J. Pharm. Pharmac., 1970, **22**, 265–269 *Received September* 18, 1969

Simultaneous determination of the effect of a non-ionic surfactant on the dissolution rate and biological activity of tablets of chlorpromazine hydrochloride

A. T. FLORENCE

School of Pharmaceutical Sciences, University of Strathclyde, Glasgow, C.1, U.K.

The effect of a non-ionic surfactant, polysorbate 80, on the biological activity of chlorpromazine hydrochloride in solution was investigated using the goldfish, *Carassius auratus*. Below a certain critical concentration the activity was enhanced in unbuffered drug solutions, but above this concentration the activity was diminished, possibly due to some association between surfactant micelles and drug molecules. The rate of solution of chlorpromazine hydrochloride from coated tablets was increased by 2% polysorbate 80, but the activity was decreased when compared with that in a simple aqueous dissolution medium. This is direct evidence of the erroneous conclusions that can be obtained if dissolution measurements alone are used in assessing the effect of additives on drug performance.

It is now well established that the presence of surfactants can drastically alter both the physical and biological behaviour of drugs in solution (Elworthy, Florence & Macfarlane, 1968). The rates of dissolution of a number of compounds, including griseofulvin (Elworthy & Lipscomb, 1968), hexoestrol (Bates, Gibaldi & Kanig, 1966) and prednisolone (Taylor & Wurster, 1965) are increased by the presence of surfactants in the dissolution medium. The biological activity of penicillins (Ullmann, 1961) and secobarbitone (Levy, Miller & Reuning, 1966) is increased below the critical micellar concentration (CMC) of the added surfactants and reduced above the CMC, the latter effect probably being due to the solubilization of the drug in the surfactant micelles.

It appears that no work has been done using simple systems in which both physical and biological parameters are measured in the same experiment.

The purpose of this paper is to present results which illustrate the necessity of making both biological and physical determinations to assess the effects of additives such as surfactants on the behaviour of organic biologically active substances. In this work goldfish are used as the test animals as described by Levy & Gucinski (1964), and the time of death noted as the end point of the experiment. The dosage form was placed in a beaker of surfactant solution containing the fish, the amount of drug released was measured spectrophotometrically at intervals and the biological effect on the fish determined by noting the time of death.

Chlorpromazine hydrochloride was chosen because it is surface active and because its solution properties are being studied in detail in this laboratory.

EXPERIMENTAL

Materials

Chlorpromazine hydrochloride was a commercial sample (Largactil, May & Baker) used without further purification. 100 mg chlorpromazine hydrochloride tablets were also of this brand. Polysorbate 80 (Tween 80), a polyoxyethylene sorbitan monooleate (Atlas Chemical Company), was used as received. Water was once distilled. Goldfish (*Carassius auratus*) were 2·5–3·5 g in weight.

Methods

Biological activity. The time of death of at least four goldfish immersed each in 200 ml (pH 5·74–5·76) of test solution at room temperature was noted, the end point being the cessation of mouth and gill movements and the lack of response of the fish to stimulation. The overturn point used by Gibaldi & Nightingale (1968) was unreliable at the concentrations of drug used in these experiments (up to 0·1%). The reciprocal of the death time (min^{-1}) was the index of activity used. A calibration curve of reciprocal death time versus concentration of chlorpromazine was obtained and found to be linear up to 0·1% (Fig. 1).

Drug release was measured by removing samples (1 ml) from the beaker at intervals, diluting 1 in 100 with water and determining the extinction at 255 nm using an SP500 (Unicam) spectrophotometer, using water or suitably diluted surfactant solution as blank. No extraneous material absorbing at this wavelength was released from the tablet coat nor did fish excretions interfere. In these experiments there was no stirring of the solution, except that caused by the movement of the fish, which, though random, was often vigorous in the small amount of solution in which the fish swam.

RESULTS

The reciprocal death time of goldfish as a function of the concentration of chlorpromazine HCl is shown in Fig. 1. Each point is the mean of at least four determinations. The effect of polysorbate 80 concentrations on the reciprocal death time

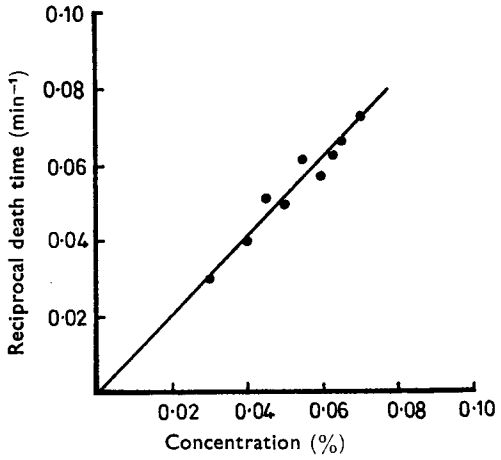

FIG. 1. Reciprocal death time of goldfish immersed in 200 ml of chlorpromazine hydrochloride solution as a function of the percentage concentration of drug. Each point is the mean of at least 4 results.

of the fish when immersed in 0·05, 0·065 and 0·1% chlorpromazine hydrochloride solutions (pH 5·75) is shown in Fig. 2. These results show the typical behaviour described by e.g. Levy, Miller & Reuning (1966); that is an increased activity at low concentrations of surfactant and a reduction in activity on increasing the surfactant concentrations to 1 and 2% which are greatly in excess of the CMC.

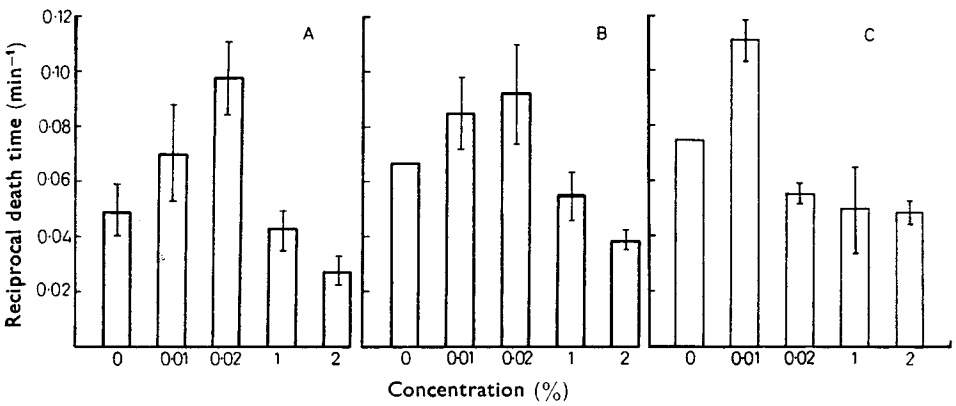

FIG. 2. The effect of polysorbate 80 on the time of death of goldfish immersed in chlorpromazine hydrochloride solutions (unbuffered) ± 1 standard deviation shown as vertical bars. A 0·05%, B 0·065%, C 0·10% chlorpromazine hydrochloride.

The significance of these results is shown by the representative figures presented in Fig. 3A for the rate of release of chlorpromazine from two 100 mg sugar coated tablets in the presence of polysorbate 80. In these experiments an increase in chlorpromazine release rate was observed with increase in surfactant concentration yet the biological activity of the solution in the 2% polysorbate is almost identical with that in water, although the fish has been in contact with about three times the amount of drug in the surfactant solution. Because drugs which are reasonably soluble in water are not thought to be significantly solubilized by surfactant micelles, it is perhaps unlikely that the reduction in activity noted is a true solubilization effect although there is possibly some association between the surfactant and the chlorpromazine, resulting if not in a decreased molecular mobility in solution then in a reduced rate of diffusion across the fish membrane. The nature of the interaction between phenothiazine derivatives and non-ionic surfactants is being investigated by nuclear magnetic resonance and pH measurements to differentiate between solubilization effects and the effect on the ionization of the chlorpromazine. The addition of non-ionic surfactants such as cetomacrogol lowers the pH of chlorpromazine HCl solutions suggesting a greater dissociation which is reminiscent of the micellar form of the chlorpromazine. The same experiments were made in phosphate buffer at pH 6·0 to determine whether bulk changes were responsible for the observed effects. However, the same general results were obtained (Table 1) although there was no evidence for the enhancement of activity at any of the concentrations of polysorbate used. Drug release and activity in stirred buffered solutions are shown in Fig. 3B.

In Fig. 2 it can be seen that the enhancement of activity at 0·02% polysorbate 80 is more pronounced in the more dilute drug solution. As phosphate buffer at pH 6 appears to enhance the activity of the 1·0% chlorpromazine (reciprocal death time

0·128 min^{-1} compared with 0·078 min^{-1} in unbuffered solution) the results in the phosphate are perhaps a continuation of the trend in the enhancement of activity by the polysorbate.

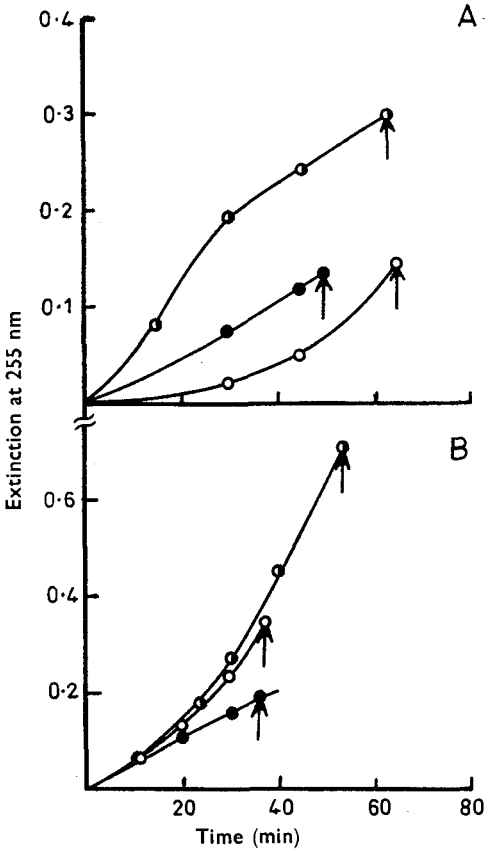

FIG. 3. Two representative results of the effect of polysorbate 80 concentration on the dissolution of drug from commercial chlorpromazine hydrochloride tablets and the biological activity in the goldfish. Death time in each denoted by an arrow. A in water (unstirred), B in phosphate buffer (stirred) at pH 6·0. Ordinate: extinction at 255 nm of a 1 in 100 dilution of sampled solution. ○ Water or buffer; ●, 0·01% polysorbate 80; ◑, 2·0% polysorbate 80. The experiments in the mechanically stirred solution (B) show the same overall effects as those in the unstirred solution.

Table 1. *Effect of polysorbate* 80 *on reciprocal death times of goldfish immersed in chlorpromazine hydrochloride solutions buffered at pH* 6·0

Polysorbate concn (%)	Chlorpromazine concn (%)	Reciprocal death time* ± standard deviation (min^{-1})
0	0·05	0·0847 ± 0·0107
0·02	0·05	0·0854 ± 0·0098
0	0·1	0·128 ± 0·039
0·01	0·1	0·104 ± 0·010
0·02	0·1	0·077 ± 0·051
1·0	0·1	0·072 ± 0·006
2·0	0·1	0·061 ± 0·009

* Results of four fish at each concentration.

DISCUSSION

In many published investigations an increased rate of solution of drug from a dosage form has been presumed to lead to more rapid absorption. Such an assumption would be erroneous in this study, which indicates that high concentrations of surfactant, that increase dissolution rates, can retard the biological effects. The tablet–water–goldfish system does not immediately appear to approximate to the human *in vivo* situation. However, Levy & his co-workers (1966) have claimed that as far as passive diffusion characteristics are concerned the fish membrane bears a similarity to human membranes.

There is a need for a simple laboratory test which has relevance to the *in vivo* performance of a formulation. Goldfish can be used with many drugs other than the phenothiazine tranquillizers: the barbiturates (Levy, Miller & Reuning, 1966), alcohol (Gibaldi & Nightingale, 1968) and an analgesic (Flanagan, Broad & others, 1969) are recent examples. The system described here has the advantage of great simplicity and goes one stage toward the development of a relevant routine test. That pretreatment of fish with polysorbate does not affect the subsequent activity of chlorpromazine by having a direct effect on the membrane requires investigation. Low concentrations of polysorbate may make the membrane more permeable whilst high concentrations might make it less permeable. This could explain the results.

Acknowledgement

I thank Mrs. Christine Selkirk for technical assistance.

REFERENCES

Bates, T. R., Gibaldi, M. & Kanig, J. L. (1966). *Nature, Lond.*, **210**, 1331–1333.

Elworthy, P. H., Florence, A. T. & Macfarlane, C. B. (1968). *Solubilization by Surface-Active Agents*, Chapter 4. London: Chapman & Hall.

Elworthy, P. H. & Lipscomb, F. (1968). *J. Pharm. Pharmac.*, **20**, 923–933.

Flanagan, T. H., Broad, R. D., Rubinstein, M. H. & Longworth, A. R. (1969). *Ibid.*, **21**, *Suppl.*, 129S–134S.

Gibaldi, M. & Nightingale, C. H. (1968). *J. pharm. Sci.*, **57**, 226–230.

Levy, G. & Gucinski, S. P. (1964). *J. Pharmac. exp. Ther.*, **146**, 80–86.

Levy, G., Miller, K. E. & Reuning, R. H. (1966). *J. pharm. Sci.*, **55**, 394–398.

Taylor, P. W. & Wurster, D. E. (1965). *Ibid.*, **54**, 1654–1658.

Ullmann, E. (1961). *Proc. 21st Intern. Pharmaceutical Conference*, Pisa.