Formulation Development and In Vitro and In Vivo Evaluation of Membrane-Moderated Transdermal Systems of Ampicillin Sodium in Ethanol: pH 4.7 Buffer Solvent System

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ABSTRACT

The objective of the present study was to develop membrane-moderated transdermal systems of ampicillin sodium and to evaluate them with respect to various in vitro and in vivo parameters. The membrane-type transdermal systems were prepared using a drug with various antinucleant polymers—hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), cellulose acetate phthalate, chitosan, sodium alginate (SA), and sodium carboxymethylcellulose—in an ethanol: pH 4.7 buffer volatile system by the solvent evaporation technique with HPMC as the rate-controlling membrane for all the systems. The swelling properties of the polymers were studied, and drug-polymer interaction studies were performed. The patches were subjected to various physicochemical studies, in vitro release studies, permeation studies, and skin irritation studies. The best patch among the formulations was selected for further in vivo studies. Compared to the other patches, SA exhibited the highest moisture content at 16%; a 21% moisture uptake was found with MC. The release and permeation of the drug from the SA patch was found to be the maximum. The in vivo study of the SA patch exhibited a peak plasma concentration C_{max} of 126 μg/mL at T_{max} 4 hours. Hence, it can be concluded that hydrophilic ampicillin sodium can be developed as a transdermal delivery system with SA that is an alternative to intravenous administration and has minimal adverse effects.

KEYWORDS: Membrane controlled, hydrophilic polymer, swelling ratio, hydrophilic drug, in vivo study.

INTRODUCTION

Ampicillin, a potent antibiotic with relatively short-termed stability in aqueous solutions,[1,2] is used clinically to treat a broad range of bacterial infections.[3-5] With parenteral injection, ampicillin is distributed rapidly and widely, resulting in a high concentration of the drug in bile.[6] From bile it is excreted into the gut and is known to cause disruption of the normal intestinal microflora by diminishing the main flora and increasing the presence of yeast as well as inducing a high risk of *Clostridium difficile* colitis.[7]

A significant improvement in antibiotic activity and a reduction of the allergic and toxic reactions to ampicillin[8,9] have been obtained by topical formulations.[10,11] The advantage of transdermal delivery of hydrophilic drugs versus oral delivery lies in the molecular nature of the gastrointestinal tract (GIT).[12] As a lipid membrane, the GIT possesses hydrophobic properties; thus, the more hydrophilic a drug is, the more likely it is to be absorbed poorly through the GIT. Moreover, the amino group in ampicillin confers[13] an ability to cross the cell wall barrier that is impenetrable to other types of penicillin. Owing to the above conditions, ampicillin developed as a membrane-moderated transdermal patch with a hydrophilic membrane was found to increase the permeation of hydrophilic drug; it was reported that the hydrophilic matrix modified with hydrophilic membrane.[14]

MATERIALS AND METHODS

Sodium carboxymethylcellulose (CMC) 1500 ± 400 cps, sodium alginate (SA) 3500 mps, chitosan (CS) 500 cps, hydroxypropyl methylcellulose (HPMC) 4000 cps, methylcellulose (MC) 3000 to 5000 mPas, and cellulose acetate phthalate (CAP) were obtained from Bharat coats, Chennai, Tamil Nadu, India. A gift sample of ampicillin sodium was obtained from Ranbaxy Laboratories (Gurgaon, India). All other chemicals and solvents used were of analytical grade.

Drug-Polymer Interaction Studies

Infrared (IR) spectroscopy (using IR spectrophotometer FTIR-8300, Shimadzu [Kyoto, Japan], by the KBr pellet method) was performed on pure substances and their physical mixtures to investigate the interaction between ampicillin sodium and various polymers.[15,16]

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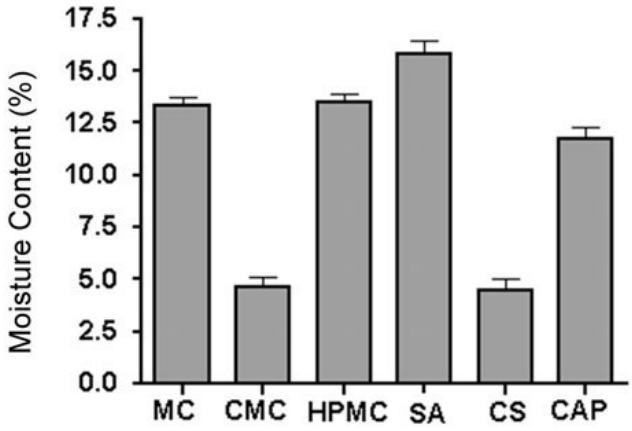

Figure 1. Percentage of moisture content from ampicillin containing different membrane-moderated film prepared by using different polymers. Data are mean ± SE (n = 3). MC indicates methylcellulose; CMC, carboxymethylcellulose; HPMC, hydroxypropyl methylcellulose; SA, sodium alginate; CS, chitosan; and CAP, cellulose acetate phthalate.

Development of Membrane-Controlled Transdermal System

The unilaminate transdermal membrane was prepared by the casting method. The polymers (3%) were dissolved in a mixture of ethanol and pH 4.7 buffer volatile solvent system (33:67) with continuous stirring at 25°C in a closed system. Ampicillin sodium was added to the polymer mixture. Glycerin (1.2% wt/wt of polymers) was added with continuous stirring. The matrix was poured into the backing membrane. The solvent was allowed to evaporate at room temperature overnight to form a dry matrix. Upon this the rate-controlling membrane of 2% HPMC was cast. Six formulations were prepared with different polymers: HPMC, CMC, MC, CAP, CS, and SA. A 20 mg amount of ampicillin sodium per 10 cm^2 matrix was incorporated.

Moisture Content

The films were weighed and kept in a desiccator containing calcium chloride at room temperature for 24 hours. The films were weighed repeatedly until they showed a constant weight. Values for the percentage of moisture content, calculated as the percentage of difference between the constant final and initial weight with respect to the initial weight,[17] appear in Figure 1.

Moisture Uptake

The weighed films were kept in a desiccator at room temperature for 24 hours. Then they were taken out and exposed to 84% relative humidity (saturated solution of potassium chloride) until a constant weight for the film was obtained. Values for the percentage of moisture uptake, calculated as the percentage of difference between the final and initial weight with respect to the final weight,[17] are presented in Figure 2.

Swelling Ratio Measurement

Preparation of Disc-Like Specimens

Discs of the cellulose derivatives SA and CS were prepared by compressing 500 mg of powder using flat-faced punches 12 mm in diameter (tableting machine, Cadmach Machinery Co, Ahmedabad, India) to yield a hardness of 100 N ± 10. Before swelling tests, the diameter and height of each tested disc were measured.

Swelling Studies

Swelling studies were performed[18] by placing the polymeric discs in test tubes and measuring their thickness as a function of time during swelling. Tubes were kept vertical at 37°C.

Skin Irritation Test

The application site was evaluated[19] for skin irritation each day using the Draize irritation score. An untreated site on the skin of a mouse acted as the control site.

Spectrofluorimetric Analysis

A 1-mL aliquot of the sample was pipetted into a 15-mL graduated glass centrifuge tube. Then 0.2 mL of trichloroacetic acid (20% wt/vol) water solution and 0.2 mL of formaldehyde (7% wt/vol) water solution were added sequentially

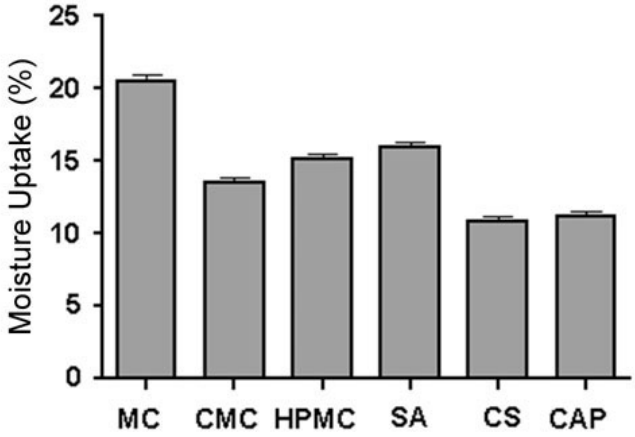

Figure 2. Percentage of moisture uptake from ampicillin containing different membrane-moderated films prepared by using different polymers. Data are mean ± SE (n = 3). MC indicates methylcellulose; CMC, carboxymethylcellulose; HPMC, hydroxypropyl methylcellulose; SA, sodium alginate; CS, chitosan; and CAP, cellulose acetate phthalate.

into the centrifuge tube, which was then vortexed for 20 seconds. The tube was capped loosely and heated in a water bath (100°C) for 30 minutes. After cooling down to room temperature, the contents were adjusted to 3 mL with 20% acetonitrite in water, mixed well, and then filtered into a sample vial and were ready for analysis. The excitation and emission wavelength were fixed at 346 nm and 426 nm, respectively.[20] The intensities of various concentrations of ampicillin sodium were used for construction of the calibration curve.

In Vitro Dissolution Studies

The in vitro drug release studies were performed using a US Pharmacopeia paddle-type dissolution apparatus (using 500 mL of phosphate buffer pH 7.4 as the dissolution medium).[21] The release rate determination is one of the most important studies to be conducted for all controlled-release delivery systems. The dissolution studies of patches are crucial because one needs to maintain the drug concentration on the surface of the stratum corneum consistently and keep it substantially higher than the drug concentration in the body, to achieve a constant rate of drug permeation.

A circular patch with an area of 10 cm^2 containing 20 mg of drug was used for the study. All dissolution studies were performed at 37 ± 2ºC, at 50 rpm, with each dissolution jar carrying 500 mL of buffer pH 7.4. Samples were withdrawn at different time intervals and analyzed spectrofluorimetrically. Cumulative amounts of drug released were plotted against time for different formulations.

In Vitro Permeation Studies

In vitro permeation studies were performed by using a modified Franz diffusion cell across a cellulose membrane using phosphate buffer pH 7.4 as the in vitro study fluid in the receptor compartment.[22] The polymeric film was placed on the cellulose membrane. The holder contains the cellulose membrane. The formulation was then placed on the receiver compartment of the modified diffusion cell containing phosphate buffer pH 7.4. The donor and receiver compartments were kept in immediate contact by wrapping parafilm at the junction. The temperature of the diffusion cell was maintained at 32 ± 0.5°C by a circulating water jacket. The whole assembly was kept on a magnetic stirrer, and solution in the receiver compartment was constantly and continuously stirred throughout the experiment using magnetic beads.

The samples were withdrawn (1 mL each time) at different time intervals and an equal amount of phosphate buffer pH 7.4 was replaced each time. The intensities of samples were measured spectrofluorimetrically. The amount of drug permeated per square centimeter at each time interval was calculated and plotted against time.

In Vivo Studies

After approval by the ethics committee, the study was conducted[23] in healthy male volunteers (weight 55-60 kg; age 25-30 years). All the participants in the study were nonsmokers and were not alcoholics. The biochemical examination of the volunteers revealed normal function of the kidney and liver. The nature and purpose of the study were fully explained to the volunteers, and an informed written consent was obtained from each one. None of the volunteers was on drug treatment within the week prior to their participation in the study. An immediate-release capsule dosage form containing 250 mg of ampicillin was chosen as the reference formulation and was administered to volunteers. The transdermal patch was applied to the anterior surface of the forearm near the elbow.

Pharmacokinetic Evaluation

Analysis of Ampicillin

Ampicillin was estimated by a carrier-reported reverse-phase High Performance Liquid Chromatography (HPLC) method.[24] A Shimadzu LC-10 VP series HPLC system (Kyoto, Japan) with 2 LC-10AT pumps, variable wavelength programmable UV/VIS detector SPD-10A VP system controller and RPC-18 column (Luna Phenomenex, Torrance, Canada, 250 mm × 4.6 mm, particle size 5 μm) was used. The system was equipped with class VP series version 6.12 software.

Chromatographic Conditions

The mobile phase consisted of water:acetonitrile:1N potassium dihydrogen phosphate:1M acetic acid (909:80:10:1). The mobile phase was filtered through a 0.4-μm membrane filter; the flow rate (2 mL/min) was monitored at 254 nm. The total run time of the method was set at 13 minutes. The peaks were well resolved, and the retention time for ampicillin and caffeine (internal standard) was 6.02 and 12.3 minutes, respectively. No interfering peaks were observed at the retention time of ampicillin and caffeine. A standard stock solution of ampicillin (100 μg/mL) was prepared in diluent as per the US Pharmacopeia. The calibration curve standard solutions were prepared by adding a known amount of ampicillin (concentration 10-50 μg/mL) and caffeine, an internal standard (5 μm/0.5 mL), to blank plasma.

Extraction Procedure

An aliquot (1 mL) of plasma sample was measured into a glass centrifuge tube followed by 0.5 mL of 10 mcg/mL caffeine (internal standard). To the above plasma 1 mL of methanol was added, vortexed for 10 minutes, and centrifuged at 5000 rpm for 15 minutes. The supernated liquid was

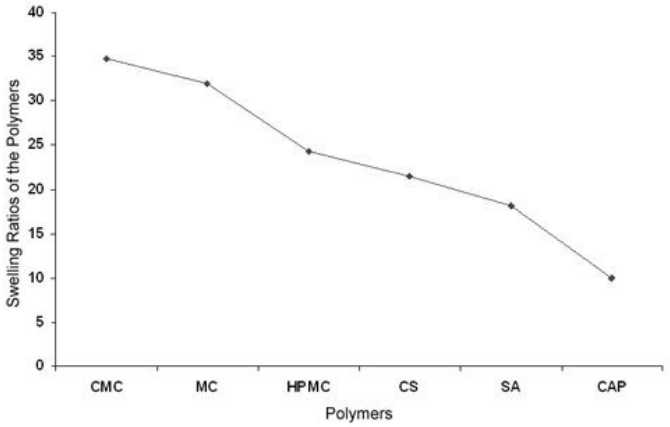

Figure 3. Swelling ratios of different polymers.

separated and nitrogen gas was passed through it until the sample was evaporated. To the residue 0.5 mL of diluent was added and the residue was dissolved in it. Then the samples were injected into the HPLC column and detected by a UV detector at 254 nm.[25]

Calibration Curve

The calibration curve was obtained by plotting peak area ratios of ampicillin-caffeine on the Y-axis against various concentrations of ampicillin on the X-axis. The pharmacokinetic parameters were calculated using noncompartmental pharmacokinetics data analysis.[23]

Results and Discussion

Moisture Content Studies

The SA membrane modified was found to have the highest moisture content of 16.5%. The HPMC and MC membrane modified exhibited moisture content of around 13.6%. The CAP membrane modified patch exhibited around 12.4%. The CMC and CS exhibited around 4%.

Moisture Uptake Studies

The membrane patches developed with MC and CAP were found to have the highest and lowest moisture uptake values, respectively. SA, which had the highest moisture content already, absorbed only a negligible amount of moisture. The moisture uptake of HPMC was around 2%. CS had around a 6% uptake of moisture. CMC, which had a low moisture content, was found to have an uptake of around 10%. Hence, when the moisture content and uptake characteristics of the patches were compared, CMC was found to have the highest uptake (around 10%), while CAP had the lowest (not even 1%).

Volume Swelling Ratio

The volume-swelling ratio, Q, of various polymers was calculated using the following equation:

$$Q = \frac{V_s}{V_d}, \tag{1}$$

where V_s is the volume of swollen gel and V_d is the initial volume of the dry disc. Results of the dynamic swelling behavior of various polymers are presented in Figure 3. CMC exhibited the most swelling and CAP the least. MC, which had a moisture content similar to that of HPMC, was found to have more swelling than HPMC, as it had more moisture uptake compared with other patches. Although the moisture content in SA was greater than CS's, the uptake of moisture was found to be around 6% more in the case of CS. Hence, CS swelled more than SA did.

Skin Irritation

The visual score was 0 (none) on both the erythema scale and the edema scale. There was no sign of skin irritation.

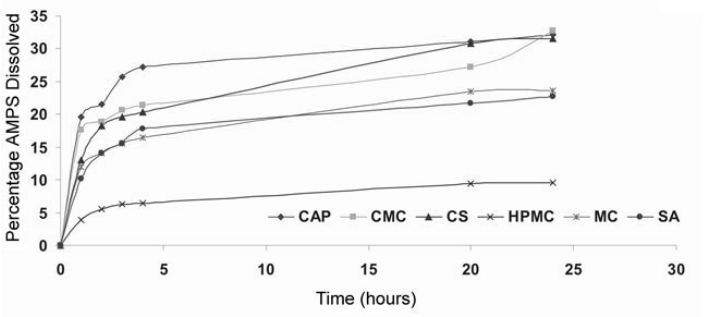

Figure 4. In vitro drug dissolution profile of ampicillin sodium membrane-moderated film from different polymers using US Pharmacopeia paddle dissolution apparatus in phosphate buffer pH 7.4. Data are mean ± SE (n = 6).

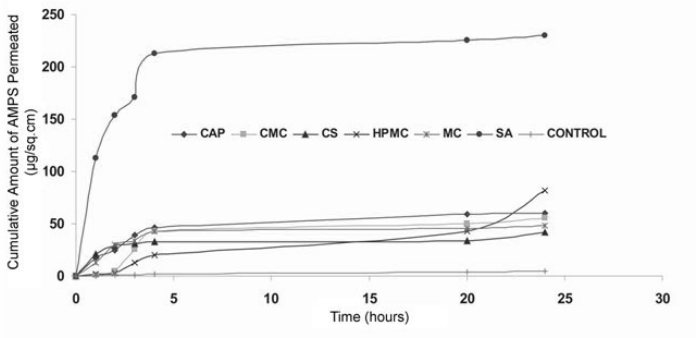

Figure 5. In vitro drug permeation profile of ampicillin sodium membrane-moderated film from different polymers using Franz diffusion cell in phosphate buffer pH 7.4. Data are mean ± SE (n = 6).

Table 1. Mean (±SD) Pharmacokinetic Parameters of Ampicillin Following Oral Administration of Tablet Dosage Form (250 mg) and Application of Sodium Alginate–Based Transdermal Patch in Human Volunteers (n = 6)*

Formulation	C_{max}	T_{max}	Relative Bioavailability (%)	MRT	$AUC_{0–24}$
Capsule dosage form	51.6	3	—	11.85	865
Sodium alginate–based transdermal patch	126	4	143	6.11	1236

*MRT indicates mean residence time; AUC, area under curve.

In Vitro Dissolution Studies of pH 4.7 Membrane-Moderated Patches

The release from SA was observed to be the highest because the pH 7.4 medium was favorable for release in this case and because SA swelled less than the other patches did. The swelling ratio for CMC was the highest, which may have affected the release of the drug. The release from CS was found to be the lowest, perhaps because CS is already insoluble in a pH 7.4 medium. CS's swelling ratio of 20 further delayed the release.

CAP may have had the lowest release of drug, in spite of having more solubility at alkaline pH and having minimum swelling, because of the HPMC membrane, which has swelling and hence delays release in pH 7.4 medium. The patches showed drug release in the following order: SA > MC > HPMC > CAP > CMC > CS, as depicted in Figure 4.

In Vitro Permeation of pH 4.7 Membrane Patches

The permeation of ampicillin from volatile vehicles (ethanol/pH 4.7 buffer, 33:67 vol/vol) was examined. The volatile vehicle without polymers (control group) showed a gradual increase in ampicillin permeation, followed by a constant low level of permeation.

In the case of patches developed with membrane in ethanol and pH 4.7 buffer, SA exhibited the highest permeation. The patch made with HPMC exhibited the lowest permeation, as shown in Figure 5. The release of the drug from CMC and MC was delayed because of their swelling characteristics, and hence their permeation was decreased as expected. Although CAP had greater stability at pH 7.4 than HPMC did, CAP's permeation was not greater than HPMC's because of the rate-controlling membrane made with HPMC. The permeation of the drug was found to be higher from CS than from CMC and MC. The permeation of drug from SA was found to be the highest, which was expected, since the release of the drug from SA was the highest. CS had the lowest release of the drug from the patch, and hence only less amount of the drug was available for permeation and thus permeation is delayed. Permeation of CMC was also delayed since it had maximum swelling ratio, which was expected, as its moisture uptake capacity was the highest and CAP's was the lowest.

Pharmacokinetic Evaluation

The oral administration of the drug as capsule yielded 51.6 μg/mL, whereas the patch yielded 126 μg/mL, as presented in Table 1. The relative bioavailability of the drug from the patch, calculated with respect to the drug administered orally, was 143%. The mean residence time was found to be 6.11 hours. The maximum drug release reaching the systemic circulation is more from a patch containing only 20 mg of the drug from an area of 10 cm^2 than from an oral dosage form containing 250 mg of the drug, possibly because, since it is a very hydrophilic drug, it has difficulty crossing the lipophilic membrane of the gut. The excess drug that is administered to compensate for the loss produces the microbial flora disturbances. The area time curves (AUC_{0-24}) for the capsule and transdermal patch of ampicillin were 865 and 1236, respectively.

CONCLUSION

Ampicillin sodium can be developed as a transdermal patch with hydrophilic SA polymer as the reservoir and HPMC as the rate-controlling membrane. The patch can duplicate an IV dosage form without causing skin irritation and hence can increase patient compliance.

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