J. Pharm. Pharmacol. 1981, 33: 408–410
Communicated December 3, 1980

0022–3573/81/060408–03 $02.50/0

Interaction of propranolol hydrochloride with montmorillonite

M. J. SÁNCHEZ MARTIN, M. SÁNCHEZ CAMAZANO, M. T. VICENTE HERNÁNDEZ†, A. DOMINGUEZ-GIL*†, *Centro de Edafologia y Biologia Aplicada del C.S.I.C. Apdo. 257, Salamanca and †Practical Pharmacy Department, University of Salamanca, Spain*

The use of drugs in the form of adsorption complexes with clay minerals may cause a decrease in the bioavailability within the organism. As a consequence there exists the possibility of achieving a sustained drug action through a slow but progressive release of the drug, thus increasing its efficiency and reducing dosage. Montmorillonite, among other clay minerals, has structural properties that favour the intercalation of organic molecules within the interlayer space, giving rise to the formation of adsorption complexes.

There are numerous reports dealing with the use of clay minerals in pharmacy (Pinck et al 1961; Barr 1964; Wai et al 1966; McGinity & Lach 1976); but only Porubcan et al (1978) and Sánchez Camazano et al (1980a,b) have examined the drug interaction mechanism with clays.

Our aim has been to study the interaction of montmorillonite with (±)-propranolol hydrochloride to see if in the form of a montmorillonite-propranolol interlayer complex a sustained release of drug was possible.

The $< 2\,\mu$m fraction of Albagel montmorillonite was used with the methodology previously described (Sánchez Camazano et al 1980a,b). Propranolol hydrochloride was B.P. and U.S.P. quality; it was assayed by u.v. spectroscopy (absorption maximum at 293 nm).

* Correspondence.

From the study of the influence of pH (between 3·0 and 8·0) on adsorption of the drug by sodium montmorillonite, the results show that the amount adsorbed is independent of the pH of the solution, which in all cases was 78 mequiv/100 g, in value close to that necessary to satisfy the exchange capacity of the clay (80 mequiv/100 g). In the adsorption process, the principal reaction that must take place is exchange of the sodium ions of montmorillonite with those of the propranolol-ammonium ions of the solution. At low pH the adsorption does not decrease which indicates that the substitution of sodium by hydrogen ions does not occur. At pH 9·0, the amine is released by hydrolysis and is precipitated because of its low solubility; this impedes the study of the adsorption of propranolol in its neutral state by montmorillonite. However, the X-ray study (see below) shows that by treating sodium montmorillonite with a highly diluted solution of propranolol hydrochloride at pH 9·0, there is an additional adsorption of the free amine as well as of the cationic form. This phenomenon has been reported by Grim et al (1947) and Kurilenko & Mikhalyuk (1959). The adsorption reaction takes place within 15 min. From the adsorption isotherm (Fig. 1) it may be seen that the maximum amount adsorbed is 78 mequiv/100 g, a value which is close to the exchange capacity of the clay, and which does not increase with increase in drug concentration. If the adsorption data are plotted according to the Langmuir equation, a straight line is obtained; according to Giles et al (1960), the approxi-

Table 1. Spacing d_{001} of sodium-montmorillonite treated with solutions of propranolol hydrochloride at different concentrations.

Propranolol hydrochloride						
Initial concentration mequiv/50 ml	Equilibrium concentration mequiv/50 ml	adsorbed mequiv/100 g	Unwashed samples dried at vacuum		Washed samples dried at vacuum	
			d_{001}	ΔÅ	d_{001}	ΔÅ
0·020	0·003	17	15·49	5·89	15·35	5·75
0·040	0·008	32	16·35	6·75	16·35	6·75
0·060	0·011	49	16·98	7·38	16·98	7·38
0·080	0·019	61	17·31	7·71	17·31	7·71
0·100	0·031	69	17·31	7·71	17·31	7·71
0·120	0·047	73	17·31	7·71	17·31	7·71
0·150	0·074	76	17·31	7·71	17·31	7·71
0·200	0·122	78	17·31	7·71	17·31	7·71
0·250	0·172	78	17·31	7·71	17·31	7·71
0·300	0·222	78	17·31	7·71	17·31	7·71
0·350	0·272	78	17·31	7·71	17·31	7·71
0·400	0·322	78	17·31	7·71	17·31	7·71

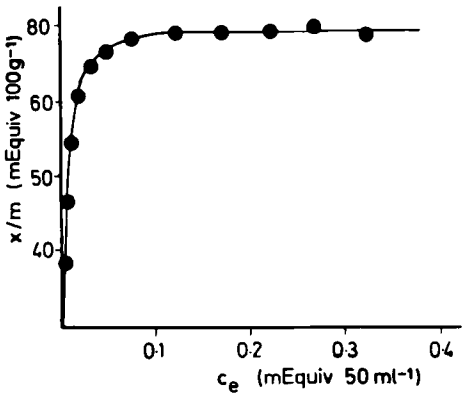

FIG. 1. Adsorption isotherm of propranolol hydrochloride by sodium montmorillonite (0·02; 0·04; 0·06; 0·08; 0·100; 0·120; 0·150; 0·200; 0·250; 0·300; 0·350 and 0·400 mequiv of propranolol hydrochloride in 50 ml. Temp. 40 °C and time of treatment 30 min.

mation between the data and this equation suggest a chemi-sorption reaction.

The results of X-ray diffraction (Table 1) are in agreement with the adsorption data and show that the organic cations penetrate into the interlayer space of montmorillonite causing an increase in the basal spacing of the silicate independent of the pH of the propranolol solution (pH 3·0–8·0), but there is dependence on concentration. Thus with solutions at different pH, the d_{001} spacing obtained is always 17·31 Å which represents an increase of 7·71 Å with respect to the value of 9·6 Å of dehydrated montmorillonite. The diagram of montmorillonite treated with solutions of propranolol at pH 9·0 gives a higher basal spacing (18·01 Å) which shifts to 17·31 Å when the complex is washed with water. This shows that at high pH values, the existing free amine together with the amine in cationic form, is also adsorbed by weak van der Waals forces, given the ease with which it is displaced. Upon treating montmorillonite with different concentrations of propranolol hydrochloride, the basal spacing increases with drug concentration until a value of 17·31 Å is reached. The low values are due to the partial replacement of the inorganic cations by the organic cations, giving rise to interstratified phases. Neither the spacings nor the amounts adsorbed vary when the complexes are washed with distilled water, at pH between 3·0 and 8·0. Nor are the spacings modified when the complexes are vacuum treated at 0·1 mm Hg. This shows that there can be no physical adsorption of molecules, i.e. molecules retained by van der Waals forces, and that the organic cations are strongly adsorbed into the interlayer space.

The study of the i.r. spectra (Fig. 2) shows the existence of the organic compound within the interlayer space of the montmorillonite, as may be seen from the presence on absorption bands of propranolol in the

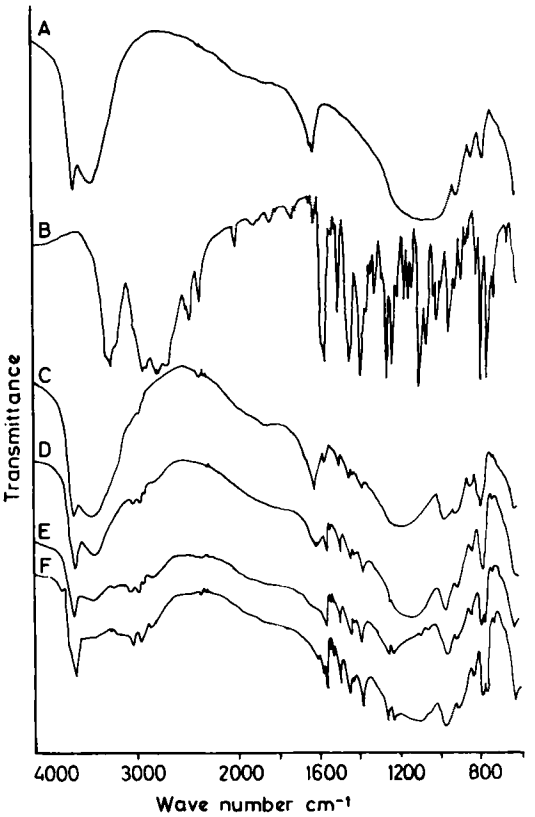

FIG. 2. I.r. spectra of (A) sodium montmorillonite (B) propranolol hydrochloride and sodium montmorillonite treated with solutions of propranolol hydrochloride of different concentrations (mequiv/50 ml) and pH (C) 0·02, pH 7; (D) 0·04, pH 7; (E) 0·10, pH 7; (F) 0·20, pH 9.

spectrum of the complex washed several times with distilled water. The variation in intensity of the bands shows that the amount of organic cation in the interlayer space increases with drug concentration. In agreement with this, the intensity of the stretching and the deformation vibration bands of the associated water of the montmorillonite at 3420 and 1630 cm^{-1}, respectively, also decrease with increase in drug concentration, since the water within the interlayer space of the montmorillonite is progressively replaced by the organic cations. In the spectrum of montmorillonite treated with the drug at pH 9·0, when, according to the X-ray results, there is adsorption of the molecules both in the cationic form and in the neutral state, these bands practically disappear because of the almost total replacement of the interlayer water.

In agreement with the molecular dimensions of propranolol and the spacing increase (Δ = 7·71 Å) which montmorillonite undergoes in the expansion, the complex must be monolayer, with a perpendicular

disposition of the naphthalene to the surface of silicate oxygen atoms. The surface occupied by the organic cation with this disposition is close to that available by exchange cation.

Therefore it may be concluded that upon treating montmorillonite with aqueous solution of propranolol hydrochloride the propranolol-ammonium cations are adsorbed into the interlayer space of the silicate, giving rise to the formation of a definite complex of 17·31 Å basal spacing corresponding to the intercalation of a monomolecular cation layer. The formation of this complex is independent of the pH of the solution, within a pH margin of 3·0–8·0, but does depend on the concentration of the solution. The adsorption mechanism is one of cation exchange and the maximum amount adsorbed is 78 mequiv/100 g.

These results are considered a valid base for the initiation of desorption studies in vitro and, later in vivo.

We would like to thank Mr N. Skinner for his invaluable help in translating the manuscript.

REFERENCES

Barr, M. (1964) J. Am. Pharm. Assoc. N S4, 4–9

Giles, C. H., MacEwan, T. H., Nakhwa, S. N., Smith, D. (1960) J. Chem. Soc. 3973–3993

Grim, R. E., Allaway, W. H., Cuthbert, F. L. (1947) J. Am. Chem. Soc. 30: 137–142

Kurilenko, O. D., Mikhalyuk, R. V. (1959) Kolloidn. Zh. 21: 181–184

McGinity, J. W., Lach, J. L. (1976) J. Pharm. Sci. 65: 896–902

Pinck, L. A., Holton, W. F., Allison, F. E. (1961) Soil Sci. 91: 22–28

Porubcan, L. S., Serna, C. J., White, J. L., Hem, S. L. (1978) J. Pharm. Sci. 67: 1081–1087

Sanchez Camazano, M., Sánchez Martin, M. J., Vicente Hernández, M. T., Dominguez-Gil, A. (1980a) Int. J. Pharm. 6: 243–251

Sánchez Camazano, M., Sánchez Martin, M. J., Vicente Hernández, M. T., Dominguez-Gill, A. (1980b) J. Pharm. Sci. 69: 1142–1144

Wai, K., Dekay, G., Banker, G. S. (1966) J. Pharm. Sci. 55: 1244

J. Pharm. Pharmacol. 1981, 33: 410–411
Communicated September 18, 1980

0022–3573/81/060410–02 $02.50/0

Entrapment of proteins as disulphide cross-linked thiolated macromolecules within cross-linked dextran ("Sephadex") gels

S. M. ALWAN, H. J. SMITH*, *Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff, S. Glamorgan, Wales, U.K.*

The entrapment of two proteins, α-chymotrypsin and bovine serum albumin (BSA), within cross-linked dextran ("Sephadex") gels as their disulphide cross-linked thiolated macromolecules is described. This type of preparation could have a use in medicine as a depot for the slow release of a protein drug (Mahbouba et al 1974).

Methods and results

Thiolated BSA (10·4–13·8 SH group mol^{-1}) was prepared from crystalline albumin (Koch-Light) by the silver ion-imidazole catalysed reaction with *N*-acetyl homocysteine thiolactone (AHTL) (Mahbouba et al 1974) the reaction being complete in 10 min. Thiolated α-chymotrypsin (see below) from α-chymotrypsin (thrice recrystallized; Koch-Light) contained 3–5·5 SH groups mol^{-1}.

The molecular size of the macromolecules formed by ferricyanide oxidation (see below) of thiolated BSA (1·4–13 thiol groups mol^{-1}) was largely independent of the thiol titre and dependent on the concentration of thiolated BSA present in contrast to cross-linking of other thiolated proteins (Mahbouba & Smith 1977). Low concentrations (0·36–0·43% w/v) with 1·35–9·5 thiol groups mol^{-1} gave n (aggregation number) = 1·0–2·0; intermediate concentrations (0·9–1·4) with 3–10·8 thiol groups mol^{-1} gave n = 1·1–5·8; high concentrations (1·8–3·1 (limiting solubility)) with 4·8–12·8 thiol groups mol^{-1} gave n = 2·2–11·1.

Cross-linked dextran (Sephadex G-100, Pharmacia; 0·5 g dry weight) was soaked with a solution of phosphate buffer (3·5 ml, 0·1 M) pH 8·0 containing thiolated α-chymotrypsin (25–100 mg) for 3 days at 4 °C. Potassium ferricyanide solution (1·8%, 1 ml diluted with water to the volume required for complete swelling of the gel) was then added and after storage (4 °C) for a further day to ensure oxidation of the thiolated α-chymotrypsin and complete swelling of the gel, the mixture was placed in a column (30 × 1·5 cm i.d.) and the excess reagent and the untrapped disulphide cross-linked thiolated protein washed out. The protein fraction was separated from the reagent using a column (45 × 2·5 cm i.d.) of cross-linked dextran (G-25) in phosphate buffer (0·1 M) pH 8·0, the concentration of the protein fraction determined (Lowry et al 1951) and the approximate amount of protein entrapped in the gel (33–40%; Table 1) calculated from the difference between the initial amount used and the excess protein washed out from the column. The molecular size of the untrapped macromolecule (n = 10–11) was measured by light-scattering (Mahbouba et al 1974) to indicate the approximate size

* Correspondence.