biodegradability, high biocompatibility, drug compatibility and permeability (Fattal et al., 1997). Thus, the capability of defeating the tumor resistance is much higher if the antineoplasic drug is incorporated not only on the particle surface, but also in the polymeric network.

With respect to their toxicity, although relatively low (Page et al., 1996; Vauthier et al., 2003b), it is important if compared to that of other polymers used in drug delivery, such as poly(lactic acid) and its co-polymers with glycolic acid, that degrade relatively slowly *in vivo*. For instance, Lherm et al. (1992) mentioned that implants made from these polymers degraded in the body after several weeks or even 1 year, depending on the composition of the polymer (lactic acid to glycolic acid ratios). On the contrary, the degradation of PACAs take place in typically a few hours to at most 3 days, depending in this case on the chain length. Moreover, the toxicity of PACA polymers grows with the degradation kinetics, which is inversely related to the alkyl chain length (Lherm et al., 1992; Müller et al., 1992). However, results from a phase I trial reveal a good tolerance of these polymeric drug carriers, as only side effects due to the associated active agent were observed (Kattan et al., 1992); in addition, PACA nanoparticles have reached a phase II clinical trial for resistant cancer (Vauthier et al., 2003a). Furthermore, exposed cells are able to recover their normal function after metabolization of the degradation products under *in vivo* conditions, especially in chronic administration. These degradation products should also be eliminated from their degradation site, and hence the contact time with the cells would be considerably lower than *in vitro*. It has been suggested that in chronic treatments, rapidly degrading PACAs seem more adequate in avoiding the overloading of cells with slowly degrading polyesters (Lherm et al., 1992; Vauthier et al., 2003b).

The present contribution will focus on the preparation and characterization of PE-2-CA and PBCA nanospheres, loaded with ftorafur [Tegafur, 5-fluoro-1-(tetrahydro-2-furyl)uracil, see Fig. 1], a highly lipophilic nucleoside (with a water solubility of 8 mg/mL at room temperature, according to data by Leodinov, 2003) extensively used in cancer treatment due to its broad spectrum of antitumor activity, administered either intravenously or orally. We also investigate the amount of ftorafur that the polymeric nanoparticles are capable to load, both on the surface and in the polymeric matrix, and the factors that influence their matrix loading efficiency; in particular, the type of monomer and its concentration, surfactant and drug concentrations, and pH. The *in vitro* release profiles, and the influence of the type of monomer and the amount of drug loaded, were also evaluated. The analytical technique used, spectrophotometry, was validated and used successfully to determine both drug loading and release.

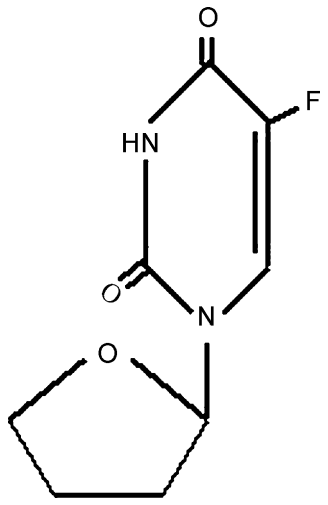


Fig. 1. Chemical structure of ftorafur.

## 2. Materials and methods

### 2.1. Materials

All chemicals used were of analytical quality from Panreac, Spain, except for ethyl-2-cyanoacrylate and butylcyanoacrylate (gifts from Henkel Loctite, Ireland) and ftorafur (purchased from Sigma–Aldrich, Germany). Water used in the experiments was of Milli-Q quality (Milli-Q Academic, Millipore, France).

### 2.2. Methods

#### 2.2.1. Preparation of poly(ethyl-2-cyanoacrylate) and poly(butylcyanoacrylate) nanospheres

PE-2-CA and PBCA colloidal nanospheres were prepared by the emulsion/polymerization method, in which the mechanism of reaction is an anionic process initiated by covalent bases present in the medium (*e.g.*, $OH^-$ ions deriving from water dissociation). The polymerization terminates by the addition of cations that neutralize such chains. The technique involves the polymerization of the monomer in an aqueous solution (of a pharmaceutical drug, in most instances) (Fattal et al., 1997; Vauthier et al., 2003b).

Briefly (Arias et al., 2001, 2006), 0.5 mL of monomer was added dropwise, under mechanical stirring (1200 rpm), to 50 mL of an aqueous polymerization medium containing $10^{-4}$N HCl and the stabilizing agent dextran-70 (1% (w/v)). After the polymerization reaction (2 h), the neutralization of the medium was achieved with sufficient amount of a KOH $10^{-1}$ M solution to ensure the end of the polymerization. A whitish suspension was obtained, which was subjected to a cleaning procedure that included repeated cycles of centrifugation (20,000 rpm, Centrikon T-124 high-speed centrifuge, Kontron, France) and redispersion in Milli-Q water. In order to ensure that the suspension was sufficiently clean, the electrical conductivity of the supernatant was checked after each cycle; it was assumed that most of the non-reacted chemicals were eliminated once the con-

ductivity approached the value corresponding to the water used in the cleaning cycles (less than 1 $\mu S\,cm^{-1}$).

The specific surface area of the particles was obtained from nitrogen adsorption using the BET method. In the device used (Quantasorb Jr., Quantachrome, USA), three mixtures of nitrogen (10, 20, and 30%) with the carrier gas, helium, are employed in order to apply the multipoint method. The sample mass was in all cases 0.5 g.

### 2.2.2. Optical characterization of ftorafur solutions

Assessment of the drug concentration in all systems investigated was performed by means of UV–vis absorption measurements at a wavelength of 271 nm in a 8500 UV–vis Dinko spectrophotometer (Dinko, Spain), using quartz cells of 1 cm path length. An optical absorbance–concentration calibration curve was obtained for ftorafur aqueous solutions with concentrations ranging between $10^{-5}$ and $10^{-2}$ M, always in the presence of $10^{-4}$N HCl, as this concentration of hydrochloric acid was required for the synthesis of the polymeric particles. Absorbance spectra were recorded 24 h after the preparation of the solutions, using a $10^{-4}$N HCl solution as a blank.

In order to study the ftorafur spectrum for the wide acid pH range to be used in the spectrophotometric assay of the drug loaded, and also at the pH = 7.4 of the release studies, we investigated the influence of $H^+$ concentration and NaOH–$KH_2PO_4$ buffer (pH = 7.4) on the optical absorbance properties of the anticancer drug solutions. A battery of $10^{-4}$ M ftorafur solutions was prepared, both in the NaOH–$KH_2PO_4$ buffer, and in solutions with increasing HCl concentrations to obtain pH values ranging from 1 to 5. After 24 h, the determination of the absorbance spectrum was carried out according to the procedure described in the previous paragraph (the corresponding HCl solutions or the pH 7.4 buffer were used as blanks).

The spectrophotometric method of analysis of the amount of drug loaded or released, was previously validated and verified for accuracy, precision and linearity (Caraballo et al., 1998). As ftorafur solutions are stable for all the pH values investigated and the validity of the molar absorption coefficient at 271 nm is demonstrated for all these pH's (see below), six standard ftorafur solutions, with concentrations ranging from $10^{-5}$ to $10^{-2}$ M, were prepared in the presence of either $10^{-4}$N HCl or NaOH–$KH_2PO_4$ buffer (pH = 7.4). Concentrations above $3 \times 10^{-4}$ M were appropriately diluted to $10^{-4}$ M, as was carried out in the loading and releasing experiments, in order to stay in the absorbance–ftorafur concentration linear range.

All solutions were protected from ambient light by wrapping all containers with aluminum foil.

### 2.2.3. Determination of the amount of drug incorporated

The loading of the nanoparticles can be achieved by two methods: drug addition during the process of generation of the nanospheres and hence trapping of the drug in the polymeric matrix, or surface adsorption on already formed particles after incubation in the drug solution.

Many authors have carried out drug loading determinations in particles of different nature by application of Beer's law to the optical absorbance of solutions containing a given initial drug concentration after carrying out a synthesis of the polymeric particles in the drug solution (Müller et al., 1991; Fawaz et al., 1997; Arias et al., 2005). As will be justified later, the drug loading procedure includes: (i) preparation of the aqueous polymerization medium with the appropriate amounts of HCl and surfactant (dextran-70); (ii) dropwise addition of the ethyl-2-cyanoacrylate or butylcyanoacrylate monomer; (iii) incorporation of adequate amounts of the drug at different time intervals; (iv) stirring of the medium for a specified period of time; (v) stopping the reaction by addition of proper amounts of KOH in order to achieve the total monomer consumption; (vi) centrifugation of the solids (13,500 rpm for 15 min); (vii) determination of the ftorafur loaded by measuring the drug concentration in the supernatant. All the experiments were carried out in triplicate.

These estimations are based on optical absorbance determinations of the polymerization medium as compared to that of the original ftorafur solution. For the method to be accurate, sources of absorbance changes other than variations in ftorafur concentration were previously identified (Arias et al., 2005). One possible source is that the drug loading influences the particle formation by changing, for instance, the size or composition. We can neglect this possibility, as electron miscroscope observations demonstrate that the particle shape and size are indistinguishable from that found in the absence of ftorafur.

The most important reason for absorbance changes is the presence of unreacted monomer and byproducts of the polymer degradation in the medium. Much smaller absorbance changes were observed when the concentrations of either HCl or dextran-70 were modified in the polymerization medium. This justifies the procedure used to estimate the drug loading (Müller et al., 1991; Fawaz et al., 1997; Arias et al., 2005): the amount of ftorafur present in solution after particle synthesis was obtained from the absorbance of the solution at a wavelength of 271 nm, after substracting the absorbance of the supernatant produced in the same conditions, but without drug in solution. In fact, we validated the method by comparing the evaluation of drug concentration in two instances: a certain amount of drug was dissolved in supernatants of polymer syntheses (carried out in the absence of drug), and the same amount was dissolved in equal amounts of water. We found that the concentrations estimated (in the first case from the difference between the absorbances of the drug plus supernatant solution and that of the supernatant) were identical to within the experimental uncertainty. These tests were carried out by sextuplicate, and demonstrated the reproducibility of the method, and the absence of molecular interactions.

We also investigated the possibility of ftorafur surface adsorption on the PE-2-CA and PBCA particles as a route to drug loading. Two procedures were followed with that aim. The first one involved a qualitative follow-up of the adsorption process, by means of electrophoretic mobility ($u_e$) determinations of the particles in dilute suspensions [0.1% (w/v)] with different drug concentrations, using a Malvern Zetasizer, 2000 (England) electrophoresis device, after 24 h equilibration. In this case, in order to evaluate the effect of ionic strength variations, we performed the experiments both with and without 1 mM $KNO_3$ in solution. The second procedure consisted of the determination of the optical absorbance of supernatants after contacting the synthesized

solids [0.3% (w/v)] with solutions of specific ftorafur concentrations, during 24 h, at 25.0 ± 0.5 °C and under mechanical stirring. Spectrophotometric determinations of the drug remaining in the supernatant solutions helped us, as above described, in the estimation of the adsorbed drug amount. The supernatants were obtained after 15 min centrifugation at 13,500 rpm. In both procedures, all solutions contained 0.1 mM HCl and 1% (w/v) dextran-70. All the experiments were carried out in triplicate.

#### 2.2.4. *In vitro ftorafur release*

The ftorafur release determinations were carried out using nanospheres with the drug loaded after an adsorption process (described above) with solutions of $10^{-2}$ or $10^{-3}$ M ftorafur concentrations; and also in nanoparticles obtained after a polymerization procedure (also described above) of its monomers in a polymerization medium containing $10^{-2}$ or $10^{-3}$ M ftorafur concentrations.

In both cases, the nanosphere suspensions were centrifuged at 13,500 rpm during 3 min in order to eliminate the non-loaded ftorafur. The particles (1.5 g) were then suspended in 10 mL of a NaOH–$KH_2PO_4$ buffer and stirred at 50 rpm. The temperature was maintained at 37.0 ± 0.5 °C during all the release experiments, which were performed in triplicate. 1.5 mL samples of the medium were withdrawn at specified times, and centrifuged at 13,500 rpm for 15 min, for determination of their optical absorbance at 271 nm. An equal volume of buffer, maintained at the same temperature, was added after sampling to ensure sink conditions. Any particles present in the samples withdrawn were returned to the medium after centrifugation and analysis of the drug concentration. The same measurement procedure used in the estimation of drug loading (Müller et al., 1991; Fawaz et al., 1997; Arias et al., 2005), was followed in the release studies.

## 3. Results and discussion

### 3.1. *Particle size and morphology*

Fig. 2 shows transmission electron microscopy (TEM) photographs of the poly(ethyl-2-cyanoacrylate) and poly(butylcyanoacrylate) particles; as observed, they are rather spherical and moderately monodisperse, forming a network of spherical particles upon drying. This polymeric network (absent in solution) was previously observed and is a consequence of the adhesive properties of the PACA family (McCarron et al., 2000; Arias et al., 2001, 2006). The sizes estimated from the photographs (average ± standard deviation; sample size: 150 particles) are 80 ± 30 nm (PE-2-CA, Fig. 2a) and 90 ± 15 nm (PBCA, Fig. 2b). The specific surface areas of the particles, obtained from nitrogen adsorption by the BET method (Quantasorb Jr., Quantachrome, USA), were very similar: 0.51 ± 0.23 and 0.48 ± 0.18 $m^2/g$ for PE-2-CA and PBCA, respectively.

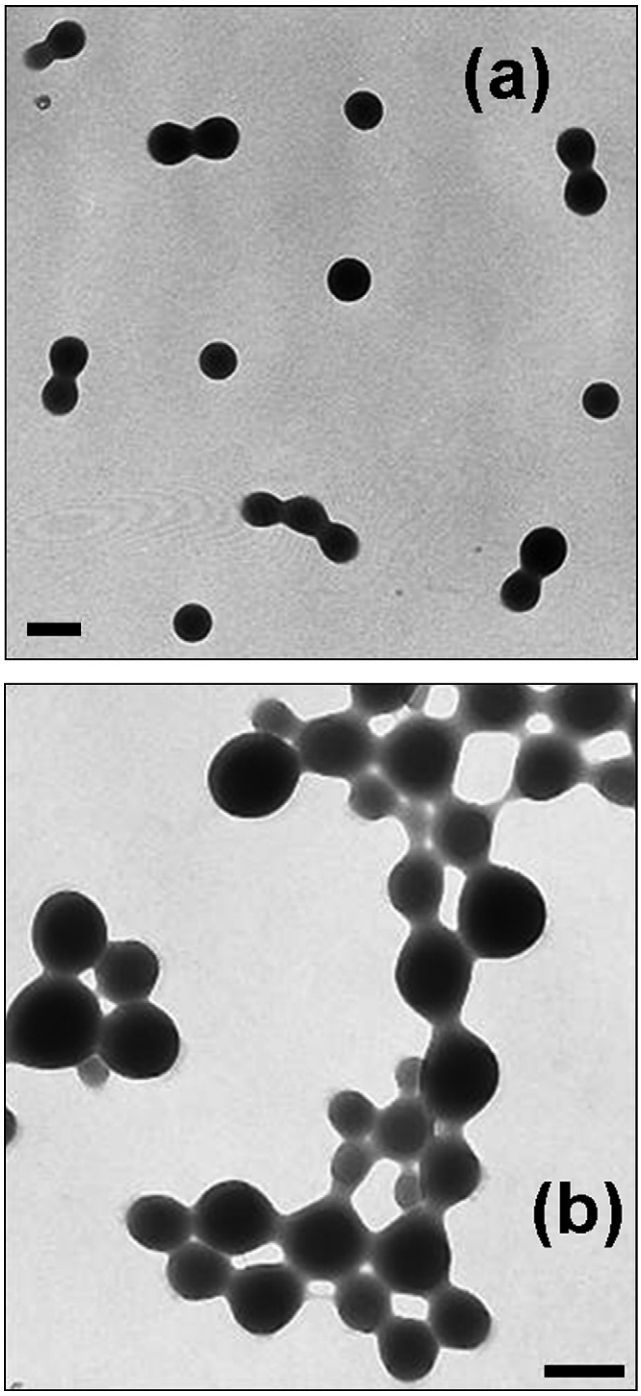


Fig. 2. Transmission electron microscopy pictures of poly(ethyl-2-cyanoacrylate) (a) and poly(butylcyanoacrylate) (b). Bar lengths: 100 nm.

### 3.2. *Optical absorbance of ftorafur solutions*

Fig. 3 shows the UV–vis absorbance spectra of ftorafur solutions, that display absorption bands only for wavelengths below ~320 nm. Although they show two maxima, only the one at 271 nm remains at a stable wavelength in a wide concentration range (up to ~$3 \times 10^{-4}$ M). Therefore, the peaks at smaller wavelength will not be used in this work; furthermore, a change in the shape (and not only in the height) is observed for the 271 nm band, that tends to disappear and merge with the low-wavelength band for concentrations over 0.3 mM (Fig. 3). We have no clear explanation for the observed deformations of the spectra: although molecular interactions typically lead to

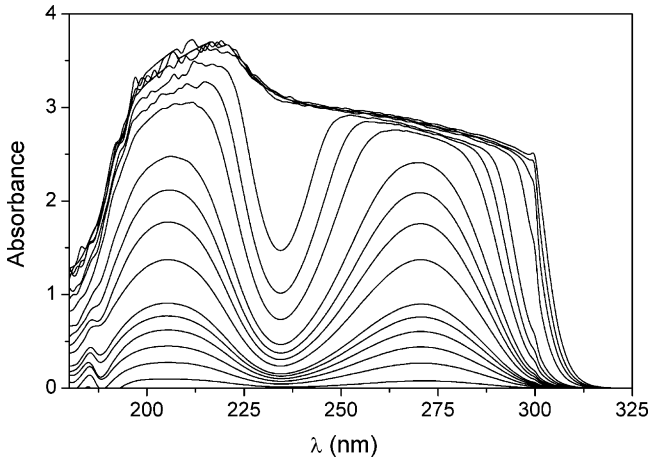


Fig. 3. UV–vis spectrum of ftorafur solutions at a pH = 4 ($10^{-4}$ M HCl). The molar concentrations in growing order of absorbance are: $10^{-5}$, $3 \times 10^{-5}$, $5 \times 10^{-5}$, $7 \times 10^{-5}$, $8.5 \times 10^{-5}$, $10^{-4}$, $1.5 \times 10^{-4}$, $2 \times 10^{-4}$, $2.5 \times 10^{-4}$, $3 \times 10^{-4}$, $5 \times 10^{-4}$, $7 \times 10^{-4}$, $10^{-3}$, $3 \times 10^{-3}$, $5 \times 10^{-3}$, $7 \times 10^{-3}$ and $10^{-2}$.

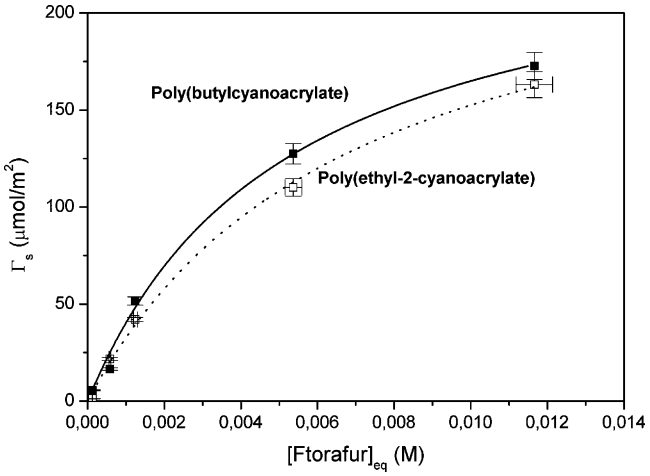


Fig. 4. Ftorafur adsorption density ($\Gamma$) to poly(ethyl-2-cyanoacrylate) (open symbols) and poly(butylcyanoacrylate) (full symbols), as a function of the equilibrium drug concentration.

departures from Beer's law for concentrations above $10^{-2}$ M, exceptions to this rule can also be found (Olsen, 1975; Arias et al., 2005). A good linearity exists between absorbance and ftorafur concentration up to $3 \times 10^{-4}$ M. From the least-squares fitting of the data, the molar absorbance coefficient was estimated to be $8070 \pm 140$ L mol$^{-1}$ cm$^{-1}$. Since we found that 0.1 M (20 mg/mL, i.e., above the drug solubility), solutions of ftorafur showed formation of crystals prior to the end of the 24 h observation period, we decided not to conduct any experiments with ftorafur solutions above 0.01 M (2 mg/mL) concentration.

The analysis of the spectra obtained at the acid pH range studied and at NaOH–$KH_2PO_4$ buffer (pH = 7.4), demonstrated that the ftorafur solutions were unaltered by pH, for all the pH values investigated, and that the molar absorption coefficient was also independent of pH.

Evaluation of the linearity, precision and accuracy parameters was carried out in order to validate the spectrophotometric method proposed to quantify ftorafur. These parameters were determined in standard solutions in six replicates. Similar results were obtained with the six standard solutions of NaOH–$KH_2PO_4$ buffer (pH = 7.4) and in six calibration standards containing known ftorafur concentrations added to supernatants of the syntheses performed without drug.

### 3.3. Surface adsorption of ftorafur

The results obtained in the spectrophotometric determination of the surface incorporation of the anticancer drug show an increase in the amount adsorbed when the drug concentration in the medium is increased. Fig. 4 displays the amount of ftorafur adsorbed by PE-2-CA and PBCA nanoparticles, as a function of the equilibrium drug concentration. As observed, the loading of ftorafur increases with the amount of drug in solution. In fact, the data seem to be well described by a Langmuir adsorption isotherm,

$$\Gamma_s = \frac{\Gamma_{max} k C}{1 + kC} \quad (1)$$

where $\Gamma_s$ is the amount adsorbed per unit area, $C$ the equilibrium concentration, $\Gamma_{max}$ the maximum drug adsorbed (equivalent to a monolayer coverage), and $k$ is the dissociation constant of the adsorption sites. The fitting parameters (±95% confidence intervals) were:

- PE-2-CA: $\Gamma_{max} = 257 \pm 15$ μmol/m$^2$; $k = 145 \pm 18$ L/mol
- PBCA: $\Gamma_{max} = 251 \pm 19$ μmol/m$^2$; $k = 190 \pm 30$ L/mol

These data indicate that there is no statistically significant effect of the type of polymer used for ftorafur adsorption. However, this water-soluble anticancer drug is known to be highly lipophilic (Calvo et al., 2001) and its adsorption on hydrophobic surfaces should be slightly favored. Hence, such adsorption might be increased onto PBCA because of its higher hydrophobicity, demonstrated in previous studies (Müller et al., 1992; Arias et al., 2001, 2006), and in fact a consequence of the raise in hydrophobicity of PACA's with the alkyl chain length (Kreuter, 1983; Gibaud et al., 1998; McCarron et al., 2000).

Given that electrophoresis is most sensitive to minute changes in the surface by adsorption of, mainly, charged entities, even at rather small amounts, we carried out determinations of the electrophoretic mobility ($u_e$) of the polymer particles in solutions of the drug. The data are shown in Fig. 5: it can be seen that $u_e$ displays a general trend to raise (towards progressively less negative values) as the concentration of ftorafur is increased. There are, however, small differences between the two types of particles, as well as a clear effect of the addition of $KNO_3$. The originally negative charge is reduced by the electrostatically favored adsorption of ftorafur (positively charged species, presumably coming from the protonation of the –NH group of the drug molecule). The presence of $KNO_3$ yields a mobility reduction because of double layer compression. In addition, $KNO_3$ screens the attraction between drug molecules and particles thus leading to the fact that the reduction of $u_e$ when ftorafur concentration increases, is more significant when there is no $KNO_3$ in the medium.

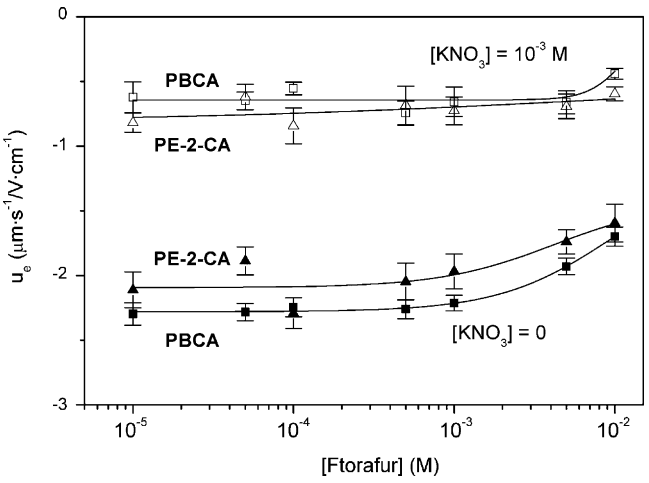


Fig. 5. Electrophoretic mobility of poly(ethyl-2-cyanoacrylate) (▲, △) and poly(butylcyanoacrylate) (■, □) particles as a function of drug concentration, in the presence (open symbols) and absence (full symbols) of $10^{-3}$ M $KNO_3$.

### 3.4. Effect of the polymerization conditions on ftorafur loading

#### 3.4.1. Kinetic aspects

The effects of both the time of drug addition and the duration of the polymerization on the ftorafur loading to PE-2-CA and PBCA nanospheres, were studied while keeping all other parameters of the synthesis constant [1% (w/v) monomer concentration, 1% (w/v) dextran-70 concentration, $10^{-4}$ M ftorafur, 0.1 mM HCl]. It was found that for both types of nanospheres the drug loading was absolutely independent of the polymerization duration, as long as it is allowed to proceed between 1 and 3 h.

Concerning the time of drug addition, as previously observed (Soma et al., 2000), it does influence the drug loading: because of the rapid polymerization kinetics of the monomers, the drug must be added to the polymerization medium as soon as possible, and preferably before the addition of the monomer, in order to obtain a maximum drug incorporation. In fact, the drug loading falls to almost zero, if it is added at any time after the first 20 min of polymerization (average time needed to observe the formation of the whitish suspension under all the synthesis conditions studied).

#### 3.4.2. Effect of monomer concentration

In the investigation of the effect of monomer concentration on the loading of ftorafur to PE-2-CA and PBCA nanospheres, all other parameters of the synthesis were kept constant [1% (w/v) dextran-70 concentration, $10^{-4}$ M ftorafur, 0.1 mM HCl, drug addition before the incorporation of the monomer and 2 h of polymerization]. The results obtained showed that the absolute amount of drug incorporated increases from ∼0.45 to ∼1.25 μmol when ethyl-2-cyanoacrylate concentration rises between 1% and 4% (w/v). Similar results were obtained in the case of butylcyanoacrylate under the same conditions (∼0.3 to ∼1.05 μmol). The relative, or specific, loading (amount absorbed by unit weight of particles), on the contrary, is essentially independent of the monomer added, staying constant at ∼0.9 and ∼0.6 μmol/g in the case of PE-2-CA and PBCA, respectively. Therefore, the only effect that the increase in added monomer concentration induces is a growth in the amount of nanospheres obtained, which is responsible for the higher absolute loading, but it will not induce a larger drug loading by the nanospheres. It can be concluded that one does not need to increase the monomer concentration to attain a better loading, as previously found with the related chemotherapeutic drug 5-fluorouracil (Arias et al., 2005). The fact that the amounts of drug loaded are higher for PE-2-CA than for PBCA, whatever the monomer concentration used, can be explained if we take into account the faster polymerization kinetics of the ethyl-2-cyanoacrylate monomer, that induces a larger mechanical entrapping of the drug (de Verdière et al., 1997; Gibaud et al., 1998).

#### 3.4.3. Effect of surfactant concentration

In this paragraph we describe our data on the role of changes in dextran-70 concentration [from 0% to 2% (w/v)] on drug loading, keeping all other parameters of the synthesis constant [1% (w/v) monomer concentration, $10^{-4}$ M ftorafur, 0.1 mM HCl, drug added before the monomer incorporation and 2 h of polymerization]. No significant influence on drug loading was found, as the surfactant concentration rises; however, its role in the generation of polymeric nanospheres is crucial, due to the combination of its initiating and stabilizing actions, similar to other frequently used agents, such as polyethylenglycols. If dextran-70 concentrations below 1% (w/v) are used, a fast coagulation of the suspensions occurs, and the formation of macroaggregates and large solid particles is observed. However, dextran-70 concentrations equal to or higher than 1% (w/v) yield very stable whitish dispersions, where sedimented macroaggregates are not observable. The addition of this stabilizing agent generates homogeneous distributions of nanospheres with reduced size and great uniformity, without negatively affecting the drug loading efficiency (Llovet et al., 1995; Stolnik et al., 1995; Arias et al., 2005).

#### 3.4.4. Effect of HCl concentration

The polymerization of an alkylcyanoacrylate monomer is an anionic process initiated by covalent bases, and hence the kinetics is governed by the relative amounts of the alcoholic –OH groups of the surfactant, and $OH^-$ ions from water dissociation. As the acidity of the medium increases, the polymerization rate decreases; as a result of this, it can be concluded that the $H^+$ concentration determines both the polymerization rate and the drug absorption (Fattal et al., 1997; Fawaz et al., 1997; Peracchia et al., 1997). Since ftorafur is essentially non-ionic, a fast polymerization mechanically entraps as much antineoplasic drug as possible; however, a very fast polymerization generates a large proportion of bulk polymer in the form of solids or macroaggregates (Llovet et al., 1995; Arias et al., 2005). The experiments were carried out for HCl concentrations between $10^{-5}$ and $10^{-2}$N, with 1% (w/v) monomer, 1% (w/v) dextran-70, ftorafur concentrations ranging from $10^{-4}$ to $10^{-2}$ M, drug incorporation to the polymerization medium before the monomer addition, and 2 h polymerization.

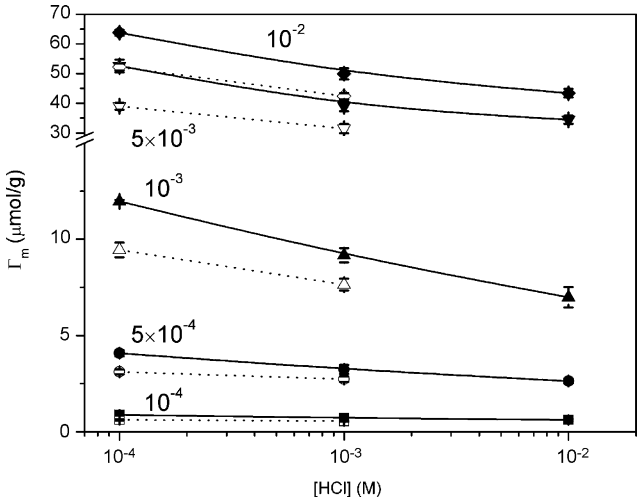


Fig. 6. Ftorafur absorption density ($\Gamma$) as a function of HCl concentration. The ftorafur molar concentrations are indicated. Poly(ethyl-2-cyanoacrylate): full symbols; poly(butylcyanoacrylate): open symbols. The lines are guides to the eye.

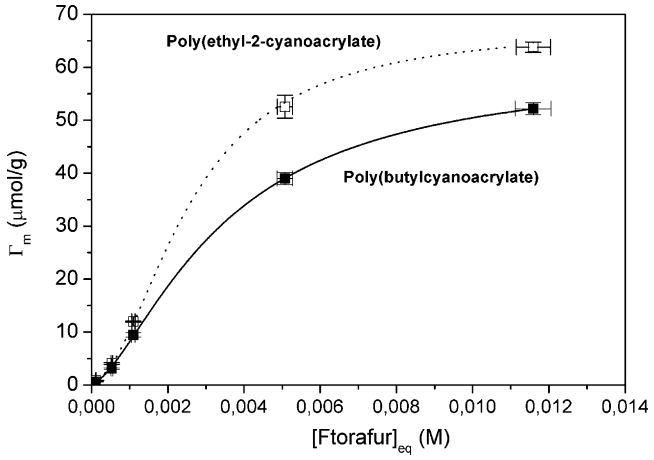


Fig. 7. Ftorafur absorption density ($\Gamma$) to poly(ethyl-2-cyanoacrylate) (open symbols) and poly(butylcyanoacrylate) (full symbols), as a function of the equilibrium drug concentration.

Fig. 6 shows that ftorafur loading to PE-2-CA and PBCA nanospheres is clearly affected by the $H^+$ concentration of the polymerization medium, whatever the drug concentration used: as $OH^-$ concentration decreases, the absorption falls. This is explained on the basis of the polymerization mechanism previously described: a fast polymerization (high $H^+$ concentration) allows an easier mechanical trapping of ftorafur. Our data indicate that absorption is largest at $[H^+] \sim 10^{-4}$ M; and, although a higher absorption is expected in more basic solutions, syntheses carried out at [HCl] < $10^{-4}$N yielded nanospheres unsuitable for parenteral administration. This is concluded from simple visual inspection of the synthesis done at $[H^+] = 10^{-5}$ M: a slightly whitish suspension is obtained, with many macroaggregates and a comparatively large amount of solid polymer precipitates. On the other hand, no formation of PBCA nanospheres is observed at $[H^+] = 10^{-2}$ M, as an almost transparent suspension is obtained after 2 h polymerizaton. This can be attributted to a very slow polymerization rate, that is probably due to the very low concentration of covalent bases at this $H^+$ concentration and to the slower polymerization kinetics of the butylcyanoacrylate monomer, in comparison to ethyl-2-cyanoacrylate, because of its longer alkyl chain length (de Verdière et al., 1997; Gibaud et al., 1998). Finally, whatever the pH and drug concentration used, the absorption density is higher in PE-2-CA than in PBCA, because of the faster polymerization kinetics of ethyl-2-cyanoacrylate monomer, as pointed above. Similarly, higher absorption values in more basic media were also obtained with other drugs, such as ciprofloxacin and 5-fluorouracil (Fawaz et al., 1997; Arias et al., 2005).

### 3.4.5. *Effect of ftorafur concentration*

Drug loading to poly(alkylcyanoacrylate) nanoparticles is an extensively studied phenomenon for a wide group of active drugs. In general, a positive effect of the increment in drug concentration is observed on the loading efficiency (Fawaz et al., 1997; Fontana et al., 1998; Arias et al., 2005).

Fig. 7 shows the amount of ftorafur absorbed by PE-2-CA and PBCA nanoparticles as a function of the equilibrium drug concentration. All the data in this Figure were obtained by varying the amount of drug added before the monomer incorporation to the polymerization medium, in the following conditions: 0.1 mM HCl, 1% (w/v) dextran-70, 1% (w/v) monomer and 2 h of polymerization. As observed, ftorafur loading increases with the drug concentration in solution, suggesting a trend toward saturation for the maximum concentrations investigated. As explained above, the amount of drug loaded to PE-2-CA is higher than to PBCA nanospheres. This conclusion can be quantitatively demonstrated by fitting the loading isotherms to sigmoidal dependences of the type:

$$\Gamma_m = \Gamma_{max} - \frac{\Gamma_{max}}{1 + (C/C_{1/2})^p} \quad (2)$$

where $\Gamma_m$ is the absorbed amount per unit mass of polymer, $\Gamma_{max}$ is its saturation value, and $C_{1/2}$ is the drug concentration at half the maximum absorption. The fitting parameters were:

- PE-2-CA: $\Gamma_{max} = 67.7 \pm 0.4$ μmol/g; $C_{1/2} = 2.57 \pm 0.03$ mM; $p = 1.92 \pm 0.03$
- PBCA: $\Gamma_{max} = 60.2 \pm 1.5$ μmol/g; $C_{1/2} = 3.40 \pm 0.16$ mM; $p = 1.52 \pm 0.07$

### 3.5. *Drug release from poly(ethyl-2-cyanoacrylate) and poly(butylcyanoacrylate) nanospheres loaded with ftorafur under optimal conditions*

The study of the effect of the polymerization conditions on ftorafur loading to PE-2-CA and PBCA nanospheres, allowed us to determine the ideal conditions to obtain the best drug loading: (i) drug incorporation to the polymerization medium before the addition of the monomer, to account for its fast polymerization kinetics; (ii) extension of the polymerization reaction during 2 h to ensure the adequate formation of the drug-loaded polymers; (iii) a monomer concentration of 1% (w/v) (larger concentrations only induce an increase in the amount of nanoparticles obtained);

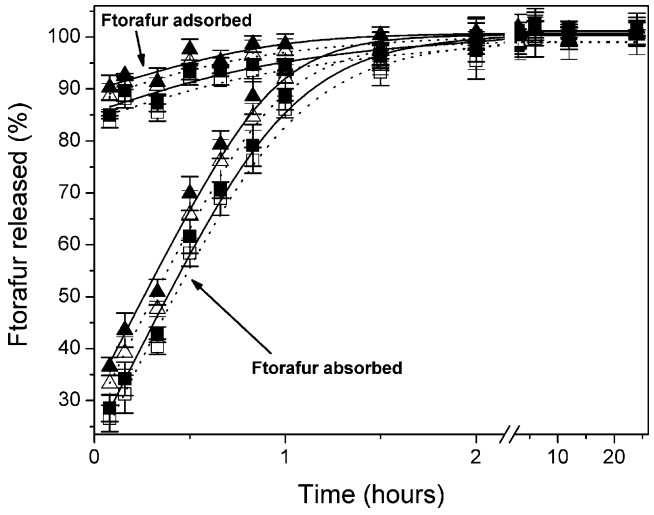


Fig. 8. Release of ftorafur, adsorbed or absorbed, from poly(ethyl-2-cyanoacrylate) (▲, △) and poly(butylcyanoacrylate) (■, □) as a function of the incubation time in NaOH–$KH_2PO_4$ buffer (pH = 7.4) at 37.0 ± 0.5 °C. The contact medium of adsorption, or the polymerization medium in which the syntheses were carried out, was $10^{-4}$ M [open symbols and dotted lines] or $10^{-2}$ M [full symbols and solid lines] in drug concentration.

(iv) a dextran-70 concentration of 1% (w/v) (its presence plays a decisive role in the generation of well-stabilized nanospheres with spherical shape and narrow size distribution); (v) $10^{-4}$N HCl concentration (lower concentrations induce the formation of nanoparticles unsuitable for parenteral administration, and an excessively acid pH lowers the drug loading); (vi) a ftorafur concentration of $10^{-2}$ M. Under these conditions, the amount of ftorafur absorbed to PE-2-CA was 64 ± 1 and 52 ± 1 μmol/g to PBCA. However, in order to study the influence of the amount of drug loaded in the release kinetics, nanospheres obtained after polymerization in a $10^{-3}$ M ftorafur medium were also investigated; the amount of ftorafur absorbed to PE-2-CA and PBCA nanospheres, under these conditions, was 12.2 ± 0.1 and 9.3 ± 0.4 μmol/g, respectively.

Fig. 8 shows the ftorafur release from PE-2-CA and PBCA nanospheres as a function of the incubation time. In the case of the polymeric nanospheres obtained after monomer polymerization in a medium with either $10^{-3}$ or $10^{-2}$ M drug concentration, the release of ftorafur from nanoparticles was observed to occur as a biphasic process: first, an early rapid release of around 70% took place within 40 min, while the remaining 30% is slowly liberated during the next 80 min. The rapid release probably represents the loss of surface-associated and poorly entrapped (adsorbed more deeply into the surface pores) ftorafur (Brasseur et al., 1991). The drug release during the slower release phase may result from particle disintegration, from drug diffusion through the polymeric matrix, or both. This biphasic profile, typical of this polymer family, suggests that the major fraction of ftorafur was adsorbed onto the surface of the nanoparticles rather than entrapped into the core of the polymeric network, as previously observed in drugs of different nature (Radwan, 1995; Némati et al., 1996; González-Martin et al., 1998; Soma et al., 2000). The release process should be a direct consequence of nanosphere disintegration by surface erosion, started in turn by hydrolysis of the polymer chains with subsequent formaldehyde formation (Lenaerts et al., 1984; Müller et al., 1992; Page et al., 1996).

With respect to the influence of the type of polymer on the release rate, as can be seen in Fig. 8, ftorafur was released slightly faster in the case of PE-2-CA than of PBCA nanospheres, probably due to the higher degradation rate associated to its shorter alkyl chain length (Müller et al., 1992; Némati et al., 1996; de Verdière et al., 1997). The results also show that the cumulative release is slightly enhanced by an increase in the drug loaded (Brasseur et al., 1991).

In the case of release of adsorbed drug, the loading of the particles was 81 ± 3 μmol/g for both polymers if the adsorption medium had a ftorafur concentration of $10^{-2}$ mol/L, and 7.7 ± 0.3 (10.8 ± 0.4) μmol/g for PBCA (PE-2-CA) in $10^{-3}$ mol/L drug solutions. Fig. 8 shows that the release of adsorbed ftorafur was almost finished after 50 min. The complete and rapid drug release suggests that ftorafur is likely adsorbed only on the external surface of nanoparticles. Furthermore, an increase in the drug loaded also enhanced the cumulative ftorafur release (Brasseur et al., 1991). The comparison between the two polymers shows that Ftorafur release is slightly faster from PE-2-CA than PBCA nanospheres, most likely due to the above-mentioned slightly stronger interaction of this lipophilic drug with the more hydrophobic surface of PBCA (Kreuter, 1983; Gibaud et al., 1998; McCarron et al., 2000).

## 4. Conclusions

In this work, attention has been paid to reproducible procedures that appear optimal to enhance the ftorafur loading by PE-2-CA and PBCA particles. The influence of the polymerization conditions and the contributions of both the surface and the polymer network to the overall drug loading were investigated by means of electrophoretic mobility and optical absorbance determinations. Among the factors affecting the ftorafur incorporation, we have found that the type of polymer, the pH and the drug concentration are the main determining ones. With respect to the release profiles, ftorafur surface adsorption onto nanospheres led to a very rapid drug release in sink conditions. However, the drug incorporation into the nanoparticles permitted a larger loading and a slower ftorafur release.

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