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Research paper

Magnetite/poly(alkylcyanoacrylate) (core/shell) nanoparticles as 5-Fluorouracil delivery systems for active targeting

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Abstract

In this article, a reproducible emulsion polymerization process is described to prepare core/shell colloidal nanospheres, loaded with 5-Fluorouracil, and consisting of a magnetic core (magnetite) and a biodegradable polymeric shell [poly(ethyl-2-cyanoacrylate), poly(butylcyanoacrylate), poly(hexylcyanoacrylate), or poly(octylcyanoacrylate)]. The heterogeneous structure of these carriers can confer them both the possibility of being used as drug delivery systems and the responsiveness to external magnetic fields, allowing an active drug targeting without a concurrent systemic distribution. Zeta potential determinations as a function of ionic strength showed that the surface behaviour of the core/shell particles is similar to that of pure cyanoacrylate particles. The first magnetization curve of both magnetite and magnetite/polymer particles demonstrated that the polymer shell reduces the magnetic responsiveness of the particles, but keeps unchanged their ferrimagnetic character. Two drug loading mechanisms were studied: absorption or entrapment in the polymeric network, and surface adsorption. We found that the acidity of the medium had significant effects on the drug absorption per unit mass of polymer, and needs to be controlled to avoid formation of macroaggregates and to reach significant 5-Fluorouracil absorption. The type of polymer and the drug concentration are also main factors determining the drug incorporation to the core/shell particles. 5-Fluorouracil release evaluations showed a biphasic profile affected by the type of polymeric shell, the type of drug incorporation and the amount of drug loaded.

Keywords: Active targeting; Controlled release; Drug delivery; 5-Fluorouracil; Magnetic carrier technology; Poly(alkylcyanoacrylates)

1. Introduction

Conventional cancer treatments, including surgery, radiation, chemotherapy, and biologic therapies (immunotherapy), are limited by the accessibility to the tumor, the risk of operating on a vital organ, the spread of cancer cells throughout the body, and the lack of selectivity towards tumor cells. Nowadays, multimodal therapy that uses radiotherapy, chemotherapy, immunotherapy, and other forms of treatment in combination with surgery provides a better chance of survival that, however, for lots of patients is insufficient.

One of the problems associated with chemotherapy is the inability to target a specific area of the body. To reach an acceptable therapeutic level at the desired site, high dosages of the drug must be administered even though only a fraction of the dose will actually reach the intended disease site, and toxic side effects will be caused at the non-target organs. One solution to this problem is to develop colloidal drug delivery systems that can physically direct the drug to the desired site. Targeting specific sites in the body simplifies drug administration procedures, reduces the quantity of drug required to reach therapeutic levels, decreases the drug concentration at non-target sites (possibly reducing side effects) and, essentially, increases the concentration of

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the drug at target sites. In addition, the possibility of reversion of the multidrug resistance might be achieved by using cytostatic-loaded delivery systems [1,2].

Because of their peculiar properties (controllable shape and sizes, responsiveness to magnetic fields, biocompatibility, non-immunogenicity, non-toxicity, etc.), an increasing number of formulations based on magnetic composite particles have been developed for biomedical applications, particularly, drug delivery [3,4]. These are systems consisting of either a magnetic core coated with a biocompatible polymer, or magnetic nuclei deposited on the polymeric surface or precipitated inside the polymer matrix [5,6]. Among the large variety of magnetic materials that may be used in these formulations [7], magnetite is a frequent choice because of its low toxicity [8,9]. In addition, polymer matrices of diverse nature have been used, both biodegradable and non-biodegradable [3,5,10–14]. Finally, clinical phase I trials have clearly shown the low toxicity of these selective drug delivery systems, as the inclusion of magnetic particles in polymeric substrates does not affect the toxicity of the latter [15–17].

For drug delivery applications, one of the most important features in the design of core/shell carriers is their final size. This property not only affects their magnetic moment (and, hence, their response to applied magnetic fields), but also their biological fate once they are administered to the patient. Nevertheless, if the particles are retained in the target tissue capillaries by the magnetic field, drug diffusion through the capillary wall (step limited by the molecular weight of the drug) will induce the therapeutic action [18]. Moreover, if this action requires extravasation, sizes even between 0.5 and 5 μm may also be suitable for that process, despite the magnetic field removal, as the permeability of these vessels is increased in the presence of solid tumors [19–22].

The main limitation of magnetic drug delivery relates to the strength of the external magnetic field that can be applied to obtain the necessary magnetic gradient to control the residence time of nanoparticles (NPs) in the desired area or which triggers the drug desorption. As a means to elude the limitations of using external magnetic fields, internal magnets can be located in the vicinity of the target by using minimally invasive surgery. Moreover, the simultaneous use of an external magnetic field and a magnetic implant to direct the magnetic NPs is also a promising strategy [5,23].

In this paper, we investigate the capabilities of poly(ethyl-2-cyanoacrylate) (PE-2-CA), poly(butylcyanoacrylate) (PBCA), poly(hexylcyanoacrylate) (PHCA) and poly(octylcyanoacrylate) (POCA) nanospheres with a magnetic core (magnetite) as delivery systems for the antitumor drug 5-Fluorouracil [5-FU, 5-fluoro-2,4-pyrimidinedione]. This active agent is extensively used in cancer treatment due to its broad spectrum of activity against solid tumors, although its therapeutic efficacy can be improved, while its undesired toxic effects are reduced [24,25] by loading this hydrophilic drug to a carrier system.

The choice of the biodegradable polymeric shell, namely poly(alkylcyanoacrylates) or PACAs, was based on their well-demonstrated therapeutic results in the treatment of both non-resistant and resistant cancers of a wide range of cell lines [21,26,27], and their low toxicity levels, as results from phase I and II clinical trials have revealed the good tolerance of these drug carriers [26,28].

We investigate the amount of 5-FU that the composite particles are capable to load, both on the surface and in the polymeric matrix, and the factors that influence the loading efficiency; in particular, the type of polymeric shell, the drug concentration and the pH. The in vitro release profiles and the factors influencing this process (type of polymer shell, type of drug incorporation and amount of drug loaded) were also evaluated. The analytical technique used, spectrophotometry, was validated and used successfully to determine both drug loading and release.

2. Materials and methods

2.1. Materials

Water used in the experiments was deionized and filtered (Milli-Q Academic, Millipore, France). All chemicals were of analytical quality from Panreac, Spain, except for ethyl-2-cyanoacrylate, butylcyanoacrylate, hexylcyanoacrylate and octylcyanoacrylate (gifts from Henkel Loctite, Ireland), and 5-Fluorouracil (purchased from Sigma–Aldrich, Germany).

According to a method originally proposed by Sugimoto and Matijević [29], based on the crystallization of a gel of amorphous ferrous hydroxide, we prepared magnetite (Fe_3O_4) particles considerably monodisperse and in the colloidal size range (average diameter ± standard deviation: 80 ± 15 nm). PACA NPs (average diameter ± standard deviation: 350 ± 100 nm) were prepared by following an emulsion/polymerization method, in which the mechanism of polymerization is an anionic process initiated by covalent bases present in the medium (e.g., OH^- ions deriving from water dissociation) [21]. In previous works [30–33], we have also described procedures to obtain and characterize such kind of particles.

2.2. Methods

2.2.1. Preparation of magnetite/PACA composite particles

The procedure followed to obtain the core/shell particles was described before and is based on the emulsion/polymerization method used in the synthesis of the pure PACA polymer, except that the polymerization medium was a magnetite suspension [30,32].

In brief, under mechanical stirring (1200 rpm), the alkylcyanoacrylate monomer (0.5 mL) was added dropwise to 50 mL of an aqueous magnetite suspension [0.75% (w/v)], containing 10^{-4} N HCl and the stabilizing agent dextran-70 [1% (w/v)]. Mechanical stirring was maintained during 6 h and the polymerization reaction was terminated

by adding 1 mL of a 0.1 N KOH solution to ensure the end of the process. In order to clean the suspensions from unreacted chemicals and non-magnetic solids, they were magnetically decanted, and the supernatant liquid substituted by water. The process was repeated until the supernatant was transparent and its conductivity (less than 1 $\mu S\ cm^{-1}$) indicated that the suspensions were clean. It took typically five cycles to reach this stage.

2.2.2. Characterization methods

2.2.2.1. Particle size and shape. Size and shape of the synthesized particles were deduced from TEM pictures obtained using a Zeiss EM 902 (Germany) transmission electron microscope set at 80 kV accelerating voltage. Prior to observation, a dilute (approx. 0.1% w/v) suspension of the particles was sonicated for 5 min, and drops of the suspension were placed on copper grids with formvar film. The grids were then dried at 40 °C in a convection oven. In order to confirm these results, the mean particle diameters were determined at 25.0 ± 0.5 °C by Quasi-Electric Light Scattering (QELS) using a Nanosizer (Coulter® N4MD, Coulter Electronics, Inc., Hialeah, FL, USA). The selected angle was 90° and the measurement was made after dilution of the aqueous suspensions.

2.2.2.2. Specific surface area. Specific surface areas of the solids were obtained by multipoint B.E.T. nitrogen adsorption in a Quantasorb Jr. of Quantachrome (USA). The carrier gas in this device was helium, and adsorption experiments were performed with 10%, 20% and 30% nitrogen/helium mixtures. The sample mass used was 0.6 g and the experiments were repeated at least three times on independent samples in all cases.

2.2.2.3. Zeta potential determination. In order to ascertain the efficiency of the coating, the surface electrical properties of the different particles were analyzed, by electrophoresis measurements, as a function of KNO_3 concentration using a Malvern Zetasizer 2000 (England) electrophoresis device. Measurements were performed at 25.0 ± 0.5 °C. The theory of O'Brien and White [34] was used to convert the electrophoretic mobility (u_e) into zeta potential (ζ) values.

2.2.2.4. Magnetic properties. The magnetic properties of the magnetite and the composite particles (first magnetization curve) were determined by means of a Manics DSM-8 vibrating magnetometer, at room temperature.

2.2.2.5. Drug concentration. In order to assess the drug concentration in all systems investigated, UV–Vis absorption measurements were performed in an 8500 UV–Vis Dinko spectrophotometer (Dinko, Spain), using quartz cells of 1 cm path length, at a wavelength of 266 nm. Since we previously found that 0.1 M 5-FU solutions showed formation of crystals prior to the end of the 24 h observation period [32], we decided not to conduct any experiments with drug solutions above 0.01 M concentration. The antitumor drug solutions were stable for all the pH values investigated (pH ranging from 1 to 4, fixed with HCl, and pH 7.4, fixed with $NaOH–KH_2PO_4$ buffer), and their molar absorption coefficients were also independent of pH [32]. The spectrophotometric method of analysis of the amount of drug loaded or released was validated and verified for accuracy, precision and linearity [32,33].

2.2.3. Determination of the 5-Fluorouracil loading

Two methods were followed in order to achieve the loading of the composite NPs: surface adsorption on already formed particles after incubation in the drug solution, or trapping of the drug in the polymeric shell upon drug addition during the generation of the core/shell particles. The factors that influence the loading efficiency were investigated; in particular, the type of polymeric matrix, the drug concentration and the pH. The effect of the modification of the monomer or surfactant concentration on drug loading was not studied, as no significant influence was previously found of either of these parameters on the loading [32,33]. However, a 1% (w/v) dextran-70 concentration ensures very stable whitish dispersions of nanospheres with reduced size and great uniformity, where sedimented macroaggregates are not observable [32,33,35].

2.2.3.1. 5-FU loading. 5-FU loading was investigated 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 carriers in the drug solution [32,33,36–38]. The procedure includes: (i) preparation of a 0.75% (w/v) magnetite aqueous polymerization medium, containing a 1% (w/v) of the stabilizing agent dextran-70, with appropriate amounts of 5-FU and HCl; (ii) dropwise addition of the alkylcyanoacrylate monomer (1% w/v); (iii) mechanical stirring of the medium (1200 rpm) for 6 h; (iv) stopping the reaction by addition of proper amounts of a 0.1 N KOH solution; (v) centrifugation of the solids (13,500 rpm for 15 min); and (vi) determination of the drug loaded by measuring its 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 5-FU solution. For the method to be accurate, sources of absorbance changes other than variations in drug concentration were previously identified [32,33]. One possible source is that drug loading influences the particle formation by changing, for instance, the size or composition. We can neglect this possibility, as electron microscope observations demonstrate that the particle shape and size are indistinguishable from those found in the absence of 5-FU.

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 5-FU concentration in solution. The most important reason for absorbance changes is the pres-

ence of unreacted monomer and byproducts of the polymer degradation in the medium. This justifies the procedure used to estimate the drug loading [32,33,36–38]: the amount of 5-FU present in solution after particle synthesis was obtained from the absorbance of the solution at 266 nm, after subtracting the absorbance of the supernatant produced in the same conditions, but without drug in solution.

2.2.3.2. Validation. We validated the method [32] by comparing the evaluation of drug concentration in two instances: a certain amount of drug was dissolved in supernatants of composite 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. However, since part of the anticancer drug could be loaded by PACA NPs not including a magnetic nucleus, we first evaluated gravimetrically the amount of polymer actually bounded to the core/shell particles: this was the difference between the initial mass of magnetic particles and that of the composite ones. Then, we estimated how much of the drug would have been trapped by the non-magnetic colloids, using our data on pure polymer particles [32]; the remainder of the drug (as compared to the initial amount) was actually ascribed to the absorption by the composite particles.

2.2.3.3. Surface adsorption. Drug surface adsorption on the core/shell particles was also investigated as a route to drug loading. Two procedures were followed with that aim. The first one involved the determination of the optical absorbance of supernatants after contacting the synthesized solids [0.3% (w/v)] with solutions of specific 5-FU concentrations, during 24 h, at 25.0 ± 0.5 °C and under mechanical stirring (50 rpm). Spectrophotometric determinations of the drug remaining in the supernatant solutions helped us, as above described, in the estimation of the adsorption. 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.

The second procedure consisted of 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. Measurements were performed at 25.0 ± 0.5 °C, after 24 h of contact at this temperature. The experimental uncertainty of the measurements was below 5%. 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.

2.2.4. In vitro 5-Fluorouracil release

The optimum loading conditions were used to perform drug release evaluations from core/shell particles. 5-FU release determinations were carried out using drug-loaded composite NPs obtained after an adsorption process with solutions of 10^{-2} or 10^{-3} M drug concentrations and, after a synthesis procedure in a polymerization medium containing 10^{-2} or 10^{-3} M drug concentrations. In both cases, the suspensions were magnetically separated in order to eliminate the non-loaded 5-FU and the non-magnetic solids. 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. Samples (1.5 mL) of the medium were withdrawn at specified times, and centrifuged at 13,500 rpm for 15 min, for determination of their optical absorbance at 266 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 [32,33,36–38] was followed in the release studies.

3. Results and discussion

3.1. Particle size and morphology

Magnetite/PACA NPs, in agreement with previous studies [30,32], were rather spherical, in the colloidal size range, and moderately monodisperse (average diameter ± standard deviation): Fe_3O_4/PE-2-CA (160 ± 15 nm), Fe_3O_4/PBCA (140 ± 20 nm), Fe_3O_4/PHCA (150 ± 30 nm) and Fe_3O_4/POCA (155 ± 20 nm). The magnetite particles are covered by a polymer shell ≈40 nm thick, in all cases.

The specific surface areas of the particles were very similar: 0.47 ± 0.11, 0.54 ± 0.17, 0.51 ± 0.15 and 0.46 ± 0.17 m^2/g for Fe_3O_4/PE-2-CA, Fe_3O_4/PBCA, Fe_3O_4/PHCA and Fe_3O_4/POCA, respectively.

3.2. Electrokinetic characterization

Fig. 1 shows the zeta potential (ζ) values of magnetite, poly(alkylcyanoacrylates) and composite particles as a function of KNO_3 concentration at a constant pH 5 (in this and the following figures, the error bars correspond to the standard deviations of replicated experiments). This figure clearly shows the similarities between the electrokinetics of polymer and core/shell particles, and their differences with magnetite are clearly observed. Therefore, the polymer shell very efficiently hides magnetite, rendering the surface of each kind of composite particle indistinguishable from that of the corresponding PACA particles. Because of such large differences between the electrophoresis of nuclei and polymer, this electrokinetic technique is a very useful tool for qualitatively checking the efficiency of the coating.

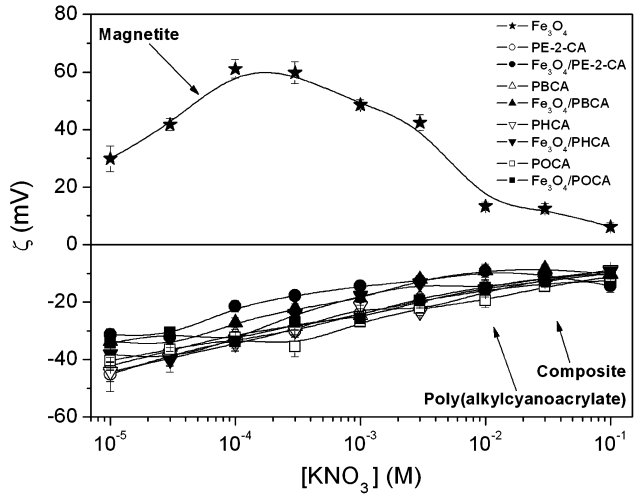

Fig. 1. Zeta potential of Fe_3O_4 (★), PE-2-CA (○), Fe_3O_4/PE-2-CA (●), PBCA (△), Fe_3O_4/PBCA (▲), PHCA (▽), Fe_3O_4/PHCA (▼), POCA (□) and Fe_3O_4/POCA (■), as a function of the concentration of KNO_3 at pH 5. The lines are guides to the eye.

These results are in agreement with our previous studies [30–32].

3.3. Magnetic properties

The magnetic responsiveness was determined by the characteristics of the first magnetization curve, shown in Fig. 2 for both the magnetite nuclei and the composite particles. As previously observed [11,31,32], the magnetic behaviour of composite NPs is similar to that of the nuclei, except that the polymeric matrix reduces the magnetization of the sample. From the linear portions (low field) of the curves in Fig. 2 we could estimate the initial susceptibility, $\chi_i = 5.24 \pm 0.01$ for magnetite and, 0.58 ± 0.04, 0.51 ± 0.03, 0.53 ± 0.01 and 0.66 ± 0.02, for the Fe_3O_4/PE-2-CA, Fe_3O_4/PBCA, Fe_3O_4/PHCA and Fe_3O_4/POCA particles, respectively. It is also significant the reduction of saturation magnetization by all of the polymeric layers: 403.9 ± 1.1 kA/m for magnetite, and 54.9 ± 0.3 kA/m, 47.8 ± 0.4 kA/m, 50.5 ± 0.3 kA/m, and 63.6 ± 0.4 kA/m, for the Fe_3O_4/PE-2-CA, Fe_3O_4/ PBCA, Fe_3O_4/PHCA and Fe_3O_4/POCA particles, respectively. In spite of this, core/shell particles meet the pursued requirements: their surface is comparable to that of the pure polymer, but they have the property of being magnetizable, so they constitute an ideal carrier for targeted drug delivery.

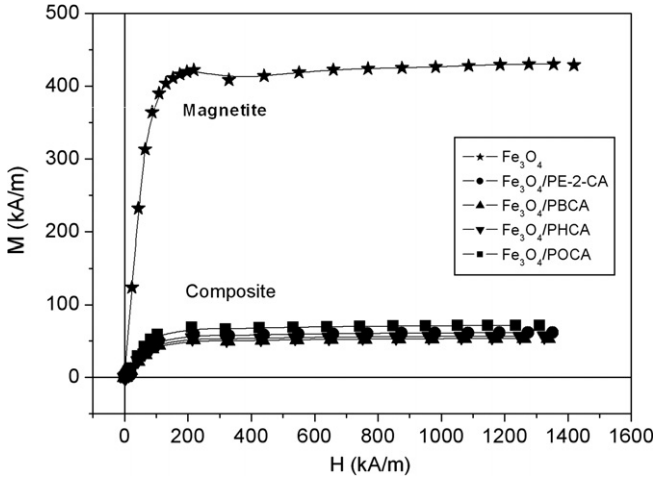

Fig. 2. First magnetization curve of the Fe_3O_4 (★), Fe_3O_4/PE-2-CA (●), Fe_3O_4/PBCA (▲), Fe_3O_4/PHCA (▼) and Fe_3O_4/POCA (■) particles. The lines are guides to the eye.

3.4. Effect of the polymerization conditions on 5-Fluorouracil loading

3.4.1. Effect of HCl concentration

The kinetics of the anionic polymerization process of an alkylcyanoacrylate monomer 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 decreases, the polymerization rate increases; therefore, it can be concluded that the H^+ concentration determines both the polymerization rate and the drug absorption [21,37,39]. Since 5-FU is essentially non-ionic, a fast polymerization mechanically entraps as much drug as possible; however, a very fast polymerization generates a large proportion of bulk polymer in the form of solids or macroaggregates [32,33,35].

Table 1 shows the drug loading to core/shell particles for HCl concentrations between 10^{-4} and 10^{-2} N and 5-FU concentrations ranging from 10^{-4} to 10^{-2} M. This process is affected by the H^+ concentration of the polymerization medium, whatever the drug concentration used and is largest at pH ~ 4 (10^{-4} N HCl): as the OH^- concentration decreases, the polymerization rate becomes slower and the absorption falls. This can be explained on the basis of the polymerization mechanism previously described: a fast polymerization (high pH) allows an easier drug mechanical trapping. Similarly, higher absorption values in more basic media were also obtained with other drugs [32,33,37]. A one-way ANOVA carried out on the data indicates, however, that the effect of pH is not statistically significant. Although a higher drug loading might be expected in more basic solutions, syntheses carried out at $[HCl] < 10^{-4}$ N yielded particles unsuitable for parenteral administration [32,33,35].

Finally, whatever the pH used, the fact that the amount of drug loaded is higher as the alkyl chain length of the polymeric matrix is shorter can be explained if we take into account the faster polymerization kinetics of monomers with shorter alkyl chain length, that induces a larger drug mechanical entrapping [27,33,40]. This tendency is statistically significant, with $p < 0.05$, as well as the increase in drug uptake upon increasing its concentration in the medium.

Table 1
5-Fluorouracil absorption density (Γ_m) to core/shell particles as a function of HCl concentration (between 10^{-4} and 10^{-2} N)

5-FU (M)	HCl (M)	Γ_m (μmol/g)			
		Fe_3O_4/PE-2-CA	Fe_3O_4/PBCA	Fe_3O_4/PHCA	Fe_3O_4/POCA
10^{-4}	10^{-4}	17.3 ± 0.6	9.8 ± 0.2	7.1 ± 0.3	4.9 ± 0.1
	10^{-3}	15.4 ± 0.8	9.1 ± 0.3	6.2 ± 0.2	3.8 ± 0.2
	10^{-2}	14.2 ± 0.8	8.5 ± 0.3	5.1 ± 0.3	3.1 ± 0.2
5×10^{-4}	10^{-4}	74.9 ± 1.4	62.7 ± 0.8	53.1 ± 0.6	30.9 ± 0.5
	10^{-3}	47.8 ± 1.3	39.1 ± 0.9	33.4 ± 0.5	17.3 ± 0.5
	10^{-2}	38.2 ± 0.9	26.2 ± 0.5	15.1 ± 0.5	7.8 ± 0.3
10^{-3}	10^{-4}	144.8 ± 2.7	118.7 ± 1.4	89.4 ± 1.4	65.9 ± 1.3
	10^{-3}	134.2 ± 2.1	111.1 ± 1.9	71.8 ± 1.4	53.4 ± 1.9
	10^{-2}	123.8 ± 2.6	102.2 ± 1.4	55.7 ± 1.1	37.3 ± 2.2
5×10^{-3}	10^{-4}	255.4 ± 6.2	215.2 ± 7.1	175.8 ± 7.1	135.2 ± 5.4
	10^{-3}	218.1 ± 7.8	172.2 ± 9.9	112.9 ± 4.9	84.6 ± 6.7
	10^{-2}	159.1 ± 7.5	123.1 ± 7.8	69.1 ± 6.6	55.2 ± 4.2
10^{-2}	10^{-4}	295.2 ± 9.9	245.4 ± 7.1	206.6 ± 9.8	162.9 ± 5.1
	10^{-3}	264.5 ± 7.8	204.2 ± 6.6	127.7 ± 7.8	93.9 ± 3.7
	10^{-2}	240.2 ± 5.6	151.4 ± 10.2	100.6 ± 4.5	66.7 ± 3.8

The anticancer drug molar concentrations ranged from 10^{-4} to 10^{-2} M.

3.4.2. Effect of anticancer drug concentration

Drug loading to PACA matrices is an extensively studied phenomenon for a wide group of drugs. Commonly, a positive effect of the increment in drug concentration is observed on the absorption efficiency [32,33,37,41]. Fig. 3 shows the 5-FU absorption by core/shell NPs as a function of the equilibrium drug concentration. All the data in this figure were obtained by varying the amount of anticancer drug added to the polymerization medium, in the following conditions: 0.1 mM HCl, 1% (w/v) dextran-70 and 1% (w/v) monomer. As observed, 5-FU loading increases with the drug concentration in solution, suggesting a trend towards saturation for the maximum concentrations investigated [33]. Finally, whatever the concentration used, the absorption density is higher in the polymeric matrices of shorter alkyl chain length, because of the faster polymerization kinetics of their corresponding monomers, as pointed above.

3.5. Surface adsorption of 5-Fluorouracil

As observed from the results obtained in the spectrophotometric determination of the 5-FU adsorption to the composite NPs (Fig. 4), the loading increases with the amount of drug in solution. In fact, the data seem to be well described by a Langmuir adsorption isotherm,

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

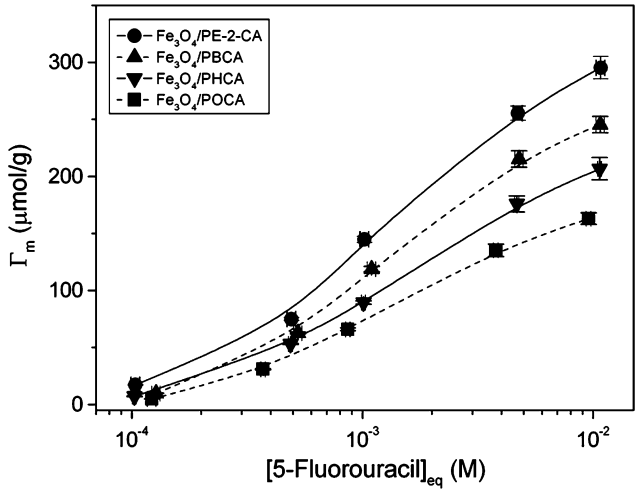

Fig. 3. 5-Fluorouracil absorption density (Γ_m) to Fe_3O_4/PE-2-CA (●), Fe_3O_4/PBCA (▲), Fe_3O_4/PHCA (▼) and Fe_3O_4/POCA (■) nanoparticles, as a function of the equilibrium drug concentration. The lines are guides to the eye.

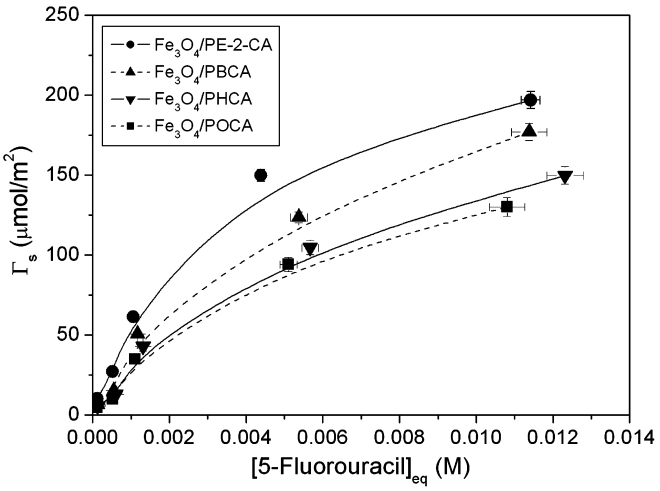

Fig. 4. 5-Fluorouracil adsorption density (Γ_S) to Fe_3O_4/PE-2-CA (●), Fe_3O_4/PBCA (▲), Fe_3O_4/PHCA (▼) and Fe_3O_4/POCA (■) nanoparticles, as a function of the equilibrium drug concentration.

where Γ_s is the amount adsorbed per unit area, C is the equilibrium concentration, Γ_{max} is 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:

$$Fe_3O_4/PE\text{-}2\text{-}CA: \quad \Gamma_{max} = 268 \pm 8\ \mu mol/m^2; \quad k = 254 \pm 21\ l/mol$$

$$Fe_3O_4/PBCA: \quad \Gamma_{max} = 249 \pm 18\ \mu mol/m^2; \quad k = 214 \pm 41\ l/mol$$

$$Fe_3O_4/PHCA: \quad \Gamma_{max} = 212 \pm 16\ \mu mol/m^2; \quad k = 182 \pm 34\ l/mol$$

$$Fe_3O_4/POCA: \quad \Gamma_{max} = 187 \pm 13\ \mu mol/m^2; \quad k = 163 \pm 31\ l/mol$$

Data indicate that the adsorption of this hydrophilic anticancer drug on hydrophobic surfaces is not considerable, as the approximation of this active agent from the aqueous phase to the hydrophobic particle surface is not favoured. Moreover, there is no statistically significant effect of the type of PACA shell on 5-FU surface incorporation. However, such adsorption is slightly increased as the hydrophobicity of the polymer becomes smaller, and in fact is a consequence of the decrease in hydrophobicity as the alkyl chain length is smaller [30,31,33,40,42,43].

The electrophoretic mobility (u_e) determinations can be used to qualitatively confirm the 5-FU surface incorporation to composite NPs, if we take into account that electrophoresis can be considered as most sensitive to surface modification by adsorption of, mainly, charged entities, even at rather small amounts. u_e data of the core/shell particles in 5-FU solutions are shown in Fig. 5 and, as it can be seen, u_e displays a general trend to raise (towards progressively less negative values) as the drug concentration is increased. There are, however, small differences between the different types of composite particles, as well as a clear effect of the addition of KNO_3. The electrostatically favoured adsorption of 5-FU (positively charged species, presumably coming from the protonation of the –NH group of the drug molecule) induces a reduction in the originally negative charge of the particles. This also explains the effect of the indifferent electrolyte: the presence of KNO_3 yields a mobility reduction because of double layer compression. In addition, this electrolyte screens the attraction between drug molecules and NPs, thus leading to the fact that the reduction of u_e when 5-FU concentration increases is more significant when there is no KNO_3 in the medium [32,33].

3.6. 5-Fluorouracil release from composite particles

The Fe_3O_4/PACA NPs used in the release investigations were obtained under the best drug loading conditions studied: (i) a 10^{-4} N HCl concentration (lower concentrations induce the formation of particles unsuitable for parenteral administration, and an excessive acid pH lowers the drug loading) and (ii) a 10^{-2} M 5-FU concentration. Furthermore, in order to study the influence of the amount of drug loaded in the release kinetics, composite particles obtained after using a 10^{-3} M drug concentration in the medium were also investigated (see Table 1 to check the amount of anticancer drug absorbed under these conditions). 5-FU release experiments were also carried out with the core/shell particles obtained after a surface adsorption process in a 10^{-2} M (10^{-3} M) drug medium. 5-FU adsorption to Fe_3O_4/PE-2-CA, Fe_3O_4/PBCA, Fe_3O_4/PHCA and Fe_3O_4/POCA NPs was 419.2 ± 11.6 (130.9 ± 2.8) μmol/g, 327.9 ± 9.9 (93.9 ± 2.1) μmol/g, 293.9 ± 10.8 (84.2 ± 5.9) μmol/g and 283.1 ± 12.8 (75.7 ± 6.8) μmol/g, respectively.

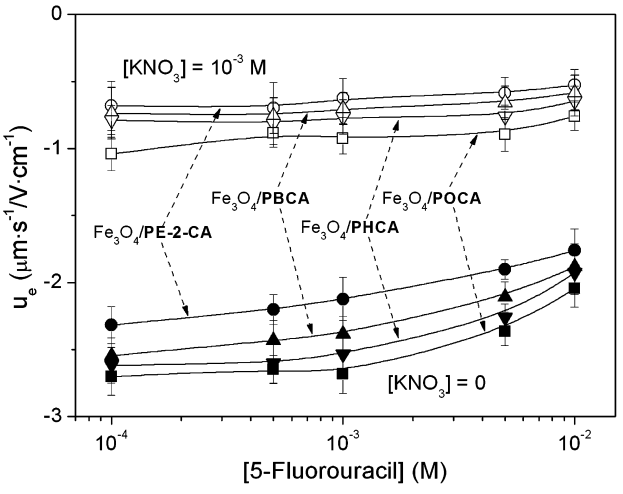

Fig. 5. Electrophoretic mobility of Fe_3O_4/PE-2-CA (●, ○), Fe_3O_4/PBCA (▲, △), Fe_3O_4/PHCA (▼, ▽) and Fe_3O_4/POCA (■, □) particles as a function of 5-Fluorouracil concentration, in the presence (open symbols) and absence (full symbols) of 10^{-3} M KNO_3.

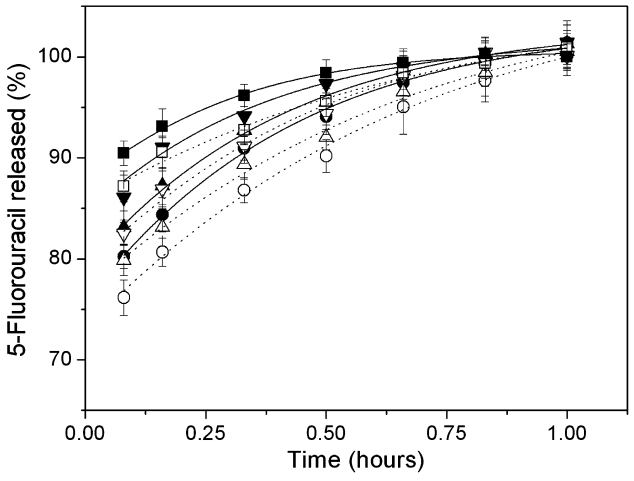

Fig. 6. Release of 5-Fluorouracil adsorbed from Fe_3O_4/PE-2-CA (●, ○), Fe_3O_4/PBCA (▲, △), Fe_3O_4/PHCA (▼, ▽) and Fe_3O_4/POCA (■, □) particles as a function of the incubation time in NaOH–KH_2PO_4 buffer (pH 7.4) at 37.0 ± 0.5 °C. The contact medium of adsorption was 10^{-3} M [open symbols and dotted lines] or 10^{-2} M [full symbols and solid lines] in drug concentration.

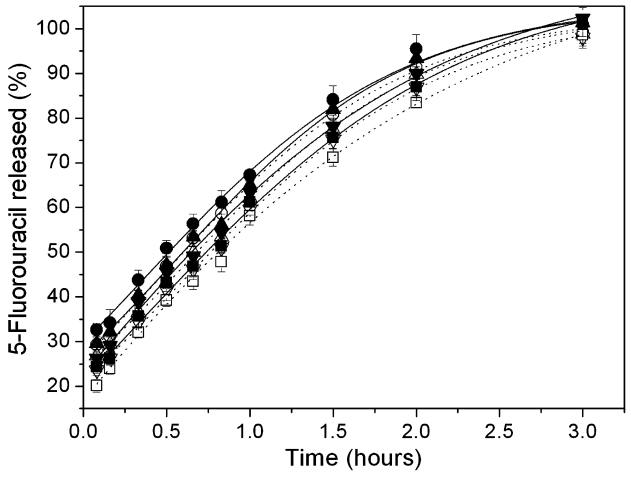

Fig. 7. Release of 5-Fluorouracil absorbed from Fe_3O_4/PE-2-CA (●, ○), Fe_3O_4/PBCA (▲, △), Fe_3O_4/PHCA (▼, ▽) and Fe_3O_4/POCA (■, □) particles as a function of the incubation time in NaOH–KH_2PO_4 buffer (pH 7.4) at 37.0 ± 0.5 °C. The polymerization medium in which the syntheses were carried out was 10^{-3} M [open symbols and dotted lines] or 10^{-2} M [full symbols and solid lines] in drug concentration.

In the case of the release of the adsorbed 5-FU, Fig. 6 shows that this process was almost finished after 40 min. The complete and rapid drug release suggests that 5-FU is likely adsorbed only on the external surface of the NPs. Furthermore, an increase in the drug loaded also enhanced the cumulative drug release [33,44]. The comparison between the polymeric shells shows that drug release is slightly slower from polymeric matrices of shorter alkyl chain length, most likely due to the slightly stronger interaction of this hydrophilic drug with the less hydrophobic PACA surfaces [33,40,43,45].

Drug release from NPs obtained in a medium with either 10^{-3} or 10^{-2} M 5-FU concentration follows a biphasic process (Fig. 7): an early rapid release of around 65% took place within 60 min, while the remaining 35% is slowly liberated during the next 120 min. The rapid release probably represents the loss of surface-associated and poorly entrapped (adsorbed more deeply into the surface pores) drug [33,44,45]. 5-FU release during the slow phase may result from either the polymer matrix disintegration, drug diffusion through the PACA shell, or both. This biphasic profile, typical of PACA, suggests that the major drug fraction was adsorbed onto the particles rather than entrapped into the core of the polymeric shell network [33,38,46–49]. Therefore, the release process should be a direct consequence of the polymer disintegration by surface erosion, started in turn by hydrolysis of the polymer chains with subsequent formaldehyde formation [21,33,42,50,51]. With respect to the influence of the type of PACA shell on the release rate, Fig. 7 shows that 5-FU was released slightly faster from polymeric matrices of shorter alkyl chain length, probably due to the higher degradation rate associated [27,33,42,45]. The results also show that the cumulative release is slightly enhanced by an increase in the drug loaded [33,44].

4. Conclusions

In this study, we have described procedures for enhancing the 5-Fluorouracil loading by magnetite/poly(alkylcyanoacrylates) (core/shell) NPs suitable for parenteral administration. The surface of the composite particles is comparable to that of the pure polymer, and they have the property of being magnetizable, thus constituting an ideal carrier for targeted drug delivery. The influence of the polymerization conditions (pH and drug concentration) and the contributions of both the polymeric matrix and the surface to the overall drug loading were investigated by means of optical absorbance and electrophoretic mobility determinations. Finally, despite drug adsorption allowing a higher loading, 5-Fluorouracil incorporation into the composite particles permitted a slower drug release defined by a biphasic profile.

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