Departamento de Farmacia y Tecnología Farmacéutica[1], Departamento de Química Farmacéutica[2], Facultad de Farmacia, Universidad de Granada, Granada, Spain

Characterization of latex-antineoplastic drug complexes by differential scanning calorimetry and microphotography

V. Gallardo[1], M. A. Ruiz[1], E. Morales[1], J. Zouaki[1], J. Campos[2], A. Conejo-García[2], M. A. Gallo[2], A. Espinosa[2]

Received June 17, 2005, accepted October 22, 2005

Dra V. Gallardo Lara, Dept. of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Granada, Campus de Cartuja, E-18071 Granada, Spain
vglara@ugr.es

Pharmazie 61: 1009–1013 (2006)

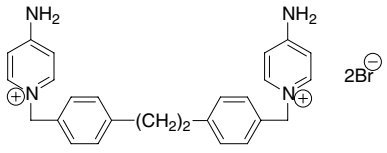
Choline kinase inhibitors have recently been identified as potentially useful antitumoral agents. Here we determine the best conditions for obtaining drug-polymer complexes with 5-fluorouracil (5-FU), and JCR791B, a new drug representing a significant advance in the development of new molecules to inhibit tumour proliferation. As polymers we used the cellulose derivatives Aquacoat® and Aquateric®. The variables in the adsorption process measured were time to adsorbent-adsorbate equilibrium, pH and concentration. The drug-polymer complexes were characterized by differential scanning calorimetry and microphotography. Our results show that adsorption of 5-FU and JCR was similar with both polymers although slightly greater with Aquacoat®. The chemical structure of the drug and its solubility in water and oil are fundamental characteristics that determine the performance of polymers as drug carriers able to provide controlled release.

1. Introduction

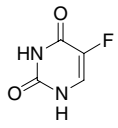
For at least 40 years, 5-fluorouracil (5-FU) has been the mainstay of treatment for patients with metastatic colorectal cancer. The mechanism of action of 5-FU involves the incorporation of fluorouridine 5′-triphosphate (FUTP) into RNAm, which interferes with gene expression, or the inhibition of thymidylate synthase (TS) by the active metabolite 5-fluorouridine monophosphate (FdUMP) (Carrico and Glazer 1997; Parker and Cheng 1990).

At present the development of new drugs to treat cancer is a fundamental task. The successful development of newer drugs requires extensive theoretical groundwork to elucidate the mechanism of action of these compounds. Choline kinase (ChoK) is responsible for the generation of phosphorylcholine, a proposed second messenger required for DNA synthesis induced by growth factors. We recently reviewed the significance of ChoK inhibitors as a novel approach to the development of antiproliferative agents. Choline kinase inhibition has a drastic inhibitory effect on cell proliferation and prevents tumour growth in mice (Campos et al. 2003; Hernández-Alcoceba et al. 1999). The compound JCR791B was recently reported to be a potentially useful antitumour agent against the HT-29 human colon cancer cell line (Campos et al. 2002).

Polymers are now widely used in the biomedical field (Donini et al. 2002), for example in the manufacture of controlled-release pharmaceuticals (Foss et al. 2004). Latexes are colloidal dispersions with particles smaller than one micron (Gurny 1983). Particular attention must be paid to how the particles for these dispersions are obtained, since the properties of the resulting dispersions will depend not only on their composition and chemical structure, but also on the method used for their manufacture, which affects both the final particle size and the stability of the system. Latex-drug preparations are based on mechanisms of adsorption of the drug on the latex surface (Zouaki et al. 1998; Llácer et al. 2000; Pamujala et al. 2004), with which it must be biocompatible. The large specific surface of these systems (Gallardo et al. 2001) makes them capable of transporting large amounts of drug, as well as providing slow, steady release of the active ingredient. To prepare the latex carriers we used the polymers Aquateric® and Aquacoat®, (cellulose derivatives) because they are approved for use in the body and are suitable drug vehicles owing to their high specific surface area, which allows the absorption of large amounts of drug compounds (Ruiz et al. 1994; Vera et al. 1996).



JCR/791B



5-FU

The aim of this study was to test polymer-drug complexes prepared by adsorption of the antineoplastic agents 5-FU and JCR791B on Aquateric® and Aquacoat® latexes. We report here the results of studies on adsorption kinetics, pH and concentration. Sediments obtained by centrifugation were characterized by differential scanning calorimetry (DSC) and microphotography to provide complementary morphological information.

2. Investigations, results and discussion

The evaluation of latex particles for possible use as a system for the transport and release of drugs (Vanderhoff and El-Aaser 1979; Llacer et al. 2000) should include, among other analyses of drug-latex interaction, direct measurement of adsorption on the particle surface and determination of the environmental conditions that optimize the process (Pott and Guy 1992).

The time to adsorbate-adsorbent equilibrium was determined as an element of the adsorption kinetics of the drug in different formulations. Fig. 1 shows that maximum adsorption was reached after 24 h in all cases, with no significant differences between the two types of latex carrier for 5-FU. The difference between the two types of latex was significant, however, for JCR, which was adsorbed in lower amounts on Aquacoat®. In all cases approximately 80% of the total maximum adsorption took place during the first 30 min.

The effects of drug concentration on adsorption are shown in Figs. 2 and 3. In all four preparations the amount of adsorbate (drug) retained by the adsorbent (latex) was proportional to the initial concentration of the drug.

Mathematical models were used to estimate the probable adsorption kinetics. The Table shows the equilibrium equation and approximate equilibrium constant (k_c), as well as the maximum amount of drug adsorbed per gram of adsorbent (saturation point, n^s) for each adsorption isotherm. The amounts adsorbed on the particle surface of both types of latex were greater for 5-FU than for JCR791B. This finding is not unexpected since adsorption of 5-FU (130.08 g/mol) was favoured by its lower molecular weight in comparison to JCR791B (556.34 g/mol) (McCarron et al. 2000).

In addition, 5-FU is less soluble in water than JCR791B ($\log P_{5\text{-FU}} = -0.68$, $\log P_{JCR791B} = -4.67$). Because of the lipophilic nature of the polymer particles (Mamuhold et al. 1995), drug retention is favoured as adsorption is greater between two surfaces with the same charge.

It is difficult to predict the behaviour of a potential adsorbate in response to a given adsorbent, as adsorption depends on the physicochemical properties of the adsorbent surface, and on the chemical nature of the adsorbate and solvent (Scout and Peppas 1999). According to the Rehbinder rule, the greater the difference in polarity between

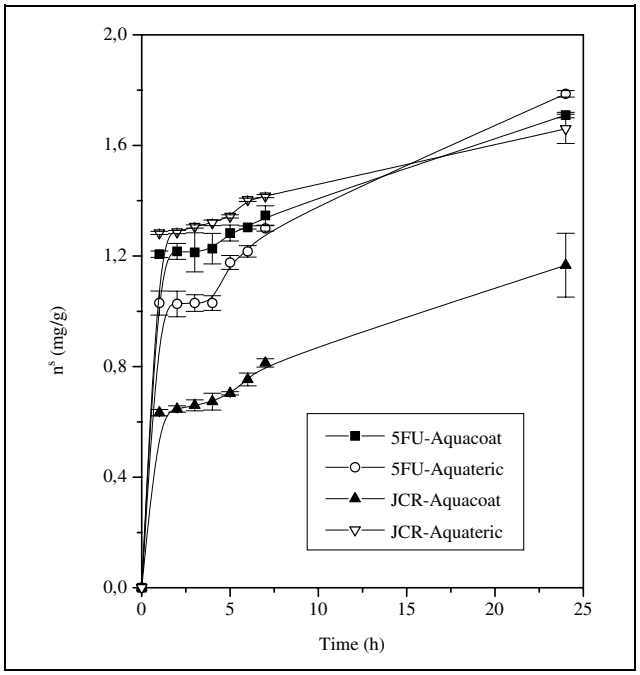


Fig. 1: Adsorption of 5-FU and JCR791B in Aquateric® and Aquacoat® over time

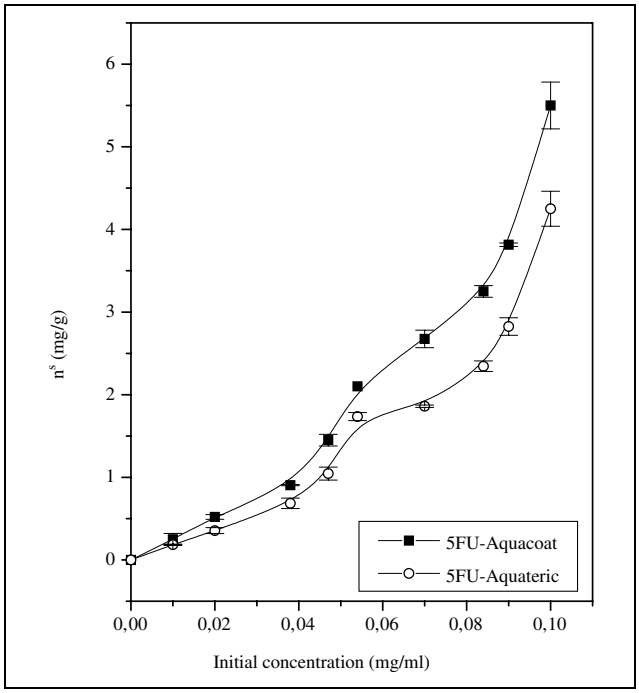


Fig. 2: Density of adsorption versus concentration for 5-FU in Aquateric® and Aquacoat®

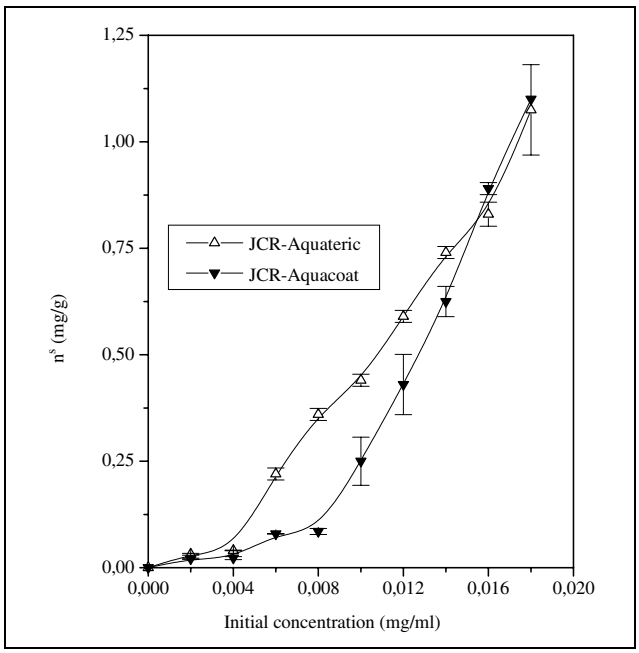


Fig. 3: Density of adsorption versus concentration for JCR791B in Aquateric® and Aquacoat®

Table: Mathematical models used to estimate adsorption kinetics

Adsorption isotherm adsorbate/adsorbent	Probable kinetics	Equilibrium equation	K_c approximate	n^s (mg/g)
5-FU/Aquacoat	Order 2	$C_n/n_s = [1/(k_c \cdot n_0^s)] + (1/n_0^s) \cdot c^n$	905	5.57
5-FU/Aquateric	Order 2	$C_n/n_s = [1/(k_c \cdot n_0^s)] + (1/n_0^s) \cdot c^n$	342	6.19
JCR791B/Aquacoat	Order 3	$C_n/n_s = [1/(k_c \cdot n_0^s)] + (1/n_0^s) \cdot c^n$	3.8×10^6	1.43
JCR791B/Aquateric	Order 4	$C_n/n_s = [1/(k_c \cdot n_0^s)] + (1/n_0^s) \cdot c^n$	2.3×10^7	3.54

adsorbate and solvent (i.e., the lower the solubility of the adsorbent), the greater the adsorption of the adsorbate (Gallardo et al. 2003; Wiechers et al. 2005). The behaviour of 5-FU was more consistent with the rule than was the behaviour of JCR791B: the difference in polarity between 5-FU and the solvent (distilled water) was greater, and this drug was adsorbed in larger amounts on both types of latex than was JCR791B.

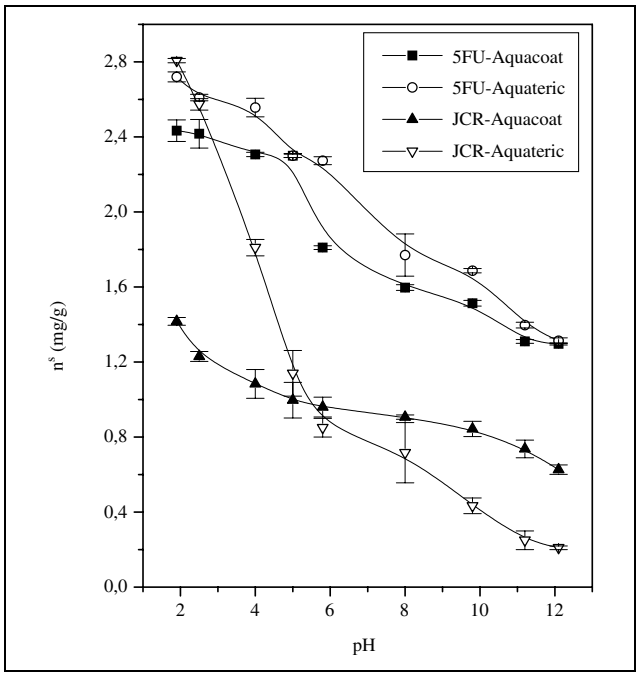


Fig. 4: Density of adsorption in Aquateric® and Aquacoat® versus pH

Other properties potentially able to influence the adsorption of the active principles tested here are:

- Flexibility of the molecule, i.e., its ability to fold into a configuration that presents a smaller surface area than in its extended state. The surface area of JCR is estimated at 424.27 Å^2, and that of 5-FU, whose structure is rigid, is estimated at 119.77 Å^2.
- Type of bonds that the JCR791B molecule can form through charge transfer and because of the presence of a polar and an apolar chain (a property lacking in 5-FU).

Despite its smaller specific surface (2 m^2/g), Aquateric® adsorbed larger amounts of the active principles than Aquacoat® (specific surface 16 m^2/g), with the difference being especially obvious for JCR791B. This finding reflected the lower lipophilicity of Aquateric® in comparison to Aquacoat® (Gallardo et al. 1996).

The effect of pH on adsorption of the two antineoplastic drugs on the two types of latex particles is shown in Fig. 4, which plots the amount of each drug adsorbed in $n^s \cdot mg \cdot g^{-1}$ against different pH values for the drug in solution.

Adsorption was similar in qualitative terms for all four latex-drug complexes: maximal adsorption was seen at acid pH values between 2 and 4, i.e., below the natural pH value of approximately 5. At higher pH values, drug retention decreased considerably, especially in Aquateric® complexes. This finding was consistent with the results of earlier electrophoretic mobility findings for these complexes (Gallardo et al. 1993).

We obtained DSC profiles for each of the two polymers separately, for each of the antineoplastic drugs, and for each of the latex-drug complexes. Fig. 5 shows the thermogram of pure 5-FU, which was characterized by an en-

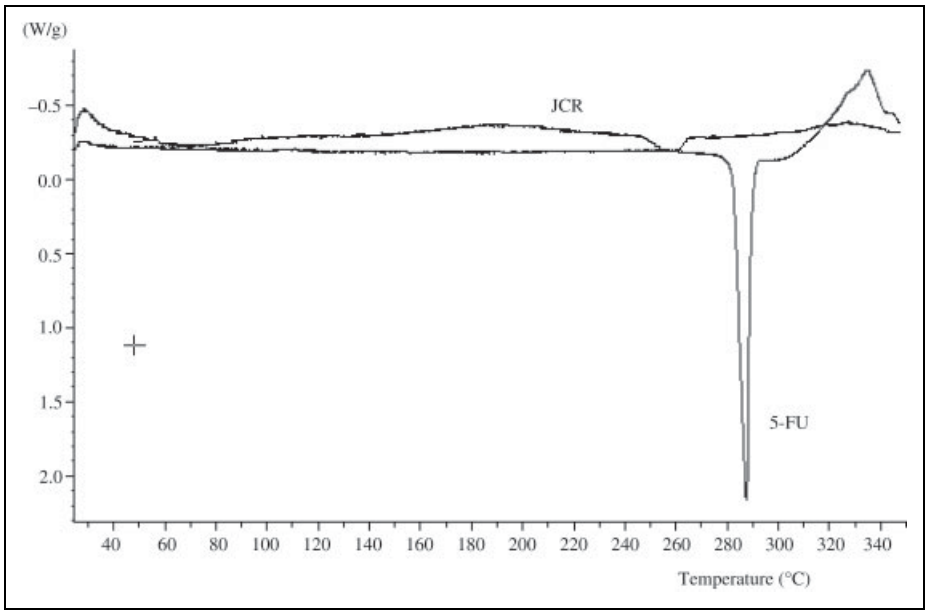


Fig. 5:
Thermograms of 5-FU and JCR791B

dothermal melting peak at 284 °C. The thermogram of JCR791B showed an endothermal melting peak at approximately 256 °C and another slightly endothermal zone at 69 °C, which may reflect the water of crystallization in this product. Fig. 6 shows the DSC profile of Aquacoat®, which produced an endothermal dip at approximately 54 °C and an exothermal peak at about 160 °C. The thermogram for Aquateric® showed an endothermal zone at approximately 69 °C and an evident exothermal peak at 173.3 °C. Fig. 7 shows thermograms for the Aquacoat® complexes. The first endothermal zone for this latex at 54 °C was shifted slightly to the left in both drug complexes, although the shift was more evident for the JCR-containing complex, where the dip in the curve appeared at approximately 39 °C. The exothermal zone at 160 °C was shifted slightly to the left (158 °C) in the JCR-containing complex, and disappeared completely in the complex that contained 5-FU.

The thermograms of the Aquacoat®-5-FU and Aquacoat®-JCR791B complexes were more similar to the DSC curves for the latex alone than to those for the drug alone.

The thermogram for Aquateric® (Fig. 8) differed from the curve obtained for Aquacoat®. In the first place an endothermal peak for Aquateric® it remains without scarcely modifications in complexes with both drugs, whereas the exothermal zone evident at 173 °C disappeared almost completely.

Analysis of scanning electron micrographs of Aquacoat® (Fig. 9A) and Aquateric® (Fig. 9B) showed Aquacoat® particles to be spherical and homogeneous in size, with a mean diameter of 339 nm (s.d. 61 nm). Aquateric® particles were of two well differentiated types: those measuring 25–30 nm in diameter, and a much smaller population of larger particles measuring approximately 4 μm in diameter. Aquateric® particles were hollow and had a rough outer surface; the inner walls appeared spongy.

Scanning electron micrographs of the two active principles are shown in Fig. 9C (5-FU) and 9D (JCR). Particles of 5-FU were irregularly shaped, and formed aggregates that varied considerably in size and overall shape. In contrast, JCR791B particles formed spongy aggregates of different sizes.

The appearance of the latex-drug complexes is illustrated in Fig. 10. The Aquacoat®-drug complexes (Fig. 10A) formed a homogeneous field of aggregates in which the latex particles themselves could not be distinguished, possibly because the drug completely coated the surface of the

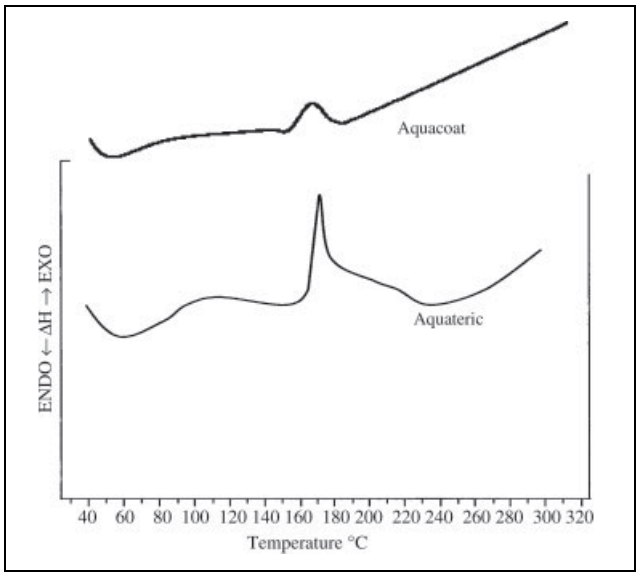


Fig. 6: Thermograms of Aquacoat® and Aquateric®

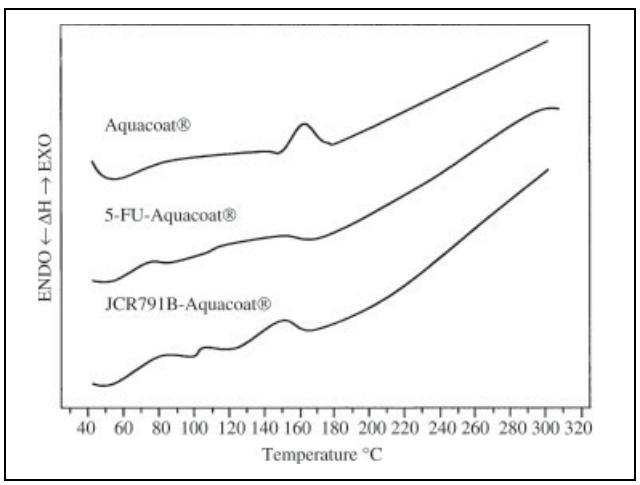


Fig. 7: Thermograms of Aquacoat® and drug-Aquacoat® complex

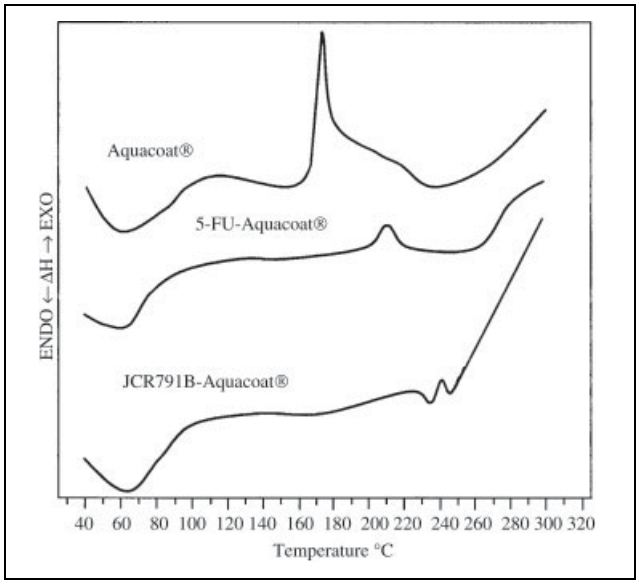


Fig. 8: Thermograms of Aquateric® and drug-Aquateric® complex

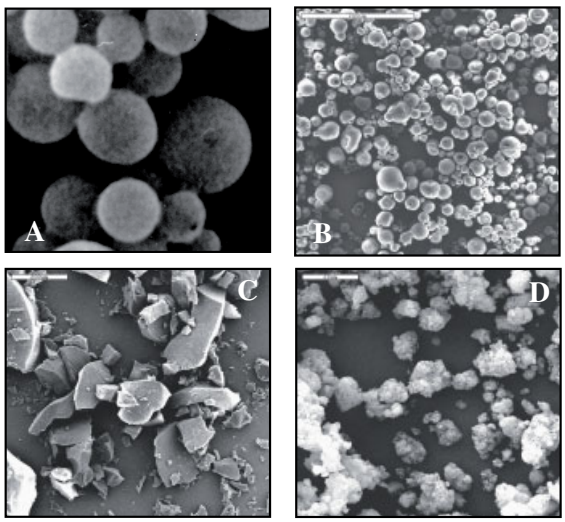


Fig. 9: Typical scanning electron micrographs of Aquacoat® latex (A), Aquateric® (B), 5-FU (C) and JCR791B (D)

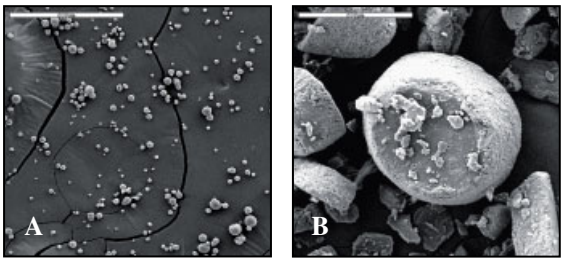


Fig. 10: Typical scanning electron micrographs of drug-latex complex: drug-Aquacoat® (A), drug-Aquateric® (B)

latex particles. In Aquateric®-drug complexes (Fig. 10B) the latex particles were clearly visible, and the drug could be seen adsorbed on the outer surface and on the inner walls of the particles.

3. Experimental

3.1. Material

5-Fluorouracil (5-FU), supplied by Sigma-Aldrich Química S.A. (Spain). JCR791B, a synthetic compound designed and supplied by the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Granada (Spain). Aquacoat®, provided by Foret S.A. (Spain), is an aqueous ethylcellulose dispersion obtained by polymerization, with a solids content of 30%. Aquateric®, provided by Foret S.A. (Spain), is a white powder insoluble in water. It consists of 69.7% cellulose acetophthalate, 20% Pluronic F-68 (cationic surfactant), 10% Myvacet 940 (monoglyceride component), and 0.3% Tween 60.

Before use both polymers were washed with distilled water to remove contaminating particles in the medium acquired during synthesis, and to achieve maximal colloidal dispersion. Washing was done by repeated centrifugation and redispersion until the supernatant had a constant conductivity.

pH was determined with 0.1 N HCl and NaOH using a Crison model MicropH 2001 pH meter.

3.2. Methods

3.2.1. Preparation of drug-latex complexes

Adsorption kinetics were studied as a function of time, pH and concentration of the drug. Each active principle was placed in contact with each latex at a constant temperature of 25 °C and with shaking at 60 rpm, followed by 30 min of centrifugation at 14 000 rpm to separate the sediment and the supernatant. Solubilized drug in the supernatant was measured by spectrophotometry at the maximum wavelength for absorption of the drug, $\lambda = 272$ (JCR791B) and 266 (5-FU) nm. The concentration of drug was calculated from calibration curves obtained with standard aqueous solutions of the drug at a known concentration. The complexes were prepared once the best adsorption conditions had been determined (time = 24 h pH = 2–4). The latex-drug complex was prepared from a solution of 30% 5-FU or JCR791B and 60% latex. However, the Aquacoat®-drug complex was prepared with only 10% latex due to the high solids content of this polymer. These complexes were then incubated in a 25 °C thermostatic bath with shaking at 30 rpm for 24 h. Subsequently, the samples were centrifuged for 50 min at 14000 rpm to separate the sediment and supernatant. Spectrophotometry was used to determine the amount of drug in the supernatant, and the amount remaining in the sediment was found by subtracting the amount in the supernatant from the initial value.

Differential scanning calorimetric analysis of the samples was performed with a Mettler FP85 DSC apparatus (Zurich, Switzerland) at a heating rate of 5 °C min^{-1} and a temperature range of 30 °C to 325 °C. Sample weight was between 5 and 6 mg.

Particle sizes in the dispersion were determined by field emission scanning electron microscopy (FESEM) (Leo 1530, Gemini, Oberkochen, Germany). Samples for micrographic examination were coated with gold, and to determine particle size the maximum horizontal diameter was measured in several photographic fields to sample a representative number of particles (at least 600).

Acknowledgements: Part of this work was supported by the Spanish Ministry of Education and Science, and the European Regional Development Fund, under Project MAT 2004–00866. We thank K. Shashok for translating parts of the original manuscript into English.

References

Campos JM, Núñez MC, Sánchez-Martín RM, Gómez-Vidal JA, Rodríguez-González A, Báñez M, Gallo MA, Lacal JC, Espinosa A (2002) Quantitative structure-activity relationships for a series of symmetrical bisquaternary anticancer compounds. Bioorg Med Chem 10: 2215–2231.

Campos J, Núñez MC, Conejo-García A, Sánchez-Martín RM, Hernández-Alcoceba R, Rodríguez-González A, Lacal JC, Gallo MA, Espinosa A (2003) QSAR-derived choline kinase inhibitors: How rational can antiproliferative drug design be? Curr Med Chem 10: 1095–1112.

Carrico CK, Glazer RI (1979) Effect of 5-fluorouracil on the synthesis and translation of polyadenylic acid-containing RNA from regenerating rat liver. Cancer Res 39: 3694–3701.

Donini C, Robinson DN, Colombo P, Giordano F, Peppas NA (2002) Preparation of nanospheres for methacrylic monomers for pharmaceutical applications. Int J Pharmacol 245: 83–91.

Foss CA, Goto T, Morishita M, Peppas NA (2004) Development of acrylic-based copolymers for oral insulin delivery. Eur J Pharm Biopharm 57: 163–169.

Gallardo V, Salcedo J, Vera P, Delgado AV (1993) Electric and adsorption properties of pharmaceutical polymers. Part I: Electrokinetics of Aquacoat. Colloid Polym Sci 271: 967–972.

Gallardo V, Ruiz MA, Vera P, González-Caballero F, Delgado AV (1996) Effect of amino acids on the stability properties of nitrofurantoin suspensions. Flocculation and redispersion as compared to interaction energy curves. J Pharm Pharmacol 48: 450–455.

Gallardo V, García E, Ruiz MA (1997) Thermoanalytical study of corticosteroid adsorption/desorption on a cellulosic polymer. React Polym 34: 127–134.

Gallardo V, García ME, Ruiz MA (2001) Design, synthesis and characterisation of ethylcellulose latex. Macromol Chem Physic 202: 2412–2416.

Gallardo V, Ruiz MA, López-Viota J, Salcedo J, Delgado AV (2003) Electrokinetic study of omeprazole drug in aqueous suspensions. Colloids Surfaces A: Physicochem. Eng Aspects 218: 21–26.

Gurny R (1983) Latex Systems. In: Breimer D, Speiser P (eds) Topics in Pharmaceutical Sciences, Elsevier Sci, Amsterdam, p. 277.

Hernández-Alcoceba R, Fernández F, Lacal JC (1999) *In vivo* antitumor activity of choline kinase inhibitors. Cancer Res 59: 3112–3118.

Llácer JM, Ruiz MA, Parera A, Gallardo V (2000) Adsorption-desorption of ondansetron on latex particles. Drug Dev Ind Pharm 26: 237–242.

Mannhold R, Rekker RF, Sonntang C, Ter Laak AM, Dross K, Polymeropoulos EE (1995) Comparative evaluation of the predictive power of calculation procedures for molecular lipophilicity. J Pharm Sci 84: 1410–1419.

McCarron PA, Woolfson AD, Keating SM (2000) Sustained release of 5-fluorouracil from polymeric nanoparticles. J Pharm Pharmacol 52: 1451–1459.

Pamujala S, Graves RA, Kishore V, Mandal TK (2004) Preparation and in vitro characterization of amifostine biodegradable microcapsules. Eur J Pharm Biopharm 57: 213–218.

Parker WB, Cheng YC (1990) Metabolism and mechanism of action of 5-fluorouracil. Pharmacol Ther 48: 381–395.

Potts RO, Guy RH (1992) Predicting skin permeability. Pharm Res 9: 663–669.

Ruiz MA, Gallardo V, Delgado AV, Vera P (1994) Study of *in vitro* release of corticoids in topical formulations,. Il Fármaco 49: 147–152.

Scott RA, Peppas NA (1999) Highly crosslinked PEG-containing copolymers for sustained solute delivery. Biomaterials 20: 1371–1380.

Vanderhoff JM, El-Aaser MS, Ugelstad J (1979) US Patent 4, 177, 177.

Vera P, Salcedo J, Delgado AV, Ruiz MA, Gallardo V (1996) Adsorption of betamethasone disodium phosphate on ethylcellulose latex: an electrokinetic study J Pharm Sci 85: 468–472.

Wiechers JW, Nelly CL, Blease TG, Federen JC (2005) Formulando con eficacia Not. Cosmet. Perfum. 282: 5–14.

Zouaki Y, Ruiz MA, Parera A, Gallardo V (1998) Adsorption-desorption of salicylic acid on latex particles. Pharm Ind 60: 468–478.