RESEARCH PAPER

Accepted 3 June 2005
Revised 17 January 2005
Received 15 May 2004
Indian J. Pharm. Sci., 2005, 67(3): 342-345

Studies on Solubility Parameter of Amoxycillin Trihydrate: Influence on *In Vitro* Release and Antibacterial Activity

H. KARANTH AND V. R. JOSYULA*
Department of Pharmacy Management, College of Pharmaceutical Sciences
MAHE, Manipal-576 104

Amoxycillin trihydrate, a broad spectrum antibiotic, is a poorly water-soluble drug and has low bioavailability on oral administration. Solubility parameter of the drug (δ_2) was evaluated in blends of ethyl acetate-propylene glycol and water-propylene glycol in the ratios 100:0, 75:25, 50:50, 25:75 and 0:100. The results obtained were compared with the δ_2 values obtained using Molar Volume method and Fedor's group substitution method. Ethyl acetate-propylene glycol (75:25) was found to give maximum solubility with an experimental value of 13.32 H in comparison to the theoretical values of 13.25 H by molar volume method and 15.46 H from Fedor's group substitution method. *In vitro* drug release studies and antibacterial effect were evaluated with an intention to study the effect of the solvent blends on the drug release and its activity. In the *in vitro* studies, the water-propylene glycol (100:0) and the ethyl acetate-propylene glycol (50:50) ratios gave maximum release of the drug in their respective blends. The results of the *in vitro* studies correlated with the antibiotic Study conducted on both binary blends.

Amoxycillin trihydrate, semi-synthetic penicillin, is a broad spectrum antibiotic used against various gram positive and gram negative organisms[1] Though the molecule is found to be effective against these microorganisms, its therapeutic efficacy is hindered due to its poor aqueous solubility[2] Co-solvency is one among the methods to improve solubility especially in case of liquid preparations[3] The choice of the appropriate co-solvent is important to obtain maximum solubility of the drug. Evaluation of solubility parameter in different solvent blends of various polarities would provide important insight about the solubility of the drug. Solubility parameter is an intrinsic physiochemical property of a substance which has been used to explain drug action[4,5], structure activity relationship[6], drug transport kinetics[7] and in situ release of drug[5]. The present study attempts to evaluate the solubility of Amoxycillin trihydrate in different blends of ethyl acetate-propylene glycol (EA-PG) and water- propylene glycol (W-PG). Experimental values obtained were compared with the theoretical values obtained bv molar volume method and Fedor's group substitution method. Water, propylene glycol and ethyl acetate were selected based on their Hildebrand values[8,9]. Water and ethyl acetate exhibit extremities of polarity and polarity of propylene glycol lies in between water and ethyl acetate[8,9]. The various blends prepared from the above solvents were analysed for its solubility, *in vitro* drug release studies and Antibacterial studies.

*For correspondence
E-mail: jvrao56@hotmail.com

MATERIALS AND METHODS

Amoxycillin trihydrate was gift sample from Brown and Burk Pharmaceutical Limited, Bangalore. Propylene glycol and ethyl acetate were purchased from Ranbaxy Laboratories, Punjab. Culture media were procured from Hi-Media Lab Pvt. Ltd., Mumbai. All other chemicals and reagents used in the study were of analytical grade and used as such.

Solubility studies:

Different blends of EA-PG and W-PG of known ratios,

100:0, 75:25, 50:50, 25:75 and 0:100, respectively, were prepared. The ratios were selected in order to conduct solubility at different values ranging 8.9 H to 23.4 H (δ_1 of ethyl acetate 8.9 H, propylene glycol 14.8 H, and water 23.4 H). Excess quantities of each of amoxycillin were added with shaking until no more solute is soluble. The conical flasks were sealed and clamped on a mechanical shaker at 25±1°, the flasks were rocked for 24h at a constant speed. After equilibrium was attained, the flasks were removed for analysis[10]. The solutions were filtered using Whatmann filter paper No 1. The filtered solution is diluted properly using 0.1N Sodium hydroxide solution and the drug content is analysed using UV spectrophotometer at 247 nm (λ_{max}).

Theoretical calculations of solubility parameter (δ_2):

Theoretically solubility parameter of amoxycillin trihydrate in different solvent blends was calculated using the molar volume method[11] and Fedor's group substitution method[11]. Equation for calculating solubility parameter using Fedor's group substitution method is, $\delta=\sqrt{\Sigma\Delta\Delta U}/\sqrt{\Sigma\Delta V}$, where $\Delta\Delta U$ represents the substituent fragment constant and ΔV the fragmental molar volume constant

Molar volume method:

The solubility parameter of amoxycillin was found out by calculating the mole fraction solubility of the blend. The number of moles of an individual solvent in the given blend is calculated and the mole fraction solubility is calculated using the equation, mole fraction solubility, $X_2=\eta_2/\eta_1+\eta_2$ where, η_1 and η_2 are the number of moles of solvent and solute, respectively. A plot of mole fraction solubilities of various ratios of the binary mixtures are plotted against (δ_1-δ_2), difference between solubility parameter of solvent and solute respectively. The peak mole fraction solubility represents the solubility parameter of the solute.

***In vitro* drug release studies:**

Stock solutions of amoxycillin trihydrate were prepared by dissolving the required amounts in all the three solvents viz., water, propylene glycol and ethyl acetate separately. The concentrations of the stock solutions were 200 µg/ml. Then the stock solutions were mixed in various proportions to vary the solubility parameter (δ_1). Five milliliters of these prepared ratios were taken in the diffusion cell (donor compartment) and 50 ml of distilled water in a beaker (recipient compartment). The sigma membrane was soaked in water over night (between 10–12h). The speed of rotation (50 rpm) was kept uniform in all the cases. Samples were withdrawn at different time interval viz., 20, 40, 60, 120, 150, 180, 240, 360 min and analyzed using a spectrophotometer at 228 nm (λ_{max}).

Antibacterial studies:

Two milliliters of prepared assay medium C[12] was taken in test tubes plugged with non-absorbing cotton and sterilised by autoclaving at 15 lbs/sq.in. at 121° for 20 min. The test tubes were labeled with concentration of drug and organism (*E. coli*). 2 ml stock solution of drug (20 µg/ml) was added to the first tube from this, 2 ml was transferred to the second tube. In the same manner rest of the dilutions were done. At each dilution the concentration of the drug gets reduced by half. All the tubes were then inoculated with the same amount of inoculum and then incubated for 24 h. Minimum inhibitory concentration (MIC) values were noted down. Reciprocal of MIC values obtained were plotted on Y-axis versus the difference between the solubility parameter of the solvent and solute (δ_1-δ_2).

RESULTS AND DISCUSSION

Solubilization of a poorly soluble drug is important in the formulation of liquid dosage forms. One of the important methods to improve the solubility of a drug with poor water solubility is by the use of cosolvents. Solubility predictions are valuable for obtaining the optimum concentration of the cosolvent in preparing a liquid dosage form of a drug especially in preformulation studies where a small amount of drug is available to the formulator.

Solubility studies were carried out in different solvent blends using mixtures of EA-PG and W-PG. The solubility was found to be maximum when the δ_2 values were closer to δ_1. The mole faction solubility and the solubility parameter of the various blends are tabulated in Table 1 and the graphical calculation of the solubility of the drug is represented in fig. 1 and 2. Experimentally, the peak solubility parameter (δ_2) of Amoxycillin trihydrate was found to be 13.33 H. This value was compared with the values calculated by Fedor's group substituent constant method (15.46 H) and calculations using molar volume (13.24 H). The experimentally calculated value (13.33 H) was used for rest of the studies.

The blends prepared from (W-PG) showed higher drug release as the water proportions increased. W-PG (100:0) gave the maximum release, whereas in blends containing EA-PG (50:50) gave maximum release when compared to other ratios in the binary system. The faster releases of the various blends were observed to be due to higher (δ_1-δ_2) values. The blend W-PG (0:100) gave minimum release,

TABLE 1: MOLE FRACTION SOLUBILITY OF AMOXYCILLIN TRIHYDRATE IN DIFFERENT SOLVENT BLENDS

Solvent ratio	δ_1	$(\delta_1-\delta_2)$	Mole fraction solubility, $X_2 \times 10^{-4}$
W-PG (100:0)	23.40	+10.08	1.263
W-PG (75:25)	21.25	+7.925	1.786
W-PG (50:50)	19.10	+5.775	2.640
W-PG (25:75)	16.95	+3.625	3.619
W-PG (0:100)	14.80	+1.475	4.042
EA-PG (25:75)	13.33	+0.000	13.56
EA-PG (50:50)	11.85	-1.475	12.28
EA-PG (75:25)	10.38	-2.950	4.267
EA-PG (100:0)	8.900	-4.425	0.220

δ_1 = Solubility parameter of solvent blend , δ_2 = Solubility parameter of drug in solvent blend

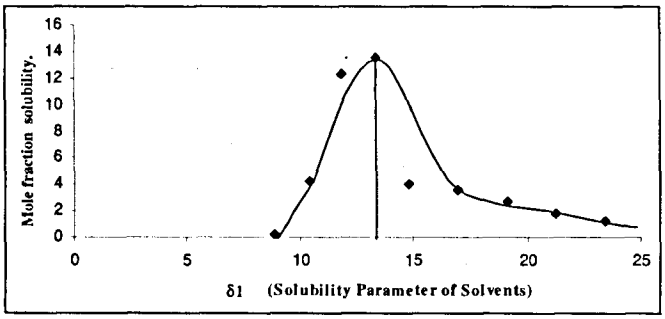

Fig. 1: Solubility Parameter of Amoxycillin Trihydrate

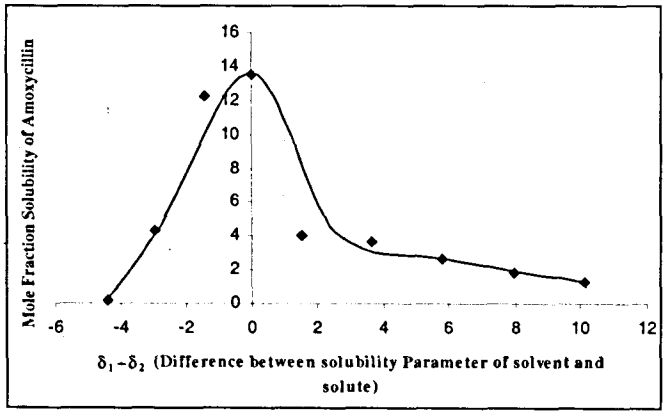

Fig. 2: Solubility Parameter by mole fraction solubility.

which may be attributed to the affinity of the solute molecules with the solvent molecules. The other factors, which also might have influenced are, viscosity of the solvent and solubility parameter of the membrane (δ_0). A plot of cumulative drug release (μg) v/s $(\delta_1-\delta_2)$ of both blends combined gave a parabolic relationship (fig. 3).

In vitro drug release studies alone would not help to predict the release pattern in a biological system. In the present study, *in vitro* release studies indicated that when the δ_2 value matches with δ_1 value there would be more solubility of drug, where as the release would be minimum. Hence to promote the release of drug from the solvent system and to permeate across the membrane of bacteria, it is required to see that δ_2 and δ_0 (membrane solubility parameter) are as similar as possible. As it is not possible to calculate the δ_0 due to the complex membrane chemistry, indirectly through the antimicrobial studies, it may be possible at least to study the release pattern if not finding δ_0 exactly.

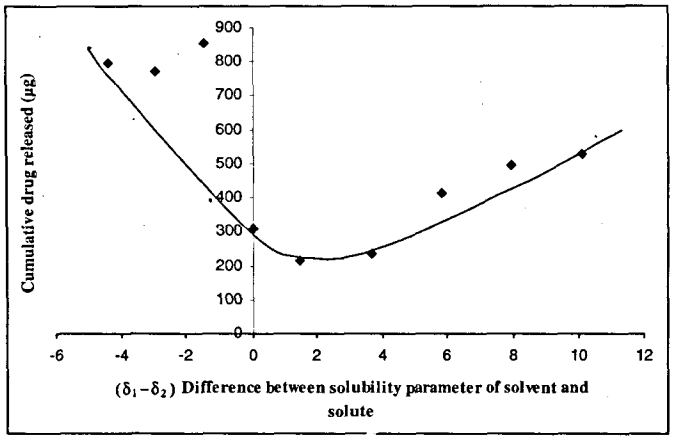

Fig. 3: Release Studies of Amoxycillin from solvent blends

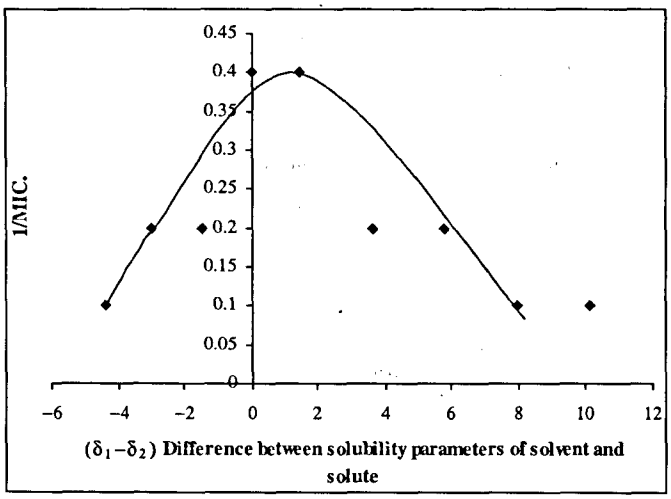

Fig. 4: Antibacterial activity of Amoxycillin in binary solvent blends

The present study indicated a parabolic relationship between (δ_1-δ_2) and 1/MIC in case of Amoxycillin trihydrate (fig. 4). The W-PG (0:100) and EA-PG (75:25) required the least amount of drug 2.5 μg/ml to elicit its antibacterial activity. Here the EA-PG (75:25) had given the maximum release of the drug by the virtue of its low (δ_1-δ_2) value in comparison to its other ratios in the binary blends. Whereas, W-PG (0:100) gave similar results as it had low (δ_1-δ_2) values in comparison to the ratios in its binary system. Although the blend had least *in vitro* release, the solvent itself may have contributed the antibacterial activity. The *in vivo* studies should be carried out further to confirm this trend. The present study could provide a lead in that direction.

To conclude, a drug to be formulated as a dosage form, the preformulation studies should be conducted and the solvent and solute characteristics should also be considered. For each drug depending upon its characters, the formulator should choose an appropriate solvent or method to develop a dosage form. The formulator must also correlate the *in vitro* and *in vivo* pattern while developing a dosage form and there should not be a generalized pattern. In that context, solubility parameter may be considered as a useful indicator to correlate *in vitro* studies with *in vivo* studies and to assist the formulator to choose a proper solvent blend in formulating a liquid dosage form.

REFERENCES

1. William A. Petri, Jr., In; Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, W.R. and Gilman, A.G., Eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Edn., McGraw-Hill Companies, Inc., New York, 2001, 1189.
2. Yalkowsky S.H., In; Eds. Techniques of Solubilization of Drugs, 12th Edn., Marcel Dekker Inc., New York, 1981, 92.
3. Krause, G.M. and Cross, J.M., **J. Amer. Pharm. Assoc. Sci. Ed.,** 1951, 40, 137.
4. Martin A., Bustamante, P. and Chun, A.H.C., In; Eds. Physical Pharmacy, 4th Edn., Waverly International, Maryland, 1994, 212.
5. Mullins, L., **Chem. Rev.,** 1954, 54, 289.
6. Khalil, S.A. and Martin A.N., **J. Pharm. Sci.,** 1967, 56, 1223.
7. Adjei, A., Newberger, J., Stavchansky, S. and Martin, A., **J. Pharm. Sci** 1984, 73, 742.
8. Hildebrand, J.H., Prausnitz, J.M. and Scott, R., In; Eds. Regular and Related Solutions, Van Nostrand Reinhold, New York, 1970, 22.
9. Hansen, C. and Beerbower, A., In; Eds., Encyclopedia of Chemical Technology, Suppli. Vol. 1, 2nd Edn., Wiley, New York, 1971.
10. Subrahmanyam, C.V.S. and Sarasija. S., **Eur. J. Pharm. Biopharm.,** 1999, 47, 289.
11. Fedors R.F., **Polym. Eng. Sci.,** 1974, 14, 147.
12. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Govt. of India, Delhi, 1996, 50.