Interaction Between Pharmaceuticals and Sodium Polyethylenesulfonate

By G. LEONARDI, M. SOLINAS, and C. BOTRÉ

Several drugs, mainly in the field of local anesthetics, were studied with a synthetic polyelectrolyte, sodium polyethylenesulfonate, by means of membrane equilibrium dialysis measurements. Data on equilibrium constants of the association between the drugs considered, as hydrochlorides, and the polyelectrolyte are reported.

THE STUDY of interactions between drugs and macromolecules has received increasing interest recently (1–5).

The result of such interaction, in general, is the formation of association complexes between the drug and the macromolecule, which may lead to unusual features, both from physicochemical and biological points of view.

In some cases, for instance, the pharmacological activity of the complex is improved with respect to the single drug, either in the sense of the intensity and/or the duration of action.

This paper reports data about the interaction observed between several local anesthetics and a synthetic polyelectrolyte, *i.e.*, sodium polyethylenesulfonate.

This study was performed mainly by means of membrane equilibrium dialysis according to a procedure proposed by Klotz *et al.* (6).

The equilibrium constants of associations between drugs and the polyelectrolyte are reported.

EXPERIMENTAL

The sample of sodium polyethylenesulfonate (NaPES) was prepared according to Breslow and Kutner (7). Its molecular weight was about 7,000. The dissociation degree (α) of the NaPES was determined potentiometrically in an aqueous solution by using a cationic glass electrode (Beckman catalog No. 39728) and saturated calomel electrodes connected to the solution by means of an agar bridge. The procedure and the apparatus were extensively described in a previous paper (8). The experimental value of α was 0.25, in good agreement with the theoretical value calculated according to Oosawa (9).

All the pharmaceuticals were used as hydrochlorides. They are listed in Table I together with the wavelength corresponding to a maximum of absorbance. Table I also reports association constants and pKa values at 25° (10).

Conductivity measurements were performed in a thermostatic bath at 25 ± 0.02° using a WTW bridge model LF 3.

The dialysis experiments were carried out with cellophane bags (HMC type). These were previously treated with boiling water for about 5 min., washed with cold water, wiped, filled with a known volume of the NaPES solution, and then dipped into a known volume of drug solution.

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The polymer concentration was 10^{-3} *M;* the drug concentration was 10^{-4} *M*. The equilibrium across the membrane was reached at room temperature after about 48 hr. No change in volume inside or outside the bag was observed.

The concentration of the drug in solution was determined with a grating spectrophotometer (Hitachi, Perkin-Elmer, model 139). The measurements of the absorbance were performed in each case at the wavelength corresponding to the maximum value of the absorption of the drug. Quartz cells, 1 mm. optical path, were employed.

At each concentration a blank test was set up. In this case, water was placed inside the bag instead of polyelectrolyte solution. The amount of drug bound to the polyelectrolyte was determined by measuring the concentration of the solution outside the bag containing the polyelectrolyte as well as the outside concentration of the drug in the blank.

RESULTS AND DISCUSSION

In order to prove that in the range of concentration considered no aggregation takes place in solution of the drugs considered, specific conductivity was measured at different concentrations. In each case the plots of specific conductivity *versus* the concentration gave straight lines. On the other hand, it was shown that when aggregation of drug molecules in solution occurs, a break in the plot of specific conductivity *versus* concentration may be detected (2).

The data obtained from the membrane equilibrium dialysis were treated according to the method of Klotz *et al.* (6). Such treatment, in fact, may be applied to a general association equilibrium between a polyelectrolyte (P) and a drug (D), for which

$$P + D \rightleftharpoons PD \qquad \text{(Eq. 1)}$$

The equilibrium constant for the ith association, K_i, is:

$$K_i = \frac{n - (i - 1)}{i} k \qquad \text{(Eq. 2)}$$

When $i = 1$, first equilibrium constant, Eq. 2 reduces to

$$K = nk \qquad \text{(Eq. 3)}$$

where n = number of sites available along the polymer
K = equilibrium constant
k = constant which depends on the nature of the anion

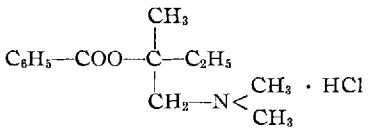
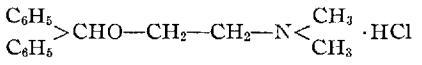
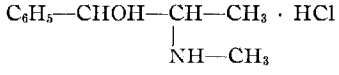
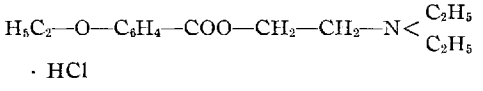
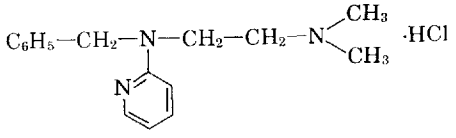
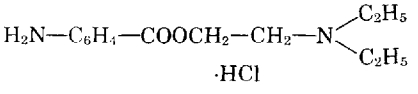
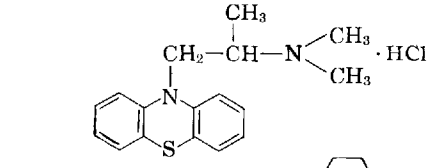
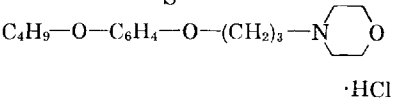
The relationship which correlates the number of sites available along the polymer to the interaction with the molecules of the drug can be written as follows:

$$\frac{1}{r} = \frac{1}{n\,k\,D} + \frac{1}{n} \qquad \text{(Eq. 4)}$$

where r is the ratio between the number of moles of bound drug and total moles of polymer.

By plotting $1/r$ *versus* the reciprocal of nonbound

TABLE I.—ASSOCIATION CONSTANTS FOR DRUG–POLYELECTROLYTE SYSTEM

Hydrochlorides of	Formula	λ mμ	Association Constant $K \times 10^{-4}$	pK_a at 25°
Amylocaine		232	7.94	8.40
Benzocaine	$H_2N—C_6H_4—COOC_2H_5 \cdot HCl$	235	...	2.43
Diphenhydramine		250	6.52	9.00
d-Ephedrine		210	25.00	9.55
Parethoxycaine		260	6.25	...
Tripelennamine		305	7.60	8.96
Procaine		290	3.33	8.95
Promethazine		248	16.95	8.65
Pramoxine		224	5.91	...

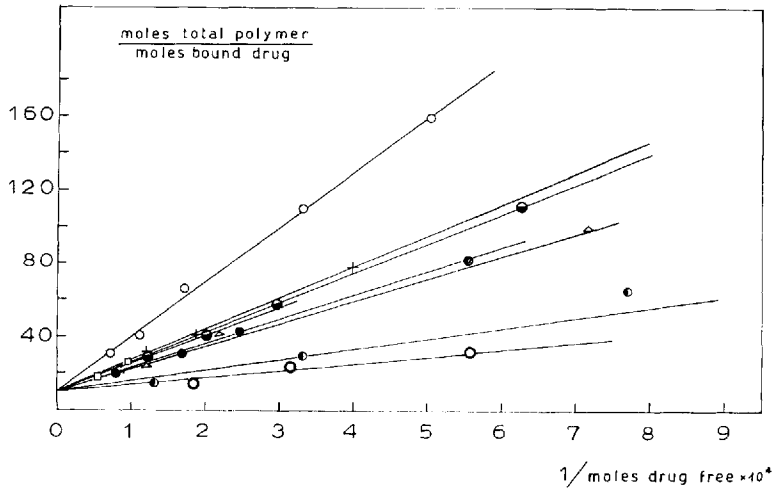


Fig. 1.—Ratio between total moles of polymer and moles of bound drug *vs.* the reciprocal of free drug molecules. Key: ○, procaine; ◐, promethazine; +, pramoxine; ○, *d*-ephedrine; ●, tripelennamine; ◒, parethoxycaine; △, amylocaine; □, diphenhydramine.

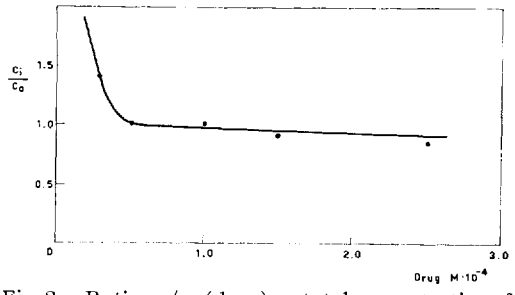


Fig. 2.—Ratio, c_i/c_0, (drug) *vs.* total concentration of the drug.

By solving the system one obtains,

$$(0.5\,a_0 - a_i)x_0^2 + (Da_i - Pa_i + a_0a_i - 0.5\,a_0^2)x_0 + (DPa_i - Da_0a_i) = 0 \quad \text{(Eq. 5)}$$

By substituting in Eq. 5 the values of D, P, a_0, and a_i, respectively, the values of x_0 and x_i are obtained.

In Fig. 2, the ratio between inside molar concentration of the drug, c_i, and outside molar concentration, c_0, is plotted against the total concentration of the drug. Figure 3 reports the ratio, c_i/c_0, respective to the chloride ions as a function of the total concentration of the drug.

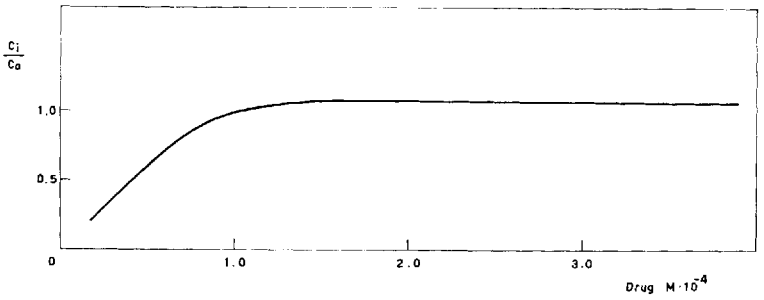


Fig. 3.—Ratio, c_i/c_0 (chloride ion) *vs.* total concentration of the drug.

moles of drug (Fig. 1), straight lines are obtained, showing that the electrostatic effect can be neglected with respect to the statistical effect. This is reasonable since the concentration of the solution is very low. The number of free sites of the polymer was, under our conditions, roughly 10 times the concentration of the drugs considered.

Under the experimental conditions of this work, the Donnan effect may be neglected, since we have

$$\begin{cases} \dfrac{x_i}{v_i} \cdot \dfrac{a_i}{v_i} \cdot \dfrac{y_i}{v_i} = \dfrac{x_0}{v_0} \cdot \dfrac{a_0}{v_0} \cdot \dfrac{y_0}{v_0} \cdot \\ \dfrac{y_0}{v_0} = \dfrac{1}{v_0}(a_0 - x_0) \end{cases}$$

and

$$\begin{cases} \dfrac{D - x_0}{v_i} \cdot \dfrac{a_i}{v_i} \cdot \dfrac{P - y_0}{v_i} = \dfrac{x_0}{v_0} \cdot \dfrac{a_0}{v_0} \cdot \dfrac{y_0}{v_0} \\ y_0 = a_0 - x_0 \end{cases}$$

where D = mmoles of total drug
P = mmoles of polymer (inside)
a_0 = mmoles of chloride outside
a_i = mmoles of chloride inside [this value was obtained by Kennon *et al.* (11)]
x_0 = mmoles of drug outside
x_i = mmoles of drug inside
v_0 = outside volume of solution (20 ml.)
v_i = inside volume of solution (10 ml.)
v_i/v_0 = 0.5
y_0 = mmoles of sodium outside
y_i = mmoles of sodium inside

The trend of the values, c_i/c_0 *versus* the concentration of the drug, shows that in the considered range of concentrations, they may be considered roughly constant and near unity. Therefore, under the above-mentioned conditions the Donnan effect can be neglected.

The association constants between the drugs and polyelectrolyte reported in Table I are approximately higher for monomethyl derivatives (*d*-ephedrine), followed by dimethyl and diethyl derivatives, while no significant differences can be observed in their respective pKa values.

Further studies are in progress to evaluate the role of the steric hindrance on such interactions.

REFERENCES

(1) Kennon, L., and Higuchi, T., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 21(1957).
(2) Botré, C., and Riccieri, F. M., *J. Pharm. Sci.*, **52**, 1011 (1963).
(3) Blaug, S. M., and Ebersman, D. S., *ibid.*, **53**, 35 (1964).
(4) Lach, J. L., and Chin, T. F., *ibid.*, **53**, 69(1964).
(5) Patel, N. K., and Foss, N. E., *ibid.*, **53**, 94(1964).
(6) Klotz, I. M., and Walter, F. M., *J. Am. Chem. Soc.*, **70**, 943(1948).
(7) Breslow, D. S., and Kutner, A., *J. Polymer Sci.*, **27**, 295(1958).
(8) Ascoli, F., Botré, C., and Liquori, A. M., *J. Mol. Biol.*, **3**, 202(1961).
(9) Ascoli, F., Botré, C., Crescenzi, V., Liquori, A. M., and Mele, A., *J. Polymer Sci.*, **40**, 169(1959).
(10) Perrin, D. D., *et al.*, *Quart. Rev.*, **18**, 295(1964).
(11) Kennon, L., and Higuchi, T., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 157(1956).