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Original Article

FREEZE-DRIED POLYMERIC NASAL INSERTS FOR ANTIHYPERTENSIVE DRUG DELIVERY

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ABSTRACT

Objective: The aim of this study was to investigate the ability of polymers to form verapamil HCl nasal inserts.

Methods: Theinserts were prepared by lyophilization technique using bioadhesive polymers as chitosan, sodium carboxymethyl cellulose, hydroxyl propyl methyl cellulose,sodium alginate and xanthan gum. The prepared inserts were characterized by different parameters as bioadhesion potential, wetting time, water uptake behavior, drug release and permeation.

Results: Verapamil HClnasal inserts prepared with 2% chitosan polymer showed a good bioadhesive, water uptake and release within 6 hrs properties as well as the highest drug contents and permeation within 8 hrs.

Conclusion: Nasal inserts could be a good alternative route to avoid the first pass metabolism of verapamil HCl.

Keywords: Intranasal delivery, Verapamil HCl, Inserts, Lyophilization.

INTRODUCTION

In recent decades nasal delivery has been paid attention as an attractive alternative dosage form. It has seriously emerged as a therapeutically viable and lucrative route for both topical and systemic therapies due to high permeability, high vascular and low enzymatic environment of nasal cavity [1]. Also, it has become a desirable alternative to the parental medication since it is amenable to self-medication and virtually painless, does not require sterile technique, and does not contribute to the bio hazardous waste and risk of accidental sticks [2-4]. Intranasal drugs are transported along the olfactory sensory neurons to yield significant concentrations in the cerebrospinal fluids [5].

However, the nasal cavity presents a number of limitations for drug absorption including enzymatic degradation, low intrinsic permeability for hydrophilic drugs, and rapid mucociliary clearance. Therefore, in order to overcome these barriers a number of strategies can be applied by modifying three components: the drug, delivery carrier, and administration device like inserts [6]. The production of this new promising drug delivery inserts required only conventional techniques like lyophilization of polymer through freeze-drying technology [7]. Nasal inserts consist of a sponge-like hydrophilic polymer matrix, in which the drug is embedded. They allow easy dosing with a high potential for systemic administration that helps prevent hepatic first pass metabolism. When the nasal insert comes into contact with the highly vascularized nasal mucosa, it absorbs water and swells. The polymer gel releases the active ingredient in a controlled manner [8].

Verapamilhydrochloride (VRP) is calcium channel blocker antihypertensive drug. It is one of the mostly affected drugs by the first pass metabolism since its bioavailability is not more than 20% and the rest is converted to the inactive metabolite nor-verapamil [9].

In this study, a bioadhesive VRP nasal insert based on water soluble polymers was developed, which allows easy administration and exact dosing due to its solid, single dose character. The in situ gelling, solid insert was designed to hydrate rapidly in the nasal cavity into a bio adhesive gel with increased residence time and extended release. The production of this new delivery system required only conventional techniques like lyophilization. The aim of this study was to investigate the ability of polymers to form inserts by lyophilization and to characterize the inserts with respect to bio adhesion potential, wetting time, water uptake behavior, drug release and permeation.

MATERIALS AND METHODS

Materials

Verapamil HCl(VRP) wasgenerously supplied from Abbot Co.,Egypt. Chitosan (CS), mucin, agar (Sigma-Aldrich, St Louis USA), sodium carboxymethyl cellulose (NaCMC), hydroxyl propyl methyl cellulose (HPMC), sodium alginate, xanthan gum (FlukaChem., Switzerland), all other chemicals used were of analytical grade.

Viscosity measurement of polymer solutions

Solutions of all types of the differentpolymers, hydroxyl propyl methyl cellulose (HPMC), chitosan (CS), Xanthan gum, sodium alginate and sodium carboxymethyl cellulose (NaCMC)were prepared in concentration as seen in table 1and left un agitated over night. They were equilibrated to 25 °C for 1 hr before measurement in a water bath. The viscosity of the polymer solutions was measured with Brookfield viscometer (DV-III ultra programmable cone and plate, USA). The measurements were made at 25°C using spindle 52 at 5rpm. All measurements were done in triplicate.

Nasal insert preparation

Different concentrations of polymers and drug as seen in table 1 were dissolved in distilled water. Drug was added in two different concentrations (20% and 40%) to show loading drug ability of inserts formed from different polymers. The formed polymeric solutions were sonicated to remove air bubbles. Aliquots of 1 ml were placed into eppendorf tubes and frozen at -25°C for 1 hr. The samples were then freeze dried using Bench Top Manifold Freeze Dryer (Millrock Technology, Inc, USA). The inserts were stored in desiccators until use [10-11].

Determination of surface pH

Agar solution was prepared by dissolving 2%w/v agar in simulated nasal electrolyte solution (SNES) by heating under stirring, then poured into a Petri dish to solidify at room temperature. The plain inserts were left to swell for 2 hrs on the surface of agar plate. Surface pH was measured by means of a pH paper placed on the surface of the swollen inserts. The measurements were performed in triplicate [12]. The SNES was composed of 7.45 mg/mlNaCl, 1.29

mg/ml KCl and 0.32 mg/ml $CaCl_2.2H$ 2O and PH were adjusted to 5.5[13].

Water uptake and mass loss of the inserts

A sponge with dimensions of 5 cm ×6.5 cm ×3 cm was fully soaked in the hydration medium SNES and placed in a petri -dish filled with the same medium to a height of 1 cm to keep the sponge soaked during the experiment. Round filter paper (d=55 mm, Schleicher Schuell GmbH, Germany) was also soaked in medium and positioned on the top of the sponge. The experimental set up was equilibrated for 30 min. Accurately weighed inserts(V=0.5 ml) were placed on the filter paper and the water uptake was determined as weight increase of the insert (weight of hydrated insert and wet filter paper minus weight of wet filter paper) over time normalized to the initial dry insert weight [14].

Bio adhesive potential of inserts

Adhesion studies were performed by adapting Bertram and Bodmeier method [11]. One hundred grams of a hot agar/mucin solution(1and2 % w/w, respectively, in phosphate buffer PH 7.4 were poured in a petri- dish (10 cm diameter)and left to gel at 4-8 °C for 3 hrs. The gel was equilibrated for 1 hr to the test conditions of 22 °C and 79% relative humidity in a chamber. The inserts, which were placed on top of the gel, moved downward due to gravity after the petri-dish was turned into a vertical position. The displacement in cm was measured as a function of time. The adhesion potential was inversely related to the displacement of the insert. The measurements were performed in triplicate.

Drug content of inserts

The uniformity of the inserts content was determined by spectrophotometrically method. The inserts were individually dissolved in 100 ml SNES and the formed solution was shaken for 1 hr then sonicated for 2 hrs. Samples (3 ml) were withdrawn and analyzed for VRP content spectrophotometrically at 278 nm using plain SNES as a blank. Drug content was calculated using standard calibration curve and the mean percent of drug content was calculated as an average of 3 readings [15].

Scanning electron microscopy (SEM)

The shape and surface morphology of all nasal inserts was performed by SEM (JXA-840A, Japan). Inserts were cut with a razor blade to expose the inner structure, fixed on supports and coated with gold-palladium under an argon atmosphere using a gold sputter module in a high vacuum evaporator then the surface was examined [16].

***In vitro* drug release**

The *in vitro* release of VRP from different nasal inserts was carried out using a USP dissolution tester (Apparatus II, Hanson SR6, USA). The lower end of the baskets was closed with a tightly stretched thin sponge. The baskets were placed vertically into release medium and adjusted exactly on the surface to wet the sponge but not submerse it. The release medium was 100 ml SNES with pH 5.5. The baskets shafts were rotated at 50rpm at 35± 0.5°C. At predetermined time intervals, samples (3 ml) were withdrawn and analyzed for VRP content spectrophotometrically at 278 nm using plain SNES as a blank withdrawn at respective time intervals. Every withdrawal was followed by replacement with fresh medium to maintain a constant volume. The results were the mean value of 3 runs [15].

***In-vitro* permeation studies across sheep nasal mucosa**

Sheep nasal mucosa was obtained from local slaughterhouses. The mucosa was carefully removed from the underlying bone by cutting with haemostatic forceps and pulling the mucosa off. To maintain the freshness of the specimen as far as possible, permeation studies were started immediately after the mucosa samples were excised [17]. The permeation study was conducted in a franz-diffusion cell (Hanson, Microvette plus, USA) with a diffusion area of 1.5 cm^2. At zero time, VRP loaded inserts were placed in the donor compartment with their lateral surface in contact with the mucosa. The receiver phase was phosphate buffer PH 5.5 and the temperature of the receptor compartment was maintained at 35±0.5 °C with an external constant temperature circulator water bath and the receiver medium was continuously stirred with a small magnetic bar in order to prevent any boundary layer effects.

Control experiments without VRP inserts were carried out simultaneously to ensure the non-interference of mucosa leaching. At predetermined time intervals, samples (0.5 ml) were taken from the receptor compartment and the cell was refilled with an equivalent amount of fresh buffer solution. Each permeation experiment was replicated three times and from the concentration of VRPin the receiving solution the amount permeated through the mucosa was calculated. The cumulative amount of VRPpermeated into the receptor compartment was plotted against time to obtain a percentage permeation profile. The steady state flux Jssq (µg/cm/h) was calculated from the linear portion of the plot of the cumulative amount permeated vs, time and expressed as Jss =Q/t = Kp C donner [18]. Where Q is the amount of VRP permeated through mucosa in (µg/cm^2) in experimental time t in (h), C donner is the concentration of VRP in the donner chamber in.

(µg/$cm^{2)}$ and Kp in (Cm h^{-1}) is the permeability coefficient of VRP through the mucosa.

RESULTS AND DISCUSSION

Determination of surface pH

Table 1 shows that the prepared inserts pH values were in the range 5.9-6.9. Such pH is very close to human nasal mucosa (5-6.5) to avoid any probable mucosal irritation [19].

Drug content of inserts

As seen in table 1, inserts had drug contents ranging from 64.27 to 99.6%. Inserts prepared with 40% VRP loaded in 1% xanthan gum is the least drug content in both 2 different VRP concentrations (65.0 and 64.27 %), followed by Na alginate then HPMC and NaCMC while that prepared with 2% CS is the highest one (99.6% and 98.4%).

Table 1: Concentration and viscosity of the corresponding polymer solutions, pH, muco adhesion and drug contents of different prepared inserts

formula	Polymer concentration (% w/w)	Polymerviscosity(cP) Mean±S. D (n=3)	VRP concentration (% w/v)	pH Mean±S. D (n=3)	Mucoadhision Displacing (cm) Mean±S. D (n=3)	Drug content (%)
F1	2% HPMC	158.83±1.46	20	6.6±0.1	0	95.56
F2	2% HPMC		40		2±0.2	90.13
F3	2% CS	474.0±5.44	20	5.95±0.2	0.5±0.1	99.6
F4	2% CS		40		0	98.4
F5	1%Xanthan gum	1572.33±5.7	20	6.8±0.09	0	65.0
F6	1%Xanthan gum		40		0	64.27
F7	2% Na CMC	319 ±6.03	20	6.9±0.25	Dissolved	94.28
F8	2% Na CMC		40		4 ±0.3	93.15
F9	2% Na alginate	375±8.46	20	6.4±0.42	4±0.2	73.37
F10	2% Na alginate		40		0.5±0.03	94.36

Water uptake of the inserts

The water uptake ability of all the nasal inserts is summarized in fig. 1 and Figure2. All the nasal inserts hydrated over a period of 6 hrs. The uptake of water by the inserts is a crucial step for the transformation into gel and for adhesion to the mucosa. The ability of hydrogels to absorb water is due to the presence of hydrophilic groups such as –OH,-COOH and $-OSO_3H$. The hydration of these functional groups results in water entry into the polymer network, which leads to expansion and consequently an ordering of the polymer chains [20]. The water uptake of inserts depended on the type of polymer used.

The balance of water uptake, polymer mass loss during the hydration, and the molecular weight of the polymer defined the viscosity of the resulting gel. Low viscosity polymers (table 1) dissolved like HPMC and NaCMC, while high viscosity polymers like xanthangum, CS and Na alginate showed good water uptake ability. Maximum water uptake is reached when the osmotic forces of functional groups are balanced by the restrictive forces of the higher ordering of the polymer chains. Swelling is a function of the presence of ionized functional groups. Also the charged polymers let to a higher extent of water uptake as in case of CS, NaCMC, and Na alginate compared to neutral polymers like HPMC [21].

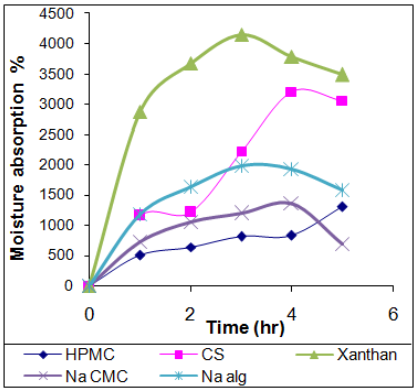


Fig. 1: Water uptake of 20% VRP loaded nasal inserts

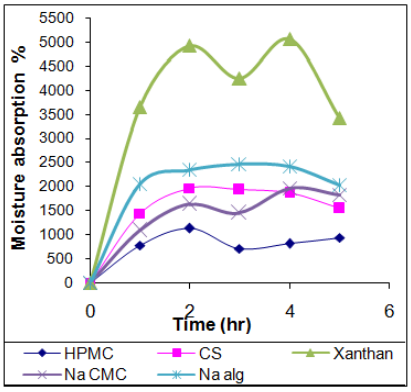
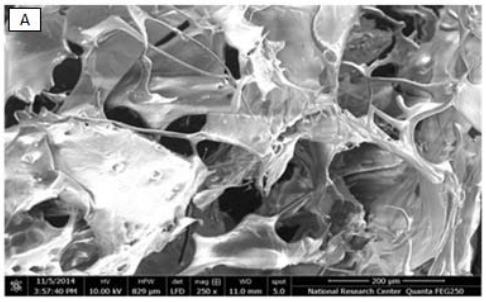
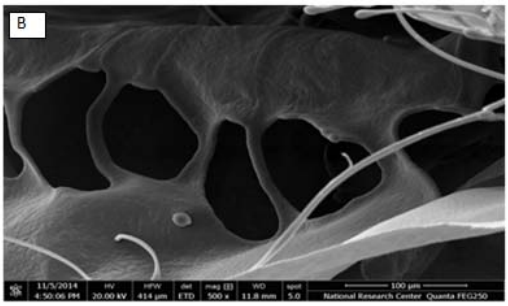


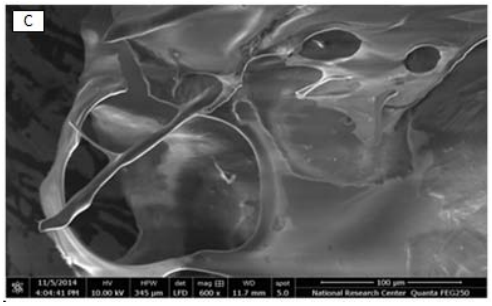
Fig. 2: Water uptake of 40% VRP loaded nasal inserts



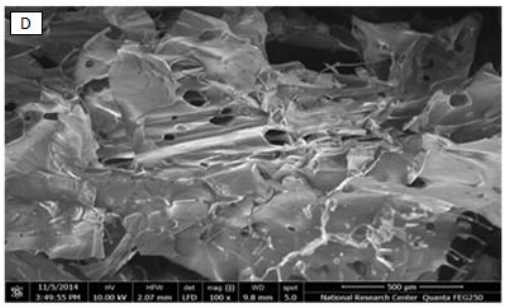
HPMC



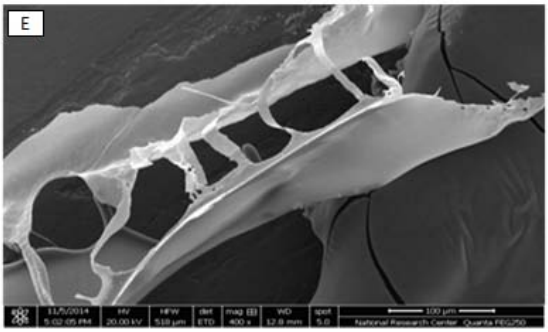
Chitosan



Xanthan gum



NaCMC



Na alginate

Fig. 3: Scanning electron micrographs of the different polymeric inserts

Bioadhesive potential of inserts

The presence of water is a prerequisite for bioadhesion, which is a key factor for a successful prolonged nasal drug delivery [22]. Once administered into the nasal cavity, the inserts have to adhere to the nasal mucosa to take up water and transform into a gel. Freeze dried insert hydration produces gelling networks able to interact with mucous as a result of physical entanglement and secondary bonding (H-bonding and Van der Waals attraction). In fact, polymers-water uptake ability, increasing the mobility of molecules, facilitates interpenetration and interaction with the mucous layer [16]. As seen in table 1, an intermediate detachment was measured for HPMC as it is neutral polymer cannot interact electrostatically with the negatively charged mucin, it may do so by entanglement due to its high molecular weight since there was general agreement that adhesion increases with high molecular weight polymers [23]. On the other hand, CS a positively charged polymer with low viscosity showed a relatively good adhesion due to electrostatic interaction and formation of thin film with the opposite charge mucin which probably would allow a prolonged contact with the mucosa [22]. No or very little displacement was observed for xanthan gum inserts and this is due to its relatively high viscosity and its good bio adhesion property [24]. A low bioadhesion potential was obtained with negatively charged Na alginate and NaCMC inserts due to their inability to interact with mucin either electro statically or by entanglements because of their rather low molecular weight and low solution viscosity [25].

Scanning electron microscopy (SEM)

Fig. 3 (A-E) shows the morphology of the nasal inserts observed by (SEM). The process of freeze-drying is based on sublimation of the frozen water leading to the formation of pores or channels in the polymer that led to physically cross-linked hydrogel. All the samples were characterized by a sponge-like structure with presence of large voids that is an important parameter to ensure rapid hydration and gelation of the insert resulting in a larger surface/contact area and increased water uptake by capillary forces and to a reduced foreign body sensation when compared to other solid dosage forms, such as tablets [26]. Furthermore, fig. 4 shows the homogenous distribution of VRP on CS sponge like structure.

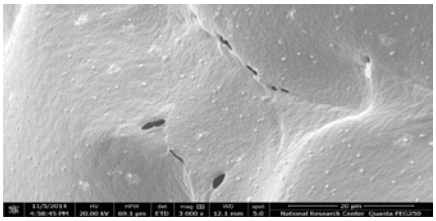


Fig. 4: Scanning electron micrograph of chitosan loaded VRP insert

***In vitro* drug release**

Release profiles of both 20% and 40% VRP loaded inserts are shown in fig. 5 and fig. 6. The release of drug from nasal inserts prepared from different polymers is a complex phenomenon composed of multiple single processes such as drug-polymer interactions, viscosity of the hydrated inserts resulting from polymer molecular weight, water uptake and polymer mass loss during hydration, and spreading of the gel with subsequent increase of the release area [21]. As a consequence of the high polymeric chain mobility (mentioned earlier in mucoadhesion) leading to a great entry of water in the nasal inserts, thus forming a viscous network, this may explain the relatively slow drug release from xanthan gum inserts [21]. On the other hand, inserts prepared fromHPMC, NaCMC, CS then Na alginate polymers released the drug rapidly and this is due to the low viscosity of the gel formed after hydration and partly due to its water soluble nature which allowed more rapid penetration of fluid into the inserts initiating dissolution of the gel matrix [21].

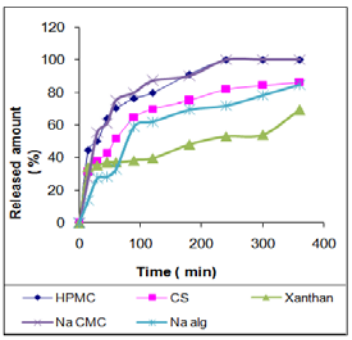


Fig. 5: Release profile from 20% VRP loaded nasal inserts

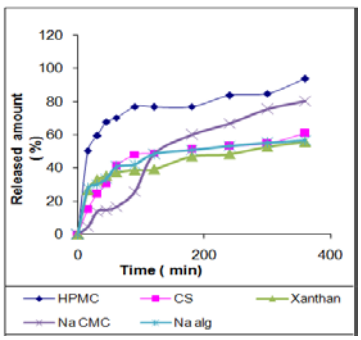


Fig. 6: Release profile from 40% VRP loaded nasal inserts

***In-vitro* permeation studies across sheep nasal mucosa**

The *in-vitro* drug permeation through mucosal membrane was performed to ensure drug absorption in biological system. The profile is depicted in fig. 7. The initial slower permeation of VRP attributed to time required for wetting, swelling and gelation of nasal inserts. The high permeation rate and short lag time were due to the nasal cavity that characterized by high surface area [27] and rich vascularized with blood supply [28]. The permeation rate of VRP from NaCMC, CS, xanthan gum, HPMC and Na alginate was (7.72, 14.03, 3.81, 12.64, 7.014 µg/cm^2. min) respectively.

Among the low permeation the viscous xanthan gum inserts showed the lowest permeation and this attributed to the same reason mentioned before in release study. At the same time, low permeation of both NaCMC and Na alginate may be attributed to salt formation between the sodium of NaCMC and Na alginate and chloride of the drug [21]. On the contrary to the positively charged CS and neutral HPMC that showed the highest permeation and this could be attributed to the uniform dispersion of the drug into the polymeric network as a result of lyophilization process[29]as seen in Fig. 4 that show the well distribution of VRP particles on CS sponge like structure.

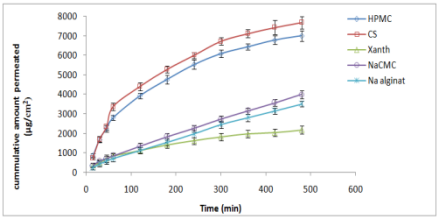


Fig. 7: Permeation profile from 40% VRP loaded nasal inserts (mean±SD; n=3)

CONCLUSION

From the present study, different polymeric in situ gelling inserts were developed for nasal delivery of VRP. The selected polymer

dictates important insert properties such as water uptake behavior, bio adhesion potential. VRP release and permeation profiles were dependent on the used polymer. The results in this study indicated that CS is a promising polymer for bioadhesive nasal insert for VRP delivery as it showed the highest drug content and permeation in 8 hrs. The present work will be furthered by performing *in vivo* absorption studies in animal models.

CONFLICT OF INTERESTS

Declared None

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