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Evaluation of carrier capacity and release characteristics for poly(butyl 2-cyanoacrylate) nanoparticles

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Summary

The characteristics of incorporation and adsorption of drugs onto poly(butyl 2-cyanoacrylate) nanoparticles has been studied using two model compounds, rose bengal and adriamycin. The maximum drug carrier capacity was found to be dependent on the method employed for loading of the compound, the nanoparticle composition, the compound itself and the pH of the solution. A linear correlation was found between the amount of compound incorporated into the nanoparticles and the time for 50% release. A novel bathocromic shift method (spectroscopy) has been used which enabled the concurrent determination of both free and nanoparticle bound compound without prior separation.

Introduction

Polyalkylcyanoacrylate nanoparticles have been investigated as potential carrier systems for drug targeting (Couvreur et al., 1980; Kreuter et al., 1983; Illum et al., 1984). The particles are claimed to be biocompatible and biodegradable; the rate of degradation being a function of the alkyl chain length with, for example, the methyl and ethyl chains degrading faster than the longer butyl chains (Pani et al., 1968). Polyalkylcyanoacrylate nanoparticles can be produced by a dispersion polymerization process carried out in an aqueous phase at a low pH and employing a polymeric stabilizing agent. The molecular weight, the concentration and nature of the stabilizing agent have been shown to be important factors in controlling the diameter of the nanoparticles with the range of 20–770 nm (Douglas et al., 1984, 1985).

Nanoparticles used as drug carrier systems appear to be capable of modifying the tissue distribution of a variety of antitumour agents and in some cases this results in an increase in the therapeutic index (Kreuter and Hartman, 1983; Kante et al., 1980; Brasseur et al., 1980). The drug can either be incorporated into the nanoparticles during the polymerization process or be adsorbed onto the surface of preformed particles. The carrier capacity is highly dependent upon factors such as the nature of polymer, pH of the sorption medium and the drug. Though a larger amount of drug would be expected to be taken up by the nanoparticles when the incorporation technique is used the literature seldom differs between these

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nanoparticles when the incorporation technique is used the literature seldom differs between these two methods when discussing carrier capacity. Mostly the carrier capacity is given as the percentage of drug associated with a given amount of nanoparticles for a given initial concentration of drug without considering the characteristics of the adsorption isotherm (Couvreur et al., 1979a and b, 1982; Kreuter et al., 1983; El-Samaligy and Rohdewald, 1982).

A drug following a constant partitioning model would have very different incorporation characteristics to one following a high affinity Langmuirian profile (Giles and Smith, 1974) (Fig. 1). A high loading level obtained at low concentrations of added solute cannot necessarily be extrapolated to higher levels of solute. In drug therapy using colloidal carriers the ratio of drug-to-carrier is probably a more meaningful quantity. Thus, ideally when designing and evaluating nanoparticle systems for drug delivery, the drug should be well associated with the nanoparticles with little drug in the unbound form. A successful nanoparticle system may well be one which has a high percentage of uptake *and* a high loading capacity, thereby minimizing the quantity of carrier required for administration. Consequently it is important to characterize the loading capacity for the drug and nanoparticle system in question.

In the present study we have investigated the incorporation and adsorption characteristics of two model compounds (rose bengal and adriamycin) associated with poly(butyl 2-cyanoacrylate) nanoparticles prepared using different stabilizing systems. Factors influencing the release of the compounds from the nanoparticles have also been studied. The investigations have been performed mainly by means of spectroscopy using a novel bathochromic shift method which enables the concurrent determination of both free and bound compound without prior separation of the nanoparticles as is normally required.

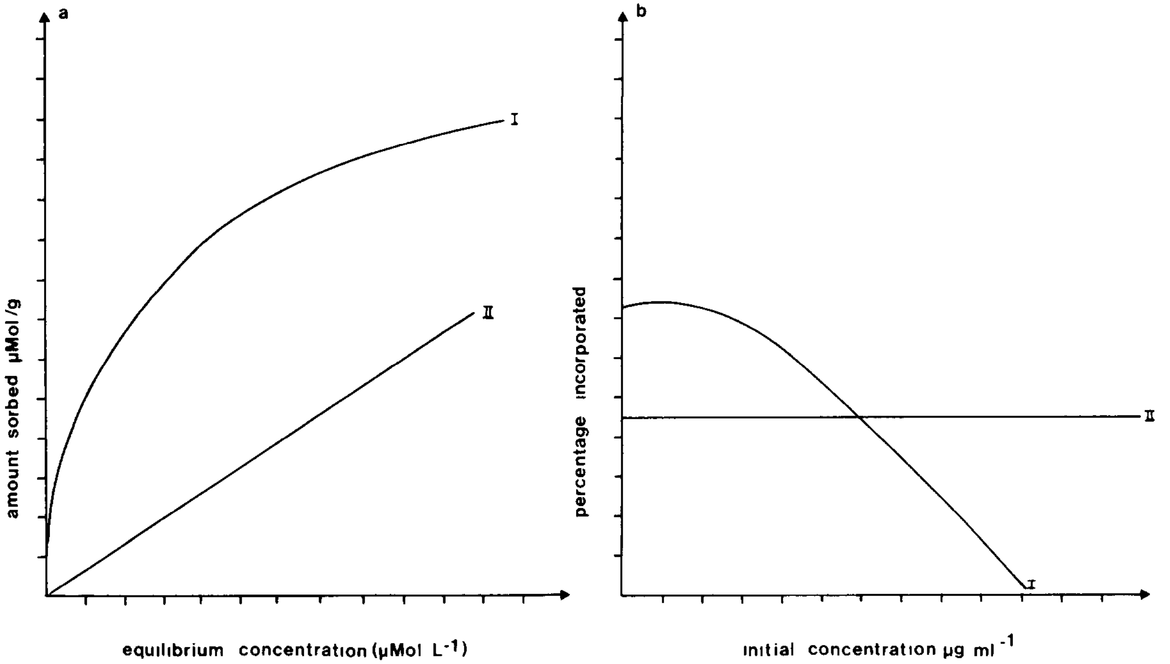

Fig. 1. Theoretical curves showing the adsorption isotherms (a) and percentage incorporation (b) for compounds following the Langmuirian type (I) and the constant partitioning type (II) isotherms

Materials and Methods

Materials

Rose bengal, dextran 70 and adriamycin were purchased from Sigma Chemicals, Dorset, U.K. and poloxamer 338 was a gift from Pechiney Ugine Kuhlmann Chemicals Division, Bolton, U.K. Butyl 2-cyanoacrylate was a gift from Sichel-Werke (F.R.G.). All other chemicals were of reagent grade.

Methods

Preparation of nanoparticles

A 1% v/v nanoparticle suspension was prepared by adding butyl 2-cyanoacrylate monomer to a filtered (0.2 μm membrane filter) solution of 0.01 N hydrochloric acid and 0.5% w/v of either dextran 70 or poloxamer 338 in distilled water. The polymerization media was stirred continuously during the process and the temperature was maintained at 20°C. After the polymerization was complete (4 h) the suspension was filtered through a sintered glass filter (grade 4, pore size 11–16 μm) and diluted accordingly for particle size measurements or drug adsorption experiments.

Particle size analysis

Particle size measurements were performed by photon correlation spectroscopy as outlined previously (Douglas et al., 1984). Results are quoted as the z-average particle diameter (d_z) and the polydispersity index (Q).

Adsorption isotherms and incorporation of solute

Adsorption isotherms for rose bengal and adriamycin onto dextran 70 or poloxamer 338 stabilized nanoparticles were determined at 20°C and pH 7.0 in a phosphate buffer using spectroscopic methods. For most experiments with rose bengal the nanoparticles were mixed with solutions of rose bengal to give a final concentration of nanoparticles in the range 0.01–0.1% v/v and adsorbate concentration within the range of 0.5–90 μg/ml. For studies on the adsorption of adriamycin the final nanoparticle concentration was 0.005% v/v and the concentration of the adsorbate was within the range of 0.25–10 μg/ml. In comparative experiments, where separation by centrifugation was also performed, the final nanoparticle concentration was 0.1% v/v and the adsorbate concentration was within the range of 5–90 μg/ml. The mixed nanoparticles and drug suspension were left overnight at 20°C before determining the amount of drug adsorbed by spectroscopy.

The influence of pH on the adsorption of rose bengal onto dextran 70 stabilized nanoparticles were determined using low ionic strength buffers. Each 10.0 ml sample contained 4.0 ml of 1% v/v dextran 70 stabilized nanoparticles, 5.7 ml of the appropriate buffer and 0.3 ml of a rose bengal stock solution. The total initial rose bengal concentration in each sample was 300 μg/ml. The samples were shaken in a water bath at 20°C overnight and the amount of rose bengal adsorbed determined by spectroscopy.

The direct incorporation of the model compounds into the nanoparticles was performed at pH 2.0 by adding different amounts of a stock solution of the solute to the polymerization medium. In these experiments the final volume of nanoparticle suspension was 10 ml and hence the amount of added monomer was 100 μl. The polymerization process was performed as for the simple nanoparticles. The concentration of the added model compound, used to follow incorporation, was within the range of 100 to 6000 μg/ml. The samples were stirred for 4 h. The samples were diluted 50 times with phosphate buffer (pH 7.4) before fluorescence or UV-visible spectroscopic determination of the incorporated compound.

Release of drug from nanoparticles

The desorption of the compounds from the nanoparticles into phosphate buffer at pH 7.4 was studied for both adsorbed and incorporated materials and for nanoparticles stabilized with dextran 70 or poloxamer 338.

For the rose bengal the desorption was followed using a flow through technique by means of a conventional dissolution apparatus (Erweka, Copley Instruments, Nottingham, U.K.) connected via a peristaltic pump to a computerized UV/visible spectrophotometer (Kontron, St. Albans, U.K.). The desorption process was followed for loading concentrations within the range of 2–10 mg of compound per 100 mg of nanoparticles to ensure that the initial amount of compound added to the nanoparticle suspension was originally completely adsorbed or incorporated. The nanoparticle suspension was diluted with phosphate buffer (pH 7.4) to provide a final volume of 500 ml, thereby giving sink conditions for rose bengal. (A typical dilution factor would be within the range of 80–250 times.) The diluted nanoparticle suspension was stirred continuously in a beaker at constant temperature (37°C). Absorbance readings were taken every 3 min for 9 h. The concentration of rose bengal in solution was calculated using the bathochromic shift method (see below).

Analytical measurements

Centrifugation method. Nanoparticle suspension was centrifuged at 33 600 *g* for 90 min using an ultra centrifuge (MSE Scientific Instruments, Crawley, U.K.) to separate free from bound rose bengal. The rose bengal remaining in the supernatant was determined by UV-visible spectroscopy at 540 nm (Kontron, St. Albans, U.K.).

Bathochromic shift method. The incorporation of a compound into nanoparticles has to be performed during the polymerization process at a pH of about 2. Consequently, an acidic compound like rose bengal ($pK_{A_1} = 3.72$) is only poorly soluble in the polymerization medium and undissolved and unincorporated solute could sediment out as particles together with the nanoparticles during the normal centrifugation process (90 min at 33 600 *g*) used to assess the extent of incorporation. To prevent a misleading overevaluation of incorporated rose bengal the pH could be raised to 7 before the centrifugation step. However, this would inevitably result in a disturbance of the equilibrium conditions and the release of incorporated rose bengal from nanoparticles to solution during the long period of centrifugation. Consequently a novel spectroscopic method has been developed for the adsorption, incorporation and release studies employing rose bengal, whereby it has been possible to determine concurrently both the free and bound rose bengal without prior separation of particles. The method is based on the fact that that when the rose bengal molecules are adsorbed onto the nanoparticles a bathoachromic shift is observed in the absorption spectrum of rose bengal (from 540 nm for free rose bengal to 548 nm for bound rose bengal (Fig. 2)). This phenomenon will be reported in detail elsewhere (Davis, Yanuka, Illum and Khan, to be published). The bathochromic shift for adsorbed rose bengal occurs for two reasons: (i) a shift in the pK_a values of the adsorbed molecule; and (ii) a more coplanar conformation of rose bengal at the surface of the particle.

The adsorption of a rose bengal molecule to a charged interface will result in a shift in the pK_a value(s) of the molecule (Lukac, 1983). The direction of the shift and the magnitude is determined by the sign and magnitude of the charge on the particle. Polybutylcyanoacrylate nanoparticles carry a negative surface potential of approximately − 40 mV (Douglas et al., 1985). This charge is expected to change the highest pK_a value of adsorbed rose bengal from 3.7 (bulk) to about 4.5 (surface) (Lukac, 1983). Therefore, in order to measure the bathochromic shift associated only with the conformational change for rose bengal molecules adsorbed on the surface of the nanoparticle, analytical measurements were made at pH 7, a value well above both the bulk and surface pK_a values. At this pH the absorption spectrum of the mixed nanoparticle–rose bengal system will consist of three components: (i) scatter due to suspended nanoparticles; (ii) free rose bengal; and (iii) bound rose bengal. The nanoparticle scatter can be eliminated using the appropriate dilution of the nanoparticle suspension as the reference in a double beam spectrophotometer. Thus we can write:

$$A_{\lambda_1} = C_F(\epsilon_F)_{\lambda_1} + C_B(\epsilon_B)_{\lambda_1}$$

$$A_{\lambda_2} = C_F(\epsilon_F)_{\lambda_2} + C_B(\epsilon_B)_{\lambda_2}$$

where C_F is the free and C_B is the bound rose bengal concentration; $(\epsilon_F)_{\lambda_1}$ and $(\epsilon_F)_{\lambda_2}$ are the molar absorption coefficients of free rose bengal at a concentration C_F at the analytical wavelengths λ_1 and λ_2; and $(\epsilon_B)_{\lambda_1}$ and $(\epsilon_B)_{\lambda_2}$ are the molar absorption coefficients of bound rose bengal at a concentration C_B at the analytical wavelengths λ_1 and λ_2. λ_1 (560 nm) and λ_2 (528 nm) were chosen to give the largest difference in ab-

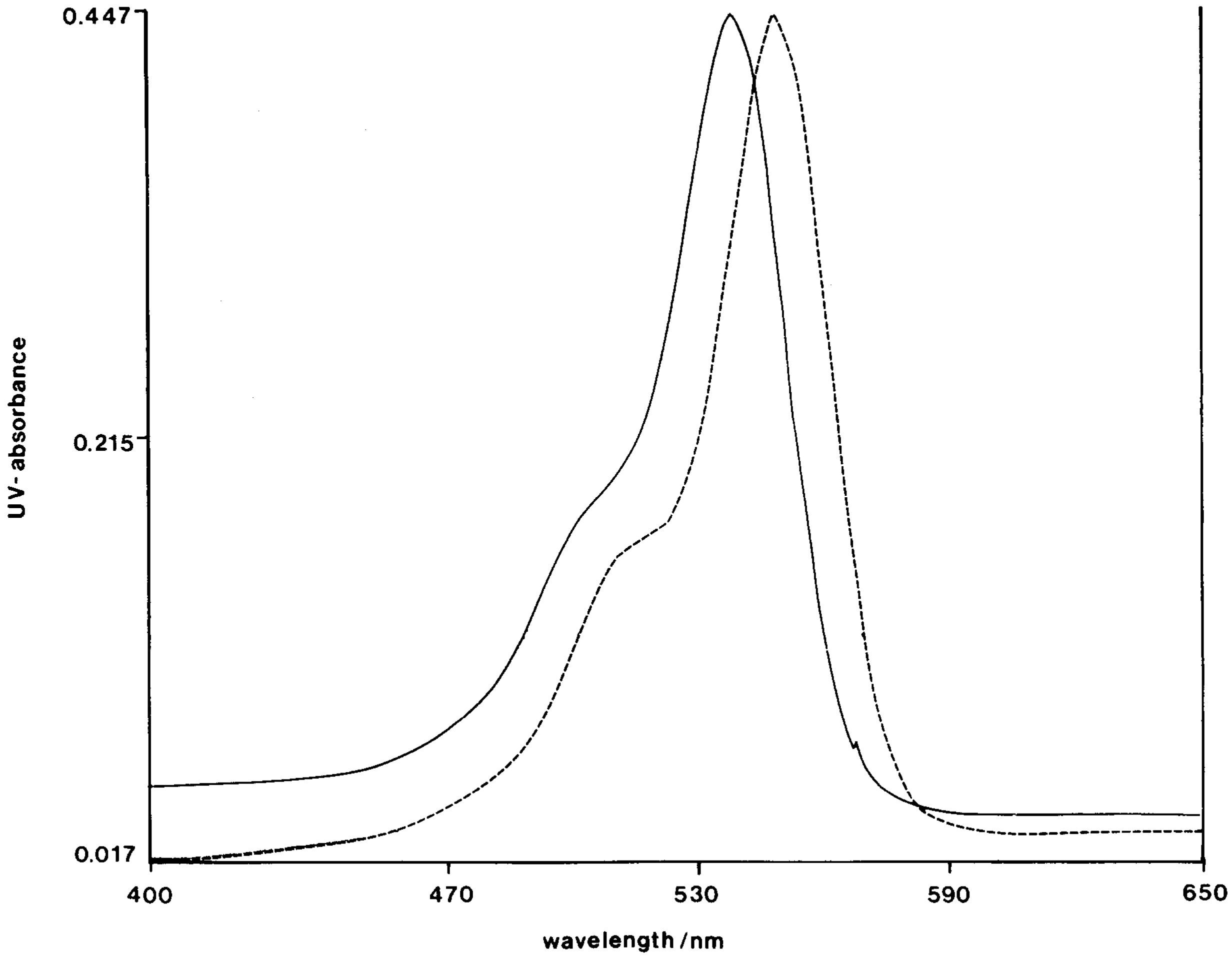

Fig. 2. Bathochromic shift in the absorption spectrum of rose bengal free and bound onto poly(butyl 2-cyanoacrylate) nanoparticles. ———, rose bengal in phosphate buffer pH 7.0 (peak at 540 nm); - - - -, rose bengal adsorbed onto nanoparticles (peak at 548 nm).

sorption between the two spectra. $(\epsilon_F)_{\lambda_1}$ and $(\epsilon_B)_{\lambda_1}$ were found to be 14698 $mol^{-1} \cdot l \cdot cm^{-1}$ and 36680 $mol^{-1} \cdot l \cdot cm^{-1}$, respectively at 560 nm and $(\epsilon_F)_{\lambda_2}$ and $(\epsilon_B)_{\lambda_2}$ were found to be 32664 $mol^{-1} \cdot l \cdot cm^{-1}$ and 22427 $mol^{-1} \cdot l \cdot cm^{-1}$, respectively, at 528 nm.

Thus for studying the adsorption and incorporation of rose bengal with nanoparticles the absorbances of the dilute nanoparticle–rose bengal systems were determined at 528 nm and 560 nm using a UVikon UV-visible spectrophotometer interfaced to a Commodore PET microprocessor (Commodore Business Machines, Santa Clara, U.S.A.). The results are expressed as concentration of free and nanoparticle-bound rose bengal. For the release studies similar measurements were performed using the computerized Kontron UV-visible spectrophotometer.

Fluorescence spectroscopy. A novel approach to a fluorescence spectroscopy method was used to determine the adsorption isotherm and incorporation of adriamycin within nanoparticles as well as the relevant release profiles. Using this method it was possible to determine the free and bound adriamycin without a prior separation process. When the emission spectra from various concentrations of adriamycin added to nanoparticle suspensions were compared with the emission spectra of supernatants from identical centrifuged mixtures the ratios were found to be 1.09 ± 0.08

(S.D.). Thus, the results indicate that once the adriamycin molecules are adsorbed onto the nanoparticles they lose their ability to fluoresce and hence when measuring an adriamycin–nanoparticle system the results directly express the concentration of free adriamycin.

The studies were performed using a Perkin-Elmer 3000 fluorescence spectrophotometer (Perkin-Elmer, Beaconsfield, U.K.), with $\lambda_{excitation}$ = 480 nm and $\lambda_{emission}$ = 558, both slits at 5 nm and a path length of 10 mm. A nanoparticle suspension of 0.005% v/v was used as the reference to correct for scatter from the particles.

Results

Particle size analysis

The z-average particle diameters of the poly(butyl 2-cyanoacrylate) nanoparticles stabilized with dextran 70 or poloxamer 338 with no material incorporated or adsorbed were found to be 130 nm (Q = 0.062) and 71 nm (Q = 0.056), respectively.

The carrier capacity of nanoparticles for rose bengal

Adsorption

The isotherms for the adsorption of rose bengal at pH 7.0 onto poly(butyl 2-cyanoacrylate) nanoparticles stabilized with dextran 70 are shown in Fig. 3a and b. The data obtained using the bathochromic shift method based on UV-visible spectroscopy are in very good agreement with the data obtained using the conventional centrifugation method. The isotherm obtained for rose bengal adsorbed onto nanoparticles stabilized by poloxamer 338 was essentially identical to that found for nanoparticles stabilized by dextran 70.

The data were fitted to the Langmuir isotherm equation in its linear form:

$$c/(x/m) = 1/ab + c/a$$

where x is the amount of solute adsorbed by a weight, m of adsorbent, c is the concentration of solution at equilibrium, b is a constant related to the enthalpy of adsorption and a is related to the surface area of the solid. Values of a and b were determined from the intercept and slope of a plot of c/(x/m) against equilibrium concentration (c) for the isotherms and mean values (n = 3) for a, b were found to be 53.03 μmol/g and 0.160 l/μmol, respectively. Correlation coefficients in the range 0.9950–0.9966 showed that the derived isotherms were indeed Langmuirian in nature.

The maximum carrier capacity for both dextran 70 and poloxamer 338 stabilized nanoparticles was found to be in the order of 5 μmol/100 mg nanoparticles (5 mg/100 mg nanoparticles).

The pH profile for the adsorption of rose bengal onto dextran stabilized nanoparticles was S-shaped (Fig. 4). This shows that the degree of ionization of the rose bengal molecule (hydroxyl group, $pK_a \sim 3.7$) influences the amount of rose bengal adsorbed onto the surface of the nanoparticles. The less ionized the molecule the higher the amount of solute adsorbed.

Incorporation

When rose bengal is incorporated into nanoparticles stabilized either by dextran 70 or poloxamer 338 the size and the polydispersity of the

TABLE 1

THE SIZE AND POLYDISPERSITY OF POLY(BUTYL 2-CYANOACRYLATE) NANOPARTICLES AFTER INCORPORATION OF DIFFERENT AMOUNTS OF ROSE BENGAL

Initial rose bengal concentration (μg/ml)	mg of rose bengal incorporated/100 mg NP		Nanoparticle diameter (nm) (polydispersity) (z-average)	
	Dextran 70 Stab.	Poloxamer 338 Stab.	Dextran 70 Stab.	Poloxamer 338 Stab.
0	0	0	130.1 (0.062)	71.0 (0.056)
100	1.0	1.0	131.5 (0.089)	88.0 (0.018)
500	5.0	3.9	145.5 (0.103)	88.0 (0.109)
1000	10.0	5.8	158.0 (0.138)	92.0 (0.209)
2000	19.8	8.6	205.0 (0.371)	123.5 (0.356)

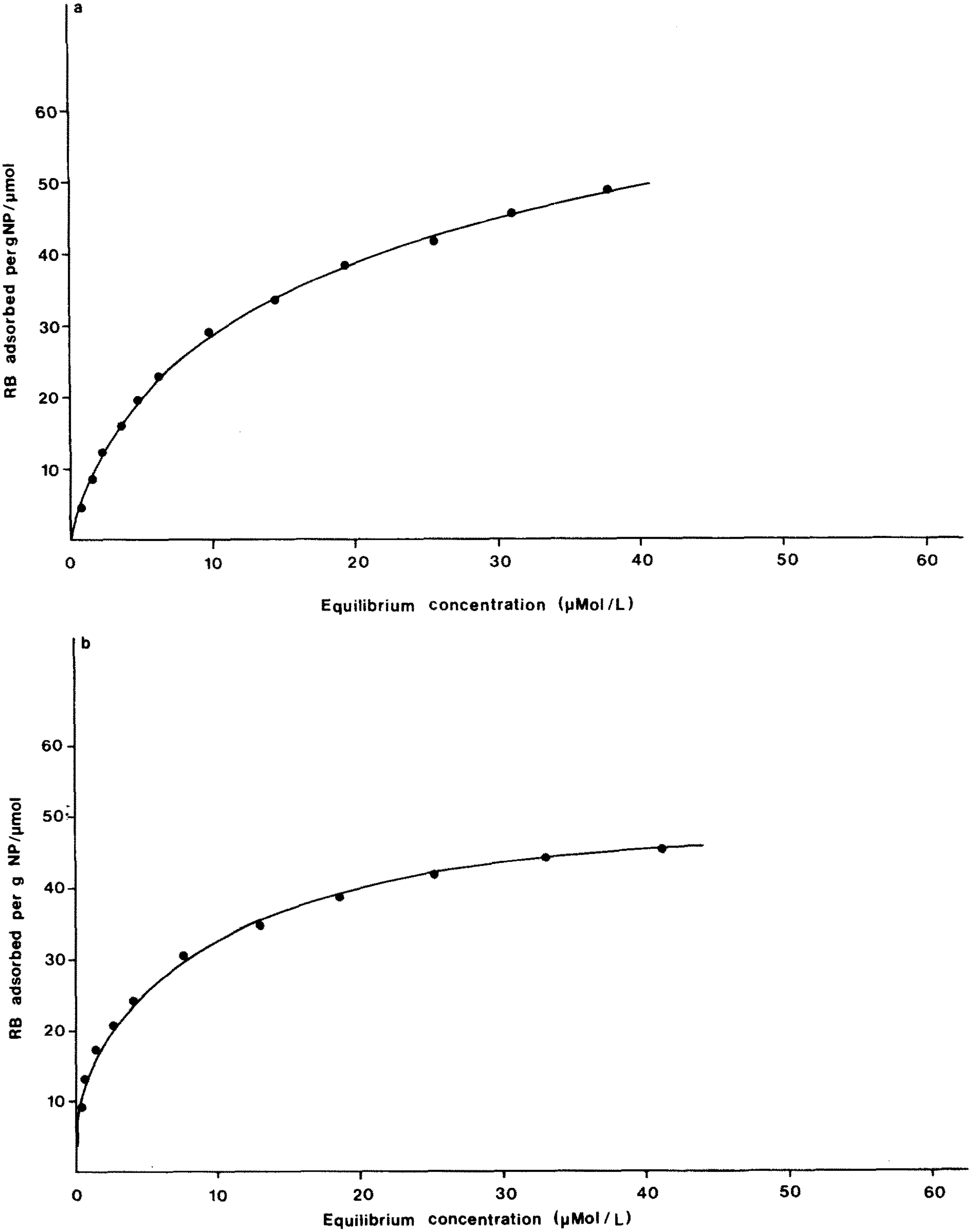

Fig. 3. Adsorption isotherms for the adsorption of rose bengal onto dextran 70-stabilized poly(butyl 2-cyanoacrylate) nanoparticles at pH 7.0. The data were obtained using the centrifugation method (a) and the bathochromic shift method (b) (n = 2).

particles increase for larger amounts of rose bengal incorporated (Table 1). More importantly, the incorporation of rose bengal into the nanoparticles during the polymerization process leads to a much greater carrier capacity than found for the simple adsorption method. Dextran 70 stabilized nanoparticles could be loaded with 37.3 μmol/100 mg nanoparticles (38 mg/100 mg nanoparticles) and poloxamer 338 stabilized nanoparticles could be loaded with 10.8 μmol/100 mg nanoparticles (11 mg/100 mg nanoparticles) (Fig. 5).

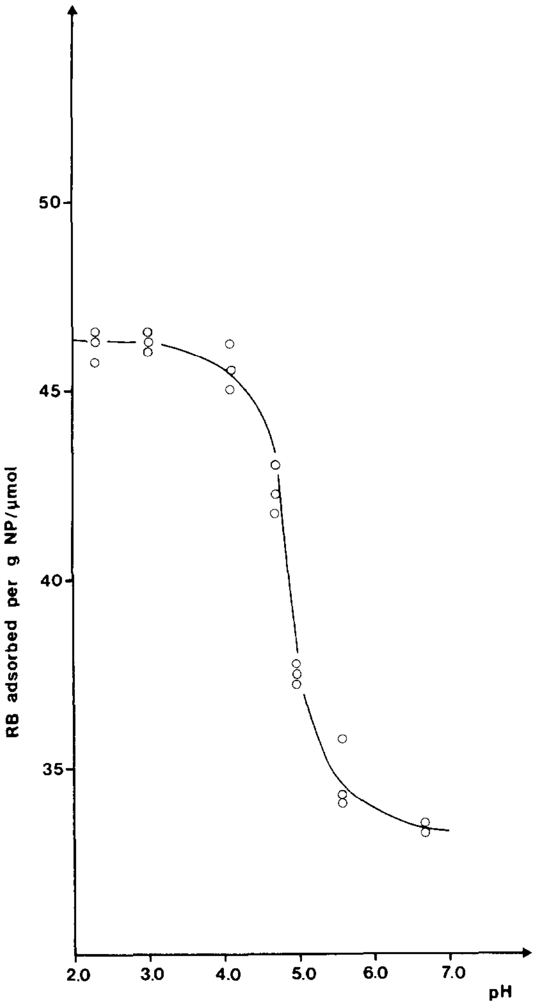

Fig. 4. The pH-profile for the adsorption of rose bengal onto dextran 70-stabilized poly(butyl 2-cyanoacrylate) nanoparticles (n = 2).

The maximum initial concentration of rose bengal that could be added during the polymerisation process such that the particles would maintain their unloaded size (130 nm and 71 nm, respectively, for the dextran 70 and poloxamer 338 stabilized systems) and an acceptable polydispersity (Q ≤ 0.2) was 1000 μg/ml for a 1% v/v monomer solution using either stabilizer. For dextran 70 stabilized particles this size and polydispersity requirement will result in a carrier capacity of 10 mg rose bengal/100 mg nanoparticles.

Fig. 6 shows the amount of rose bengal incorporated into dextran 70 stabilized and poloxamer 338 stabilized nanoparticles expressed as percentage of the initial rose bengal concentration. The dramatic effect of the choice of stabilizing material is clearly demonstrated. For dextran stabilized nanoparticles more than 90% incorpora-

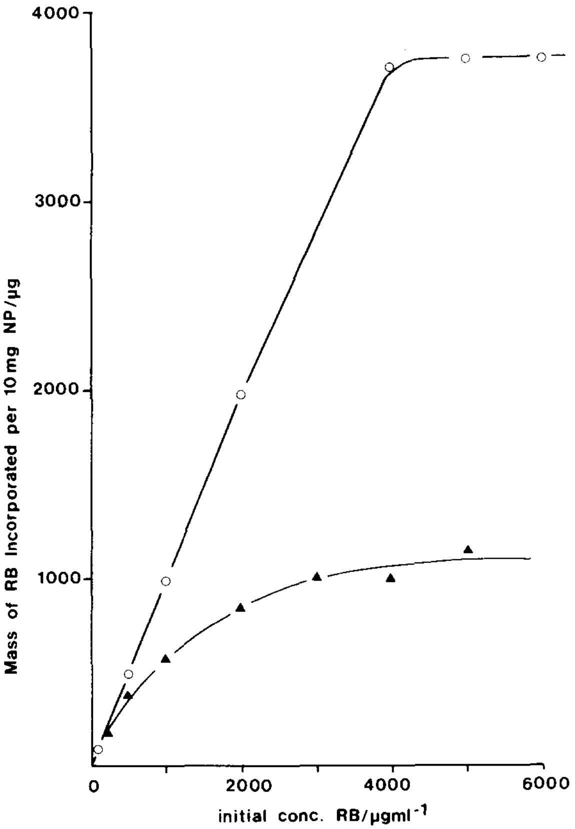

Fig. 5. The incorporation of rose bengal into poly(butyl 2-cyanoacrylate) nanoparticles stabilized with ○———○ dextran 70 and ▲———▲ poloxamer 338 (n = 2).

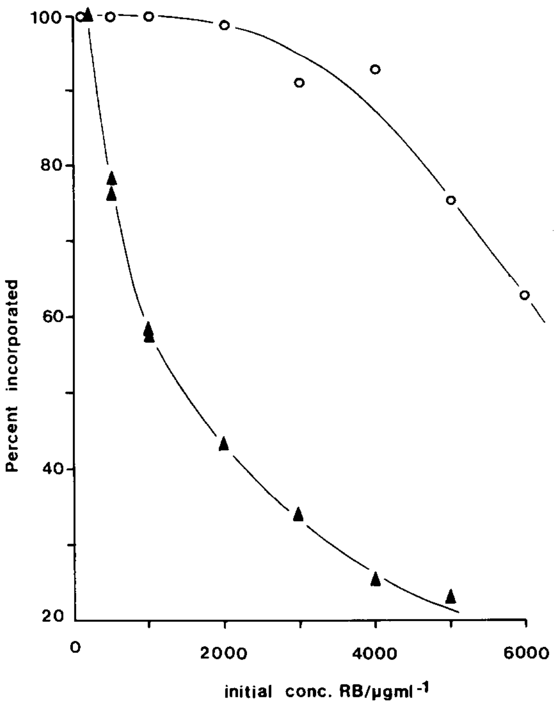

Fig. 6. The amount of rose bengal incorporated into poly(butyl 2-cyanoacrylate) nanoparticles stabilized with ○——○ dextran 70 and ▲——▲ poloxamer 338. The data are expressed as percentage of initial rose bengal concentration (n = 2).

tion can be achieved for the initial drug concentrations up to 4 mg/ml while for poloxamer 338 high incorporation can only be achieved at low initial concentration of rose bengal.

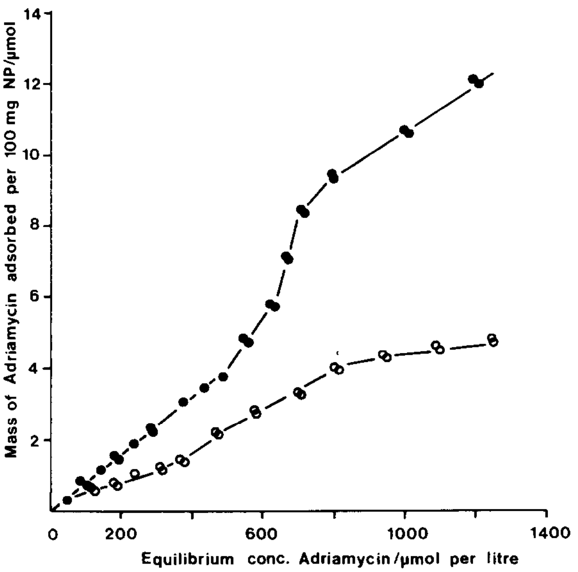

Fig. 7. The adsorption isotherms for the adsorption of adriamycin (at pH 7.0) onto poly(butyl 2-cyanoacrylate) nanoparticles stabilized with ●——●, dextran 70 and ○——○, poloxamer 338 (n = 2).

The carrier capacity of nanoparticles for adriamycin

The adsorption of adriamycin onto poly(butyl 2-cyanoacrylate) nanoparticles stabilized with dextran 70 and poloxamer 338 follows S-shaped isotherms at pH 7.0 as shown in Fig. 7. The process of incorporating adriamycin into dextran 70 stabilized nanoparticles was found to be very inefficient; for an initial concentration of 1000 µg/ml (1.72 mmol/l) only about 25% was found to be

TABLE 2

THE TIME FOR 50% RELEASE INTO PHOSPHATE BUFFER (pH 7.4) OF ROSE BENGAL ($T_{50\%}$) ADSORBED ONTO OR INCORPORATED INTO POLY(BUTYL 2-CYANOACRYLATE) NANOPARTICLES STABILIZED WITH DEXTRAN 70 (Dex-NP) OR POLOXAMER 338 (Pol-NP) (n = 2)

	Amount of RB/100 mg nanoparticles (mg)	$T_{50\%}$ (min)	
		Dex-NP	Pol-NP
Rose bengal incorporated	2.0	54.5 (±0.5)	47.0 (± < 0.05)
	4.0	–	56.5 (±0.5)
	5.0	76.5 (±1.5)	–
	8.0	99.0 (±1.0)	–
	8.0 *	6.0 *	–
	10.0	121.0 (±1.0)	
Rose bengal adsorbed	3.1	6.0 (± < 0.05)	–
	2.8	–	2.5 (± < 0.05)

* Release medium containing 0.02% w/v poloxamer 338.

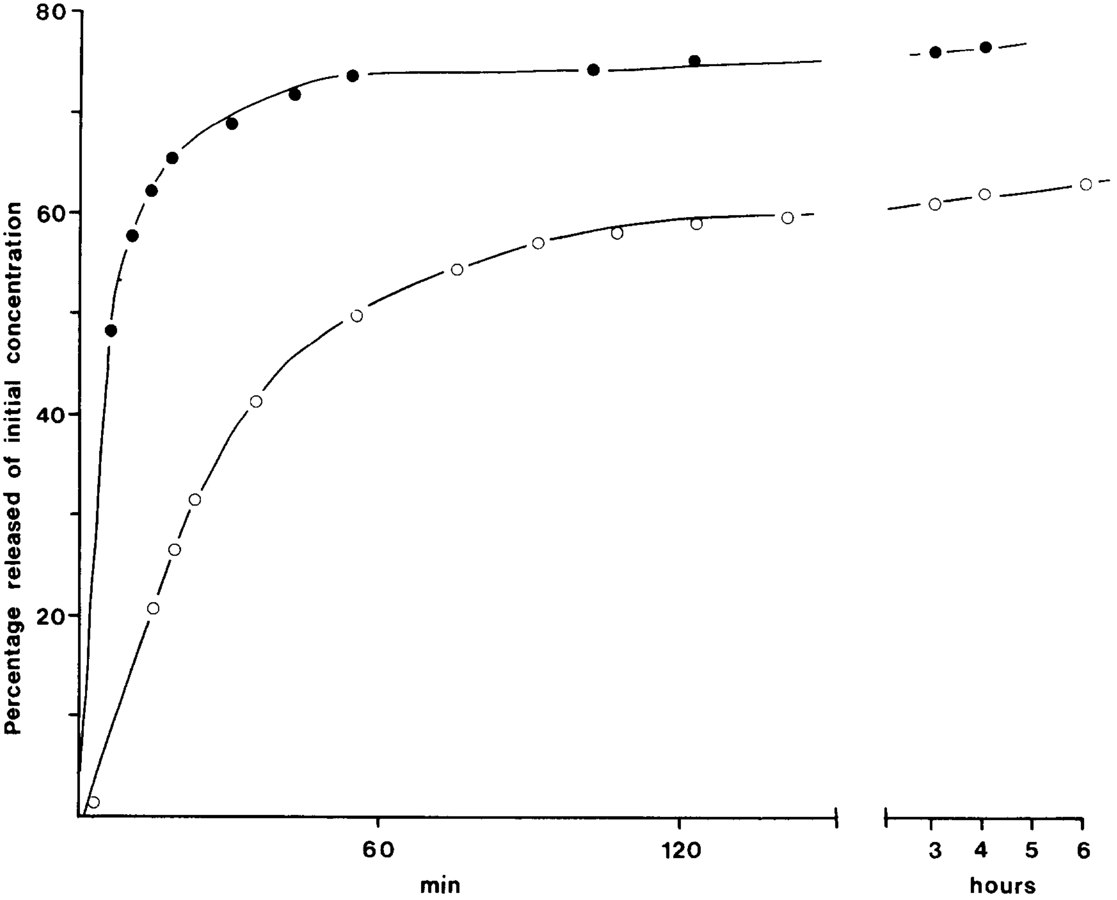

Fig. 8. The release profiles for rose bengal (RB) adsorbed onto (3.1 mg RB/100 mg NP), ●——●, and incorporated into (2.0 mg RB/100 mg NP), ○——○, poly(butyl 2-cyanoacrylate) nanoparticles (NP) stabilized with dextran 70 (n = 2).

incorporated compared to 100% for the same initial concentration of rose bengal.

The release of solutes from nanoparticles

The release of rose bengal from dextran 70 stabilized nanoparticles, after both adsorption and incorporation, is characteristically biphasic with an initial fast release phase followed by a second much slower first-order release phase. Typical release profiles are shown in Fig. 8 for adsorption–release and incorporation–release processes.

The release profiles can be described using a simple biexponential function

$$P_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where P_t is the concentration of compound remaining in the nanoparticle at time t; the constants A and B and the two rate constants α and β (obtainable from a semilogarithmic plot) can be used to characterize the release of the compound. (For adsorption $\alpha = 3.6\ \text{min}^{-1}$, $\beta = 1.1\ \text{h}^{-1}$ and for incorporation $\alpha = 1.2\ \text{min}^{-1}$ and $\beta = 1.1\ \text{h}^{-1}$.)

The initial release rate is faster for adsorption–release than for incorporation–release and a higher extent of release is attained before the initiation of the second slow release phase. The time for release of 50% of the initial concentration ($T_{50\%}$) is shown in Table 2 for rose bengal adsorbed onto or incorporated into nanoparticles stabilized with either dextran 70 or poloxamer 338. $T_{50\%}$ increased with increasing amounts of rose bengal incorporated into nanoparticles stabilized with either dextran or polo-

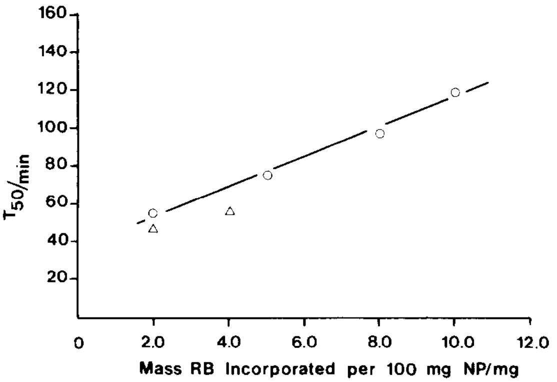

Fig. 9. The correlation between the time for 50% release and the amount of rose bengal incorporated into 100 mg of poly(butyl 2-cyanoacrylate) nanoparticles stabilized with ○——○ dextran 70 and △——△ poloxamer 338 (n = 2).

xamer, the release rate being higher for the latter stabilizer for the same amounts incorporated. Also for adsorbed rose bengal the rate of release was higher for poloxamer stabilized nanoparticles. When poloxamer 338 was added to the release medium in a concentration of 0.02% the time for 50% release of rose bengal adsorbed onto dextran 70 stabilized nanoparticles, decreased from 99 min to 6 min. A linear correlation ($r = 0.995$) was found between the time for 50% release ($T_{50\%}$) and the amount of rose bengal incorporated per 100 mg dextran 70 stabilized nanoparticles (Fig. 9).

Discussion

The results show clearly that there is a great difference in the carrier capacity of poly(butyl 2-cyanoacrylate) nanoparticles depending whether the loading is performed by an adsorption or an incorporation process. Previously, in the literature other authors have often failed to make this distinction and have referred to adsorption of a drug when the actual process of loading is by incorporation or vice versa (Couvreur et al., 1979a and b). When rose bengal is incorporated into dextran stabilized nanoparticles rather than being adsorbed an 8-fold higher maximum loading capacity is obtained.

The adsorption of rose bengal onto nanoparticles stabilized with dextran 70 or poloxamer 338 follows a Langmuirian type of isotherm (Fig. 3a and b). The initial curvature in the Langmuirian type of isotherm indicates how that as more sites on the surface are filled, it becomes increasingly difficult for a rose bengal molecule to find an available site. This could be due to either an orientation or solvent effect (Giles and Smith, 1974). However, as a bathochromic shift is seen in the absorption spectra for rose bengal adsorbed onto nanoparticles, it can be concluded that the rose bengal molecules are adsorbed flat on the surface (Davis et al., to be published).

The adsorption of rose bengal onto nanoparticles is affected by pH through the ionisation of the hydroxyl group in the molecule (Fig. 4). However, the change in adsorption in going from a pH of 2 to a pH of 7 is only about 30%.

The adsorption isotherms and the maximum carrier capacities for rose bengal for both dextran 70 stabilized and poloxamer 338 stabilized nanoparticles are essentially identical. In contrast, there are great differences between the maximum carrier capacities of the two stabilized systems when rose bengal is incorporated during the polymerization process. This difference can be readily explained by competitive solubilization of the rose bengal in micelles formed by the fraction of the poloxamer surfactant which is not incorporated (grafted) into the nanoparticles during polymerization. Poloxamers are known to form monomolecular micelles at low concentrations and multimolecular micellar aggregates at higher concentrations (Florence and Attwood, 1982). Furthermore, rose bengal in its non-ionized form (at pH 2.0) would therefore be expected to partition easily into the micelles. For the adsorption studies the nanoparticle systems were diluted upwards of a hundred times before the addition of rose bengal and consequently the solubilization effect of the surfactant was negligible. Furthermore rose bengal is in its ionized form at pH 7.0 and the affinity of the compound for the poloxamer micelles would be expected to be low.

The rose bengal, carried by the nanoparticles after an incorporation process, is believed mainly to be either dissolved, dispersed in, or adsorbed on the polymer matrix and the remainder to be adsorbed on the surface of the particles. In contrast, the rose bengal carried by nanoparticles after an adsorption process will solely occupy the external surface of the nanoparticles including minor pores and pits. This is supported by the fact that if a flat-on configuration is assumed for the rose bengal molecule, (surface area 0.98 nm^2 as calculated from crystallographic data assuming the molecule to be of an ellipsoid shape) it can be calculated that the number of molecules adsorbed at the maximum loading capacity for the adsorption process occupy an area equivalent to about 120% of the total surface area of the nanoparticles (5.3×10^4 nm^2 for the dextran stabilized and 1.5×10^4 nm^2 for the poloxamer-stabilized nanoparticles) in the system (assuming then to form a solid monodisperse system). Furthermore, the system where rose bengal is incorporated during the polymerization process, show an initial burst effect in release studies which probably reflects surface adsorbed rose bengal.

The data for adriamycin show a different type of behaviour. The initial convex curvatures of the S-shaped isotherms obtained for the adsorption of adriamycin onto the nanoparticle systems (Fig. 7) indicate that adsorption of the solute becomes easier as the concentration rises implying a side-by-side association between the adriamycin molecules (Giles and Smith, 1974). For aromatic solutes this normally means that the adsorbed molecules are oriented perpendicular to the surface. Calculations using the estimated total surface area of the nanoparticle system and for the adriamycin molecule (1.4 nm^2 calculated from crystallographic data assuming the molecule to be of an ellipsoid shape; Courseille et al., 1979) in a flat or end-on conformation at the surface show that the end-on conformation is more likely at the higher concentration since the surface of the nanoparticles would be completely covered with molecules orientated flat-on at a concentration of about 4 μmol/100 mg nanoparticles for the dextran stablized and at a concentration of about 1 μmol/100 mg nanoparticles for the poloxamer-stabilized nanoparticles.

Acknowledgement

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