

Novel iontophoretic drug delivery of estradiol with alendronate for osteoporosis treatment

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Abstract: Alendronate and estradiol are potential molecules for the treatment of osteoporosis but their use is limited by lower skin permeation and first-pass metabolism respectively. To develop a combinatorial dosage regimen of alendronate and estradiol and enhance skin permeation through iontophoresis for the treatment of osteoporosis. The alendronate and estradiol-containing gel system were developed using carbopol-940 and triethanolamine as independent variables while viscosity and *ex vivo* permeation as dependent variables. The formulated gel was evaluated for viscosity, pH and *ex vivo* permeation study with and without iontophoresis. A permeation study was performed on rat abdominal skin. The viscosities for the developed formulations were found to be in the range of 945 cp-1298 cp, The pH of the formulation was found to be 7.4 which is ideal for the ionization of most drugs. *Ex vivo* permeation ranged from 79.99 to 99.89%. The permeation of both drugs was found to be increased upon application of DC (0.25, 0.50, 0.75 mA/cm²). The maximum percentage of permeation was found to be 99.89% (F3 batch). The skin permeation of alendronate and estradiol was enhanced by the novel formulation using iontophoresis.

Keywords: Alendronate, carbopol gel, estradiol, iontophoresis, osteoporosis.

INTRODUCTION

Patients suffering from osteoporosis carry a high risk of bone fracture due to weak bone structure in elderly people. The bones of the forearms and vertebrae in the spine and hip region are most likely to be affected. This fact also leads to poor quality of life affecting both the social as well as financial aspects of the patient (Ferrari *et al.*, 2016). The bone fracture serves as the first identification mark of the disease. Even the smallest amount of force or minor stress can lead to the rupture of bones. This physical condition disturbs the ability to carry out normal day-to-day functions. The imbalance of bone remodeling as a result of the osteocatabolic process is a reason for underlying decreased trabecular density (Saul *et al.*, 2019). This factor determines the quality of bone which in turn determines the capacity of bone to resist mechanical stress. Hip and spine breakage with a mortality rate of 15%-25% is common. A decrease in bone density is said to be a major reason behind delayed or difficult healing and repair. In women, low estrogen levels after menopause and drugs used to treat some diseases can cause bone loss (Tsoi *et al.*, 2020). Some anti-seizure medications, protons pump inhibitors, serotonin reuptake inhibitors and glucocorticoids are a type of medications that initiate the bone loss process, in addition to this smoking and lack of exercise are also risk factors (Golob *et al.*, 2015). A low level of bone density is considered an osteoporotic condition and can be detected by X-ray absorptiometry at the hip. Smoking cessation, avoidance of certain medications and use of a proper diet

are some of the known preventive measures. For a disease in elderly people prevention of falling is the only option (Selby, 2015).

Treating the disease with anabolic therapies or anti-resorptive drugs (like risedronate, etidronate and alendronate) to increase bone formation are preferred techniques due to the involvement of multifunctional etiologies in the disease (Pedersen *et al.*, 2019). In one of the surveys alendronate, a second-generation nitrogen-containing drug, is found to increase Bone mineral density (BMD) by 7.48% at doses of 10mg/day and 5.81 % at doses of 5mg/day in osteoporotic postmenopausal women (Pedersen *et al.*, 2019). These drugs effectively treat osteoporosis and other bone ailments by acting as powerful inhibitors of osteoclasts-mediated bone resorption (Wade *et al.*, 2014).

Transdermal drug delivery systems or topical delivery include benefits such as increased bioavailability, controlled release rate, painless administration, and bypass to first-pass metabolism (Kolimi *et al.*, 2022). Through the use of penetration enhancers and physical procedures like sonophoresis and iontophoresis, the pace and extent of drug delivery can be improved. One of their more common techniques appears to be iontophoresis, which involves passing two electrodes with opposing charges over the skin to repel drug molecules with a comparable charge (Bakshi *et al.*, 2020). It has long been common practice to administer therapeutic molecules via the skin using sonophoresis or phonophoresis. This method uses high or low-frequency ultrasound to improve the drug's penetration of soft tissue or the skin. This

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method has been in use since 1950 and is popular due to its painless handling and ability to easily transmit both macromolecules and low molecular weight medicines over the skin (Giri *et al.*, 2017).

Estradiol is used to treat osteoporosis as hormone replacement therapy and is generally given by oral route of administration. A higher dose is required to get desired therapeutic effect as it undergoes extensive first-pass hepatic metabolism (Zhang *et al.*, 2018). The intravenous route is also not suitable as its blood concentration rapidly gets increases followed by rapid elimination (Giri *et al.*, 2017).

So transdermal route of administration is the alternate option in the treatment of osteoporosis. Similar to other leading bisphosphonate drugs, alendronate is used to treat osteoporosis, hypercalcemia in cancer patients and Paget's disease. Alendronate's strong polarity and hydrophilicity prevent it from being easily absorbed via the skin (Klara *et al.*, 2022). The use of estradiol and alendronate would be a synergistic combination for the treatment of osteoporosis. However, skin permeation enhancement of both drugs is the need for hours. So, we have attempted to develop a novel combination of alendronate and estradiol with enhanced permeation through the skin using iontophoresis.

MATERIALS AND METHODS

Materials

Estradiol and alendronate were kindly gifted by Shouguang Fukang Pharmacy Factory (Shandong, China). Carbopol 940 and Triethanolamine have been purchased from Sigma Aldrich; the USA. All other chemical agents and materials were of analytical grade and used as received. Male Wistar rats weighing about 200±25g were used.

Methods

Preparation of electrodes

The electrodes were composed as follows

a) Anode: Silver wire of 1mm diameter X 4cm length was used as the anode.

b) Cathode: Silver-silver chloride electrode of about 2 -4 cm in length was used as cathode.

By dipping the silver wire into the molten silver chloride to create a thin, even layer, a rod-shaped electrode was created. In 0.1M HCl, the electrodes were submerged to chlorinate them.

Animals

The institutional animal ethics committee gave its approval to all animal studies, which were carried out in line with the National Institutes of Health's guide for the care and use of laboratory animals. Male Wistar rats weighing about 200±25g were selected for the study. All the animals were housed individually in wire-bottomed

cages with the required temperature and humidity and a normal diet is followed with the feeding of regular tap water.

Preparation of skin

After sacrificing animals, the skin was excised carefully from the abdominal region and hairs were removed using an electrical hair clipper. The skin sample was defatted using a scalpel and cleaned using isopropyl alcohol and washed with distilled water. Physiological saline solution (0.9% sodium chloride) was used to store skin at room temperature for half an hour before use

Preparation of carbopol gel

Pre-weighed quantity of carbopol -940 and sodium CMC was added to distilled water and polymers were allowed to soak (solution A) while drugs were dissolved in distilled water (2ml) (solution B). Both the solutions were mixed with continuous shaking and subsequent addition of tri-ethanolamine. The different batches of formulations were prepared by using different concentrations of Carbopol-940 and tri-ethanolamine as shown in table 1 (Zidan *et al.*, 2017).

Evaluation of carbopol gel

Surface pH

It was determined by applying pH paper to the Carbopol gel's surface and recorded the pH.

Viscosity

The Brookfield viscometer LVDV-E model was used to test viscosity. The sampling tube was filled with the formulations. Before each measurement, the samples were examined in a circulating bath attached to the viscometer adapter at 37°C +/- 0.5°C. The spindle's angular velocity was raised by 1 to 4 while the formulation's viscosity was gauged.

Dilution pH

1gm Carbopol gel was weighed accurately and diluted to 25ml with distilled water. The pH of the solution was measured by using a pH meter.

Ex vivo drug permeation study without iontophoresis (passive study)

The drug permeation study was carried out using Franz diffusion cell (area 4.84 cm²). The gel was applied on the epidermal side of the rat skin in donor compartment. The receptor compartment was filled with pH 7.4 phosphate buffer and magnetic bead was used to agitate it continually at a speed of 50 rpm (Wade *et al.*, 2014). For about 1 hour the skin was hydrated in pH 7.4 phosphate buffer. On the skin's epidermal surface in the donor compartment, an amount of gel equal to 25 mg of the drugs estradiol and alendronate was administered. The whole assembly was kept at 37 0.5°C. The experiment was carried out for 8 hours, with aliquots of the dissolving medium being removed at 1-hour intervals and replaced

with new media in the same quantity. Using a double-beam spectrophotometer, samples were spectrophotometrically analyzed for estradiol at 260 nm and alendronate at 285 nm.

Ex vivo drug permeation study with iontophoresis

This study was conducted using a Dual Channel Pocket Transcutaneous Electrical Nerve Stimulation (TENS) iontophoretic equipment. 7.5 volts and a 500mA A.C. adapter with two copper electrodes were used to power the device. To ensure adequate attachment, the cathode was applied to the donor compartment's epidermal surface using adhesive tape, whilst the anode was introduced to the receptor compartment. For 1 hour, three different current intensities 0.5, 0.6 and 0.7mA/ cm² were used. Samples were taken out and examined using a UV spectrophotometer as previously described at predefined intervals.

STATISTICAL ANALYSIS

The statistical evaluation was done by application of Design expert software version 2.0 where the concentration of Carbopol-940 and Triethanolamine was considered independent factors while viscosity and surface pH was considered a dependent factor.

RESULTS

The Carbopol gel of alendronate and estradiol was prepared using Carbopol-940, sodium CMC and triethanolamine. The different batches of gel were evaluated for their viscosity and *ex vivo* permeation. The viscosity for the carbopol batches was found to be in the range of 945cp- 1298cp while *ex vivo* permeation ranged from 79.99 to 99.89 %. fig. 1 shows the interaction between Carbopol 940 and Tri ethanolamine for both viscosity and *ex-vivo* permeation. The general physical evaluations of gel-like pH, appearance, spreadability, and homogeneity were evaluated and are mentioned in table 2.

EX-vivo permeation study

Statistical analysis was performed using design expert software 2.0 version. Carbopol 940 and triethanolamine concentrations were considered independent variables while viscosity (Y1) and *ex-vivo* permeation (Y2) of drugs were considered dependable variables. In this design 2 variable and 3-level statistical design containing independent variables (A, B) at 3 levels (-1, 0, +1) was used to study the effect on the dependent variables (Y1, Y2). The observations from table 3 showed that *ex-vivo* permeation varied with the independent variables like (A: Carbopol-940, B: Triethanolamine).

It was observed that the percentage *ex-vivo* permeation increases by increasing the carbopol content when compared to triethanolamine whose effect was considered negligible when compared to Carbopol-940. Direct

relation was observed between polymer concentration and viscosity of the formulated gel. The viscosity of the formulation increases with the increasing concentration of carbopol-940. The viscosity of the carbopol batches was found to be in the range of 945 cp - 1298 cp while its *ex vivo* permeation ranged from 79.99 to 99.89 % (fig. 2). The summary of the response of variables Y1 and Y2 is shown in table 3. The polynomial equation of the second order for Y1 and Y2 was given by

$$Y1 = +71.71 + 4.61A + 13.53B + 1.51C - 3.01A^2 - 1.37B^2 + 0.30AB - 0.31A + 0.21B$$

$$Y2 = +68.54 + 4.78A + 16.63B + 1.11C - 3.12A^2 - 1.17B^2 + 0.23AB - 0.34A + 0.12B.$$

Ex- vivo drug permeation study with iontophoresis

The aforesaid study was performed using a TENS iontophoretic instrument which was operated at 7.5 V with a 500 mA A.C. adapter with two copper electrodes of different densities of 0.25, 0.50, and 0.75 mA/cm² applied for 90 minutes. The sample was withdrawn at different time intervals, collected, and analyzed for drug content (both alendronate and estradiol) at different absorption wavelengths. The results obtained from the experiment revealed that when DC current (0.25, 0.50, 0.75mA/cm²) was applied the rise in permeation of both drugs was observed. The reason underlying the fast permeation of molecules can be stated as electrochemical polarization in the skin (Lapteva *et al.*, 2020). table 4 shows the effect of iontophoresis which increases ($P < 0.01$) the quantity of drug penetration through the skin.

DISCUSSION

Our research group has attempted to develop a formulation containing alendronate and estradiol with enhanced skin permeation using iontophoresis that would be beneficial for the treatment of osteoporosis. Estradiol is having higher first-pass metabolism so it needs to administer other than the oral route while alendronate is a highly polar and hydrophilic molecule that doesn't easily pass through the skin. The major barrier to transdermal delivery is the stratum corneum which reduces the percutaneous absorption and metabolic activity of the skin (Shankar *et al.*, 2022). For efficient treatment of formulations containing alendronate and estradiol skin permeation enhancement of these two molecules is required. The chemical permeation enhancers are having harmful effect on the epidermis of the skin. In such situation use of iontophoresis is highly recommended. The electrical potential energy generated during application of iontophoresis is responsible for the enhancement in skin permeation. During this process ionization of the drugs takes place which acquires the negative charge on them and leads to the enhancement in skin permeation. Drugs are delivered through the skin in the form of charged ions and biological membranes or barriers, organs, or tissues are crossed using electrical current as a medium.

Table 1: Composition of Estradiol and Alendronate gel

| Batch No | Estra Diol (%) | Alendro Nate (%) | Sodium CMC (g) | Carbopol 940 (g) | Trietha Nolamine (ml) | Methyl paraben (g) | Propyl Paraben (g) | D/W (ml) |
|----------|----------------|------------------|----------------|------------------|-----------------------|--------------------|--------------------|------------------------------------|
| F1 | 0.5 | 1 | 1.25 | 1 | 0.5 | 0.020 | 0.002 | Quantity sufficient to make 100 ml |
| F2 | 0.5 | 1 | 1.25 | 1.5 | 0.5 | 0.020 | 0.002 | |
| F3 | 0.5 | 1 | 1.25 | 2 | 1 | 0.020 | 0.002 | |
| F4 | 0.5 | 1 | 1.25 | 2.5 | 1 | 0.020 | 0.002 | |
| F5 | 0.5 | 1 | 1.25 | 3 | 1 | 0.020 | 0.002 | |

Table 2: Evaluation of Gel

| Formulation | Appearance | pH | Spredability (g.cm.sec) | Homogeneity |
|-------------|-------------------|------|-------------------------|-------------|
| F1 | White transparent | 6.92 | 5.4 | Homogenous |
| F2 | White transparent | 6.78 | 5.6 | Homogenous |
| F3 | White transparent | 6.98 | 5.2 | Homogenous |
| F4 | White transparent | 6.65 | 4.0 | Homogenous |
| F5 | White transparent | 6.68 | 5.6 | Homogenous |

Table 3: Statistical analysis of Y1 and Y2

| Models | R ² | Adjusted R ² | Predicted R ² | Std. Dev | Press | Remarks |
|--------------------|----------------|-------------------------|--------------------------|----------|--------|---------------|
| Response Y1 | | | | | | |
| Linear | 0.9215 | 0.9567 | 0.9875 | 3.10 | 190.80 | Not suggested |
| 2FI | 0.9420 | 0.9235 | 0.8712 | 3.45 | 210.27 | Not suggested |
| Quadratic | 0.9967 | 0.9757 | 0.94867 | 1.16 | 150.89 | Suggested |
| Cubic | 0.9730 | 0.9675 | 0.9198 | 2.55 | 110.25 | Aliased |
| Response Y2 | | | | | | |
| Linear | 0.9435 | 0.9347 | 0.9345 | 3.00 | 176.76 | Not suggested |
| 2FI | 0.9760 | 0.9255 | 0.8872 | 3.12 | 223.21 | Not suggested |
| Quadratic | 0.9897 | 0.9877 | 0.9487 | 1.21 | 123.45 | Suggested |
| Cubic | 0.9870 | 0.9565 | 0.9378 | 2.21 | 123.89 | Not suggested |

Regression equations of the fitted models

$$Y1 = +71.71 + 4.61A + 13.53B + 1.51C - 3.01A^2 - 1.37B^2 + 0.30AB - 0.31A + 0.21B$$

$$Y2 = +68.54 + 4.78A + 16.63B + 1.11C - 3.12A^2 - 1.17B^2 + 0.23AB - 0.34A + 0.12B$$

Table 4: Comparative Evaluation of cumulative percentage drug permeated from gels at a different current density

| Current Density (mA/cm ²) | % drug release from optimized formulation (9 hrs) | | | | |
|---------------------------------------|---|-------|-------|-------|-------|
| | F1 | F2 | F3 | F4 | F5 |
| 0 | 67.98 | 67.98 | 65.87 | 68.98 | 69.76 |
| 0.5 | 89.97 | 87.96 | 86.65 | 86.98 | 86.67 |
| 0.6 | 92.43 | 97.98 | 98.95 | 98.67 | 98.78 |
| 0.7 | 87.24 | 86.23 | 87.32 | 86.54 | 85.34 |

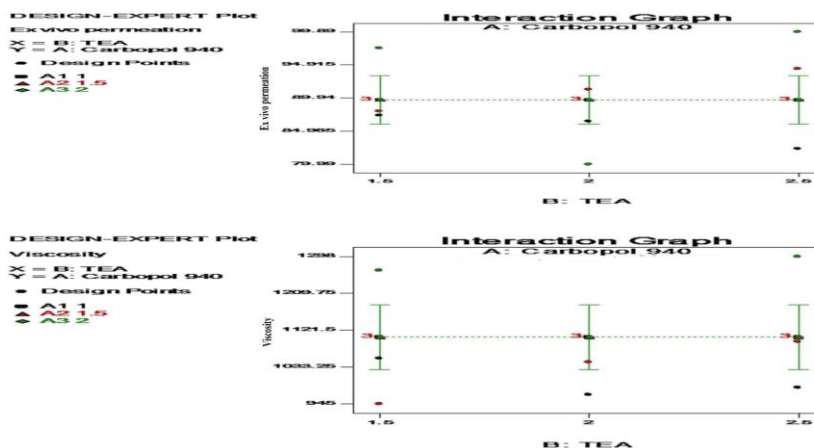


Fig. 1: Graph showing the interaction between carbopol 940 and Tri ethanolamine for both viscosity and ex-vivo permeation

Ionic migration across biological membranes increases in response to the externally applied electrical potential. The electrical charge and time spent applying it directly affect how much drug is spread throughout the skin (Datta *et al.*, 2021). In addition to the typical benefits of transdermal delivery, this electrically assisted transdermal delivery method involves the migration of ions when a low-level electrical current is passed through a formulation containing ionised species across the skin. The current input is controlled at a predetermined rate to control the drug delivery in a pulsatile manner (Bakshi *et al.*, 2020). After iontophoresis, the negatively charged cathode pushes similarly charged drug ions through the skin when compared to passive diffusion. So, theoretically, as current density increases the transport across skin also increases. The reason behind the choice of carbopol as a polymer was due to its compatibility, stability, and lesser toxicity as compared to other polymers. The ability of carbopol to decrease the surface tension of water and interfacial tension between aqueous systems results in good wetting and spreading of the drug over the skin surface to increase contact area resulting in higher permeability of the drug (Ita, 2016). The electrodes were prepared using a cathode and anode of silver-silver chloride wire. The ON-OFF system was used to avoid the continuous current. The use of DC current can cause the skin to become polarized, which would limit the effectiveness of the iontophoretic system, which is proportional to the duration of the direct current application. The pulsatile current was used to counteract this accumulation of polarized current, which may also aid to speed up penetration. The above regression equation's positive and negative values indicate, respectively, a synergistic and an antagonistic relationship between the factor and response.

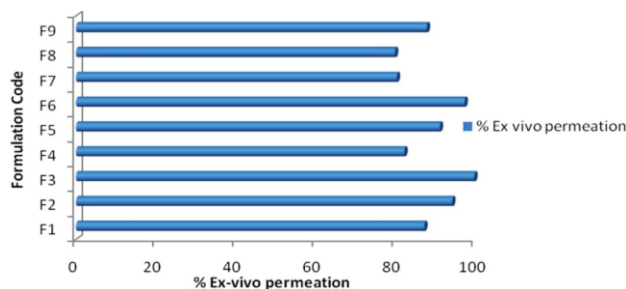


Fig. 2: *Ex-vivo* permeation study of all formulations showing the highest release from the F3 batch

As shown in table 3, R^2 was found to be 0.9816 which indicated good fit model. In regression equation terms A and B indicates the results obtained after changing at a time from lower level to higher level.

The *ex-vivo* permeation study without iontophoresis i.e. passive study has shown the difference between releases of all formulation batches. The maximum percentage of permeation was found to be 99.89% (F 3 batch). The

study was continued up to 9 hrs. The pH of the formulation was found to be 7.4 which is ideal for the ionization of most drugs. The drug that remains unionized at this pH will permeate the skin through a trans-cellular pathway as it might be more lipophilic. The graphical representation of the passive permeation of all formulations is shown in fig. 2. The current, which serves as the impetus for the passage of ions across the skin during iontophoresis, was responsible for the noticeably increased amount of drug that was transported to the receptor compartment from all gels. The effect can be seen directly from values observed in table 4 along with ionization of the drug the presence of pH around 7.4 also leads to the high conductivity of the drug at the cathode.

Different groups of researchers used iontophoresis to maximize the drug permeation through the skin. Iontophoretic delivery of transdermal patches containing ropivacaine to enhance the local anesthetic effect in children has been studied. It was proved that ropivacaine patches when coupled with iontophoresis in children, showed superiority in lowering the pain threshold and cooling sensation as compared to only patches (Yu *et al.*, 2019). Iontophoresis was used to deliver estradiol-loaded PLGA nanoparticles transdermally for the treatment of osteoporosis, and the researchers discovered that the combination of a nanoparticulate system with iontophoresis was effective. However, they demonstrated the efficacy of iontophoresis based on BMD and didn't perform the skin permeation study to prove the enhancement of the permeation of drug molecules through the skin (Takeuchi *et al.*, 2016). Similarly, the use of calcium as well as phosphates using iontophoresis was studied through topical route for the treatment of osteoporosis and concluded that calcium and phosphate donating micro particles coupled with local iontophoresis strategy is comparable with estrogen therapy for the treatment of osteoporosis (Gomez *et al.*, 2012). Enhanced transdermal permeability of estradiol nanoparticles using iontophoresis was studied and concluded that the permeation of estradiol could be enhanced by employing nanoparticles and iontophoresis (Tomoda *et al.*, 2012). All of these studies showed the value and effectiveness of iontophoresis in enhancing the permeation of active molecules in a variety of diseased conditions. In our research work, we have successfully enhanced the permeation of alendronate and estradiol using iontophoresis which could be beneficial in osteoporosis treatment. According to research, continuous application of an external current causes heat energy to be produced, which fluidizes skin lipids and alters the skin's inherent integrity, increasing skin permeability. Applying an iontophoretic drug delivery system for the treatment of osteoporosis results in this phenomenon, which sufficiently explains the cause of increased drug action. However, The use of Iontophoretic device by patients may create the handling problems and it should be done

under supervision of medical expert. This may be considered as major limitation of this research work. The scale up and clinical studies are quite essential to establish the *in vitro* and *in vivo* co relation for this research work which can be considered as a future experimental part.

CONCLUSION

The aforesaid study shows enhancement in permeation of both alendronate and estradiol through the skin using the iontophoresis technique. The maximum permeation of the drug into the body through iontophoretic drug delivery also underlines the effectiveness of formulation in the treatment of Osteoporosis.

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