DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(4), 461-472 (1993)

PHARMACEUTICAL INVESTIGATIONS OF A FILM FORMING MATERIAL ISOLATED FROM ROOTS OF SALACIA MACROSPERMA

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ABSTRACT

A film forming material (FFM) was isolated in pure form from chloroform extract of roots **Salacia macrosperma** (Hippocrataceae) and was found to be a polymer of isoprene. It was not absorbed through gastrointestinal tract of rats and non-toxic. Electronic microscopic studies on films prepared with different concentrations of FFM in various organic solvents revealed that chloroform is an ideal solvent for preparing uniform films. Use of FFM as a film coating material was evaluated.

INTRODUCTION

Salacia macrosperma (Family:Hippocrataceae) is found in the Western regions of India from Konkan southwards. In Ayurvedic System of Medicine, both root bark and leaves of **S. macrosperma** are used in the treatment of **Diabetes mellitus**, especially in south India (1). It is also used as remedy for enlargement with congestion

of liver and piles (2). Quinone compounds have been reported from the root bark (3). Magniferin has been isolated from root by Desai **et al.**(4). Quinine methide, compounds related to prestimirin and three known compounds prestimerin, tingenone and hydroxy tingenone have been isolated from the root bark by Reddy **et al**(5). Decoctions prepared from roots and leaves of **S. macrosperma** were reported to possess oral hypoglycaemic activity (6). During our detailed investigations on roots of **S. macrosperma**, a film forming material (FFM) was isolated. Present report covers the isolation, purification, chemical characterization of FFM and its utility as film coating material.

EXTRACTION AND PURIFICATION OF FFM

Powdered roots of **S. macrosperma** were extracted with chloroform by double maceration. The extract was concentrated in a rotary flash evaporator under vacuum to a semisolid consistency and air-dried. The roots yielded a yellowish solid elastic mass. The solubility of extract in different organic solvents was evaluated and it was found that pyridine could extract all phytoconstituents leaving a colourless, inert film forming material (FFM). The extract was repeatedly extracted with cold pyridine (20°C), till all the other components were extracted. The residue was dissolved in chloroform and air-dried to yield pure FFM. The substance was a colourless crystalline compound when freshly crystallized from alcohol (95%) but acquired slight stickyness on long exposure to air and light.

CHARACTERIZATION OF FFM

Elemental Analysis

The elemental analysis of FFM revealed the composition of 88.1% of carbon and 11.8% of hydrogen.

UV Spectrum of FFM

The UV spectrum of FFM in n-hexane has shown a strong absorption at 196 nm assignable to unconjugated olefines, ethylene chromophore (-C=C-C-C).

IR Spectrum (KBr γ max cm^{-1})

The IR spectrum has shown strong absorption at 2850 and 2960 cm^{-1} assignable to C-H stretching vibrations of methyl group [$-CH_3$] and at 2920 cm^{-1} assignable to C-H stretching vibrations of methylene groups [$-CH_2-$]. The strong bands appearing at 1380 and 1440 cm^{-1} can be attributed to C-H bending vibrations of methyl group [$-CH_3$]. A moderate absorption at 1660 cm^{-1} is assignable to -C=C- stretching vibrations of unconjugated linear olefines (7).

^{1}H PMR Spectrum [$CDCl_3$ δ ppm]

The PMR spectrum of FFM has displayed signals at 1.55, which can be assigned to end methyl protons [s, 3H, end methyl]. A peak recorded at 1.95 can be attributed to methylene protons [s, 4H, methylene protons]. Absorption peak at 5.1 is attributed to methine proton [m, 1H, methine proton].

Molecular Weight

The compound was found to have high molecular weight, since the instrumenthas not given an interpretable spectrum. The molecular weight of FFM was determined by Rast method.

Bromine Test

The substance rapidly decolourized bromine in chloroform to form a bromo product.

Melting Point

FFM failed to give a sharp melting point and was melted at 57-65°C; the cooled melt retained a glassy consistency for a long time.

ACUTE TOXICITY STUDIES

Acute toxicity studies of FFM were carriedout to know the toxic effects on albino rats, when administered orally (8). Oral route was choosen, since the FFM was expected to be useful as a coating material for oral solid dosage forms.

Preparation of FFM Suspension

Accurately weighed quantity of FFM (10 g) was dissolved in 10 ml of chloroform and mixed with 1 g of acacia in a glass mortar. Chloroform was allowed to evaporate after uniform mixing with acacia. Measured quantity of distilled water (10 ml) was added with continuous trituration to form uniform suspension.

Toxicity Studies

Albino rats of either sex weighing between 150-200 g were divided into seven groups each containing six animals. Control group received acacia mucilage only (1 ml of 0.1% w/v), while other groups received different, oral doses of FFM ranging from 200 mg to 2 g per kg of body weight. Animals were maintained on Lipton Rat Deit and observed for toxic symptoms for 48 hours after the administration of FFM. The faeces of the rats were collected and extracted with chloroform to findout whether FFM was absorbed through G.I.T. or not.

ELECTRON MICROSCOPIC STUDIES OF FILMS PREPARED WITH FFM

Preparation of Coating Solutions

FFM was dissolved in petroleum-ether (60°-80°), benzene and chloroform to give different concentrations (0.25, 0.5, 1.0 and 1.5% w/v), which were used as coating solutions.

Coating Methodology and Evaluation

Clean circular glass coverslip was held with a small pointed forceps and dipped into the coating solution for one minute. The covership was taken out from the coating solution and placed on a horizontal glass plate and air-dried. The lower surface of the coverslip was cleaned with respective solvent. Coverslips were coated with different concentrations of FFM in petroleum-ether (60°-80°), benzene and chloroform.

The coated surface of the coverslip was used for studies. The sample was prepared by gold plating method The samples were observed under different magnifications (180-2000 times) using electron microscope. The studies were carried to find out uniformity, continuity and pore size of films. The photographs were taken at different magnifications.

EVALUATION OF FILM COATING PROPERTIES OF FFM

Electronic microscopic studies revealed that FFM was able to form uniform and continuous films on glass coverslips with chloroform as solvent. Based on these observations, evaluation of film coating properties of FFM was attempted. Chloroform was used as a solvent for preparing various concentrations of FFM (0.25,

o.5, 1.0, 1.5 and 2.0% w/v). Marketed tablets of sulpha methoxazole (SMZ) each containing 0.5 g of drug were used, after testing for hardness, weight variation and content uniformity as per official procedure (9). The mean amount of SMZ per tablet was determined.

Coating of Tablets

Dip-coating method was employed in the present investigations (10). About 100 ml of coating solution was taken in 250 ml of beaker and a glass cylinder containing No.10 mesh at one end was used for coating of tablets. Tablets were placed in glass cylinder so as to rest on mesh and the cylinder was dipped in coating solution for one minute. The cylinder was then removed from the coating solution and the tablets were air-dried. Tablets were coated with different concentrations of FFM in chloroform.

Disintegration Time of Tablets

The disintegration time of uncoated and coated tablets was determined as per the procedure given in Indian Pharmacopoeia (9). Distilled water was used as medium and the temperature of medium was maintained at 37°C ± 1°C. The average of disintegration time for six tablets was used for interpretation of data.

Dissolution Studies

Basic unit of U.S.P. dissolution apparatus was used in the study (11). A 1000 ml capacity round bottom flask with inside diameter of 10 cms, with its sides flanged near the top was employed. A paddle formed from shaft (diameter-8 mm) was used as the stirring element. The stirring blade (thickness-3 mm) forms a section of a circle having a diameter of 80 mm and

extended by parallel chords of 40 mm and 80 mm. The shaft was positioned so as to pass through the centre of the flask and a distance of 2.0 ± 0.2 cms between the blade and inside bottom of the vessel was maintained during the studies. The shaft was rotated at r.p.m. of 50 ± 5.

The dissolution studies were performed in 900 ml of distilled water as medium at 37° ± 1°C. An aliquot of 1 ml was withdrawn from fixed position at regular intervals. After each sampling, 1 ml of distilled water was added to the flask to maintain a constant volume of the medium. The samples were analysed colorimetrically for the content of sulphamethoxazole (12). All the dissolution studies were carried out in triplicate and the average of three readings was taken. For the validation of the deviation from central tendency of the data, standard deviations were calculated.

RESULTS AND DISCUSSION

The chloroform extract of roots of **S. macrosperma** was a yellowish solid elastic mass (1.9% w/w). The percent yield of FFM from crude drug after purification with pyridine was 1.6% w/w. The molecular weight of FFM was estimated to be 1627, indicating a possibility of polymer and the elemental analysis results proved that the compound is a hydrocarbon. The UV spectrum, IR spectrum and bromine test revealed presence of unsaturation in FFM. The proton signals in the PMR spectrum of FFM suggests that it contains three types of protons and the ratio of methyl : methylene : methine proton was found to be 3:4:1 respectively. The results of all the experiments suggest that the compound is a polymer of isoprene with the following structure.

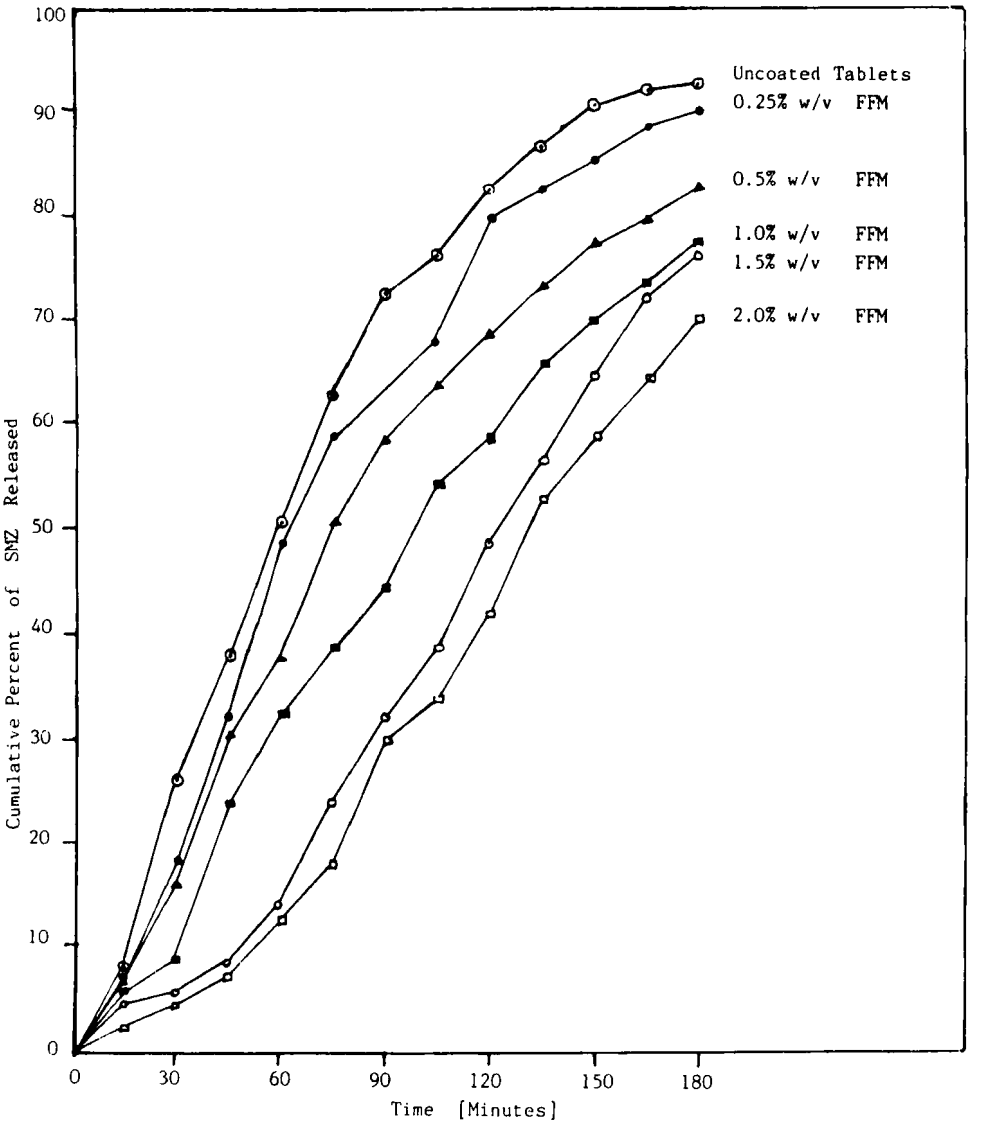

Fig. 1 . Effect of Film Forming Material (FFM) Isolated From S. macrosperma on Dissolution Profiles of Commercial Sulphamethoxazole(SMZ) Tablets.

$$(H_2C\text{-}\overset{CH_3}{\overset{|}{C}}\text{=}CH\text{-}CH_2)_n$$

The proton signals agreed with the above structure qualitatively and quantitatively and the spectrum compared well with that of the isoprenoid part in ubiquinone and Kofler's quinone (13).

None of the animals used in acute toxicity studies showed any toxic symptoms and there was no mortality in any group treated with FFM. The chloroform extract of the faeces of rats treated with FFM after evaporation yielded FFM. This clearly indicates that FFM is not absorbed through gastrointestinal tract of rats.

The electron microscopic studies were carried out on films prepared with FFM using petroleum-ether (60°-80°C), benzene and chloroform to findout the influence of solvents on the nature of the films formed it was evident from the studies that the pore size of film decreased as the concentration of FFM in coating solution was increased. The films formed with 0.25, 0.5,1.0 and 1.5% w/v of FFM in chloroform showed mean pore diameter of 10.0, 7.6, 5.1 and 4.6 microns respectively, while films formed with benzene showed mean pore diameter of 13.3, 7.7, 5.4 and 4.8 microns respectively. The films prepared with 0.25, 0.5, 1.0 and 1.5% w/v of FFM in petroleum-ether (60°-80°) have a mean pore diameter of 13.5, 10.1, 7.3 and 6.8 microns respectively. Of all the solvents investigated, chloroform formed uniform and continuous films with low pore size values when compared with other solvents. Films formed with FFM in benzene and petroleum-ether '60°-80°) were not uniform and pore sizes were found to have varied distribution. Hence, it can be concluded that chloroform is suitable solvent for good film properties of FFM.

TABLE - I

Effect of Film Forming Malerial Isolated from S. macrosperma on Disintegration Time of Commercial Sulphamethoxazole Tablets

S.No.	Conc. of FFM (% w/v)	Disintegration time* (minutes)
1.	0.25	3.50(0.14)**
2.	0.50	5.05(0.41)
3.	1.00	7.65(0.38)
4.	1.50	9.64(0.49)
5.	2.00	13.65(0.56)
6.	Uncoated	2.75(0.23)

*Values given table are average of 6 observations.
**Values in parenthesis indicates standard deviation.

The disintegration time of coated sulphamethoxazole tablets **vis-a-vis** concentration of coating material is recorded in Table.I. With an increase in concentration of FFM, there was systematic increase in the disintegration time of coated tablets. It could be due to increased thickness of coating on tablet surface and reflects upon the efficacy of FFM as coating material.

The mean amount of SMZ per tablet was estimated to be 495.6 mg. The cumulative percent of sulphamethoxazole released with respect to time in dissolution studies of coated and uncoated tablets are shown in Fig.1. The uncoated tablets released 50.05% and 91.0% drug after one hour and three hours of dissolution respectively. In case of tablets coated with 0.25, 0.5, 1.0, 1.5 and 2.0% w/w FFM, the cumulative amounts of drug released upto one hour were found to be 40.89, 38.31, 32.50, 14.41 and 14.02% respectively. The

results clearly indicate that FFM is forming uniform coat on tablet surface and hence decreasing the drug release from tablet.

FFM isolated from **S. macrosperma** is hydrophobic in nature and able to form uniform and continuous film on tablet surface. This non-absorbable film coating material may be used for the preparation of sustained action dosage forms.

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