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UPTAKE CAPACITY AND ADSORPTION ISOTHERMS OF DOXORUBICIN ON POLYMERIC NANOPARTICLES: EFFECT OF METHODS OF PREPARATION

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ABSTRACT

Nanoparticles made from biodegradable materials like polyalkylcyanoacrylates are being examined as drug delivery systems. The capacity of these particles to carry a drug can differ subject to the method of manufacture. We studied the adsorption and uptake of doxorubicin by nanoparticles when the drug was added before or after completion of formation of the isobutylcyanoacrylate nanoparticles. The uptake of the drug was measured by ultracentrifugation. The percent of the drug associated with the nanoparticles formed in the presence of the drug was twice that of the other method. However, the affinity of doxorubicin for nanoparticles of either method, determined by the Langmuir and Freundlich isotherms, was the same. The release study indicated that only 4 to 8% of the drug is released in vitro under sink condition. This may suggest that the drug adsorbs strongly on the surface and also associates with the matrix of the nanoparticles.

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INTRODUCTION

Nanoparticles are colloidal particles ranging in size from ten to a thousand nanometers in diameter(1). They have been manufactured from polymers such as polyalkylcyanoacrylates, polymethylmethacrylate(2), polyvinylpyridine(3), polygluteraldehyde, polyacrylamides(4), and from macromolecules like gelatin(5) and human serum albumin(6).

Alkylcyanoacrylates are a group of rapidly polymerizing(7), biodegradable(8) monomers that are used as sutures and in surgical implants(9). Their mixtures, used extensively in oral and general surgery to stop severe bleeding, have a maximum polymerization time of about 3 seconds(7). Nanoparticles of polyalkylcyanoacrylates were developed by Couvreur and coworkers(10). The nanoparticles have been tested as delivery systems for ophthalmic drugs(11), antibiotics(12), anticholinergics(13) and anticancer agents(10). The rate of degradation of such nanoparticles has been shown to be a factor of the length of the monomer(14).

Drugs can be incorporated into the nanoparticles during the process of polymerization or adsorbed on the surface of the nanoparticles previously formed and in suspension(15). Since the sequence of addition of a drug can affect the amount of drug associated with the nanoparticles, we investigated its effect on the uptake and release of doxorubicin from the nanoparticles.

Doxorubicin is an anthracycline antibiotic effective against a large spectrum of malignancies. Its therapy is limited by its cumulative dose dependent cardiotoxicity(16) and a limitation on high peak plasma concentrations(17). This restrictive dosing regimen makes doxorubicin an attractive candidate to be delivered in a sustained release manner. The small size and biodegradable nature of the polyalkylcyanoacrylate nanoparticles

make them promising carriers for parenteral and sustained release injections or as a site specific delivery system for cytotoxic drugs like doxorubicin.

MATERIALS AND METHODS

Preparation of Nanoparticles Associated with Doxorubicin

Polybutylcyanoacrylate nanoparticles were prepared as described by Couvreur and coworkers(10). Briefly, the nanoparticles of alkylcyanoacrylates are prepared by an aqueous anionic polymerization at a low pH in the presence of a steric stabilizing agent(18). The polymerizing medium was 1% citric acid solution with 1% dextran 70 as the stabilizing agent. Isobutylcyanoacrylate (Sigma Chemical Co., St. Louis, MO), 100μl, was added to the polymerizing medium and stirred at 2000 rpm.

Method 1: The drug, doxorubicin HCl (Sigma Chemical Co., St. Louis, MO), was dissolved in the polymerizing mixture before the monomer was added. The amount of drug added was in the range 0.1 to 10 mg. The mixture was stirred for four hours. Polymerization of the monomer is quick due to its vulnerability to nucleophilic attack from the anionic hydroxyl ions in the polymerizing mixture. It has also been suggested that doxorubicin may be involved as an initiator in the polymerization process(19).

Method 2: The monomer was added to the polymerizing mixture, which contained no drug, and was stirred for two hours. The drug, dissolved in a minimal volume of the polymerizing mixture, was added and the mixture stirred for another two hours.

Scanning electron microscopy was used to determine the shape and size of the particles. The micrographs from both preparations were similar and showed uniformly formed particles with an average diameter of 100 nm (Fig. 1).

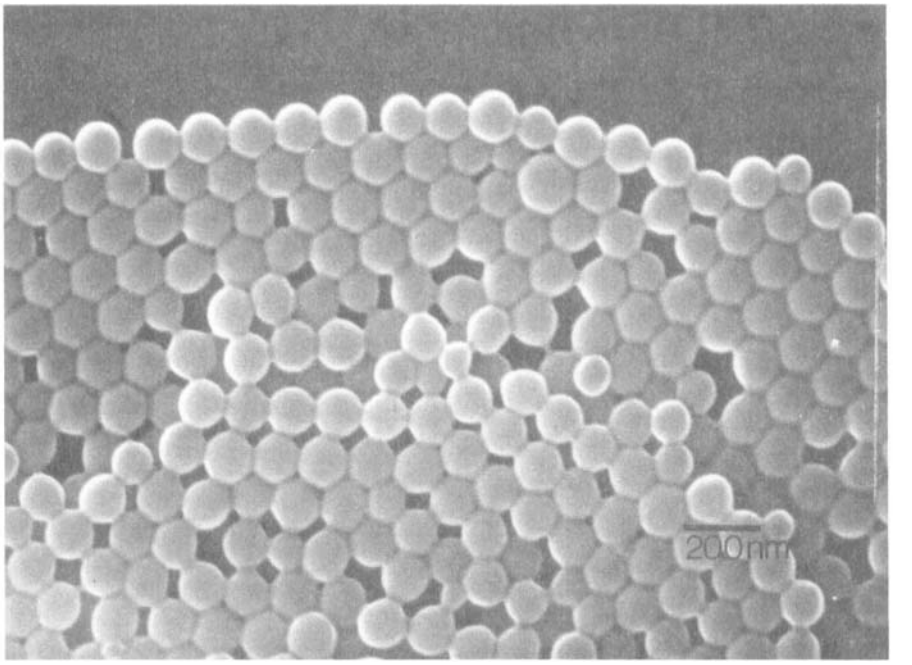

FIGURE 1
Scanning electron micrograph of isobutylcyanoacrylate nanoparticles.

Uptake Studies

The nanoparticle suspensions were centrifuged for 30 min at 100,000 g. The supernatants were separated, diluted and analyzed by HPLC. The chromatographic system consisted of a Waters C18 Novapak cartridge, Waters 6000A solvent delivery system and a Gilson 121 fluorometer. The mobile phase was methanol: ammonium formate buffer pH 4.0 (70:30), pumped at a flow rate of 2 ml/min. The solvents used were of HPLC grade. The detector excitation and emission wavelengths were set at 470 nm and 540 nm, respectively.

The amount of drug associated with the nanoparticles was determined from the difference in the initial

amount added and the free amount in the supernatant. The nanoparticle pellets were dried and weighed to determine the weight of the nanoparticles. The experiment was carried out at 25°C and repeated four times for each data point. Actual weight of the product was too small to display any significant increase in the weight due to the adsorption of doxorubicin.

Release Studies

The nanoparticles prepared with both methods were suspended in pH 7.0 phosphate buffer and at various time points the mixture was centrifuged and the supernatant was completely removed. The pellet was resuspended in fresh buffer. The samples were then analyzed by HPLC.

RESULTS AND DISCUSSION

Surface energy of solids reveals itself in many phenomena of which adsorption and wetting are the most important(20). The Freundlich and Langmuir adsorption isotherms are a popular approach for representation of adsorption data. These isotherms are a function of the amount of drug adsorbed on the adsorbent to the amount of drug remaining in solution at equilibrium(21).

In the two methods utilized to prepare the nanoparticles, the amount of drug (x) associated with unit weight of nanoparticles (m) increased as the drug concentration at equilibrium increased (Fig. 2). The plateau levels of the adsorption isotherms for the two methods were 80 μg and 40 μg of doxorubicin per mg of nanoparticles for method 1 and 2, respectively. Percent of the drug associated with the nanoparticles was 50 to 70 % of the initial amount for method 1 and 25 to 35 % for method 2.

The Langmuir isotherm assumes uniform adsorption sites and the adsorption is limited to a monolayer(22).

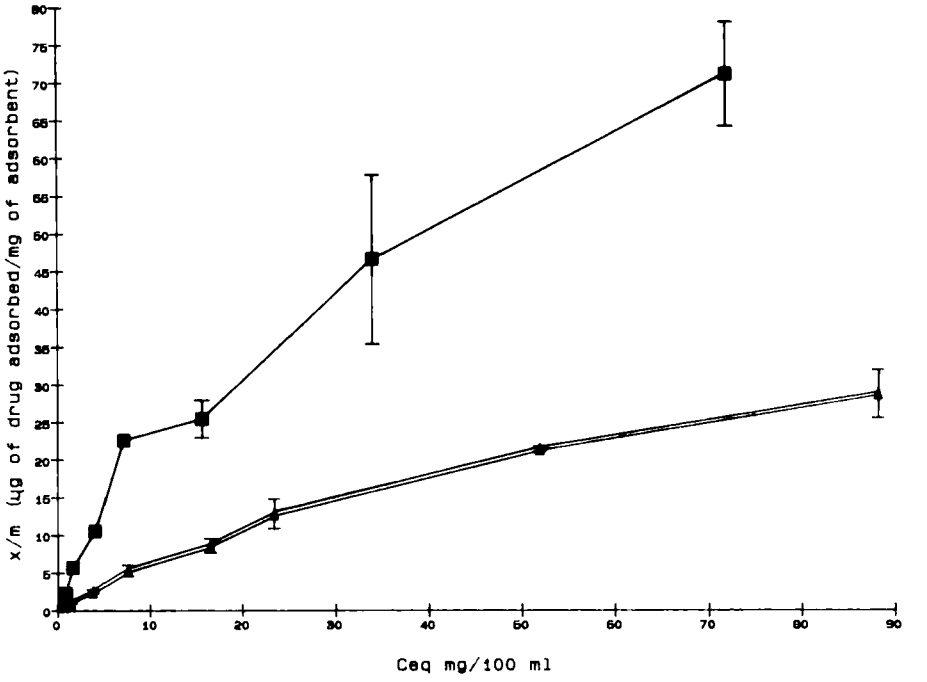

FIGURE 2
Adsorption isotherms of doxorubicin on nanoparticles prepared by method 1 (-■-) and method 2 (-▲-).

The linear form of the isotherm is:

$$C_{eq}/\ x/m = 1/\ k_1*k_2 + C_{eq}/\ k_2$$

where C_{eq} is the concentration of the drug remaining in the supernatant, in mg/100 ml, after equilibrium is obtained, x/m is the amount of drug adsorbed per mass of nanoparticles, and k_1 and k_2 are constants. The constant k_1*k_2 is a measure of the relative affinity of the adsorbate for the adsorbent while k_2 is the maximum amount of adsorbate that can be adsorbed by unit mass of adsorbent.

The uptake data from both methods of preparation gave a good fit with Langmuir equation (r^2 = 0.87 for

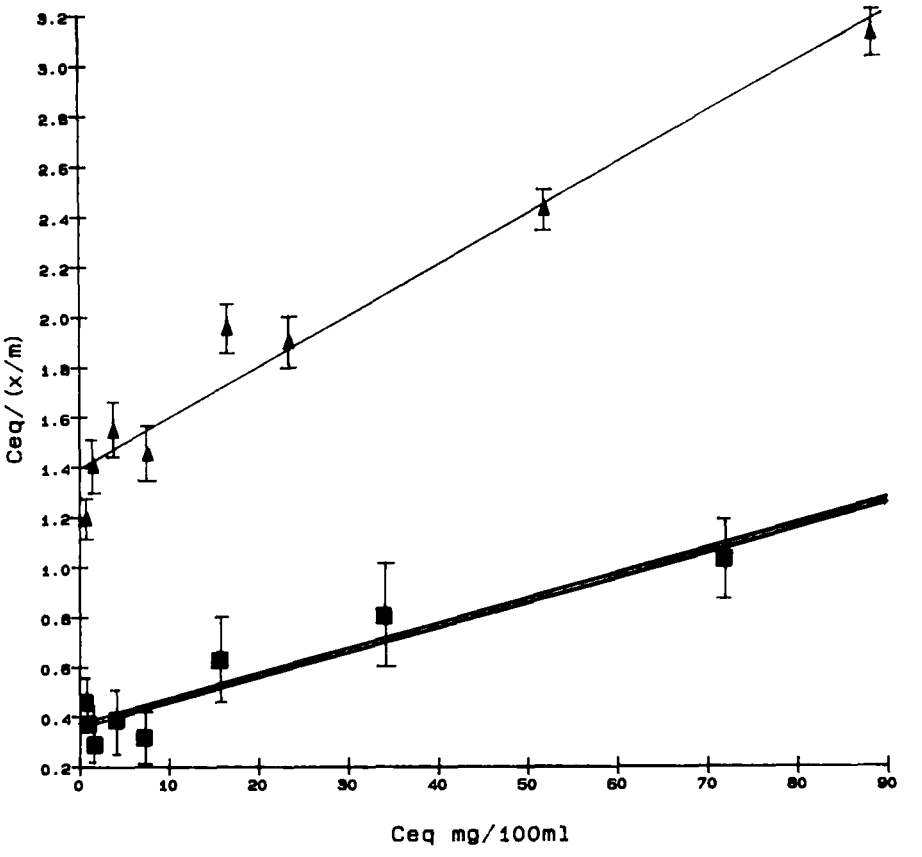

FIGURE 3
Langmuir isotherm of adsorption of doxorubicin on nanoparticles prepared by method 1 (-■-) and method 2 (-▲-).

method 1 and 0.97 for method 2) (Fig. 3). The maximum adsorption capacity for the isotherms were 100.10 μg and 49.50 μg doxorubicin per mg of nanoparticles for methods 1 and 2, respectively (Table 1). Adsorbent efficiency is dependent on the specific surface area of the adsorbent and the affinity between the adsorbent and adsorbate(20). The difference in the uptake can be explained by comparing these two factors. In the polymerizing mixture, the

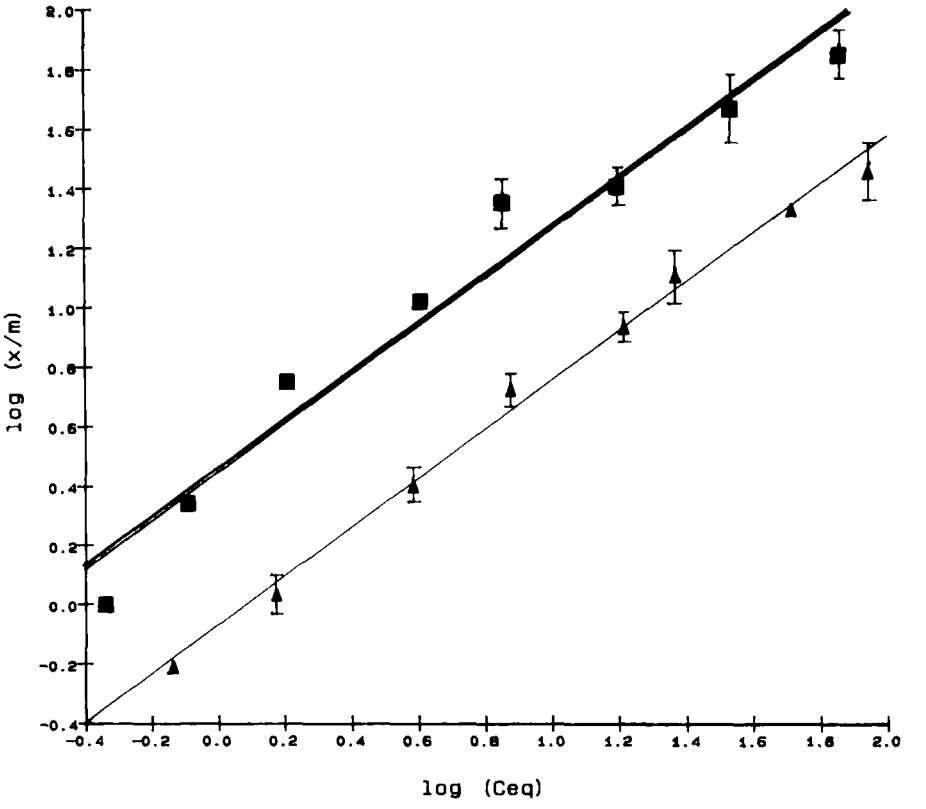

FIGURE 4
Freundlich isotherm of adsorption of doxorubicin on nanoparticles prepared by method 1 (-■-) and method 2 (-▲-).

nucleophilic attack on the monomer, isobutylcyanoacrylate, produces carbanions. These react with other monomer molecules to form oligomeric chains which in turn nucleate to form nanoparticles. Assuming doxorubicin interacts instantaneously, the molecules of the drug in the solution of method 1 would interact first with the forming polymer chains and later with the surface of the nanoparticles. However, in method 2, nanoparticle formation is complete before addition of doxorubicin,

TABLE 1
Constants from Langmuir and Freundlich Isotherms

		Method 1	Method 2
Langmuir isotherm			
Affinity constant	k_1*k_2	2.73	0.72
Maximum capacity μg/mg	k_2	100.10	49.50
	r^2	0.87	0.96
Freundlich isotherm			
Adsorptive capacity	K	2.85	0.84
Affinity constant	N	0.816	0.823
	r^2	0.96	0.99

leaving only the surface of the formed nanoparticles available for adsorption.

The Freundlich equation in the linear form is:

$$\log x/m = \log K + N \log C_{eq}$$

where C_{eq} and x/m are as defined earlier, and K and N are constants. The y-intercept is an estimate of the adsorbent capacity, and the slope represents the intensity of adsorption. If a set of data fits the equation it is likely, but not necessarily proven, that the surface of the adsorbent is heterogeneous(22).

The uptake data from both methods of preparation gave a good fit with Freundlich equation (r^2 = 0.96 for method 1 and 0.99 for method 2) (Fig. 4). The slopes for the isotherms were similar (Table 1), 0.816 for method 1 and 0.823 for method 2, indicating that the affinity of the drug for the nanoparticles is similar. The difference in the capacity of the adsorption can be attributed to the differences in the surface area available for the adsorption.

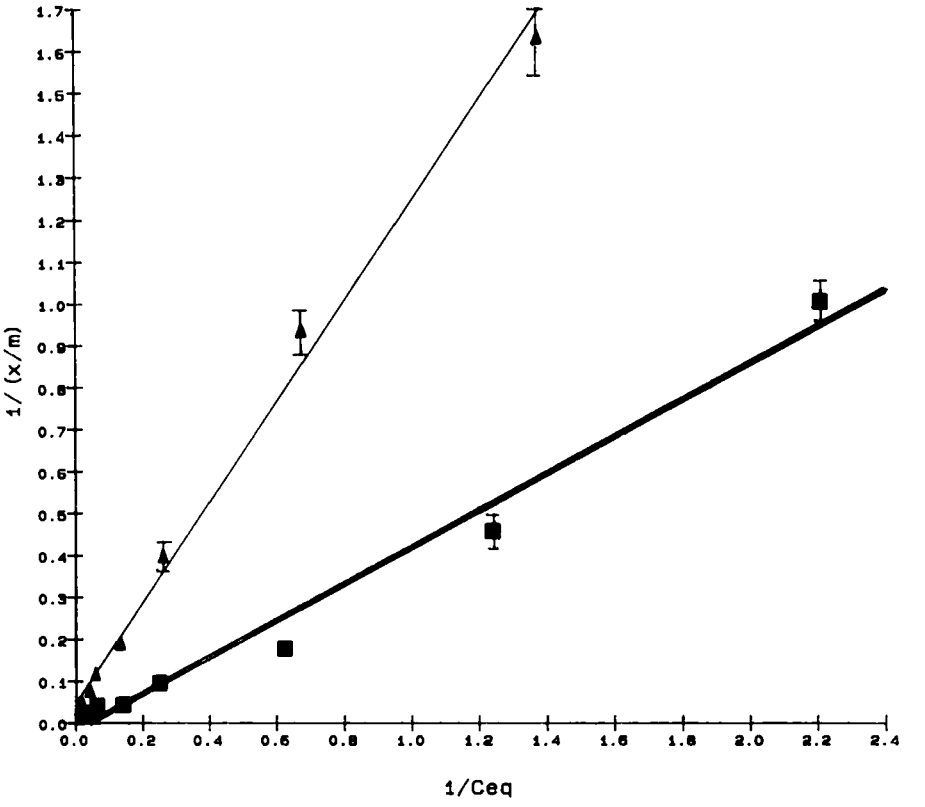

FIGURE 5
Double reciprocal plot of adsorption of doxorubicin on nanoparticles prepared by method 1 (-■-) and method 2 (-▲-).

The goodness of the fit to Langmuir or Freundlich type of isotherm was based solely on the comparison of r^2 values obtained from the linear plots using linear regression analysis of the respective isotherms. The data showed a poor fit with BET type of isotherms and a good fit to the double reciprocal equation, plotted as 1/(x/m) vs $1/C_{eq}$ (Fig. 5).

The increase in the uptake of method 1 may suggest that the drug associates with the matrix of the nanop-

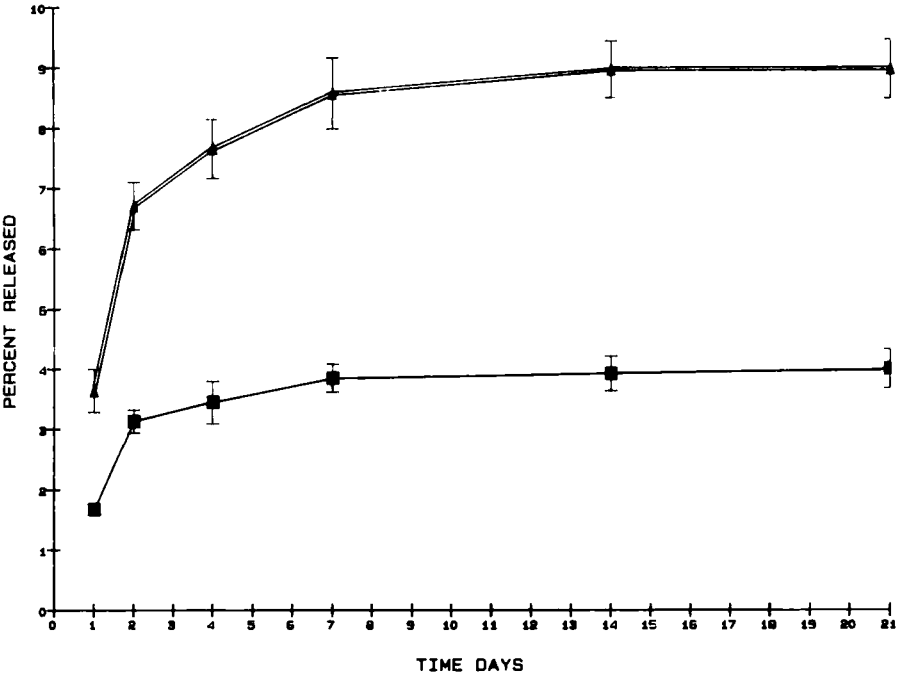

FIGURE 6
Percent of doxorubicin released versus time from nanoparticles prepared by method 1 (-■-) and method 2 (-▲-).

articles that is accessible to the drug during the formation of nanoparticles. The Langmuir isotherms indicate a greater uptake capacity for the nanoparticles of method 1 while the Freundlich isotherms suggest that the affinity of the drug for the nanoparticles of both methods is similar. Therefore, it can be concluded that the drug is strongly adsorbed on the surface and to the surface of the oligomeric chains in the matrix.

The release study, carried out under sink conditions, is presented in Figure 6. The amount of the drug released from nanoparticles was 4% of the amount of the drug adsorbed to the nanoparticles for method 1 and 8%

for method 2. The first order rate constant for release of doxorubicin from the nanoparticles was 0.380 day^{-1} and 0.384 day^{-1} for method 1 and 2, respectively. The release and the adsorption isotherms may suggest that the drug adsorbs rather strongly, for example, with chemical adsorption to the nanoparticles.

The polymers are known to be biodegradable hence erosion would first occur at the surface releasing the drug there earlier than the drug associated with the matrix. This would in turn suggest that the release of doxorubicin from the nanoparticles of method 1 would be in a sustained manner and dependent on the rate of the erosion of the particles.

ACKNOWLEDGEMENT

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