Indian Journal of Pharmaceutical Education and Research | Association of Pharmaceutical Teachers of India

Gel Based Iontophoretic Delivery System for Enhanced Transdermal Permeation of Alendronate Sodium

V B Rajendra*, M.H.G Dehghan, M Saifee, D L Dhamecha and S R Lahoti

Department of Pharmaceutics, Y.B.Chavan College of Pharmacy, Maulana Azad Education Trusts, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad, Maharashtra, India 431001

ABSTRACT

Submitted: 20/05/2011 Revised: 24/08/2011 Accepted: 28/12/2011

The purpose of the present study was to explore the passive and electrically assisted transdermal transport of Alendronate Sodium (AS) by iontophoresis in combination with penetration enhacers. For better bioavailability, better patient compliance, and prevention of gastro-intestinal irritation of AS, an iontophoretic drug delivery system of AS gel was formulated using Carbopol-940. Optimization of gel indicated the suitability of the Carbopol gel for transdermal iontophoretic delivery of AS. The study was conducted using silver–silver chloride electrodes across hairless Guinea pig skin. The effects of Current density, Pulse current and Penetration enhancer on the AS permeation were investigated. Iontophoretic transport of AS was found to increase with an increase in the current density. In case of pulsatile iontophoresis permeation of AS was observed to be higher at ON: OFF ratio of 1:1 in comparison to ON:OFF ratio 1:2, 1:4. Cathodal pulsed iontophoresis with penetration enhancer i.e Dimethyl sulphoxide (DMSO) significantly increased the AS skin permeation as compared with the passive controls.

Keywords: Alendronate Sodium, Iontophoresis, Carbopol gel.

INTRODUCTION

Alendronate Sodium (AS) is a recently approved potent drug for clinical use in treatment of osteoporosis, Paget's disease, metastatic bone disease, hypercalcemia of malignancy and primary hyperparathyroidism.[1] AS is chemically 4-amino, 1 hydroxybutylidene-1, 1-bisphosphonic acid Sodium salt trihydrate. The molecular weight is 325.12 and pKa 2.72, 8.73, 10.5, 11.6 with zwitter ion properties .[2] The sodium salt is soluble in water. Dose of AS for prevention of osteoporosis (5 mg/d), prevention and treatment of glucocorticoid induced osteoporosis (5 or 10 mg/d), treatment of male osteoporosis (10 mg/d) and treatment of Paget's disease (40 mg/d for 6 months).[3] However oral administration of AS has been linked to severe gastrointestinal side effects, including esophagitis. It is quite clear that oral delivery of AS encompasses several unattractive features that may be resolved via transdermal administration. Successful iontophoretic delivery would provide enhanced bioavailability and eliminate adverse gastrointestinal effects. The present research work deals with *In-vitro* iontophoretic delivery of AS using Guinea pig model.

Iontophoresis is a technique that facilitates movement of ions of soluble salts across a membrane under an externally applied potential difference that is induced across the skin by a low-voltage electric current.[4] The application of constant current is controlled by an electronic device that adjusts the voltage in response to the changes in skin electrical resistance. Charged drugs as well as other ions are carried across the skin as a component of induced ion flow. Iontophoresis effectively delivers a large variety of compounds across the skin.[5] Numerous factors are known to affect iontophoretic delivery.[5,6] Some important considerations include $flux_{ss}$ proportionality with respect to applied current density and the presence of ions other than drug (these decrease the efficiency of iontophoretic transport of the drug). Current up to 0.5 mA/cm^2 is believed to be tolerable for patients. The onset of action with iontophoretic treatment is rapid, in contrast to hours need to lapse for passive transdermal delivery.[7] Since drug delivery is proportional to applied current, significant advantages of iontophoresis include the possibility of preprogramming the drug delivery, dose tailoring on an individual basis, or time tailoring in a constant or pulsatile fashion.

Because of the complex nature of the drug delivery, most of the studies related to transdermal iontophoresis are focused on aqueous solutions.[8] Gels are considered to be the most suitable delivery vehicles for iontophoresis, as they can be easily amalgamated with the iontophoretic delivery system and match the contours of the skin. Gels also have other advantages over liquids, such as ease of fabrication into the device, suitability with the electrode design, deformability into skin contours, better occlusion, and better stability. Moreover, the high proportion of water employed in gel formulations can in turn provide an advantageous electroconductive base for clinical use.[9] Carbomer-940 is a synthetic high molecular weight polymer of acrylic acid that is crosslinked with either allyl sucrose or allyl ethers of

***Address for Correspondence:**

V B Rajendra, Department of Pharmaceutics, Y.B.Chavan College of Pharmacy, Maulana Azad Education Trusts, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad, Maharashtra, India 431001

E-mail: vivekrajendra@gmail.com

penthaerythritol. They contain 56 to 68 % of carboxylic acid calculated on a dry basis. The molecular weight of carbomer resin is theoretically estimated at 7×10^5 to 4×10^9. Carbopol gel shows good electrical conductivity. In addition, this property can be exploited for refillable unit dose iontophoretic drug delivery systems.[10]

The present study was undertaken to improve the delivering of AS using gel based iontophoretic delivery system. The approach involved checking the drug permeability by passive and iontophoretic transport using an *Ex-vivo* hairless pig skin model. The effects of current density, pulsed current, and penetration enhancer on the AS permeation were investigated.

EXPERIMENTAL

MATERIALS

Alendronate sodium (Cipla Mumbai) and Carbopol-940 (Research fine lab, Mumbai) were obtained as gift samples.Silver wire (1 mm diameter, 99.9% pure) was purchased from Loba Chime (Mumbai, India). Distilled water having a resistivity of more than 18 MΩ was used to prepare aqueous solutions. Other chemicals used in the study were of analytical grade and were purchased from Loba Chime.

METHODS

Preparation of Electrodes

a) Silver wire- (1mm diameter X 4 cm length) used as anode.

b) Silver- silver chloride electrode- (2cm length) used as cathode.

The rod-shaped electrode was prepared by dipping the silver wire into the molten silver chloride to form thin and uniform coat. The electrodes were chlorinated by immersing in 0.1 M HCl.[11]

Preparation of Skin

The density of the hair on human skin and pig skin is similar. Hence, guinea pig skin was chosen for the permeation studies. Guinea pigs were allowed free access to food and water. The hair of the guinea pig skin at dorsal side was removed with hair remover clipper 24 hr before experiment. Guinea pigs were sacrificed by respiratory paralysis by chloroform immediately before experiment. The skin was carefully excised; adhering fat and other visceral debris was removed manually. Separated epidermis was washed with normal saline solution before starting the experiment.[12]

***Ex-vivo* Permeation Study for optimizing the current density and pulsed current**

The hairless pig skin was mounted on vertical diffusion cells that were maintained at $37^0C \pm 1^0C$ using a hot water circulator. The skin was mounted on the diffusion cell with the stratum corneum facing the donor compartment. 35mg AS was dissolved in 3.5ml Phosphate buffer and the entire solution then transferred in to the donor compartment. The receiver solution for permeation studies contained pH 7.4 saline phosphate buffer solution. A constant direct current of 0.2, 0.4, 0.5 mA/cm^2 was applied for iontophoresis using silver–silver chloride electrodes. Pulse current with ON: OFF ratio of 1:1, 1:2, 1:4 was applied at current density 0.5mA/cm^2. The cathode was dipped in the donor solution and the anode in the receptor solution and agitated using a Teflon-coated magnetic stirrer (REMI India) at 100 rpm. Passive permeation was tested without application of any current.

Preparation of Carbopol gel

Weighed quantity of Carbopol-940 was mixed with 5 ml distilled water. In another beaker weighed quantity of Alendronate sodium were dissolved in 3 ml distilled water. Both were mixed, and to it triethionalamine was added with vigorous stirring.[10] The final weight was adjusted by using distilled water. The twelve formulations were prepared by varying the amount of Carbopol-940 and triethionalamine. After optimization, gel containing 1% of AS, Carbopol-940, triethionalamine was prepared and used for further studies.

Evaluation of Carbopol gel

The prepared Carbopol gel was evaluated for following parameters:

Surface pH: It is determine by placing pH paper onto the surface of Carbopol gel and pH was recorded.[12]

Dilution pH: Accurately weighed 1gm Carbopol gel was diluted to 25ml with distilled water and pH of solution was measured by using pH meter (EQUIP-TRONICS EQ-610).[12]

Viscosity: Viscosity of gels was measured by using Brookfield Viscometer. (Model no. CAP-2000$^+$ Spindle no. 64)

***Ex-vivo* Permeation Studies Using Carbopol Gel**

The hairless pig skin with the stratum corneum side facing the donor compartment was mounted on a vertical diffusion cell that was maintained at $37^0C \pm 1^0C$ using a hot water circulator. Exactly 3.5gm of carbopol gel containing 35 mg of AS was put into the donor compartment. A Pulse current of 0.5 mA/cm^2 with ON: OFF ratio 1:1 was applied for iontophoresis using a silver–silver chloride electrode. Passive permeation was tested without application of any current. The same experiment was repeated by use of different penetration enhancers i.e. Dimethyl Sulphoxide (DMSO), Urea, Polyethylene glycol-400 (PEG-400), Ethanol, Span-80 and Tween-80.

Sample Collection and Data Analysis

Exactly 1 ml of the sample was collected after every hour from the side arm of the diffusion cell using a syringe and was replaced with the same volume of prewarmed (37^{0}C) fresh 7.4 pH Phosphate buffer solution. The sample is diluted with 2ml $FeCl_3.6H_2O$, and 7ml 0.2 M Perchloric acid and tested for drug content at 300 nm using a UV spectrophotometer (model V-630, Jasco, Tokyo, Japan).[13]

The real steady-state situation was not observed clearly during permeation studies. For this reason the $flux_{ss}$ was calculated from the slope of the linear portion of the curve.

The enhancement ratio (ER) for the $flux_{ss}$ was calculated as follows:

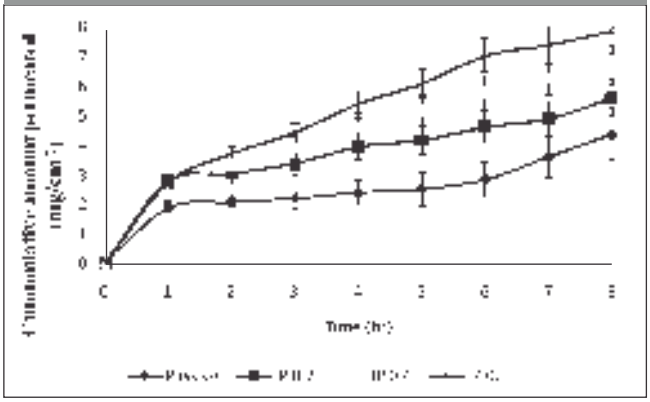
$$ER = \frac{\text{Iontophoretic Flux}_{ss}}{\text{Passive Flux}_{ss}}$$

RESULTS AND DISCUSSION

Iontophoresis markedly improved the transdermal permeation of AS. On ionization, alendronate acquires a negative charge. On iontophoresis, the negative charge of the cathode pushes negatively charged alendronate ions into the skin; that is why its transport across the skin is increased as compared with passive diffusion. As seen in Figure 1, as the current density increases, the permeation of AS is increased. With the current density at 0.5mA/cm^2, the $flux_{ss}$ was 0.8298 mg/cm^2 hr, while it was only 0.3957mg/cm^2 hr when the current density was 0.2mA/cm^2. Therefore, the remaining studies were performed using current density of 0.5mA/cm^2.

Use of continuous direct current may result in skin polarization, which can reduce the efficiency of iontophoretic delivery proportional to the length of direct current application.[14]

Fig.1: Effect current density on permeability of Alendronate sodium. IP means iontophoresis.0.2, 0.4, 0.5 mA/cm^2



The build up of this polarisable current can be overcome by using pulsed direct current that is delivered periodically.[15] Therefore, to further increase the permeation rate and the $flux_{ss}$ of AS across the skin, pulsed iontophoresis using current density of 0.5mA/cm^2 was carried out. As seen in Figure 2, the permeation profile of AS at pulsed iontophoresis of ON: OFF ratios of 1:1, 1:2 and 1:4 was similar to that of the continuous current. However, the permeation rate was significantly increased at the pulse rate of 1:1, with a $flux_{ss}$ value of 0.9669 mg/cm^2 hr and an ER of 1.16 as compared with continuous current. The use of pulse current allows the skin to depolarize and return to its initial electric condition when the current phase is put off for a fraction of time.

Gels are clinically acceptable delivery systems for iontophoresis in terms of stability and ease of handling and refilling of iontophoretic patches. Carbopol-940 was used as gelling agent because it forms the gel at 0.5-2% with enough viscosity to hold the formulation in the electrode cavity when the electrode is applied to the skin. Gel containing 1% w/w Carbopol-940, Triethanolamine and AS showed good gelling property, viscosity, and pH, it was considered optimum for iontophoretic drug delivery, and further *ex vivo* permeation studies were performed on it. The passive permeation profile of AS gel in Figure 3 shows a significant decrease in the permeation of AS with a $flux_{ss}$ of 0.0909mg/cm^2 hr, as compared to passive permeation of AS from the solution with a $flux_{ss}$ of 0.1828 mg/cm^2 hr. This indicates that viscosity of the gel significantly decreases the permeation of AS. Therefore, AS diffusion through the carbopol gel may be a rate-determining step. On iontophoresis, (0.5mA/cm^2 with ON: OFF ratio of 1:1) the permeation of AS from the gel was significantly increased with a $flux_{ss}$ of 0.4099mg/cm^2 hr. To further increase the permeation, a permeability study was performed by using different penetration enhancers.[16] Influence of chemical enhancer (5% w/w) combined with

Fig. 2: Effect of pulse current on the iontophoretic permeability of Alendronate Sodium, at current density 0.5mA/cm^2. Data 1:1, 1:2, 1:4 represents ON:OFF ratio

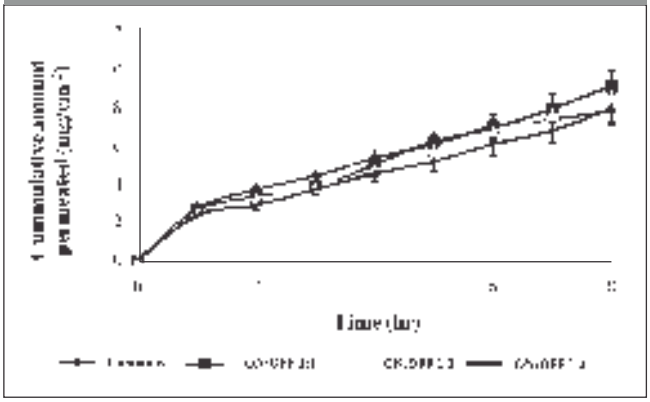


Fig.3: Effect of different penetration enhancer in combination with iontophoresis on permeability of Alendronate sodium gel.

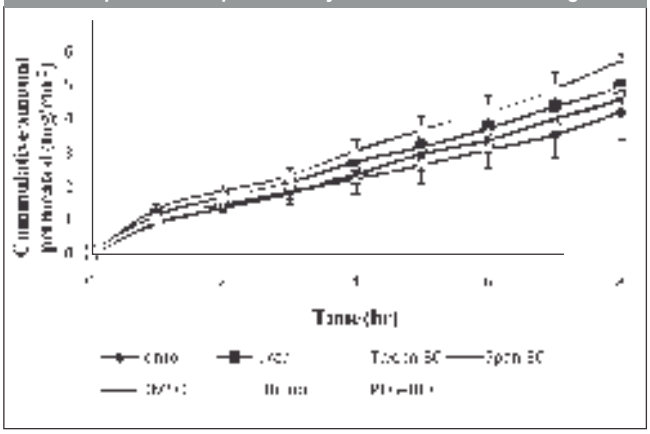
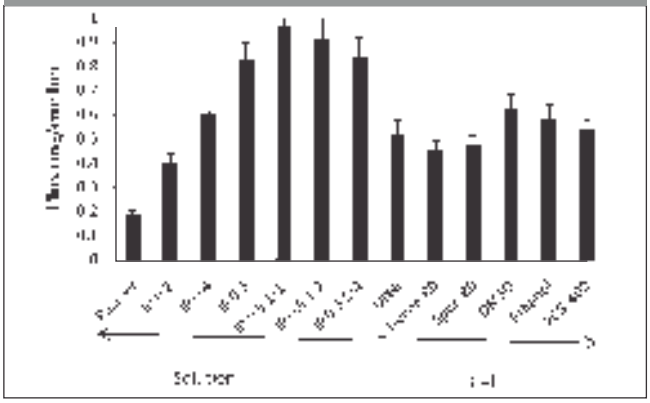


Fig.4: Effect of Current density, Pulse current, and Pentration enhancer on iontophoretic fluxss$_{ss}$ of Alendronate Sodium. IP indicates iontophoretic permeation.



iontophoresis on transdermal permeation of AS from gel base system was studied with DMSO, Urea, PEG-400, Ethanol, Span-80 and Tween-80. DMSO acts as penetration enhancer by increasing the water content of cell as well as alteration in cellular membrane. In the cell if the ratio of bound water to free water is fixed, urea convert the bound water into free water which lead to increase in water content of cell, thus resulting in penetration enhancement activity. PEG-400 and ethanol act via hydration of skin and fluidisation of cellular membrane.

As seen in Figure 3, permeation was markedly enhanced by iontophoresis with penetration enhancers as compared with iontophoresis alone. In this case Urea, PEG-400 Span-80 and Tween-80 do not significantly increase the permeation of AS in comparison to iontophoresis alone. The flux$_{ss}$ observed in case of Urea, PEG-400, Span-80 and Tween-80 was 0.5239 mg/cm^2 hr, 0.5388 mg/cm^2 hr, 0.4733 mg/cm^2 hr, 0.4544 mg/cm^2 hr with ER of 1.27, 1.31, 1.15, and 1.10 respectively. However DMSO and Ethanol significantly increase the permeation of AS with flux$_{ss}$ of 0.6134 mg/cm^2 hr, 0.5821 mg/cm^2 hr, and an ER of 1.50 and 1.41 respectively, in contrast to iontophoresis alone.

CONCLUSION

Results indicate that flux$_{ss}$ of AS solution pulsatile iontophoresis with 1:1 at 0.5mA/cm^2 is satisfactory to obtain the required flux$_{ss}$. Also AS gel containing DMSO (5%w/w) subjected for pulsatile iontophoresis at 0.5mA/cm^2, ON: OFF 1:1 showed better flux enhancement and iontophoretic transport of AS was 3.4 times to that of passive transport. This indicates the feasibility of transdermal iontophoretic delivery of AS gel in combination with penetration enhancer. Further *In-vivo* studies will be required to support in vitro conclusions and develop *In-vitro–In-vivo* correlations.

ACKNOWLEDGMENT

We would like to thank Padmashree Mrs. Fatma Rafiq Zakaria, Hon'ble Chairman, Maulana Azad Education Trust for providing us all the facilities. We are also thankful to Dr. A.R. Khan for helping in the fabrication of iontophoretic circuit. Further we are thankful to Cipla pharma Mumbai, India for providing the gift sample of Alendronate Sodium.

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