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INVESTIGATION OF THE RELATIVE AFFINITY OF DOXORUBICIN FOR NEUTRAL AND NEGATIVELY CHARGED PARTICULATE CARRIERS

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Abstract

Microparticles produced from polyisobutylcyanoacrylate (IBCA) and polyglutaraldehyde (PGA) were investigated for their relative affinity and surface characteristics using doxorubicin (DOX) as a model drug. IBCA microparticles have been reported to exhibit neutral hydrophobic surface features, whereas PGA microparticles have been shown to possess negatively charged carboxyl groups on their surface. The adsorption of DOX on the surface of these particles was studied by adding the drug to preformed microparticles. The amount of drug adsorbed was determined by centrifugation and analysis of the supernatant for the free drug by HPLC. The adsorption data was examined by Langmuir, Scatchard, and Hill equations. The results indicate that IBCA micropatricles have a higher adsorption capacity for DOX, however PGA microparticles demonstrated a higher relative affinity for the drug molecule. Additionally, both microparticles presented curvilinear Scatchard plots indicating the possibility of more than one type of binding sites for

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the drug on the surface of these particles. It appears that strong electrostatic attraction may exist between the positively charged amino group on DOX and the negatively charged carboxyl groups of PGA microparticles.

INTRODUCTION

Particulate drug delivery systems have been shown to influence the distribution of drug molecules in the body (1-4). Micro- or nano-particular systems have been formulated from a wide variety of polymers including polycyanoacrylates (4), polymethacrylates (5), and polyglutaraldehyde (6). Additionally, macromolecules such as gelatin (7) and albumin (3) have also been utilized as drug delivery systems. The drug may be physically adsorbed on or associated within the polymer chains or chemically linked to the polymer. Obviously, in vivo, the drug would be released by desorption or hydrolysis of the chemical linkage. One of the important factors influencing the interaction of the drug with the surface of the particles is the surface charge. The present study investigates the relative affinity of hydrophobic neutral polyisobutylcyanoacrylate nanoparticles (IBCA) (8) and negatively charged polyglutaraldehyde microparticles (PGA) (6) for an amphoteric, amphipathic drug.

Doxorubicin (DOX) was selected as the model drug. It is an anthracycline antibiotic with a wide spectrum of antitumor activity. The drug molecule adsorbs easily to a number of surfaces including, polyethylene, glass, steel, etc. (9). The hydrophobic anthraquinone moiety and the hydrophilic amino sugar (daunosamine) confer amphipathicity to the drug molecule. Additionally, the drug molecule exhibits amphotericity. This amphipathic and amphoteric nature of the drug molecule is expected to influence the nature and strength of interaction with charged or uncharged particulate carriers.

MATERIALS AND METHODS

Preparation of IBCA particles

The neutral hydrophobic IBCA particles were prepared by aqueous anionic polymerization at low pH in the presence of a steric stabilizing agent. The polymerizing medium was 1% citric acid solution containing 1% dextran 70 as the stabilizing agent. IBCA monomer (isobutylcyanoacrylate; Sigma Chemical Co., St. Loius, MO) was added to the polymerizing medium and stirred at 2000 rpm for two hours.

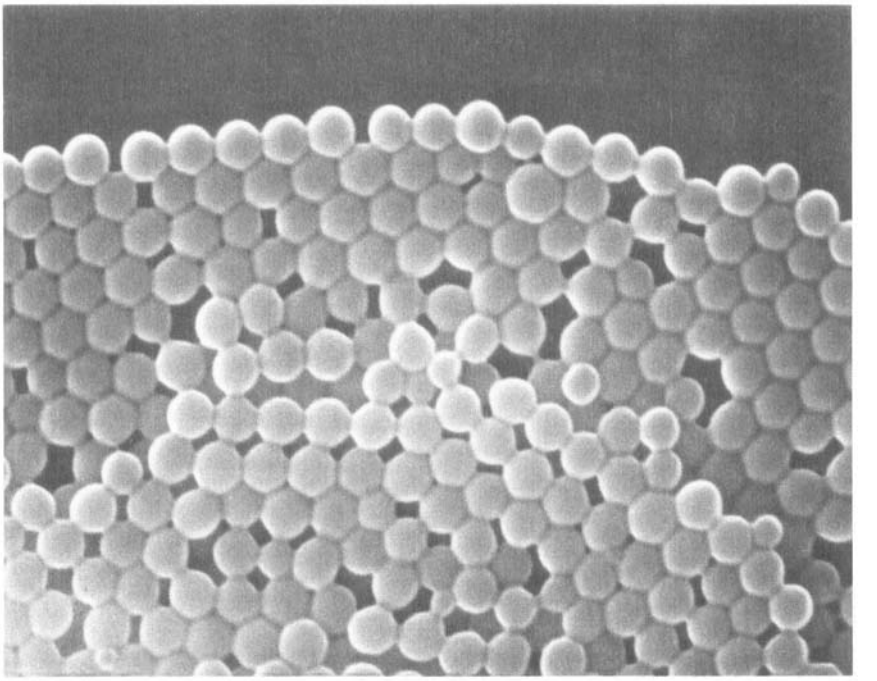
Preparation of PGA particles

The negatively charged PGA particles were prepared by adjusting the pH of a 7% solution of glutaraldehyde (containing 2.5% dextran 70), to 11 with 1N sodium hydroxide and stirring the resulting mixture at 2500 rpm for six hours at room temperature (6).

Drug Uptake Study

The drug-particle conjugates were prepared by adding varying amounts of doxorubicin hydrochloride (DOX; Sigma Chemical Co., St. Loius, MO) to suspensions of known weights of particles in water and shaking the resulting mixture at room temperature overnight. The IBCA-DOX suspension was centrifuged at 100,000 g for 30 min in an ultracentrifuge. The supernatant was separated, diluted and analyzed by HPLC. The PGA-DOX suspension was centrifuged at 5000 g for 20 min and supernatant collected for HPLC analysis. The HPLC system consisted of a Waters C_{18} Novapak column, Waters 6000A solvent delivery system, Waters U6K injector and a Gilson 121 fluorometer. The mobile phase was methanol:ammonium formate buffer pH 4.0 (70:30) at a flow rate of 2 ml/min. The excitation and emission wavelengths were 470 and 540 nm, respectively.

(a)



(b)

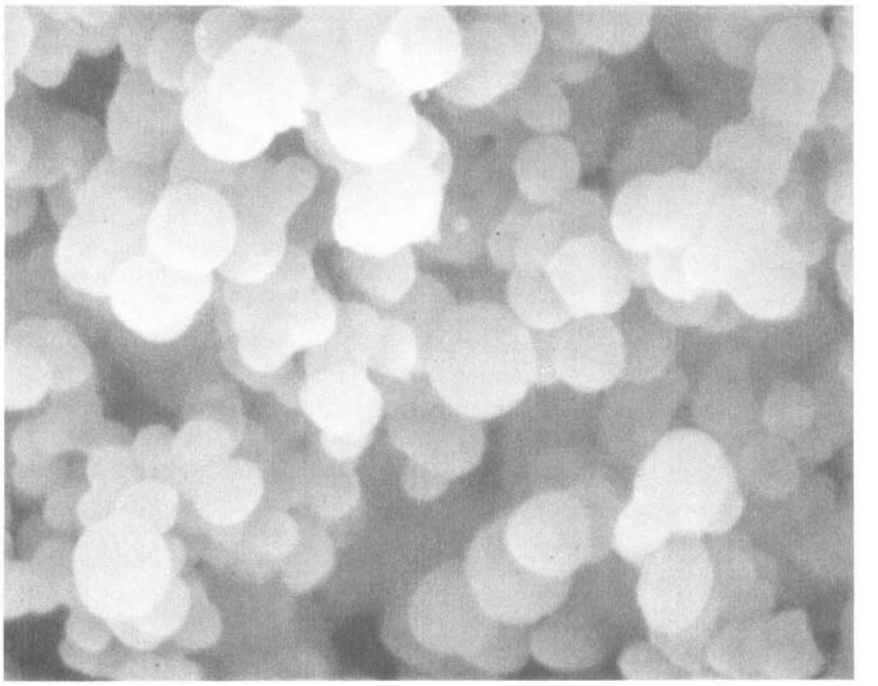


Figure 1

(a) Electron micrograph of IBCA particles (64,000x)

(b) Electron micrograph of PGA particles (14,000x)

The particle size was determined by scanning electron microscopy. The micrographs for IBCA particles showed uniformly spherical particles with an average diameter of 0.1 um (Fig. 1a). The micrographs of PGA particles showed spherical particles with an average diameter of 1 um (Fig. 1b).

The amount of drug adsorbed on the surface of the particles was determined from the difference in the initial amount added and the free amount in the supernatant. Data are shown as mean $\pm$ standard error of 4-5 experiments.

RESULTS AND DISCUSSION

The amount of drug adsorbed on the carrier was normalized by the weight of microparticles (x/m, ug/ug) and plotted against equilibrium concentration of DOX in the supernatant (Ceq, ug/ml). The adsorption isotherms for IBCA and PGA microparticles are shown in Fig. 2. The plot indicates that the particles bind increasing amounts of drug with increasing amount of DOX added. However, IBCA nanoparticles appear to be capable of adsorbing at least 3.5 times more DOX than PGA microparticles.

Fig. 3 indicates that the adsorption of DOX on the two types of microparticles follows Langmuir isotherm. The Langmuir equation for adsorption of solutes in solution to solids is:

$$\frac{Ceq}{(x/m)} = \frac{1}{k_1} + \frac{Ceq}{k_1 k_2} \qquad (1)$$

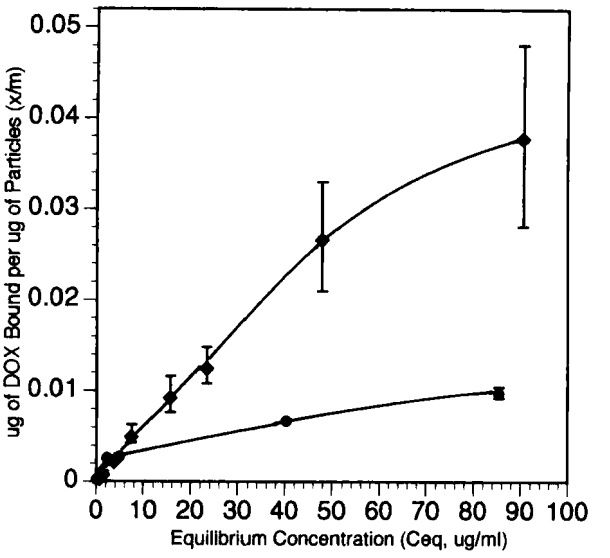


Figure 2
Adsorption isotherms for DOX on IBCA (♦) and PGA (●) particles

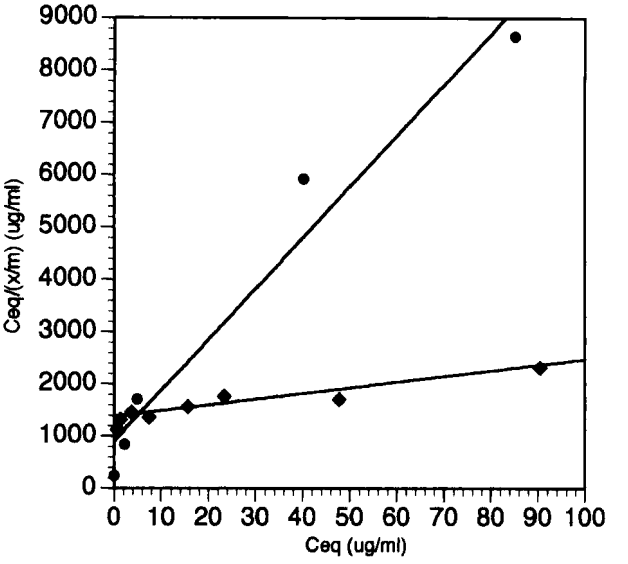


Figure 3
Langmuir isotherms of DOX for IBCA (♦) and PGA (●) particles

where k_1 represents maximum capacity of adsorbent for the adsorbate and k_1k_2 represents the relative affinity of adsorbate for the adsorbent (11). The maximum capacity of adsorbate for adsorbent (k_2) was approximately 4.5 times higher for IBCA than that for PGA particles. However, the relative affinity (k_1k_2) of DOX for PGA microparticles was about 140 times higher than that for IBCA nanoparticles, indicating the drug binds strongly to PGA microparticles but these microparticles get saturated rather easily.

In order to further investigate the mechanism of binding of DOX to IBCA and PGA microparticles, the adsorption data was subjected to Scatchard analysis (12) using the following equation:

$$\frac{(x/m)}{\text{Free}} = vK - K*(x/m) \qquad (2)$$

where "Free" is the amount of free drug at equilibrium, K is the association constant of drug for the particles, and v is the number of independant binding sites per mg of microparticles. Fig. 4 shows the Scatchard plots for the two types of microparticles. The curvature of the plots suggest at least two types of binding sites for each microparticle. The first type may be a high affinity, low capacity site and the second a low affinity, high capacity site (12). The association constant (K) of the first type for PGA appears to be

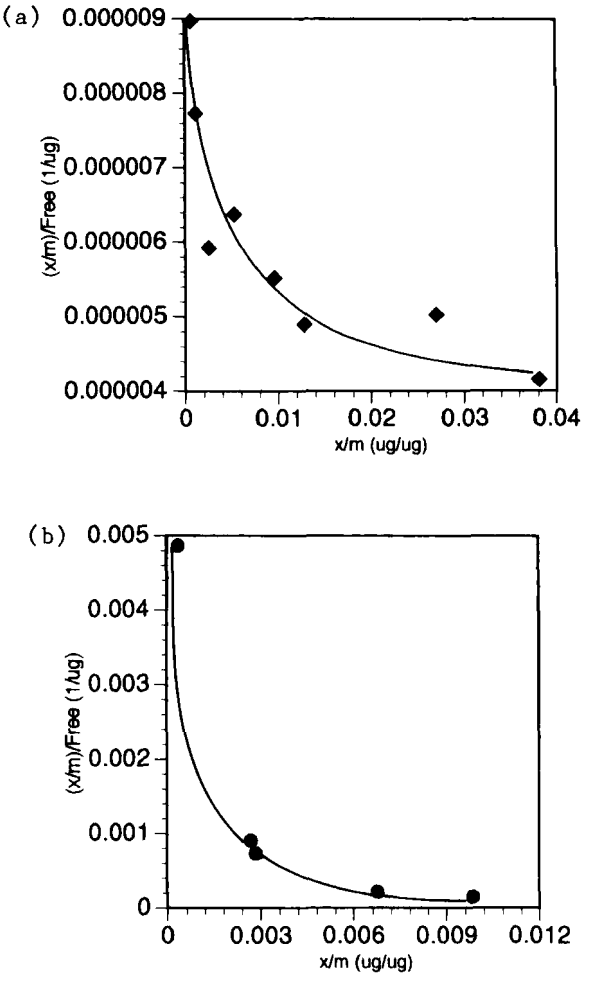


Figure 4
(a) Scatchard plot of DOX binding to IBCA particles
(b) Scatchard plot of DOX binding to PGA particles

about 80 times higher than that for IBCA, which qualitatively supports the relative affinity parameter obtained from Langmuir isotherm. Additionally, the IBCA microparticles appear to have over 8 (type I) binding sites per mg compared to about 3.5 sites per mg of PGA

microparticles. This may explain the lower adsorption plateau seen in the adsorption isotherm (Fig. 2) and the lower maximum adsorption capacity of PGA microparticles for DOX seen in Langmuir analysis (Fig. 3).

The presence of multiple binding sites on each microparticle is further suggested by Hill formula (13). This analysis has been used extensively in assessing receptor-drug binding (13). The method involves the following equation:

$$\log \frac{(x/m)}{(x/m)_{max}-(x/m)} = n*\log(Ceq) - n*\log(K_D) \quad (3)$$

where $(x/m)_{max}$ is the maximum amount of drug bound per ug of microparticle, n is Hill coefficient and is a measure of cooperativity, and K_D is the dissociation constant of the drug microparticle complex. The Hill coefficient (n) for the IBCA is slightly greater than 1 indicating the possibility of positive cooperativity in binding of drug to these hydrophobic microparticles (Fig. 5). On the other hand, the Hill coefficient is less than one for PGA microparticles suggesting multiple binding sites or negative cooperativity. Additionally, PGA microparticles exhibit a lower dissociation constant (K_D) indicating a relatively higher association constant for DOX as compared to that for IBCA microparticles.

Table I summarizes the various parameters obtained from the different analysis of binding data.

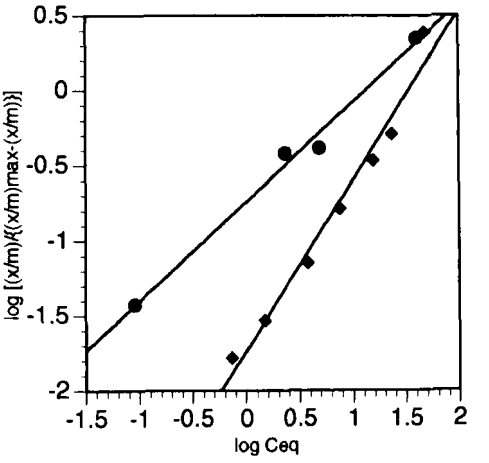
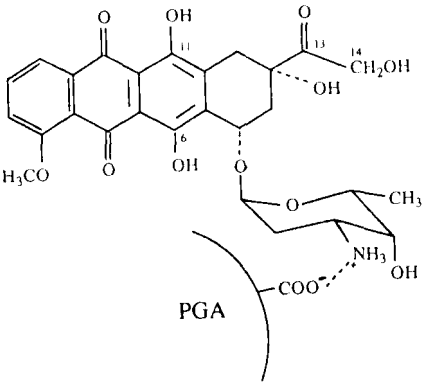


Figure 5
Hill plots for DOX binding to IBCA (♦) and PGA (●) particles

TABLE I
Summary of Various Constants for the Binding of DOX to IBCA and PGA Microparticles

Isotherm	IBCA	PGA
Langmuir		
Affinity constant (k_1k_2)	$72.637*10^{-6}$	$1.028*10^{-2}$
Maximum capacity (k_2)	$4.96*10^{-2}$	$1.104*10^{-3}$
Scatchard		
Association constant	$1.871*10^{-3}$	1.5111
# of binding sites (sites/mg)	8.028	3.499
Hill		
Hill coefficient	1.132	0.499
Dissociation constant	44.719	28.749

In conclusion, it appears that the higher binding capacity of IBCA microparticles for DOX could result from the larger number of binding sites per unit of particles. However, the drug binds much strongly to PGA microparticles which seems to suggest that simple physical adsorption may not be the only mechanism operating in the binding of DOX to these negatively charged microparticles. It is likely that electrostatically attractive forces exist between the protonated amino group on the daunosamine moiety of DOX and the negatively charged carboxyl groups on the surface of PGA microparticles, as shown below:



Similar results have been reported in a study examining the binding mechanism of DOX to ion-exchange albumin microcapsules (14). This conclusion is consistent with <u>in vitro</u> drug release studies for PGA-DOX microparticles carried out in this laboratory which resulted in a slow release rate of DOX (data not shown). IBCA-DOX microparticles gave an <u>in vitro</u> drug release

rate of 0.384 day^{-1}. Additionally, it has been suggested that association of DOX with poly-2-butylcyanoacrylate particles may involve perpendicular stacking of DOX molecules on the surface the neutral hydrophobic particles (15). This may further explain the high binding capacity of IBCA particles for DOX.

Therefore, these results suggest that the association and release of a drug from particulate carriers may be controlled by the surface characteristics of the carrier.

ACKNOWLEDGEMENT

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REFERENCES

1. N.V. Bapat, "Formulation and pharmacokinetics of doxorubicin associated with polymeric nanoparticles and oxidized dextran", Ph.D. dissertation, Northeastern University, 1991.
2. L.W. Seymour, K. Ulbrich, J. Strohalm, J. Kopecek, R. Duncan, Biochem. Pharmacol., 39, 1125 (1990).
3. P.K. Gupta, C.T. Hung, J. Pharm. Sci., 78, 745 (1989).
4. P. Couvreur, CRC Crit. Rev. Ther. Drug Delivery Sys., 5, 1 (1988).
5. V. Bentele, U.E. Berg, J. Kreuter, Int. J. Pharm., 13, 109 (1983).
6. A.D. McLeod, F.C. Lam, P.K. Gupta, C.T. Hung, J. Pharm. Sci., 77, 704 (1978).

7. J.J. Marty, R.C. Oppenheim, P. Speiser, Pharm. Acta Helv., 53, 17 (1978).
8. P. Couvreur, B. Kante, M. Roland, P. Speiser, J. Pharm. Sci., 68, 1521 (1979).
9. E. Tomlinson, L. Malspeis, J. Pharm. Sci., 71, 1121 (1982).
10. N. Bapat, M. Boroujerdi, Drug Develop. Ind. Pharm., 18, 65 (1992).
11. A.W. Adamson, "Physical Chemistry of Surfaces", Interscience, New York, 1967, p.368.
12. A. Martin, J. Swarbrick, A. Cammarata, "Physical Pharmacy", Lea & Febiger, Philadelphia, 1983, p.335.
13. W.B. Pratt, P. Taylor, "Principles of Drug Action", Churchill Livingston, New York, p. 54.
14. A. Sawaya, J.-P. Benoit, S. Benita, J. Pharm. Sci., 76, 475 (1987).
15. L. Illum, M.A. Khan, E. Mak, S.S. Davis, Int. J. Pharm., 30, 17 (1986).