

Development and Evaluation of a
Mucoadhesive Nasal Gel of
Midazolam Prepared with
Linum usitatissimum L.
Seed Mucilage

Shyamoshree BASU¹, Subrata CHAKRABARTY²,
Amal K. BANDYOPADHYAY *¹

¹ Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032, India.

² Dr. B. C. Roy College of Pharmacy and Allied Health Sciences, Dr. Meghnath Saha Sarani, Bidhannagar, Durgapur – 713206, India.

* Corresponding author. E-mail: akbju@yahoo.com (A. K. Bandyopadhyay)

Sci Pharm. 2009; 77: 899–910

doi:10.3797/scipharm.0807-10

Published: September 27th 2009

Received: July 14th 2008

Accepted: September 24th 2009

This article is available from: <http://dx.doi.org/10.3797/scipharm.0807-10>

© Basu *et al.*; licensee Österreichische Apotheker-Verlagsgesellschaft m. b. H., Vienna, Austria.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

The aim of the present study was to prepare mucoadhesive nasal gels from mucilage isolated from seeds of *Linum usitatissimum* L. containing midazolam hydrochloride. Characterization of the isolated mucoadhesive agent was carried out, including determination of pH, swelling property, viscosity and mucoadhesive strength. The results were compared with synthetic polymers (HPMC and carbopol 934). Nasal gels of midazolam were prepared with and without enhancers and the permeation study was carried out using excised goat nasal mucosa. Permeation profiles were evaluated and histological study of nasal mucosae before and after permeation study was also conducted to determine histological change, if any.

Keywords

Nasal • Midazolam hydrochloride • Mucoadhesive • Enhancers

Introduction

Nasal drug delivery system has emerged to be an important area in the field of pharmaceutical research. The nasal mucosa is considered to be a promising site for the systemic delivery of drugs where a rapid onset of action is required and for drugs (eg. peptides and proteins) that are not easily administered via other routes than by injection [1]. This route thus, serves as an excellent needle-free alternative which may improve patient compliance and allows extended use of self medication for many chronic diseases. The nasal mucosa being a highly vascularised area helps in rapid systemic absorption of the drug, thereby avoiding hepatic “first-pass effect” of drugs leading to quicker onset of action, which could be especially important in the management of crisis situations like cardiac arrest, epileptic seizures, severe nausea and vomiting [1–3].

Despite the potential advantages, there are certain factors like mucociliary clearance, mucous and epithelial barriers and enzymic activity, that adversely affect the bioavailability of drugs administered intranasally [1–3]. Rapid mucociliary clearance of drug formulations is an important factor that is responsible for the underlying low bioavailability of drugs administered via nasal route [2–6]. Hence, to overcome this problem, a platform for drug delivery is required which can be effectively provided by mucoadhesive gels. The mucoadhesive agents make intimate contact with the mucin of mucosa, thereby, prolonging residence time of the drug in nasal cavity enhancing the period of contact with nasal mucosa, which may improve drug absorption [1–4].

Midazolam hydrochloride (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine hydrochloride) is a potent water soluble benzodiazepine [7]. It has powerful anxiolytic, amnestic, hypnotic, anticonvulsant, skeletal muscle relaxant and sedative properties [8]. It is a fast-acting benzodiazepine, with a short elimination half-life that ranges between 1.5 to 2.5 hours. It is therefore very useful for short minor procedures such as dental extraction [8]. Oral bioavailability of midazolam has been reported to be very low (~36%) [8]. Midazolam administered intravenously has been associated with cardio respiratory adverse events. The parenteral formulation is very hazardous due to various reasons. Hence, formulation of midazolam as a mucoadhesive nasal gel may replace the conventional midazolam injection and widen the scope of novel drug delivery system.

Linum usitatissimum L. mucilage is a water soluble heterogeneous polysaccharide composed of xylose, arabinose, glucose, galactose, galacturonic acid, rhamnose and fucose [9–13]. It has good water-holding capacities, owing to its marked swelling capacity and high viscosity in aqueous solution [14, 15]. It has been reported that many oligo- and polysaccharides in many substances possess mucoadhesive properties [16]. Since, this mucilage is a rich source of polysaccharides and has remarkable swelling capacity and high viscosity, it was selected to prepare nasal gels of midazolam.

In the present investigation flaxseed (*Linum usitatissimum* L.) mucilage was used as a mucoadhesive agent for the preparation of nasal gels containing midazolam hydrochloride (MDZ HCl). The main objective was to prolong the residence time of midazolam in the nasal cavity. Mucoadhesive material extracted from *Linum usitatissimum* L. seeds was used in the formulation because of its edibility, biodegradability and biocompatibility [12].

Experimental

Materials

Midazolam hydrochloride was obtained as a gift from Sun Pharmaceutical Industries Ltd., Gujarat, India. *Linum usitatissimum* L. seeds were purchased from local market. Hydroxy propyl methyl cellulose (HPMC) and carbopol 934 were purchased from S.D. Fine Chemicals Ltd, Mumbai, India. Sodium thioglycollate and sodium taurocholate were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All other reagents and chemicals used were of analytical grade.

Methods

1. Extraction of mucoadhesive material from *Linum usitatissimum* L. seeds

500 g of *Linum usitatissimum* L. seeds were washed with double distilled water to remove any adherent material. About three volumes of water were added and heated at 60 °C on a water bath for about 4 h until the slurry was prepared. The viscous solution was then filtered and the filtrate was diluted with three volumes of water and kept undisturbed overnight in a refrigerator, so that most of the undissolved portion settled down. In the following day, the clear supernatant portion was decanted and concentrated at 60 ± 1 °C in a rotary vacuum evaporator. The concentrate was cooled to room temperature and precipitated in about three volumes of acetone. The precipitate was washed repeatedly with acetone and dried at 50 ± 1 °C. The dried material was ground by a mechanical grinder and passed through # 80 mesh sieve and kept in a desiccator till further use [17].

2. Characterization of isolated mucoadhesive material

Determination of pH

pH of 1 % (w/v) aqueous solution of *Linum usitatissimum* L. mucilage (LUM) was measured using a Toshniwal pH meter (Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer, India).

Swelling capacity

Swelling capacity of LUM was determined by keeping 1 g of the polymers in 20 ml water in a measuring cylinder for 24 h [18]. Swollen volume was recorded and determined as follows:

$$\text{Swollen volume} = (V_2 - V_1)$$

where V_1 and V_2 are initial volume of the material prior to hydration and volume of the hydrated material.

Determination of viscosity

Viscosity of 1% (w/v) aqueous solution of LUM was measured by TV-10 Viscometer (Toki Sangyo Co. Ltd., Tokyo, Japan) using spindle M1 and cord no. 20 at 37 ± 1 °C at five different speeds of 10, 20, 30, 60 and 100 rpm.

The above studies were conducted for synthetic polymers (HPMC and Carbopol 934) and results were compared.

Evaluation of mucoadhesive strength

The mucoadhesive strength of LUM and synthetic polymers was evaluated in terms of tensile strengths.

Tensile strength of 1% (w/v) aqueous solutions of the mucoadhesive agents was measured by Park Robinson method [19, 20].

The forces of adhesion and bioadhesive strengths were calculated as follows:

$$\text{Force of adhesion (N)} = \frac{m \cdot g}{1000}$$

where m is the weight required to detach polymer solution from the mucosa in grams and g is the acceleration due to gravity taken as 9.81m/s^2 .

$$\text{Bioadhesive strength (N/m}^2\text{)} = \frac{\text{Force of adhesion (N)}}{\text{Surface area of mucosa exposed to polymer solution (m}^2\text{)}}$$

3. Preparation of nasal gel containing midazolam

Mucoadhesive nasal gels were prepared by dissolving midazolam hydrochloride in nasal solution (0.65% NaCl, 0.04% KH_2PO_4 , 0.09% K_2HPO_4 and 0.02% benzalkonium chloride) (pH 6) [21] in a constant stirring condition. Required amounts of LUM and synthetic polymers (HPMC and Carbopol 934) were added to the solution and stirred on a magnetic stirrer until a uniform solution was obtained which was kept at 4°C overnight to allow complete swelling so that a homogenous gel was formed. Penetration enhancers (sodium taurocholate and sodium thioglycollate) were also used in the formulations at a concentration of 0.50 % (w/v).

4. Evaluation of nasal gel

In vitro permeation study

The *in vitro* permeation study was conducted using a Franz diffusion cell containing 100 ml of phosphate buffer (pH 6, 0.1M) using an excised goat nasal mucosa [2, 22]. The goat nose was obtained from local slaughterhouse. After removing the skin, the nose was stored on ice cold phosphate buffer (pH 7.4, 0.05 M) [23]. The septum was fully exposed and nasal mucosa was carefully removed using forceps and surgical scissors. The mucosal tissues were immediately immersed in Ringer's solution. The nasal mucosa was mounted on the diffusion cell displaying permeation area of 2.54 cm^2 and 0.1 g of gel containing 5 mg midazolam was placed on it. Throughout the study the buffer solution in the chamber was maintained at $37 \pm 1^\circ\text{C}$ by connecting the Franz diffusion cell with water bath. At predetermined time intervals 1 ml of the samples were withdrawn at a time, and an equal amount of phosphate buffer was replaced. The samples were diluted appropriately and filtered. Absorbances of the samples were measured spectrophotometrically at 218 nm using Jasco V-550 UV/Vis Spectrophotometer (Tokyo, Japan). The amount of drug permeated per square centimeter of mucosa at each time interval was calculated from the calibration curve. The mean cumulative percent of drug permeated was plotted against time (Fig.3).

Apparent permeability coefficient was calculated as follows:

$$\text{Apparent permeability coefficient (cm/s)} = \frac{dC}{dt} \cdot \frac{V}{C \cdot A}$$

where dC/dt ($\mu\text{gml}^{-1}\text{s}^{-1}$) is the change in concentration of drug permeated per unit area of mucosa per unit time, C (μgml^{-1}) is the initial concentration of drug in the donor compartment, A (cm^2) is the area of permeation and V (ml) is the volume of phosphate buffer in the diffusion cell.

Histological evaluation of nasal mucosa

Histological study of excised nasal mucosa was conducted after 5 h *in vitro* permeation to detect if any significant histological change has occurred during the experiment. After permeation study nasal mucosa was cleared off the gel, sectioned with a rotary microtome (Model 1090 A, The Western Electric and Scientific Works, India) and fixed in 10 % formalin solution. The sectioned tissue was then stained with hematoxylin and eosin. Another normal mucosa was taken as a control and treated similarly. Tissue sections were observed under Olympus CKX41 optical microscope (Olympus Optical Co. Ltd., Tokyo, Japan). The photographs were taken with an Olympus-SC 35 camera (Figure 4).

Results

Table 1 depicts the values of pH and swollen volumes of the mucoadhesive agents. pH of *Linum usitatissimum* L. mucilage was found to be 5.88 which is within the pH range of the nasal cavity (5.5-6.5) and also comparable with the synthetic polymers like HPMC and carbopol that are widely used in formulation of nasal gels.

Swollen volume of LUM ($8.53 \pm 0.80 \text{ cm}^3/\text{g}$) was found to be slightly greater than that of HPMC ($7.89 \pm 0.95 \text{ cm}^3/\text{g}$) but significantly less than that of carbopol 934 ($15.57 \pm 0.65 \text{ cm}^3/\text{g}$).

Tab. 1. Values of pH and swollen volumes of mucoadhesive agents.

Mucoadhesive agent	pH	Swollen volume (cm^3/g) \pm SD (n=6)
LUM	5.88	8.53 ± 0.80
HPMC	6.73	7.85 ± 0.95
CP	3.27	15.75 ± 0.65

LUM = *Linum usitatissimum* L. mucilage; HPMC = Hydroxy propyl methyl cellulose; CP = Carbopol 934

The viscosity plot depicted in Fig.1 shows that viscosity of 1 % w/v aqueous solutions of LUM, HPMC and carbopol 934 were found to be in the range of 18-36, 5-12 and 7.5-18 respectively.

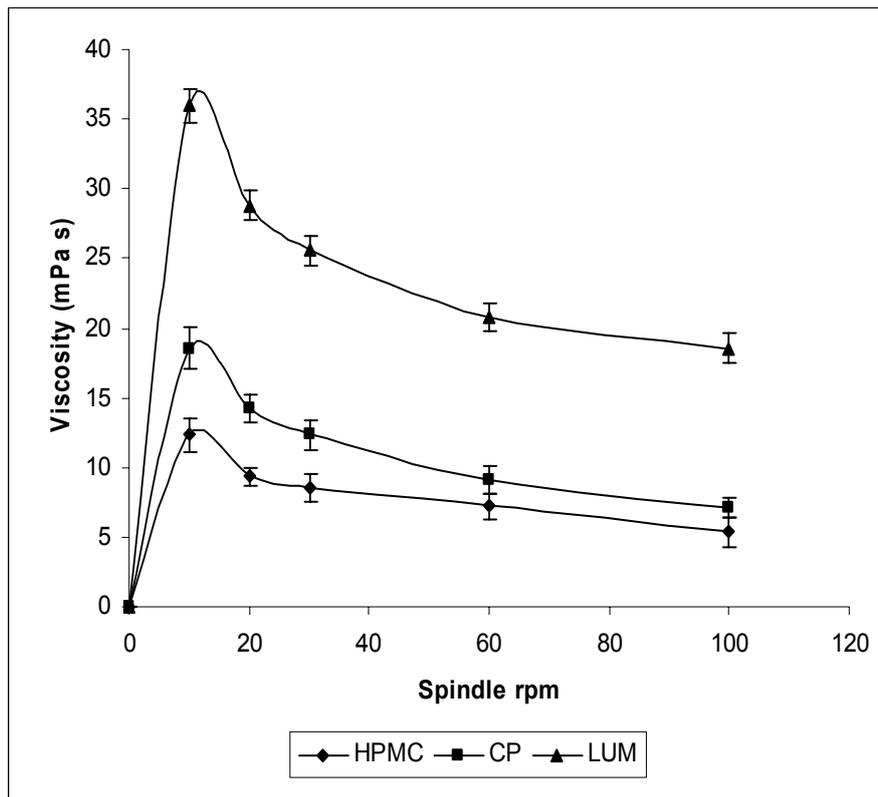


Fig. 1. Comparative study of viscosity of 1% w/v aqueous solutions of LUM and synthetic polymers (HPMC and Carbopol 934) at 37 ± 1 °C. Data show mean values \pm SD (n=6).

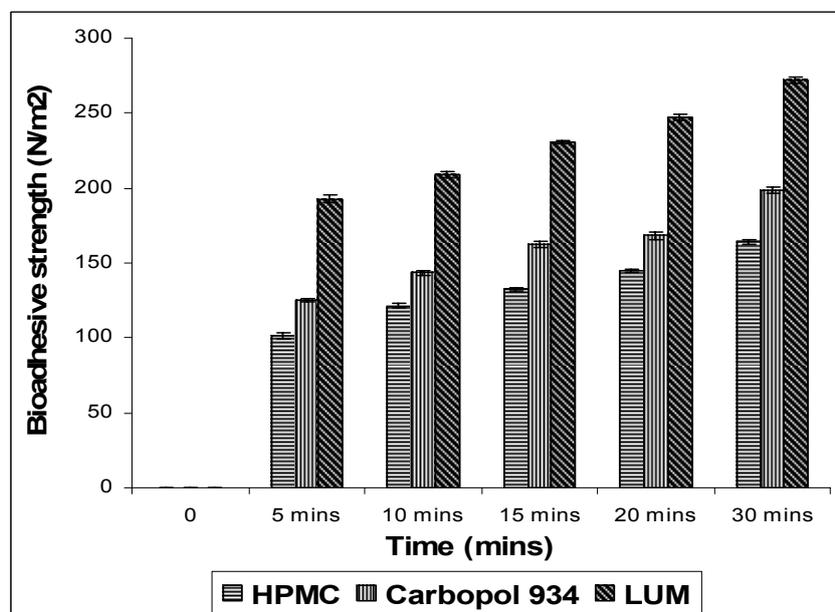


Fig. 2. Comparative study of tensile strength of 1%w/v aqueous solutions of LUM and synthetic polymers at 37 ± 1 °C. Data represent mean values \pm SD (n=6).

Figure 2 shows the results of the comparative study of the tensile strength determination of the mucoadhesive agents. It is evident from the plots that LUM exhibits greater force of adhesion and bond strength compared to HPMC and carbopol 934.

In vitro permeation profiles of midazolam from mucoadhesive nasal gels are shown in Fig.3. A permeation study was carried out across goat nasal mucosa using phosphate buffer as the medium in Franz diffusion cells at $37 \pm 1^\circ\text{C}$. Apparent permeability coefficients were calculated as per the equation mentioned above and are displayed in Table 2.

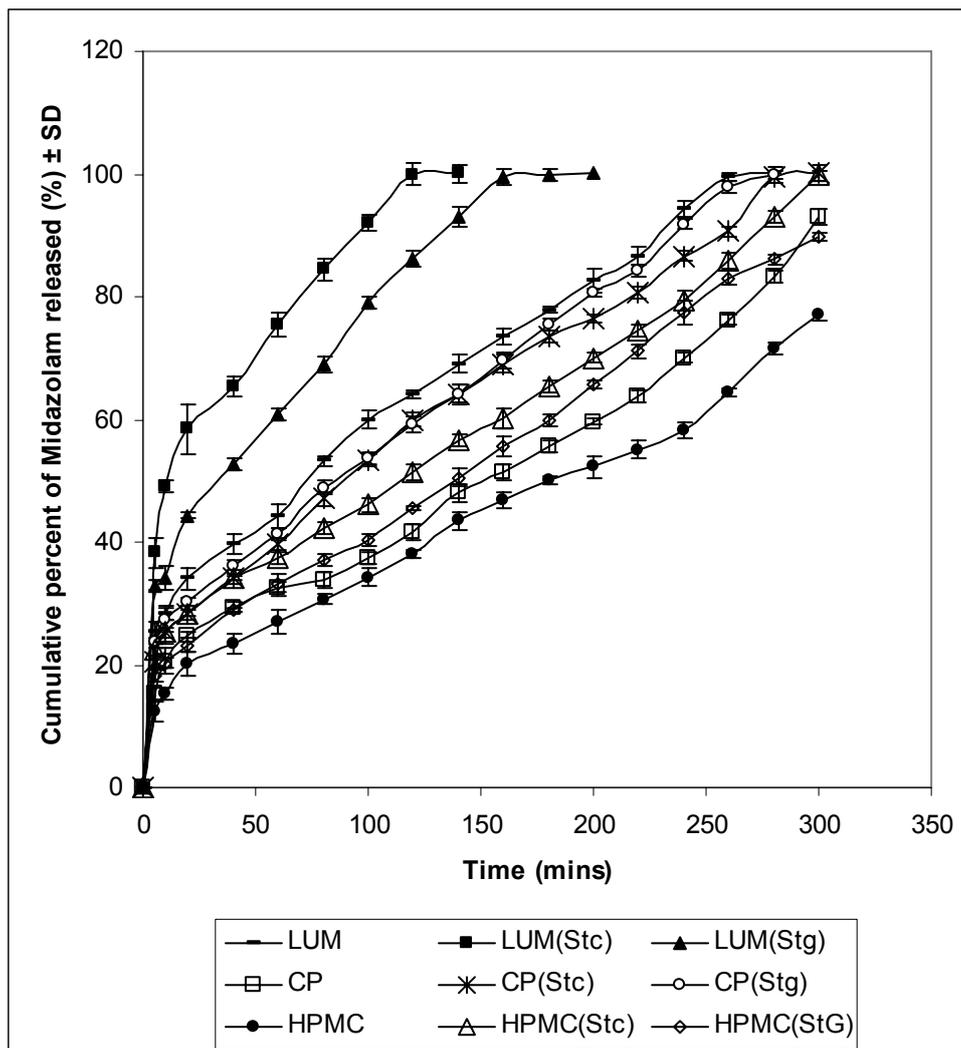


Fig. 3. Comparative result of *in vitro* release profile of midazolam hydrochloride from the nasal gels prepared with *Linum usitatissimum* L. mucilage, HPMC and Carbopol 934, with and without 0.5 % w/v of sodium taurocholate (Stc) and sodium thioglycollate (Stg) in phosphate buffer (pH 6) at $37 \pm 1^\circ\text{C}$. Data represent mean values \pm SD (n=6).

Tab. 2. Permeability coefficients of different formulations. Data represent mean values \pm SD (n=6).

Formulation code	Permeability coefficient $\times 10^{-5}$ (cm/s) \pm SD
LUM	6.13 \pm 0.64
LUM (Stc)	13.13 \pm 0.32
LUM (Stg)	9.68 \pm 0.96
CP	4.40 \pm 0.78
CP(Stc)	5.02 \pm 0.45
CP (Stg)	4.89 \pm 0.35
HPMC	3.50 \pm 0.58
HPMC(Stc)	4.11 \pm 0.93
HPMC (Stg)	4.04 \pm 0.45

The photographs of the histology of nasal mucosa before and after permeation study are shown in Figures 4(a), (b) and (c), respectively.

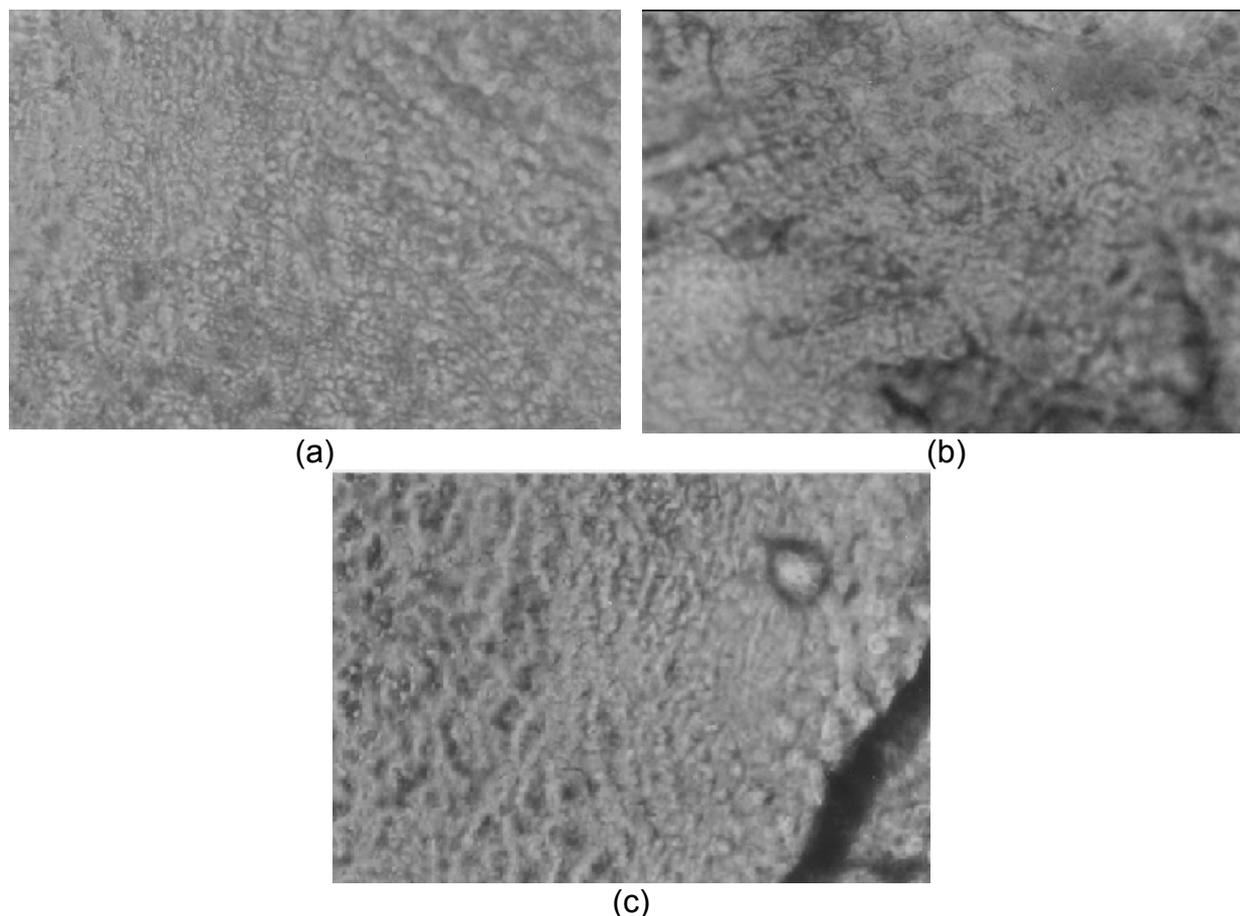


Fig. 4. Histological photomicrographs of eosin-hematoxylin stained nasal mucosa (40 \times 0.55 μ PHP magnification) (a) Control - mucosa without application of nasal gel. (b) nasal mucosa after application of nasal gel containing 0.25% sodium taurocholate. (c) nasal mucosa after application of nasal gel containing 0.75% sodium taurocholate.

Discussion

The pH of the mucilage isolated from *Linum usitatissimum* L. was found to be consistent with that of the nasal mucosa [17] as shown in Table 1. Hence, LUM can be effectively used in the formulation of nasal mucoadhesive gels.

Swellability is an indispensable property of mucoadhesive polymers. There is a critical degree of swelling that is necessary for optimum bioadhesion [1]. However, excessive swelling converts the gel network to a slippery surface that causes a decrease in bioadhesion [23]. From the result of the swelling capacity study (Table 1) it is observed that LUM exhibited moderate swellability compared to HPMC and carbopol suggesting better mucoadhesion.

Viscosity of LUM was found to be higher than that of HPMC and carbopol at the same concentration as is depicted in Figure 1. Hence, LUM can be considered to be a better gel forming agent than synthetic polymers.

Assessment of mucoadhesive strength showed that LUM has better bioadhesive strength than synthetic polymers. From the Figure 2, it is observed that greater the contact time, higher is the force of adhesion and hence greater bioadhesive strength. It can be concluded from the results that the isolated mucilage with higher viscosity and moderate swellability has better mucoadhesive property compared to synthetic polymers. This may be due to presence of certain functional groups in the mucilage as mentioned above that were able to establish a more intimate contact with mucin of the mucosa.

From the *in vitro* permeation profiles (Fig. 3) it is observed that 100 % midazolam hydrochloride was released in 5 h from gel prepared with LUM without any enhancer. Nasal gels containing enhancers showed considerable increase in the permeation rate. In case of nasal gel containing 0.5% sodium taurocholate, 100 % drug was released in 2 h 20 min. And in case of sodium thioglycollate, 100 % drug was released in 3 h 20 min.

In case of gels prepared with HPMC and carbopol 934 without any enhancer, only 77.10 % and 93.05 % drug was released in 5 h. Nasal gels containing enhancers showed considerable increase in the permeation rate. For HPMC gel containing sodium taurocholate (0.5%), 100 % drug was released in 5 h and for carbopol gel containing sodium taurocholate (0.5%), 100% drug was released in 4h 20 min. For both the synthetic polymers, sodium thioglycollate showed no significant difference in drug release from sodium taurocholate. The apparent permeability coefficients are furnished in Table 2. Permeability coefficients of the gels prepared with synthetic polymers were found to be remarkably lower than those prepared with LUM. Nasal gel prepared from LUM containing 0.5% sodium taurocholate exhibited highest permeability coefficient of $13.13 \pm 0.32 \times 10^{-5} \text{ cm/s}$.

Histological photographs (Figure 4 (a), (b) and (c)) indicate that no potential change was observed in the microscopic structure of nasal mucosae after application of nasal gels containing different concentrations of enhancers implying that the concentrations of enhancers used in the study were safe and effective.

The mucilage isolated from *Linum usitatissimum* L. seeds has proved to be a better mucoadhesive agent than the synthetic polymers like HPMC and carbopol 934, which are most frequently used for the formulation of nasal gels. Hence, the gels prepared from LUM exhibited favourable mucoadhesive properties that caused them to adhere to the nasal mucosa for a long time and hence enhance the absorption of drugs administered intranasally. Midazolam is generally used as an injection. The mucoadhesive nasal gel containing midazolam prepared from *Linum usitatissimum* L. mucilage is a new concept in the field of novel drug delivery system.

Acknowledgement

The authors are grateful to Dr. V. Ravi Chandran Endowment Fund for providing financial assistance for carrying out the work.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

References

- [1] Ugwoke MI, Agu RU, Verbeke N, Kinget R. Nasal mucoadhesive drug delivery: Background, applications, trends and future perspectives. *Adv Drug Del Rev.* 2005; 57: 1640–1665. doi:10.1016/j.addr.2005.07.009
- [2] Datta R, Bandyopadhyay AK. A new nasal drug delivery system for diazepam using natural mucoadhesive polysaccharide obtained from tamarind seeds. *Saudi Pharm J.* 2006; 14: 115–119.
- [3] Martin E, Schipper NGM, Verhoef JC, Merkus FWHM. Nasal mucociliary clearance as a factor in nasal drug delivery. *Adv Drug Del Rev.* 1998; 29: 13–38. doi:10.1016/S0169-409X(97)00059-8
- [4] Illum L. Nasal drug delivery: new developments and strategies. *Drug Discov Today.* 2002; 7: 1184–1189. doi:10.1016/S1359-6446(02)02529-1
- [5] Türker S, Onur E, Özer Y. Nasal route and drug delivery systems. *Pharm World Sci.* 2004; 26: 137–142. doi:10.1023/B:PHAR.0000026823.82950.ff
- [6] Ugwoke MI, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. *J Pharm Pharmacol.* 2001; 53: 3–22. doi:10.1211/0022357011775145
- [7] Goldman RD. Intranasal Drug Delivery for Children with Acute Illness. *Curr Drug Ther.* 2006; 1: 127–130. doi:10.2174/157488506775268470

- [8] Wikipedia. The free encyclopedia.
<http://en.wikipedia.org/wiki/>.
- [9] Erskine AJ, Jones JKN.
The structure of linseed mucilage (Part I).
Can J Chem. 1957; 35: 1174–1182.
doi:10.1139/v57-158
- [10] Hunt K, Jones JKN.
The structure of linseed mucilage.
Can J Chem. 1962; 40: 1266–1279.
doi:10.1139/v62-195
- [11] Muralikrishna G, Salimath PV, Tharanathan RN.
Structural features of an arabinoxylan and a rhamnogalacturonan derived from linseed mucilage.
Carbohydr Res. 1987; 161: 265–271.
doi:10.1016/S0008-6215(00)90083-1
- [12] Cui W, Mazza G, Biliaderiss CG.
Chemical structure, molecular size distributions, and rheological properties of flaxseed gum.
J Agric Food Chem. 1994; 42: 1891–1895.
doi:10.1021/jf00045a012
- [13] Chen HH, Xu SY, Wang Z.
Separation and purification of acidic polysaccharides and neutral polysaccharides in flaxseed gum.
Journal of Food and Fermentation Industry. 2004; 1: 96–100
- [14] Chen HH, Xu SY, Wang Z.
Study of rheological properties of flaxseed gum.
Journal of Wuxi University of Light Industry 2004; 1: 30–35
- [15] Fedeniuk RK, Biliaderis CG.
Composition and physicochemical properties of linseed mucilage.
J Agric Food Chem. 1994; 42: 240–247.
doi:10.1021/jf00038a003
- [16] Harding SE.
Mucoadhesive interactions.
Biochem Soc Trans. 2003; 31: 1036 -1041.
doi:10.1042/BST0311036
- [17] Rao YM, Vani G, Bala Ramesha Chary R.
Design and evaluation of mucoadhesive drug delivery systems.
Ind Drugs. 1998; 35: 558–565.
- [18] Park H, Robinson JR.
Mechanisms of mucoadhesion of poly (acrylic acid) hydrogels.
Pharm Res. 1987; 4: 457–464.
doi:10.1023/A:1016467219657
- [19] Lee JW, Park JH, Robinson JR.
Bioadhesive based dosage forms: the next generation.
J Pharm Sci. 2000; 89: 850–866.
doi:10.1002/1520-6017(200007)89:7<850::AID-JPS2>3.0.CO;2-G
- [20] Sudhakar Y, Kuotsu K, Bandyopadhyay AK.
Buccal bioadhesive drug delivery: A promising option for orally less efficient drugs.
J Control Rel. 2006; 14: 15–40.
doi:10.1016/S0048-721X(84)80026-3
- [21] Loyd V, Allen JR.
Compounding Nasal Preparations.
Current and Practical Compounding Information for the Pharmacist
in Secundum Artem, (www.paddocklabs.com) vol 7, no.1

- [22] Schmidt MC, Simmen D, Hilbe M, Boderke P, Ditzinger GN, Sandow JR, Lang S, Rubas W, Merkle HP.
Validation of excised bovine nasal mucosa as *in vitro* model to study drug transport and metabolic pathways in nasal epithelium.
J Pharm Sci. 2000; 89: 396–407.
doi:10.1002/(SICI)1520-6017(200003)89:3<396::AID-JPS10>3.0.CO;2-F
- [23] Metia PK, Bandyopadhyay AK.
In vitro and *in vivo* evaluation of a novel mucoadhesive buccal oxytocin tablet prepared with *Dillenia indica* fruit mucilage.
Pharmazie. 2008; 63: 1–5.
doi:10.1691/ph.2008.7248