PHYSICO-CHEMICAL STUDIES OF ASPIRIN WITH GLYCINE

By H. D. C. Rapson, D. O. Singleton, A. C. E. Stuart* and Mary P. Taylor†

From Beecham Research Laboratories Limited, Brockham Park, Betchworth, Surrey

Received May 19, 1959

The relative solubility and rate of dissolution of aspirin in water and glycine solution have been measured. A technique involving a mathematical examination of the front profile of chromatograms has been used to study the extent of the adsorption of glycine from aqueous solution on aspirin. Aspirin is more soluble and more rapidly dissolved in glycine solution than water, and glycine is found to adsorb, in significant amounts, on aspirin crystals. The findings are discussed in an attempt to explain, in physico-chemical terms, differences in taste and adhesion to the oral mucosa that are discernible when aspirin tablets compounded with or without glycine are savoured. Attention is drawn to certain aspects of the results which have a bearing on the transport of aspirin.

Compound tablets of aspirin with glycine are now an accepted form for the administration of aspirin for rheumatic conditions when plain aspirin cannot be tolerated. An editorial in the British Medical Journal[1] draws attention to this method of aspirin therapy, commenting that the physical association of the components is such that they quickly disperse in the mouth even though not taken with a draught of water. No trace of their presence or of any irritant action can be observed gastroscopically[2] and indigestion from their use has not been recorded in fifty consecutive cases. Vining and Kersley[3], examining an aspirin and glycine preparation (Paynocil), found that when this preparation was administered to rheumatic patients intolerant of aspirin, doses of 20 to 100 grains daily could be tolerated without gastric side-effects.

It is a discernible fact that should an aspirin tablet disintegrate in the mouth then the drug can be tasted and the crystalline particles may adhere to the mucosae. If, however, aspirin is compounded with glycine in such a manner that the tablet disintegrates in the mouth within a few seconds, aspirin can no longer be tasted and the particles do not adhere to the mucosae. These observations must be capable of explanation in more precise physical terms and three lines of argument suggest themselves.

The interrelationship of taste mechanism and chemical structure may partly account for the physiological differences in the behaviour of aspirin when compared with aspirin-glycine.

The solubility and rate of dissolution of aspirin in glycine solution may differ from that of aspirin in water.

The formation of adsorbed films of glycine on the aspirin crystals may modify the interaction between aspirin and the protein surfaces of the mucosa.

* Present address: William R. Warner and Co. Ltd., Eastleigh, Hants.
† Present address: The Radiological Protection Service, Clifton Avenue, Belmont, Surrey.

The first possibility is conjectural and difficult to expose to experiment. The physico-chemical mechanisms of the other two possible modes of action are readily susceptible to measurement and this paper presents the results of such measurements.

Rates of Dissolution of Aspirin in Water and Glycine Solution

Experimental

Aspirin dissolution has already been reported[4] but this did not include dissolution in glycine solutions.

The aspirin used in this work was Monsanto "Aspirgran"; the glycine was a pure recrystallised material supplied by C. L. Bencard Limited; both materials had a particle size range within the limits 18 to 100 mesh.

Two lots of 500 mg. of aspirin were accurately weighed into a pair of

TABLE I

Comparative rates of dissolution of aspirin in (A) water, (B) 0·25 per cent aqueous glycine solution

t min.	A 20°		B 20°	
	E 1 cm. 225·5 mμ	Concentration mg./100 ml.	E 1 cm. 225·5 mμ	Concentration mg./100 ml.
2	0·398	83	0·502	105
4	0·620	129	0·777	162
6	0·794	165	0·952	198
8	0·947	197	1·104	230
10	1·002	209	1·202	250
20	1·300	271	1·528	318
30	1·386	289	1·634	340
45	1·425	296	1·685	351
60	1·451	302	1·719	358
90	1·499	312	1·768	368
	A 26°		B 26°	
2	0·997	207	1·191	248
4	1·173	244	1·467	306
6	1·320	275	1·604	334
8	1·417	295	1·706	355
10	1·521	317	1·754	365
20	1·696	353	1·967	410
30	1·828	381	2·057	429
45	1·838	383	2·145	447
60	1·855	386	2·155	449
90	1·887	393	2·176	453

matching 250 ml. Quickfit conical flasks clamped in a Microid flask shaker. At zero time 100 ml. of distilled water was added to one and 100 ml. of 0·25 per cent glycine solution to the other and the shaker started. One ml. samples were withdrawn at timed intervals from each flask and run into 95 ml. of absolute ethanol in 100 ml. graduated flasks, making up to the mark with ethanol. This dilution in ethanol minimised the hydrolysis of the aspirin during the period between withdrawal of the sample and reading the extinction of the solution in a Hilger Uvispek ultra-violet spectrophotometer. The diluted samples were read as quickly as possible against the appropriate blank solution in 1 cm. or 0·5 cm. quartz cells at 225·5 mμ. The E (1 per cent, 1 cm.) for aspirin has previously been found to be at a maximum at this wavelength with a value of 480 in ethanol. The presence of the 0·0025 per cent of glycine

in the final dilution read in the spectrophotometer had no effect on the extinction of the aspirin that could be differentiated from normal experimental error.

The dissolution experiments were made at laboratory air temperature without any attempt at thermostatic control. Repeat runs were made on different days; for one set of experiments the temperature of the two solutions was 20°; on the second occasion the temperature was 26°. The results of these experiments are shown in Table I. From these it can be seen that the solubility of aspirin in 0·25 per cent aqueous glycine is some 15 to 20 per cent greater than in water at laboratory temperatures. The rates of dissolution are likewise proportionately greater.

The Adsorption of Glycine by Aspirin at the Aspirin: Water Interface

Outline of Technique

If an aqueous solution of glycine, saturated with aspirin, is allowed to flow through a column of aspirin crystals the effluent at first contains no glycine but, as more solution is passed through the column, the glycine content rises until eventually the effluent composition is the same as that of the solution entering the column. The lag in arrival of the glycine is partly due to the displacement of the water originally in the column by the glycine solution and partly to the adsorption of glycine by the aspirin crystals. The variation of glycine concentration of the effluent with volume is shown for a typical experiment in Figure 1. Point A corresponds to the volume of liquid initially in the column; the shaded area B represents the amount of glycine removed from solution by adsorption on the aspirin; in the region of C the glycine content approaches that of the inflowing solution, shown in Figure 1 by the horizontal line at 0·5 per cent glycine.

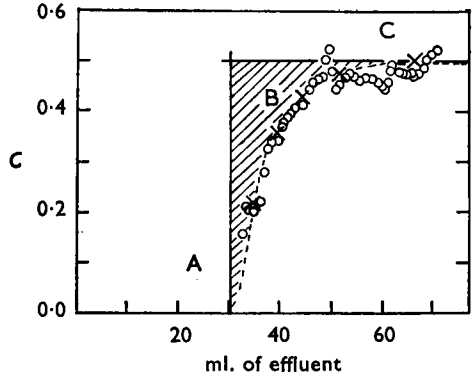

Fig. 1. The progressive change in glycine concentration, *C* in per cent weight by volume, of the effluent in a typical chromatogram. O Experimentally determined values of glycine concentration. –×– Calculated chromatogram profile.

If the shape of curve AC is analysed mathematically a relation for the adsorption isotherm can be obtained. If the size and surface area of the crystals is known this relation can be roughly expressed in terms of the number of layers of glycine molecules at the aspirin: water interface. The data from a number of experiments analysed in this manner are shown in Figure 2. It can be seen that even if the true area of the crystals is ten times the estimated area, which is calculated from the geometry

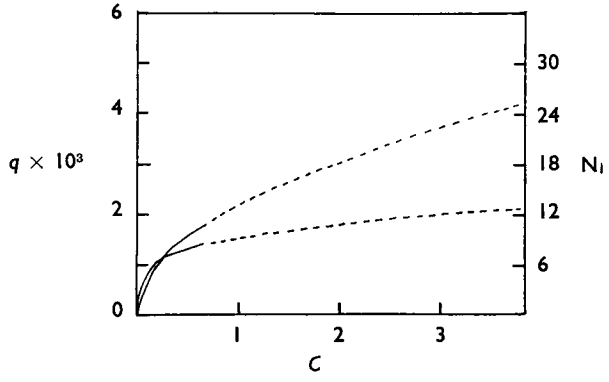

FIG. 2. Adsorption isotherm obtained by analysis of chromatogram profiles. Where q is the amount adsorbed in g./g. of aspirin, and C is the glycine concentration in per cent weight by volume.

—— Portion of isotherm covered by the glycine concentration used.
– – – Extrapolated portion of isotherm obtained by useof the equation $q = aC^p$ to which portion the isotherm at lower concentrations appears to conform.

Calculation of the number, N_l, of layers of molecules on the aspirin crystal surface is based on the mean geometric and the calculated cross sectional areas of, respectively, the crystals and the glycine molecule.

of the particles, then layers of up to 50 molecules could be obtained under the conditions expected when an aspirin: glycine tablet dissolves in the mouth. It is believed that a layer of even 10 molecules would have a physiological effect inhibiting adhesion of aspirin crystals to the mucosa of the digestive tract. The results are consistent with the observed fact that little taste of aspirin is detected when an aspirin: glycine tablet is held in the mouth before swallowing, in comparison with pronounced acid taste of the ordinary aspirin tablet made from crystals of the same particle size.

To simulate the actual conditions these adsorption measurements have been made on aspirin crystals of particle size similar to that used for preparation of the compound tablets, whereas the use of much smaller particles is to be preferred in adsorption studies on account of the larger surface area. It seems significant to find that the adsorption is measurable under these conditions.

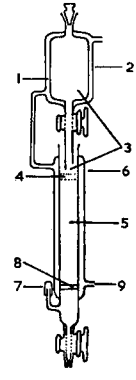

FIG. 3. Chromatography apparatus showing thermostatically controlled, jacketed, adsorption column.

1, Jacket. 2, Reservoir and constant head device. 3, Glycine solution saturated with aspirin. 4, Cotton wool. 5, Aspirin crystals. 6, Constant head. 7, Rubber cap. 8, Sintered disc. 9, Water inlet.

EXPERIMENTAL

The column (Fig. 3) was prepared by packing the aspirin crystals into a glass tube (diameter 1·45 cm., length 70 cm.) into which a sintered

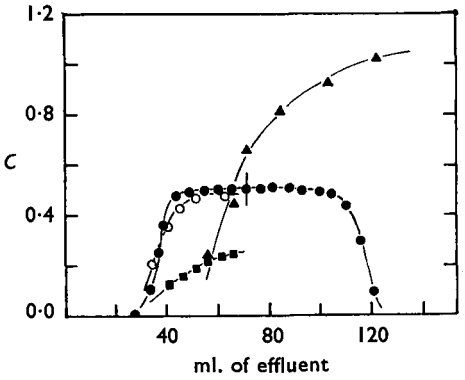

FIG. 4. Glycine chromatograms. Where C_o and C are, respectively, the inflowing and outflowing concentrations in per cent weight by volume.

■ $C_o = 0{\cdot}25$ per cent glycine; 22°, pH 3·0 (Small column).
○ $C_o = 0{\cdot}5$ per cent glycine; 22°, pH 3·0 (Small column).
▲ $C_o = 1{\cdot}0$ per cent glycine; 25°, pH 3·0 (Large column).
● $C_o = 0{\cdot}5$ per cent glycine; 35°, pH 2·0 (Small column).
Start of the elution with glycine free solvent.

glass disc of No. 2 porosity had been fused. A small pad of cotton wool at the top of the column prevented disturbance of the crystals by the inflowing solution. The stoppered reservoir, placed so that the outlet was just below the surface of the liquid in the column, acted as a constant head device, the flow rate being controlled by a tap at the bottom of the apparatus. Both reservoir and column were surrounded by a thermostatically controlled flow of water through a jacket shown in the Figure.

After introducing a weighed amount of aspirin crystals into the column, the latter was gently tapped and saturated aspirin solution was slowly drawn up to displace the whole of the air between the crystals. Saturated aspirin solution was then run in from the reservoir until steady flow conditions were established. The reservoir was then replaced by one containing the appropriate glycine solution saturated with aspirin.

Weighed quantities of effluent were taken at suitable intervals during each run, sampling more frequently when the glycine concentration was changing more rapidly. The samples were assayed for glycine by the ninhydrin method, using a spectrophotometer at a wavelength of 575 mμ.

Measurements were made with solutions containing 0·25, 0·5 and 1·0 per cent glycine at a pH of 3·0 and temperatures of 22° to 35° and with a

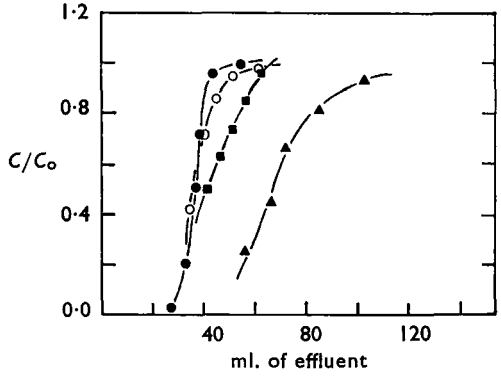

FIG. 5. Reduced glycine chromatograms. Where C_o and C are, respectively, the inflowing and outflowing glycine concentrations in per cent weight by volume.

■ $C_o = 0{\cdot}25$ per cent glycine; 22°; pH 3·0.
○ $C_o = 0{\cdot}5$ per cent glycine; 22°; pH 3·0.
● $C_o = 0{\cdot}5$ per cent glycine; 35°; pH 2·0.
▲ $C_o = 1{\cdot}0$ per cent glycine; 22°; pH 3·0.

0·5 per cent glycine solution at pH 2·0 and 35°. The experimental results are shown in Figures 4, 5 and 6. In the reduced chromatograms of Figure 5 the glycine concentration of the effluent is expressed as the ratio C/C_o where C_o is the concentration entering the column. In order to determine whether the glycine was irreversibly held by the aspirin, columns on to which glycine was adsorbed were washed (eluted) with a saturated solution of aspirin only and the effluent analysed for glycine. From the results of such an experiment illustrated in Figure 4, it would appear that the glycine is easily removed, suggesting that the binding is physical rather than chemical.

The essential points of a mathematical analysis of these measurements are now given in the following paragraphs.

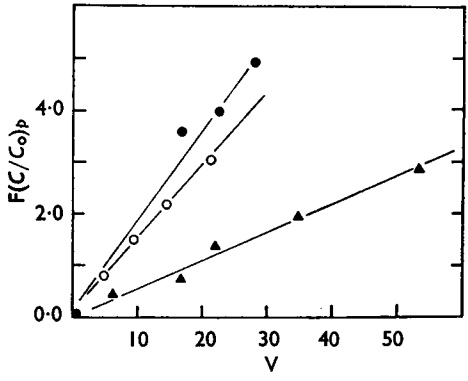

FIG. 6. Test of Gluekauf equation for adequate representation of chromatogram front profile. Values of the function $F\left(\frac{C}{C_o}\right)_p = \mathrm{Log}_{10}\left(1-\left(\frac{C}{C_o}\right)^{1-p}\right)$ for $p = 0{\cdot}25$ are plotted against the effluent volume V, which is in ml.

○ $C_o = 0{\cdot}5$ per cent glycine; 22°, pH 3·0.
● $C_o = 0{\cdot}5$ per cent glycine; 35°, pH 2·0.
▲ $C_o = 1{\cdot}0$ per cent glycine; 22°, pH 3·0.

Gluekauf[5] has deduced an equation relating the concentration to the volume of effluent for solutes having concave adsorption isotherms of the form

$$q = a\,C^p \qquad (1)$$

where q = amount adsorbed,
C = concentration of solute in solution,
p and a are constants depending on the solute, solvent and adsorbent.

The Gluekauf equation is

$$(V-V_t) = -\frac{F}{K(1-p)}\log_e\left\{1-\left(\frac{C}{C_o}\right)^{(1-p)}\right\} \qquad (2)$$

where V = volume of effluent,
V_t = threshold volume (breakthrough volume),
C_o = concentration of solute entering the column,
C = concentration of solute leaving the column at volume of effluent V,
F = flow rate,

and K is a constant, such that 1/K is the time taken for $1/e$th of the equilibrium amount of solute to adsorb on the adsorbent.

If this equation holds for a chromatogram, then a plot of $\log_e\left(1-\left(\frac{C}{C_o}\right)^{(1-p)}\right)$ against V will give a straight line whose slope m is given by:—

$$m = -\frac{K(1-p)}{F} \qquad .. \quad .. \quad .. \quad (3)$$

Calculations are made with various values for p and the value of p for the best fit is taken.

Figure 6 shows the results of such calculations for a number of chromatograms and it appears that a value of 0·25 (approx.) for p is most reasonable.

The points marked on the curve of Figure 1 have been obtained from equation (2) into which values for p and K have been inserted. It is possible to calculate K from equation (3) since the flow rate for any experiment is known and the slopes of the lines in Figure 6 are measurable.

The value for p of 0·25 has been used to calculate the isotherm shown in Figure 2. For interest the isotherm for $p = 0{\cdot}5$ is also given, since the most probable value of p is between 0·25 and 0·5. The equation to the isotherm is obtained by insertion of the value for p in equation (1). The constant a of the latter equation is then evaluated from any pair of experimental values for q and C.

Equation (1) is the well-known Freundlich adsorption isotherm. This isotherm has been used to correlate the results, with apparent success, even though it may not be strictly applicable to the adsorption phenomena studied. For this reason the extrapolated portions of the isotherms of Figure 2 are shown as broken lines; whilst the continuous line indicates the portion covered by the experimental conditions. This does not, however, detract from the fact that adsorption is taking place as evidenced by the time lag in arrival of glycine in the effluent.

Conclusions

It was suggested that the physiological differences in the behaviour of aspirin and aspirin with glycine should be capable of more precise explanation in physico-chemical terms.

It was also suggested that the basis of two possible and readily tested explanations were concerned with solubility, rate of dissolution and adsorption.

The hypotheses have been put to the test and the results show that aspirin is 15 to 20 per cent more soluble, and that it dissolves more rapidly, in the presence of glycine. Consequently it is to be expected that aspirin in the presence of the latter will be more rapidly transported from the mouth by salivation and swallowing.

The adsorption of glycine on aspirin particles has also been demonstrated. The mechanism of such adsorption is probably closely related to that of the adhesion of aspirin to the mucosal membranes. Consequently the non-adhesion of a glycine coated aspirin particle can be, quite reasonably, anticipated.

The conclusion that marked interaction between glycine and aspirin molecules occurs in solution is fully substantiated by the observed solubility and adsorption phenomena.

Edwards[4] suggests that diffusion is an important factor in determining the dissolution rate. The diffusion process would, therefore, seem to be affected by the interaction of glycine with aspirin, consequently such interactions may have an important influence on the transport and activity of aspirin in the body. Their exact nature and significance must, however, be the subject of further investigation.

Acknowledgements. The authors acknowledge with thanks the useful discussions they have had with Dr. J. Farquharson, Dr. L. J. Edwards and Mr. D. N. Gore; and the assistance they have received from their colleague Mr. D. F. Lawson.

References

1. *Brit. med. J.*, 1955, **2**, 31.
2. Douthwaite, *Lancet*, 1954, **2**, 917.
3. Vining and Kersley, *Brit. med. J.*, 1957, **1**, 444.
4. Edwards, *Trans. Faraday Soc.*, 1951, **47**, 1191.
5. Gluekauf, *J. chem. Soc.*, 1947, 1302, 1315.

After Mr. Rapson presented the paper there was a DISCUSSION. The following points were made.

The adsorption theory best explains the observed facts. Glycine is adsorbed on aspirin during manufacture of the tablets. There was no information on the particle size of the aspirin used in the final product. The proportion of glycine to aspirin was the lowest which would give the required effects. The preparation is subject to hydrolysis. Replacing glycine with sucrose does not disguise the taste of aspirin. The pH of the aspirin-glycine preparation is about pH 2. The glycine had no apparent effect on the adsorption peak of the aspirin.