
Influence of egg albumin of dissolution of Methotrexate

U. V. SINGH AND N. UDUPA*

College of Pharmaceutical Sciences
Kasturba Medical College, Manipal - 576 119

Accepted 1 August 1997

Received 14 October 1996

Solid dispersion of methotrexate (MTX) was prepared using natural polymer egg albumin. The *in vitro* release of MTX was faster from solid dispersions. The interaction in solid state as revealed by XRD and DSC showed that the crystallinity of the drug was affected. It is predicted that there may be complex formation between the two components.

THE solubilities of poorly water soluble drugs are found to be increased by their dispersion in a water soluble carrier^{1,2}. The most commonly used carriers are long chain polymers like polyethylene glycol and polyvinylpyrrolidene³⁻⁵. Solid dispersion techniques have been utilized to reduce the particle size of drugs and to increase their dissolution rates^{6,7}. From the safety point of view, a naturally occurring polymers such as proteins or polysaccharides may be a good and ideal candidate. Egg albumin is a water soluble polymer widely used in the field of food and in the pharmaceutical formulations. Very few attempts have been made to improve the dissolution of drugs employ naturally occurring polymers in contrast to synthetic polymers. The present work describes the possible utility of egg albumin as a water soluble carrier. Methotrexate was chosen because it is practically insoluble in water and by solid dispersion technique dissolution can be enhanced. Although on oral administration absorption is not a major problem yet some side toxic effects are associated with it which may be overcome due to faster dissolution. Faster dissolution may reduce the drug residence time in gastrointestinal tract and result in faster absorption. Thus solid dispersion of methotrexate was prepared and *in vitro* release and spectral analysis was carried out.

Albumin (ovalbumin, grade II, Crude dried egg white, mean molecular weight 45000) and methotrexate (molecular weight 454.4) were procured from Sigma Chemical Co., (USA) The drug albumin solid dispersion (1:1, 1:3, 1:5, 1:10, w/w) were prepared by the kneading method. The required quantity of drug and egg albumin were weighed and placed in a mortar. The mixture was kneaded with 1.4 times their amount of water for one hour and then dried at 15° under vacuum. The physical mixtures of MTX with albumin in the same ratios were prepared by simple blending in the mortar.

Dissolution rate tests were carried out at 37±0.5° and 50 RPM in 200 ml of phosphate buffer pH 7.2 according to USP-XXII, No.2 paddle method. The equivalent amount of 5 mg of drug as a 100 mesh powder was weighed and put in the dissolution cell. At different time intervals aliquot was withdrawn. The aliquot after filtration through nalgene filters was extracted with chloroform to remove egg albumin. After centrifugation at 2500 rpm for 15 minutes, the organic phase was transferred to a new tube, and the drug concentration was measured spectrophotometrically at 303 nm. The volume withdrawn from the dissolution cell was replenished with an equal amount of buffer after each sampling.

The solubility measurements were carried out according to method reported by Higuchi and Connors⁸. Excess amount of drug was added to aqueous

*For correspondence

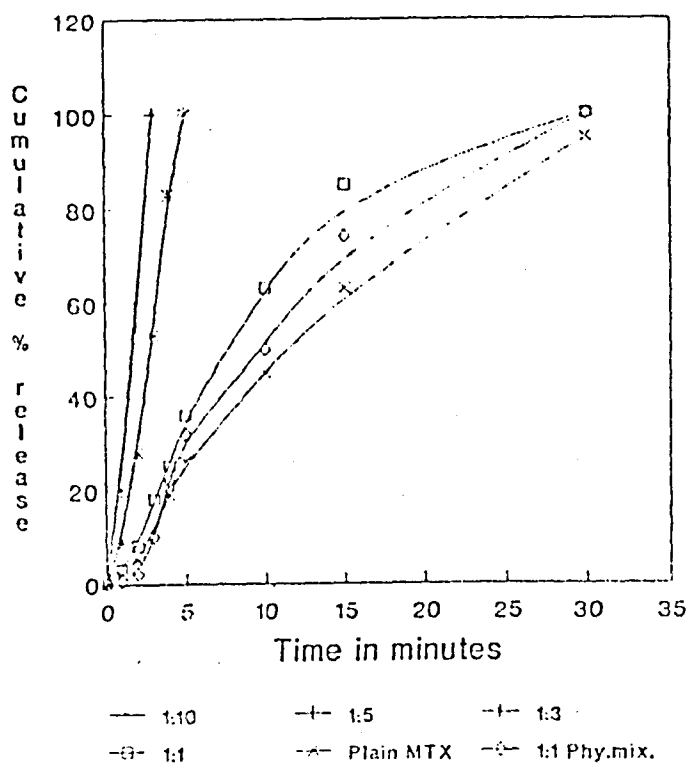


Fig. 1 : *In Vitro* dissolution profile of MTX-Albumin solid dispersion

solutions containing various concentrations of egg albumin. The flasks were vigorously shaken in a shaker bath at room temperature and protected from light for 3 hours. This short period of shaking for 3 hours was done because decomposition of albumin was observed after shaking for 8 hours. The solubility of drug in water after vigorous shaking for 3 hours was same as that after shaking for 7 days. The suspension was centrifuged and filtered through Whatman No.1 filter paper. The filtrate was extracted with chloroform and the drug content was analysed at 303 nm spectrophotometrically.

The powder X-ray diffraction (XRD) pattern of MTX, Albumin, phy. mix and solid dispersion were recorded using X-ray diffractometer Philips model PW 1140/90. Differential scanning calorimetry was recorded on V4.OB DUPONT 2000.

Fig. 1 shows the dissolution profile of MTX from its solid dispersion with albumin. The solid dispersion exhibited a significantly greater dissolution rate than that of drug or Physical mixture. The dissolution rate of the drug from the solid dispersions increased as the amount of albumin increased. In order to eluci-

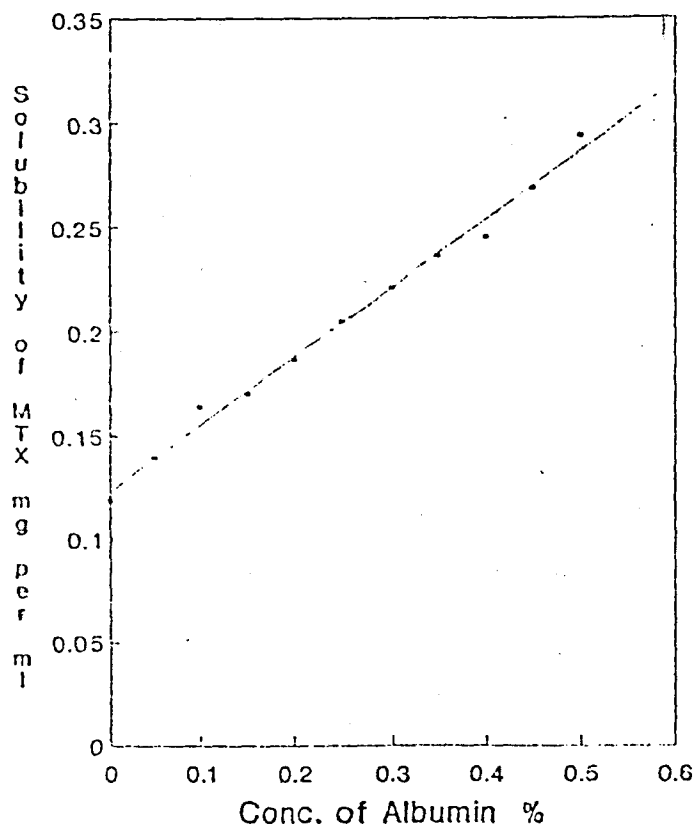


Fig. 2 : Phase solubility of MTX with Albumin

date the dissolution mechanism of drug from albumin solid dispersion, the interaction of drug with albumin was studied in aqueous solution and in the solid state.

Fig. 2 shows the phase solubility profiles of MTX with egg albumin. The drug solubility increased with increasing albumin concentration. Similar increased solubility of flurbiprofen and pindolol with egg albumin have been reported⁹. Product formed may be due to the hydrophobic and electrostatic interaction between the drug and albumin as expected from drug-serum albumin interaction which may result in increase in solubility of drug with increase in albumin content^{10,11}.

The XRD of albumin did not show any prominent peaks indicating its amorphous nature (halo pattern). MTX showed intense peaks indicating its crystalline nature. The solid dispersions were prepared in various ratios viz. 1:1, 1:3, 1:5 and 1:10. As the content of albumin increased the solid dispersions became more and more amorphous leading to the suppression of prominent peaks of MTX. In 1:1 solid dispersion, although many of the drug peaks were seen but

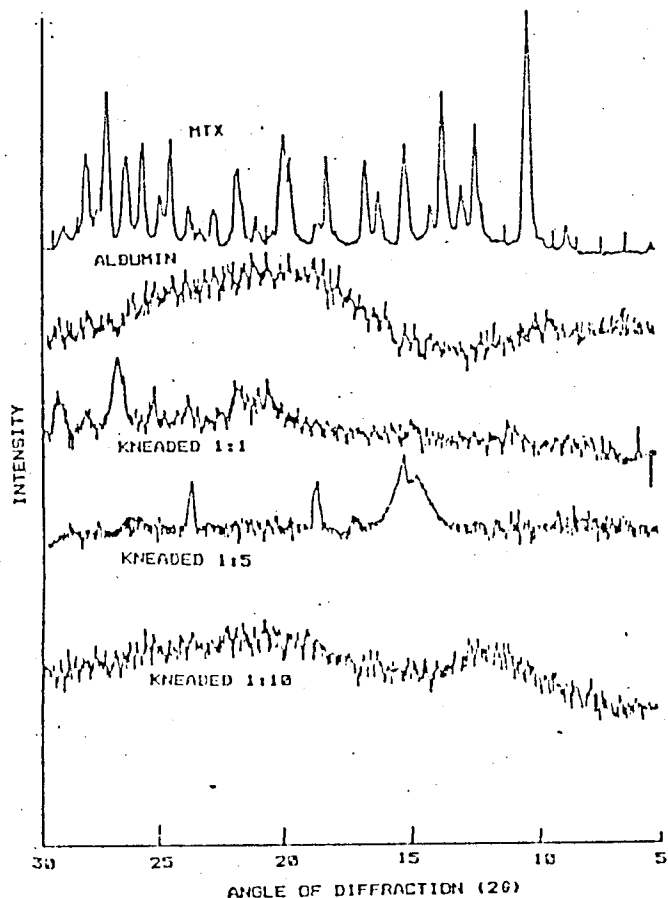


Fig. 3: X-ray diffraction pattern of MTX albumin solid dispersions

the intensity decreased and was superimposed on continuous spectra. This tendency of suppression of crystallinity of drug continued as the content of the albumin increased. The solid dispersion prepared in the ratio 1:10 was truly amorphous in nature. The drug peaks were completely suppressed resembling plain albumin. The physical mixture showed prominent peaks of the drug (Fig 3).

Fig. 4 shows the DSC thermograms of drug-albumin (1:10) solid dispersion and physical mixture (1:10). The polymorphic transformation of the drug on heating was observed due to appearance of an exotherm at 177° before melting at 182°. The DSC of albumin showed peak corresponding to the vaporization of moisture (47°). The disappearance of drug peak from the solid dispersions and the physical mixture indicated that MTX had interacted with albumin and this excluded the presence of crystalline drug in the solid dispersion and the physical mixture. How-

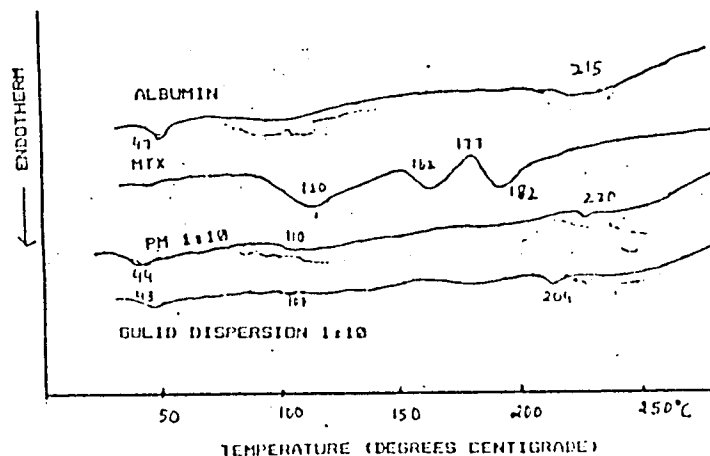


Fig. 4: DSC curves of MTX albumin solid dispersions

ever the *in vitro* dissolution studies showed faster drug release from solid dispersion than physical mixture. The preliminary results showed that MTX solid dispersion with albumin increased the dissolution rate by lowering the crystallinity.

REFERENCES

1. Chiou, W.L. and Riegelman, S., *J. Pharm. Sci.*, 1971, 60, 1281.
2. Ford, J.L. *Pharm. Acta. Helv.*, 1986, 3, 69.
3. Shefter, E. and Cheng, K.C., *Int. J. Pharm.*, 1980, 6, 179.
4. Ford, J.L., Stewart, A.F. and Dubois, J.L., *Int. J. Pharm.*, 1986, 28, 11.
5. Jachowicz, R., *Int. J. Pharm.*, 1987, 35, 7.
6. Allen, L.V., Levinson, S.R. and Martono, D.D., *J. Pharm. Sci.*, 1978, 67, 979.
7. Acartuk, F., Ksilla, O. and Celebi, N., *Int. J. Pharm.*, 1992, 85, 1
8. Higuchi, T. and Connors., *Adv. Anal. Chem. Instr.*, 1965, 4, 117.
9. Imai, T., Saito, Y., Matsumoto, H., Satoh, T. and Otagiri, M., *Int. J. Pharm.*, 1989, 53, 7.
10. Tanford, C., *The hydrophobic effect: formation of micelles and biological membranes*, Wiley, New York, 1973, 64.
11. Vallner, J.J., *J. Pharm. Sci.*, 1977, 66, 447.