produce hypotension when high doses are given initially.

In conclusions, Tweens and Spans enhanced the transdermal permeation of prazosin HCl. The intra group variation among the Spans and Tweens were insignificant. Tweens were found to be better than Spans in the enhancement of permeation of the drug through excised guinea pig skin. Therapeutic levels of the drug could be achieved through transdermal permation.

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REFERENCES

1. Tenjasla, S.N., and Tseggai, A., **J. Clin. Pharm. Ter.**, 1992, 17, 37.

2. Vollmer, U., Muller, B.W., Wilffert, B. and Peters, P.J., **Pharm. Pharmacol.**, 1993, 45, 242.

3. Moffat, A.C., Jackson, J.V., Moss, M.S., Widdop, B., and Greenfield, E.S., Eds. In, Clarke's isolation and identification of drugs in pharmaceuticals, body fluids, and post mortem material, 2nd Ed, The Pharmaceutical Press, London, 1986, 916.

4. Hoffman, B.B. and Lefkowitz, R.J., In: Gilman, A.G., Rall, T.W., Nies, A.S., Taylor, P., Eds, The Pharmacological basis of Therapeutics, 8th Ed, Pergamon Press, New York, 1990, 226.

Bioactive Polymers; Synthesis, Characterisation, Release and Antimicrobial Property of Macromolecular Prodrug of Ampicillin

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The matrix of poly (methyl methacrylate-co-maleic anhydride) with surface containing functional anhydride group of different percentage was prepared by solution polymerization and characterized. A macromolecular prodrug of ampicillin was synthesized by linking the amino group of ampicillin to anhydride group of matrix via an amide bond. The amount of ampicillin covalently bound to the matrix was spectroscopically characterized and the *in vitro* release rate in weakly basic medium was established with its antimicrobiological activity. This prodrug allows a prolonged release (7-8 days) of the drug.

MUCH attention has been lately paid to the preparation and properties of pharmacoogically active polymers[1-6] which can serve as a carrier for low molecular weight drugs to form prodrugs. The controlled slow release of pharmacologically active components in the body can be achieved from prodrugs which can be considered as a special type of drug delivery system from which drug release is

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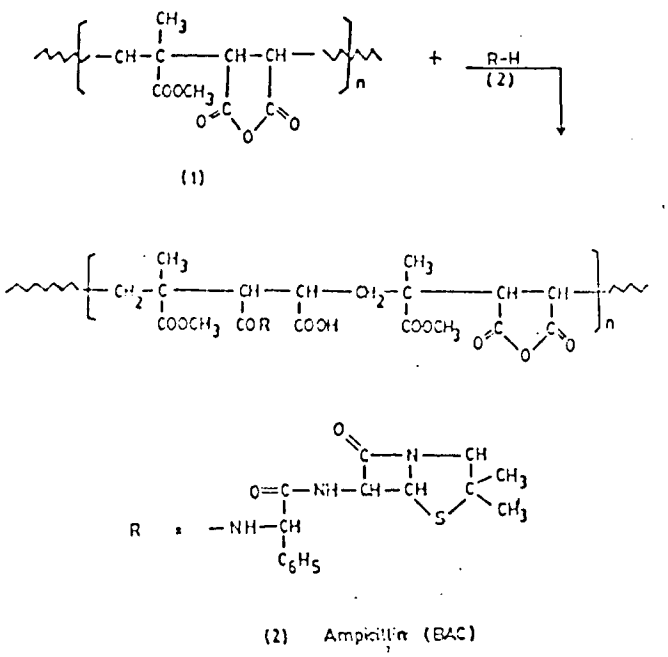
REACTION SCHEME



(1)

(2) Ampicillin (BAC)

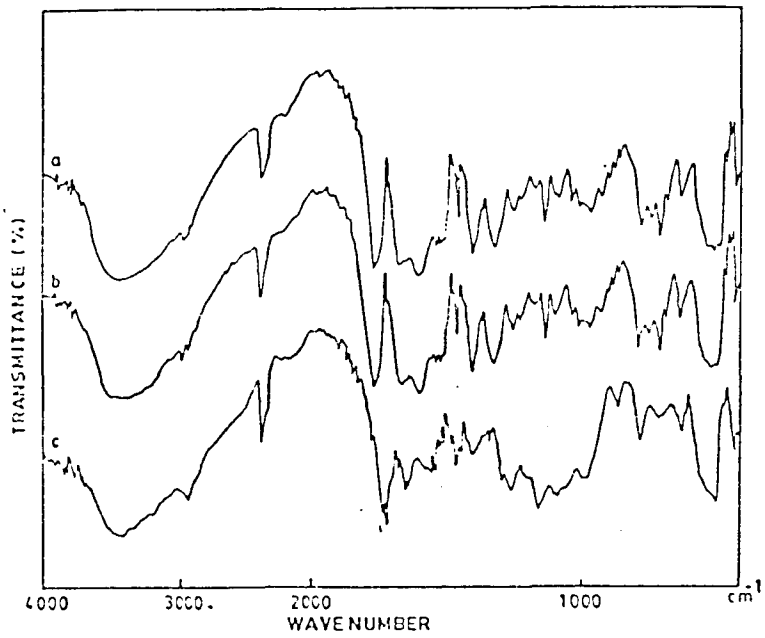


Fig. 1: I.R. Spectra of (a) methyl methacrylate-co-maleic anhydride (b) ampicillin (c) polymer bound ampicillin

accomplished by the cleavage of chemical bond[7]. The present study reports the synthesis of macromolecular prodrug of ampicilin to anhydride group of matrix of poly(methyl methacrylate-co-maleic anhydride) via amide bond. *In vitro* release rate, the amount, and the antimicrobiological activity of ampicilin covalently bound to the matrix with the surface containing different percentage of anhydride group have been studied.

Methyl methacrylate (inhibitor free) was obtained from G.S.F.C Polymers Unit, Baroda, India. Ampicilin was purchased from Central Drug House, New Delhi. All the chemicals and solvents used were of analytical grade.

The polymeric carrier with average molecular weight (16,700- 48,000) was prepared by solution polymerization of methyl methacrylate with maleic anhydride using azo catalyst at 75° in toluene as solvent by a patented method[8]. The copolymer obtained was characterised by measuring intrinsic viscosity,limiting viscosity number in dimethyl formamide (DMF) solution at 25° ± 0.1° as per ASTM-D-2837 using Ubbleohde Viscometer for dilution sequences. The intrinsic viscosities in DMF were observed to be 0.0179 $dl^{-1}g$, 0.01654 $dl^{-1}g$, 0.01649 $dl^{-1}g$ and 0.0157 dl^{-1} for PM-MA-1, PM-MA-2, PM-MA-3 and PM-MA-4. The weight average molecular weight (Mw) was determined in dioxane by GPC maxima 820.

Coupling of ampicillin (AMP) to the macromolecular carrier (1) in the presence of triethyl amine (TEA) is based on the following reaction.

Dimethyl sulfoxide (DMSO) proved to be the most appropriate solvent for the coupling reaction of AMP to MMA-MA copolymer. Coupling reaction was carried out by the following method[9]. 1g of the polymer matrix (constant in all synthesis) was dissolved in 25 ml anhydrous DMSO with stirring. TEA 0.25 ml was then added and calculated amount of AMP was dissolved in 12.5-13 ml of DMSO and added dropwise at 20-25° into previously prepared solution of methyl methacrylate-co-maleic anhydride. The reaction mixture was stirred for 1 h at 20° and

Table 1

Composition of Poly (methyl methacrylate-co-maleic anhydride) and Release Rate Data at 37°C

Polymer System	Moles of MMA : MA in feed Composition	Moles of MMA : MA in copolymer composition	Content of AMP in mg/g of copolymer	Percent Drug (AMP) Release			Time in days
				pH -7.4	pH - 7.6	pH - 7.8	
PM-MA-1	1 : 0.340	0.789 : 0.214	212.40	51.9	57.3	61.4	8
PM-MA-2	1 : 1.02	0.602 : 0.402	414.81	61.71	68.46	72.9	8
PM-MA-3	1 : 1.53	0.550 : 0.456	426.40	64.37	68.8	75.94	8
PM-MA-4	1 : 3.06	0.512 : 0.469	559.44	71.3	78.0	81.3	6

Abbreviations :
MMA : Methyl methacrylate
MA : Maleic anhydride
PM-MA-1 : Poly (methyl methacrylate-co-maleic anhydride) containing 20.30 mole % MA
PM-MA-2 : Poly (methyl methacrylate-co-maleic anhydride) containing 40.30 mole % MA
PM-MA-3 : Poly (methyl methacrylate-co-maleic anhydride) containing 44.73 mole % MA
PM-MA-4 : Poly (methyl methacrylate-co-maleic anhydride) containing 48.60 mole % MA

for variable times, depending on the experimental program at room temperature. On finishing the coupling process, 250 ml cold distilled water with 0.5 ml hydrochloric acid was introduced to precipitate the reaction product. The precipitate was filtered off, washed with two portions of distilled water of 20-25 ml each and dried over calcium chloride.

Adequate conversion was obtained at room temperature in 20 h. The optimum weight ratio of copolymer with biologically active compound (BAC) was found to be 1:0.5 and 1:1. For PM-MA-1-BAC: copolymer = 0.5:1 and for PM-MA-2, PM-MA-3 and PM-MA-4, BAC : copolymer = 1:1. Determination of ampicillin content in the coupling product (Table: 1) was conducted spectroscopically at 558 nm by Fehling's method[10].

A comparison of IR spectra of ampicillin, the polymer support and coupling product shows the occurence of reaction. As shown in (Fig: 1). IR spectra of copolymer indicates the anhydride absorption in the region 1850-1780 cm^{-1}. Absorption around 3300-3060 cm^{-1} (N-H streching of-CONH), 1680-1630 cm^{-1} corresponding to amide streching was observed.

The content of BAC in the polymer was determined spectroscopically, showing characteristic UV absorption at 252 nm since the absorption maximum of the drug has not been shifted when bound covalently. After proper calibration, the carrier MMA-MA does not have absorption in the region around 252 nm and hence it does not interfere with the absorption of the drug components. Similar observations were also made by Pitha et al[12], while working on a model drug alprenol bound to polyacrylamide carrier.

On taking a time interval of 4-20 h, the amount of AMP chemically bound increases continuously. Thus one can suppose that with increasing the reaction time, polymeric drug containing increasing amount of chemically bound AMP can be obtained until all functional group of support or acid number becomes minimum[9].

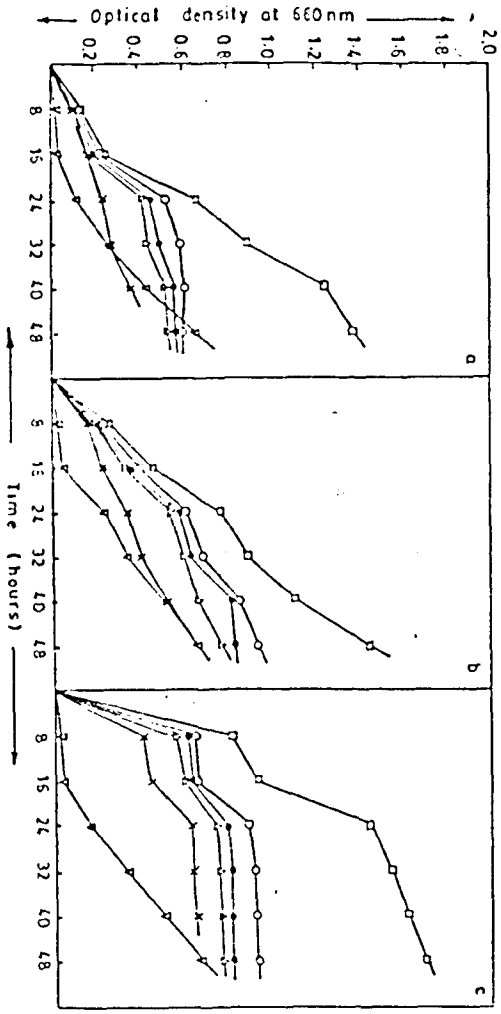


Fig. 2 : Effect of ampicillin retained from polymer complex as a function of time on the growth of (a) Bacillus subtilis (b) Staphylococcus aureus (c) Escherichia coli.
▽ Pure ampicillin ❑ Control
o 20.3% maleic anhydride O 40.3% maleic anhydride
Δ 44.7% maleic anhydride x 48.6% maleic anhydride

The *in vitro* drug release was studied spectrophotometrically[13] at 37° by keeping the polymer bound drug in contact with 0.1 M phosphate buffer of pH values 7.4 - 8.0. In a typical experiment, (25 mg) of polymer bound AMP samples were equilibrated with 5 ml of buffer for a fixed interval of time, 24 h. The supernatant was separated by centrifugation (at 3000 rpm) for 10 min. and the amount of AMP released from PM-MA-1, PM-MA-2, PM-MA-3, PM-MA-4 was determined by measuring the absorbance at 558 nm (Fehling's Test). The residues after centrifugation were again equilibrated with fresh 5 ml portion of buffer and the process was repeated. The results are shown in (Table : 1). The release behaviour depends on the kind of polymer system and in every case the amount of drug release decreases gradually with time.

For the purpose of evaluating the release behaviour of AMP from polymer matrix, the hydrolysis of PM-MA-1, PM-MA-2, PM-MA-3, PM- MA-4 was investigated *in vitro* in various buffers at 37°. The release of AMP in case of PM-MA-4 was 86.6% of the total bound drug within 6 days. On the other hand, the release of AMP is prolonged to 8 days and 84.05%, 81.7% and 66.3% of the total bound drug was released in 8 days in case of PM-MA-3, PM-MA-2 AND PM-MA-1 AT pH 8.0.

The release profiles of ampicillin with different percentage of polymeric carrier were obtained using *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus.* These have been presented in (Fig. 2).

The study indicates that the rate of drug release could be controlled by the amount of incorporated anhydride. In a control experiment, only culture without the drug shows higher growth rate than in the experiment in the presence of the drug. The presence of the drug alone reduces the growth rate from the beginning as indicated by slower increase in optical density with time. Bound drug is unable to show its effect in the beginning. However, after a lag of 16 h, bacterial growth is inhibited due to release of the drug. The antimicrobial activity was found to increase with increasing percentage of anhydride in the polymer. (Table : 1)' indicates the rate of AMP released as a function of maleic anhydride concentration. Similar observations were also made by Heller et al[14]. while working on a model drug, timolol maleate bound to maleic anhydride copolymer with varying amount of anhydride.

The active drug when bound to a polymer having varying amount of incorporated anhyride prolongs the release of model drug up to 8 days. The percent-

age cumulative release depends upon the amount of anhyride incorporated and pH of the media. Our study provides a concept of providing therapeutic level of active agent in the target site for long duration and permits to manipulate the pharmokinetic behaviour of the drug.

REFERENCES

1. Ringsdrof, H., **J. Polym. Sci., Polym. Symp.**, 1975, 51, 135.
2. Donaruma, L.G., **Prog. Polym. Sci.**, 1974, 4, 1.
3. Lymann, D. J., **J. Makromol. Sci., Rev. Macromol. Chem.**, 1966, 1, 335.
4. Mark, H.F., **Pure and Appl. Chem.**, 1968, 16, 201.
5. Batz, H.G., **Adv. Polym. Sci.**, 1977, 23, 25.
6. Gros, L. and Ringsdrof, H., **Angew, Chem.**, 1981, 93, 311.
7. Azori, M., Pato, J. and Feheravari, F., **Makromol. Chem.**, 1986, 187, 303.
8. Asahi Chemical Co., Jap. Pat., 1979, 23, 037.
9. Dumitriu, S. and Popa, M., **Makromol. Chem.**, 1988, 189, 103.
10. Sethi, P.D., "Quantitative Analysis of Drugs in Pharmaceutical Formulations" 2nd Ed, CBS Publishers, 1993, 162.
11. Dyer, J.R., Application of Absorptions spectroscopy of Organic compounds. Prentice Hall of India Pvt. Ltd., New Delhi, 1978, 22.
12. Pitha, J. and Zjawiony, J., **Makromol. Chem.**, 1981, 182, 1943.
13. Vasudevan, P., Singh, M. and Bala, K., **Makromol. Chem.**, 1982, 183, 1897.
14. Heller, J. and Himmelstein, K.J., **Methods in Enzymology.**, 1985, 112, 432.

Studies on Lipids on some varieties of Linseed (Linum usitatisimum) of Vidarbha Region

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The seeds of C-429, R-552, RLC-4, RLC-6 and T-397 varieties of Linseed, were extracted with chloroform-methanol (2:1, v/v) to yield the total lipids (TL) in 43.9, 46.3, 42.1, 43.5 and 45.2 percent respectively. The TL were fractionated by silicic acid column chromatography into neutral lipids (NL) (87.8-89.6%), glycolipids (GL) (5.8-6.6%) and phospholipids (PL) (3.8-5.8%). The NL, upon being subjected to preparative TLC, were separated into monoacylglycerol, diacylglycerol, triacylglycerol, free fatty acids, sterols, steryl esters and hydrocarbons. The fatty acid composition of all the lipid materials, as determined by GLC, revealed the major fatty acids to be linolenic, linoleic, oleic, stearic and palmitic acids. The fatty acids reamined static qualitatively but variability between the lipids and varieties was observed.

LINSEED *(Linum usitatisimum Linn, Linaceae)* is an important rabi crop. In Maharashtra state, it is grown mainly in the Vidarbha region, where it is also used as an edible oil but the presence of cynogenic glucosides restricts the use of linseed meal.

Vegetable plant lipids have important implications for sensory quality, cell membrane biochemistry and post-harvest physiology[1,2].

Fatty acid and lipid composition of different varieties of linseed[3], groundnuts[4] and cottonseeds[5,6]