

Development of a new nasal drug delivery system of diazepam with natural mucoadhesive agent from *Trigonella foenum-graecum* L

Rimi Datta and A K Bandyopadhyay*

Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032

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A new nasal drug delivery system of diazepam has been developed with a natural mucoadhesive agent from fenugreek (*Trigonella foenum-graecum* L). The mucoadhesive strength, viscosity, gelling property and *in vitro* drug release characteristics through franz-diffusion cell using excised bovine nasal membrane of this natural mucoadhesive agent was found to be higher in comparison to synthetic polymers, hydroxy propyl methyl cellulose (HPMC) and carbopol 934, which are conventionally used for similar purpose. This patient friendly, needle free dosage form may replace the diazepam injections in future.

Keywords: Diazepam, Nasal drug, Synthetic polymers

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Introduction

Nonparenteral routes for drug delivery include nasal, buccal, pulmonary and transdermal routes. In particular, drug administration by the nasal route allows for high absorption of small molecular weight hydrophobic drugs, avoidance of first pass effects and ease in administration by patients¹. However, due to rapid mucociliary clearance, a platform for drug delivery is most essential. Highly swellable mucoadhesive gels exhibiting mucoadhesive behaviour could be extremely useful in nasal delivery applications. Mucoadhesive agents in their molecular form make intimate contact with mucin of mucosa and then make adhesion with the nasal membrane and finally the mucoadhesive carriers allow the release of drug through nasal membrane in a continuous fashion. In this investigation, extract of fenugreek (*Trigonella foenum-graecum* L) has been used as a natural mucoadhesive agent and diazepam as the model drug to evaluate the drug release pattern in *in vitro* system.

In vitro permeability studies offer advantages over *in vivo* as they can be performed more rapidly, involve fewer animals and simpler analytical procedures can be followed since the presence of plasma proteins in the samples is avoided². Additionally, since pre- and post mucosal factors are

eliminated with *in vitro* techniques, the systems are more standardized³⁻⁶.

Materials and Methods

Materials

Diazepam was obtained as a gift sample from M/S East India Pharmaceutical Works Ltd, Kolkata. Fenugreek seeds were purchased from local market. Hydroxypropylmethyl cellulose 5 cPs (HPMC) and carbopol 934 were purchased from S.D. Fine-Chemical Ltd, Mumbai. All other chemicals were of analytical grade.

Methods

Extraction of Mucoadhesive Agent from Fenugreek Seeds

Fenugreek seed mucilage was extracted following the established methods^{7,8} with little modifications. Fenugreek seeds (200g) were soaked in double distilled water at room temperature and then boiled with sufficient amount of double distilled water under stirring condition in a water bath until the slurry was prepared. The solution was cooled and kept in refrigerator overnight to settle out undissolved materials. The upper clear solution was decanted off and centrifuged at 500 rpm for 20 min. The supernatant was separated and was concentrated at 60°C on a water bath to one third of its original volume. Solution was cooled down to the room temperature and was poured into thrice the volume of acetone by continuous stirring. The precipitate was

*Author for correspondence
Tel: 033-30940712
E-mail: akbj@yahoo.com

washed repeatedly with acetone and dried at 50-60°C under vacuum. The dried material was powdered and kept in a desiccator. The yield of natural mucoadhesive fenugreek extract (NMFE) was 20-25 percent. The mucoadhesive property of NMFE was determined^{9,10} and results were compared with synthetic polymers.

Determination of pH and Viscosity

The pH and viscosity of 1% w/v solution of NMFE were measured in Toshcon pH Meter and Toki sangyo Viscometer TV-10, respectively and values were compared with 1% w/v solutions of the synthetic polymers HPMC and carbopol 934.

Release Studies of the Drug

Preparation of Excised Tissue—Bovine nasal mucosa was obtained from the local slaughterhouse. The skin covering the nose was removed and stored on ice-cold buffer solution during transport to the laboratory¹¹. The septum wall was fully exposed by a longitudinal incision through the lateral wall of the nose. The septum mucosa was carefully removed from the underlying bone by cutting along the whole septum and pulling the mucosa off the septum with homeostatic forceps. The cavity mucosa was also carefully removed from the conchae and lower cavity using homeostatic forceps after exposing the cavity each side of the septum. The mucosal tissues were then immediately immersed in Ringer's Solution.

Preparation of Diazepam Solution—The diazepam solution was prepared by dissolving the drug in a little amount of chloroform and diluting the solution with the nasal solution (0.65% NaCl, 0.04% KH₂PO₄, 0.09% K₂HPO₄ and 0.02% benzalkonium chloride).

Formation of Drug-Polymer Gel—Diazepam (5 mg) was dissolved in 0.1 ml of chloroform. Nasal solution (5 ml) was added to the drug in a constant stirring condition. The required amount of NMFE or synthetic polymer (HPMC or carbopol 934) was added to the drug solution and stirred on a magnetic stirrer until the gel is formed.

Release Pattern of Drug from Gel—Franz diffusion cell¹² was used for drug release study. The diffusion chamber (exposed tissue surface area, 2.54 cm²) is filled with 100 ml of phosphate buffer solution (pH 6.0). Excised nasal mucosal membrane was secured over the mouth of upper tube keeping mucus side exposed to gel. For equilibration, mucosae were preincubated with preheated buffer for ~30 min.

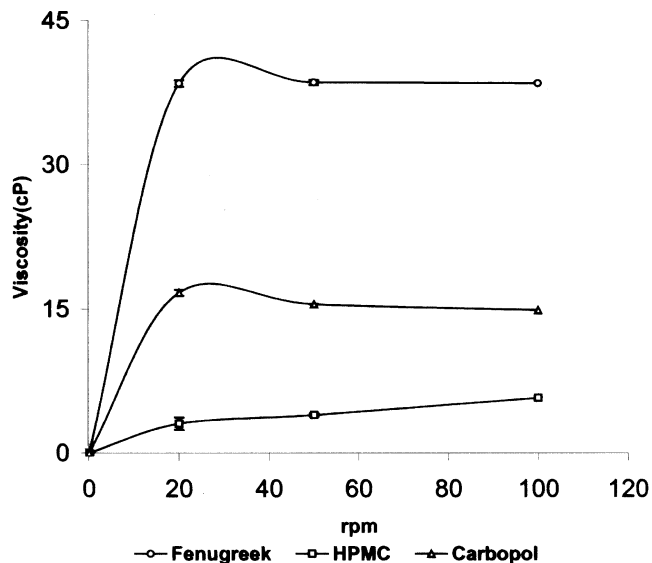


Fig. 1—Comparative viscosities of natural mucoadhesive extract of fenugreek, HPMC and carbopol 934 in 1% w/v solution at 37°C±1

Diazepam containing gel (1 mg/ml) placed on the membrane were dispersed in 100 ml of phosphate buffer solution (pH 6.0) and stirred constantly by a PTFE-coated magnetic bar at 600 min⁻¹. Cells were kept under a constant oxycarbon flow (O₂, 95%; CO₂, 5%). Throughout studies, buffer solution in the chamber was maintained at 37°C connecting franz diffusion cell with water bath. At predetermined period, 1 ml of sample was taken and simultaneously replaced with same volume of prewarmed (37°C) fresh buffer solution. Collected samples were diluted suitably and drug concentration estimated using Jasco V-530 UV/VIS Spectrophotometer at 240 nm wavelength.

Results and Discussion

All values were expressed as mean of 6 observations.

pH and Viscosity Measurement Studies

The pH of materials were as follows: NMFE, 7-7.5; HPMC, 6.5; and carbopol 934, 3.0. As the pH of nasal mucosa varied (5.5-6.5), all tested materials were found to be more or less suitable for preparing nasal gels. Viscosities (Fig. 1) of different materials (1% w/v) were found as follows: NMFE, 37-40; HPMC, 3-6; and carbopol 934, 14-17cp. NMFE exhibited a higher viscosity than synthetic polymers (HPMC and carbopol 934) at same concentration.

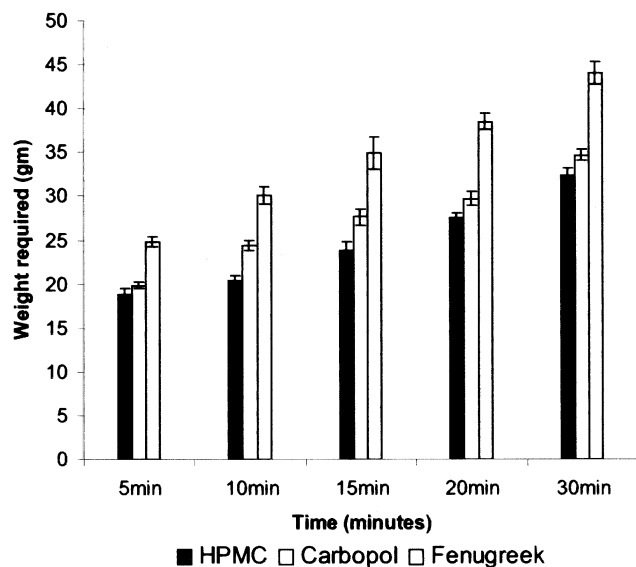


Fig. 2—Comparative bioadhesive properties of natural mucoadhesive extract of fenugreek, HPMC and carbopol 934 by shear stress method in 0.5% w/v solution at 37°C±1

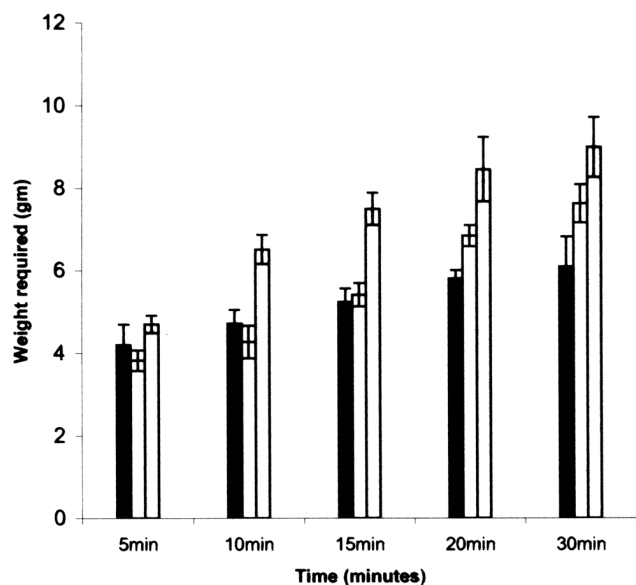


Fig. 3—Comparative mucoadhesive properties of natural mucoadhesive extract of fenugreek with HPMC and carbopol 934 by Park & Robinson method in 1% w/v solution at 37°C±1

Mucoadhesive Property Measurement Studies

The results of the mucoadhesive property were recorded for various polymers with different contact times (Figs 2 & 3). In this study, 0.5 percent polymers were used for shear stress method⁹ and 1 percent for Park & Robinson method¹⁰. It is observed that increased contact time increased the adhesion strength, allowing for more adhesion. Probably

increasing contact time might be reducing hydration due to evaporation facilitating higher adhesion.

NMFE having high molecular weight and high viscosity exhibited higher adhesion and better mucoadhesive property in comparison to synthetic polymers at the same concentration. This may be due to presence of numerous carboxyl and hydroxyl groups, which adopts more favorable macro molecular confirmation and accessibility of its hydrogen-binding group, while compared with other polymers. HPMC was reported to form weaker bondage with mucus, which may be due to decrease in available hydrogen binding sites or an unfavorable contamination for entanglement to occur.

In vitro Release Study

In vitro dissolution studies of diazepam, with NMFE and synthetic polymers showed drug release (98 %) in 5-6 h in case of synthetic polymers and within 3 h 45 min in case of NMFE (Fig. 4). The full diazepam was released from fenugreek in 4 h. Several enhancers (0.5 % conc.) like sodium taurocholate, sodium tauroglycocholate, bile salt and sodium thioglycollate were used to evaluate their effects on drug release characteristics from nasal gels containing natural and synthetic mucoadhesive agents. Sodium taurocholate gave the best result (100% drug release in 90 min from NMFE). In case of HPMC and carbopol 934, no significant change of drug release was observed with enhancers.

Conclusions

Fenugreek polymer is a better mucoadhesive agent than HPMC and carbopol 934 in respect of mucoadhesive strength, gelling property and drug release. Permeation studies through bovine nasal mucosa suggested that sodium taurocholate is a better penetration enhancer than others for the release of diazepam from fenugreek polymer based nasal gel. Since this natural mucoadhesive agent is edible, it is easily biodegradable and also non-allergic. Diazepam is routinely administered parenterally. Since injections are the most hazardous and have least patient compliance, this nasal drug delivery system may substitute the conventional diazepam injection. This work will definitely add a new dimension in the area of novel drug delivery research.

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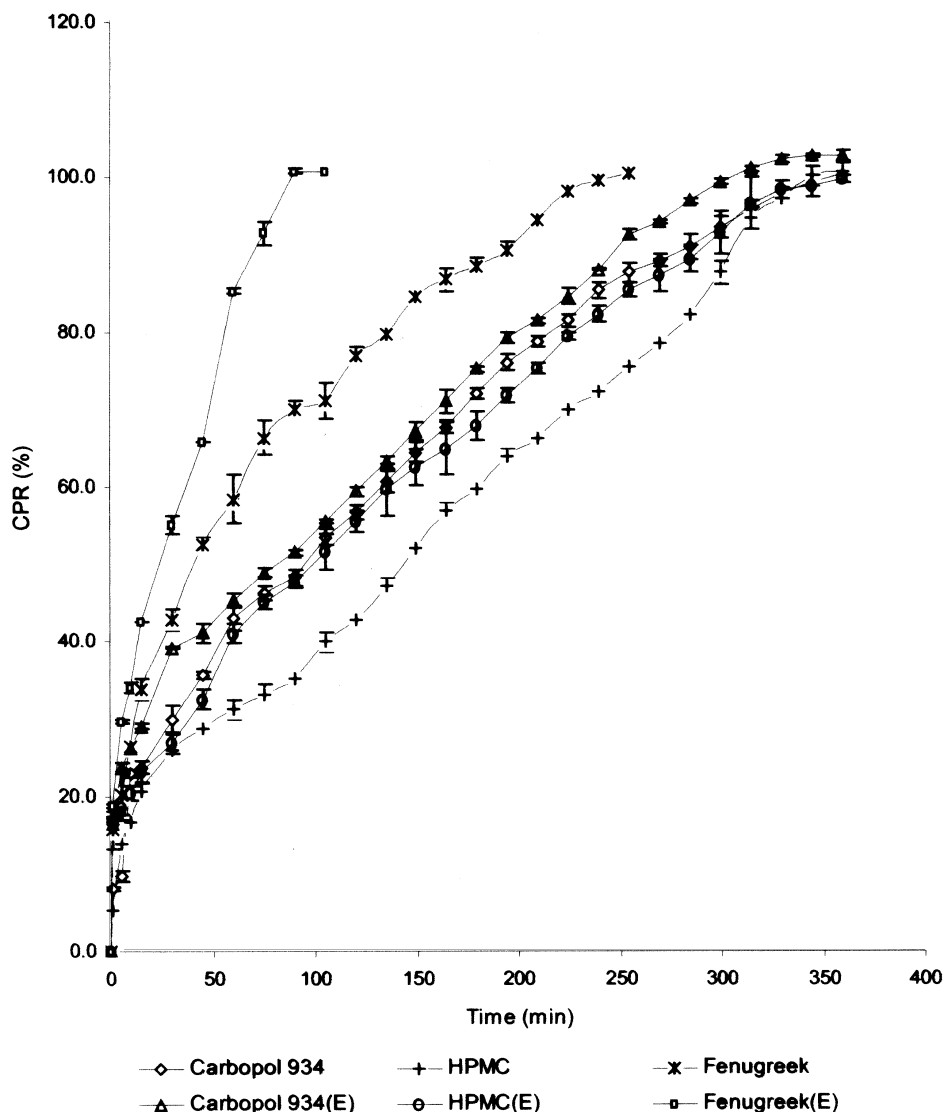


Fig. 4—Comparative result of cumulative percentage release of diazepam from the natural mucoadhesive extract of fenugreek, HPMC and carbopol 934 with and without enhancer sodium taurocholate. The phosphate buffer solution in the diffusion chamber was maintained at pH 6.0, at $37^{\circ}\text{C}\pm 1$ and stirred at a constant rate by a PTFE-coated magnetic bar at 600 min^{-1} . Cells were kept under a constant oxycarbon flow (95% O_2 , 5% CO_2). Drug solution = 1mg/ml; E = enhancer

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