

## ***In Vitro* Evaluation of Novel Mucoadhesive Buccal Tablet of Oxytocin Prepared with *Diospyros Peregrina* Fruits Mucilages**

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Novel mucoadhesive buccal tablets (NMBTs) of oxytocin were prepared as cores in cup fashion to release and permeate the drug unidirectionally toward the buccal mucosa to reach the systemic circulation directly. Adhesive cups for NMBTs were prepared with mucilage (DPM) isolated from edible *Diospyros peregrina* fruit. Mucoadhesive properties like shear and tensile and peel strengths of the adhesive cups were estimated on freshly excised bovine buccal mucosa. Core tablets were formulated with oxytocin using two penetration enhancers, sodium taurocholate and sodium thioglycollate. *In vitro* permeability studies of NMBTs were conducted in a Franz diffusion cell containing 50 ml of phosphate buffer, pH 6.6, at  $37 \pm 0.2^\circ\text{C}$  through excised bovine buccal mucosa, and the amount of drug permeated was estimated at 220 nm on reverse-phase HPLC using a BDS Hypersil C<sub>8</sub> column with acetonitrile and potassium dihydrogen orthophosphate buffer 0.05 M, pH 6.6, (20 : 80 v/v) as the mobile phase, at flow rate of 1.25 ml/m. The NMBTs containing 0.75% w/w sodium taurocholate showed 26% permeability without damaging the histology of the buccal mucosa. The results suggest that this formulation may be a suitable alternative to oxytocin injections.

**Key words**—oxytocin; mucoadhesive; buccal permeation; permeation enhancer

### **INTRODUCTION**

In the field of pharmaceutical technology there is an urgent need for nonparenteral routes of administration of potentially active peptides and proteins, which are being developed currently in genetic engineering and biotechnology.<sup>1)</sup> Delivery of macromolecular drugs like oxytocin offers many challenges to pharmaceutical scientists, as they are ineffective by the oral route due to gastrointestinal degradation and extensive hepatic first-pass metabolism. Further, due to the poor permeability of oxytocin through the skin and mucosa, it is administered through the less patient-friendly parenteral route.<sup>2,3)</sup> Its very short biological half-life necessitates repeated injections, causing pain and discomfort to patients.

Oxytocin is used clinically for the induction and augmentation of labor, mid-trimester abortion, and the cessation or prevention of postpartum hemorrhage.<sup>4)</sup> It is estimated that 30% of all deliveries in the USA and other countries use oxytocin to induce and augment labor.<sup>4)</sup> Among various transmucosal routes like nasal, rectal, vaginal, ocular, pulmonary, and buccal evaluated for peptide/protein absorption, the

nasal and buccal routes have shown considerable advantages.<sup>5–10)</sup> Moreover, the nasal route has distinct limitations due to interference with the mucociliary activity by the penetration enhancers added to nasal formulations and irreversible peptide/protein degradation caused by enzymes (proteases and peptidases) present in the nasal mucosa.<sup>11,12)</sup> On the other hand, the buccal mucosa has several advantages<sup>13)</sup> such as rich vascularity, moderate permeability, suitability for both local and systemic drug delivery, less enzymatic activity, and avoidance of first-pass hepatic metabolism. The buccal mucosa is less sensitive than the nasal mucosa and less prone to irritation or injury despite being regularly exposed to a multitude of foreign compounds.<sup>14)</sup> The accessibility of the buccal cavity makes the application of drugs easy and acceptable to patients, while permitting easy removal in the event of adverse reactions.

Conventionally used buccal dosage forms have serious drawbacks like the salivary scavenging effect leading to a reduction in drug concentration as well as retention time of the dosage forms at the site of application.<sup>15)</sup> However, the structural integrity of the buccal mucosa also decreases the permeability of macromolecular drugs.<sup>16)</sup>

In the present investigation, the main objectives

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were to prevent the salivary scavenging effect by designing a mucoadhesive dosage form that delivers the drug unidirectionally and improve the rate of penetration of oxytocin by the inclusion of safe, effective permeation enhancers like sodium taurocholate and sodium thioglycollate. The mucoadhesive material (DPM) isolated from ebony fruit (*Diospyros peregrina*) was used in the formulation because of its edibility, biodegradability, and biocompatibility.

## MATERIALS AND METHODS

**Materials** Oxytocin was a gift from Hemmo Pharma, India. Ebony fruit was purchased from local vendors. Hydroxypropylmethyl cellulose (HPMC), carbopol 934 p (CP), microcrystalline cellulose (MCC), magnesium stearate, talc, and acetone were purchased from S. D. Fine-Chem. Ltd., India. All HPLC-grade solvents were purchased from Merck Ltd. (Mumbai, India). All other reagents and chemicals used were of analytical grade. Special punches and dies were designed and fabricated by Sigma Engineering Corporation (Sonarpur, India).

**Extraction of Mucoadhesive Material from Edible Ebony Fruit** Ebony fruit (200 g) were washed with double-distilled water to remove any adherent materials. Approximately three volumes of double-distilled water were added and heated at 60°C in a water bath for 4 h. The thick viscous solution obtained was strained through muslin cloth. The filtrate was diluted with three volumes of double-distilled water and kept undisturbed overnight in a refrigerator. The following day, the upper clear supernatant portion was decanted and concentrated at 60 ± 1°C in a rotary evaporator. The concentrate was cooled to room temperature and precipitated in three volumes of acetone. The precipitate was washed three times with acetone and dried at 50 ± 1°C. It was powdered in a mechanical grinder and screened through an 80-mesh sieve.<sup>17)</sup>

### Evaluation of Mucoadhesive Agents

**Physical Parameters** The pH of 1% w/v solution of DPM was measured using a Toshniwal pH meter (Toshniwal Institute Manufacturing, Pvt. Ltd., Ajmer, India). Swellability studies of DPM were conducted by storing DPM 1 g with 20 ml of distilled water in a measuring cylinder for 24 h.<sup>18)</sup> The swollen volume was recorded. All the above parameters were measured with the commercially available polymers HPMC and CP and compared

(Table 1).

**Shear Strength** Mucoadhesive strengths of 0.5%, 1.0%, and 1.5% w/v aqueous solutions of DPM were measured and compared with HPMC and CP following standard shear stress methods (Fig. 1).<sup>19)</sup>

**Tensile Strength** The force required to separate two freshly excised bovine buccal mucosa perpendicularly affixed with mucoadhesive polymer was determined using the Park and Robinson method (Fig. 1).<sup>20)</sup>

### Formulation of Novel Mucoadhesive Buccal Tablet Preparation of Adhesive Cups

Granules for adhesive cups were prepared by mixing the respective mucoadhesive agents with microcrystalline cellulose at ratios of 1 : 3, 1 : 1 and 3 : 1 with the wet granulation method and then passed through a #18 sieve. Granules were dried in a tray drier at 50 ± 1°C for 6 h, passed through a #22 sieve, and mixed with magnesium stearate and talc. Granules were compressed in a 10-station rotary mini press (Rimek, Karnavathi Industries, India) using a specially fabricated lower flat punch 4.4 mm in diameter and a projected upper punch 2.8 mm in inner diameter and 4.4 mm in outer diameter, as shown in Fig. 2(a).

Table 1. Study of Physical Parameters of Mucoadhesive Agents

| Mucoadhesive agent | pH   | Swellability (vol/g) <i>n</i> =3 |
|--------------------|------|----------------------------------|
| DPM                | 6.31 | 8.50 ± 0.11                      |
| HPMC               | 7.39 | 7.43 ± 0.08                      |
| CP                 | 2.87 | 17.90 ± 0.05                     |

DPM : *Diospyros peregrina* mucilage, HPMC : hydroxypropylmethyl Cellulose, CP : carbopol 934 p.

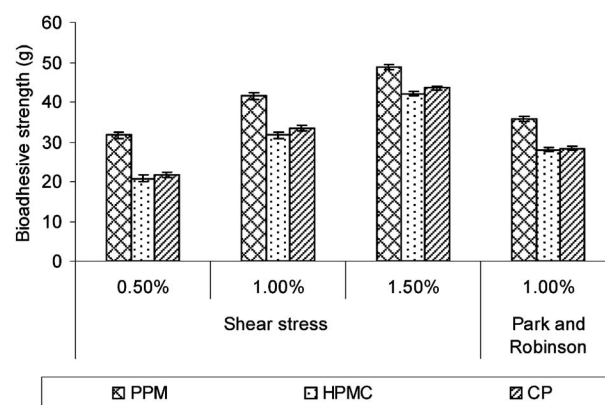


Fig. 1. Comparisons of Shear Strength of 0.5%, 1.0% and 1.5% w/v Aqueous Solutions and Tensile Strength of 1.0% w/v Aqueous Solutions of DPM with HPMC and CP

**Preparation of Core Tablets** Core tablets were formulated with oxytocin using penetration enhancers such as sodium taurocholate and sodium thioglycollate (Table 2) and compressed by direct compression using 2.8-mm flat punches (Fig. 2(b)). Compression force was adjusted to obtain tablets 0.8 mm thick.

**Preparation of Novel Mucoadhesive Buccal Tablets** Novel mucoadhesive buccal tablets (NMBTs) were prepared by inserting core tablets manually into adhesive cups and compressing in the 10-station mini press using 4.5-mm flat punches (Fig. 2(c)).

**Measurement of *In Vitro* Mucoadhesive Strength of Adhesive Cups**

**Shear Strength** The shear strength of the adhesive cups was calculated by measuring the force required for parallel detachment from freshly excised bovine buccal mucosa using a specially designed apparatus, as represented in Fig. 3(a). The back of a mucoadhesive cup was fixed to a movable plastic strip with synthetic adhesive. The other side of the cup was pressed over excised bovine buccal mucosa for 30 sec applying constant pressure. After 5 m, the weight required to detach the novel adhesive cup from the mucosa was recorded.<sup>19)</sup>

The force of adhesion and the bond strength were calculated as

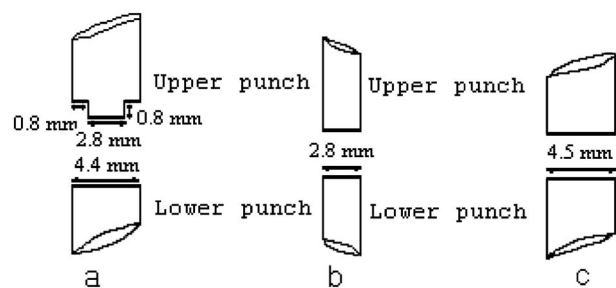


Fig. 2. Specially Designed Punches for the Preparation of a) Cups, b) Core Tablets and c) NMBT

$$\text{Force of adhesion (N)} = \frac{\text{Weight (g)}}{1000} \times 9.81 \quad (1)$$

$$\text{Bond strength (N/m}^2\text{)} = \frac{\text{Force of adhesion (N)}}{\text{Surface area of cup (m}^2\text{)}} \quad (2)$$

and given in Table 3.

**Peel Strength** A similar experiment was conducted to determine the force required for tangential detachment of adhesive cups from freshly excised bovine buccal mucosa, as represented schematically in Fig. 3(b), and calculated in Table 3.<sup>21)</sup>

**Tensile Strength** The method for the measurement of tensile strength, *i.e.*, the strength required to detach the adhesive cups perpendicularly from freshly excised bovine buccal mucosa is shown in Fig. 3(c).<sup>19,22)</sup> The results are shown in Table 3.

***In Vitro* Retention Time of Adhesive Cups** The *in vitro* retention time is one of the most important physical parameters of an adhesive cup. An adhesive cup was pressed over the excised bovine buccal mucosa for 30 sec after previously being secured on a glass slab and was immersed in a beaker containing 500 ml of isotonic phosphate buffer, pH 6.6, at 37±0.2°C. One stirrer was fitted at a distance of 5 cm from the

Table 2. Compositions of Core Tablets

| Formulation | Oxytocin (mg) | MCC (mg) | STc (mg) | STg (mg) | Total (mg) |
|-------------|---------------|----------|----------|----------|------------|
| F1          | 0.032         | 9.968    | —        | —        | 10.000     |
| F2          | 0.032         | 9.943    | 0.025    | —        | 10.000     |
| F3          | 0.032         | 9.943    | —        | 0.025    | 10.000     |
| F4          | 0.032         | 9.918    | 0.050    | —        | 10.000     |
| F5          | 0.032         | 9.918    | —        | 0.050    | 10.000     |
| F6          | 0.032         | 9.893    | 0.075    | —        | 10.000     |
| F7          | 0.032         | 9.893    | —        | 0.075    | 10.000     |
| F8          | 0.032         | 9.868    | 0.100    | —        | 10.000     |
| F9          | 0.032         | 9.868    | —        | 0.100    | 10.000     |

MCC : microcrystalline cellulose, STc : sodium taurocholate, STg : sodium thioglycollate.

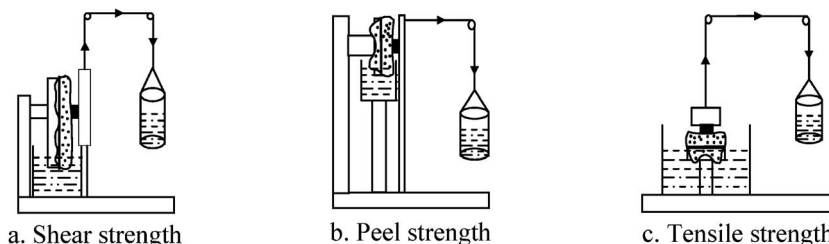


Fig. 3. Schematic Diagram of a) Shear b) Tensile and c) Peel Forces

Table 3. *In Vitro* Shear, Peel, and Tensile Strengths of Adhesive Cups

| Formulations         | Shear strength      |                       |                                   | Peel strength       |                       |                                   | Tensile strength    |                       |                                   |
|----------------------|---------------------|-----------------------|-----------------------------------|---------------------|-----------------------|-----------------------------------|---------------------|-----------------------|-----------------------------------|
|                      | Weight (g)<br>(n=3) | Force of adhesion (N) | Bond strength (N/m <sup>2</sup> ) | Weight (g)<br>(n=3) | Force of adhesion (N) | Bond strength (N/m <sup>2</sup> ) | Weight (g)<br>(n=3) | Force of adhesion (N) | Bond strength (N/m <sup>2</sup> ) |
| DPM : MCC<br>=1 : 3  | 2.53±0.059          | 0.0248                | 2744.526                          | 2.89±0.122          | 0.0284                | 3135.052                          | 3.27±0.161          | 0.0321                | 3547.273                          |
| DPM : MCC<br>=1 : 1  | 3.48±0.063          | 0.0341                | 3775.080                          | 3.79±0.162          | 0.0372                | 4111.365                          | 4.24±0.130          | 0.0416                | 4599.522                          |
| DPM : MCC<br>=3 : 1  | 3.76±0.097          | 0.0369                | 4078.822                          | 4.12±0.132          | 0.0404                | 4469.347                          | 4.48±0.157          | 0.04439               | 4859.873                          |
| HPMC : MCC<br>=1 : 3 | 2.40±0.100          | 0.0235                | 2603.503                          | 2.53±0.115          | 0.0248                | 2744.526                          | 2.70±0.100          | 0.0265                | 2928.941                          |
| HPMC : MCC<br>=1 : 1 | 3.23±0.115          | 0.0317                | 3503.881                          | 3.33±0.115          | 0.0327                | 3612.361                          | 3.76±0.057          | 0.0369                | 4078.822                          |
| HPMC : MCC<br>=3 : 1 | 3.56±0.152          | 0.0349                | 3861.863                          | 3.56±0.152          | 0.0349                | 3861.863                          | 3.93±0.057          | 0.0386                | 4263.236                          |
| CP : MCC<br>=1 : 3   | 2.46±0.100          | 0.0241                | 2668.591                          | 2.56±0.057          | 0.0251                | 2777.070                          | 2.83±0.115          | 0.0278                | 3069.964                          |
| CP : MCC<br>=1 : 1   | 3.33±0.057          | 0.0327                | 3612.361                          | 3.40±0.152          | 0.0334                | 3688.296                          | 3.96±0.057          | 0.0388                | 4295.780                          |
| CP : MCC<br>=3 : 1   | 3.50±0.152          | 0.0343                | 3796.775                          | 3.63±0.152          | 0.0356                | 3937.799                          | 4.06±0.057          | 0.0398                | 4404.260                          |

tablet and rotated at 25 rpm (Fig. 4).<sup>19)</sup> The time for complete erosion or detachment of the tablet from the mucosa was recorded (Table 4).

#### Evaluation of Oxytocin NMBTs

***In Vitro* Permeation Study** *In vitro* permeation studies of NMBTs were carried out in a Franz diffusion cell containing 50 ml of phosphate buffer, pH 6.6, at 37±0.2°C at 50 rpm using freshly excised bovine buccal mucosa (Fig. 5).<sup>23)</sup> Immediately after collecting the buccal membrane from the local slaughterhouse, it was placed in ice-cold phosphate buffer 0.05 M. The connective tissue of the membrane was carefully removed using fine-point forceps and surgical scissors to adjust to a uniform thickness of 1.0±0.1 mm measured with an absolute digimatic caliper (Mitutoyo Corporation, Japan). The mucosa was mounted in the Franz diffusion cell. Samples were collected at several time intervals up to 4 h and analyzed with a Jasco UV 1575 HPLC detector using a BDS Hypersil C<sub>8</sub> column (250 mm×4.6 mm×5 μm) with a mobile phase of acetonitrile and potassium dihydrogen orthophosphate buffer 0.05 M, pH 6.6 (20 : 80 v/v), at a flow rate of 1.25 ml/m. The detection wavelength was 220 nm. Chromatographic peaks were automatically integrated and recorded on a Data Apex Chromatographic Station for Windows 1.7 data

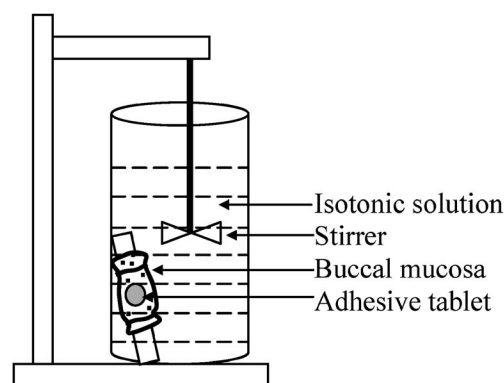


Fig. 4. Schematic Representation of *In Vitro* Retention Time Measuring Instrument

In 500 ml of isotonic phosphate buffer, pH 6.6, at 37±0.2°C, and stirrer speed of 25 rpm.

Table 4. *In Vitro* Retention Time of Adhesive Cups

| Adhesive cups    | Retention time (h) (n=3) |
|------------------|--------------------------|
| DPM : MCC=1 : 3  | 2.35±0.187               |
| DPM : MCC=1 : 1  | 3.46±0.262               |
| DPM : MCC=3 : 1  | 4.08±0.234               |
| HPMC : MCC=1 : 3 | 1.56±0.348               |
| HPMC : MCC=1 : 1 | 2.27±0.267               |
| HPMC : MCC=3 : 1 | 2.47±0.452               |
| CP : MCC=1 : 3   | 2.36±0.267               |
| CP : MCC=1 : 1   | 2.58±0.612               |
| CP : MCC=3 : 1   | 3.11±0.534               |

module (Data Apex; Prague, Czech Republic) (Fig. 6).

**Histologic Study** An experiment was conducted to examine any potential alteration in the histology of the buccal mucosa that may have arisen due to the application of NMBTs. An NMBT was carefully placed on the buccal mucosa and at the end of 4-h *in vitro* permeation, the NMBT was removed using forceps. The mucosa was then sectioned so that the outermost epithelium layer was about 0.2 mm thick using a rotary microtome. Another fresh buccal mucosa was used as a control and similarly sectioned. Those two epithelial layers were then immediately fixed in 10% formalin solution. The tissue was sectioned, stained with hematoxylin, and eosin and examined under an Olympus CKX41 microscope (Olympus Optical Co.

Ltd., Tokyo, Japan). Photographs were taken with an Olympus SC 35 camera (Fig. 7).

**RESULTS AND DISCUSSION**

The pH value of 1% w/v solution of DPM was found to be 6.37, near the buccal pH (6.6), suggesting its nonirritability and biocompatibility with the buccal mucosa (Table 1). The swollen volume of 1 g of DPM ( $6.82 \pm 0.10$ ) was slightly less than that of HPMC ( $7.43 \pm 0.08$ ), but less than that of CP ( $17.90 \pm 0.05$ ), suggesting its moderate swellability. Swelling is one of the primary characteristics for a polymer to be bioadhesive. However, excessive swelling due to overhydration always leads to the formation of a slippery surface. Hence retention of dosage forms at the site of application is practically impossible. Further, it is not suitable for solid adhesive dosage forms because of the loss of mechanical strength and structural integrity due to excessive swelling.<sup>24)</sup>

The shear strengths of 0.5%, 1.0% and 1.5% w/v aqueous solutions of DPM, HPMC and CP showed that the adhesive strength of DPM was greater than that of HPMC and CP and increased with increasing concentration. The tensile strength of 1% w/v aqueous solution of DPM was determined using the Park and Robinson method and was found to be comparable with that of the other polymers studied.

Shear, tensile, and peel strengths of the adhesive cups prepared were measured to evaluate their bio/mucoadhesiveness. Freshly excised bovine buccal mucosa was used as the substrate to assess the *in vitro* mucoadhesive strength. The force of adhesion and

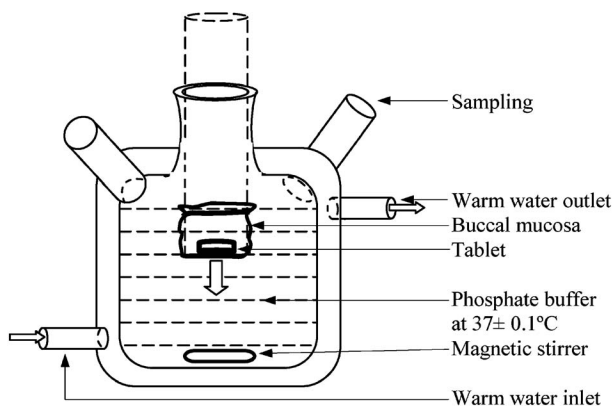


Fig. 5. Schematic Diagram of Franz Diffusion Cell for Permeation Study of NMBTs  
In 50 ml of phosphate buffer, pH 6.6, at  $37 \pm 0.2^\circ\text{C}$ , and magnetic stirrer speed of 50 rpm.

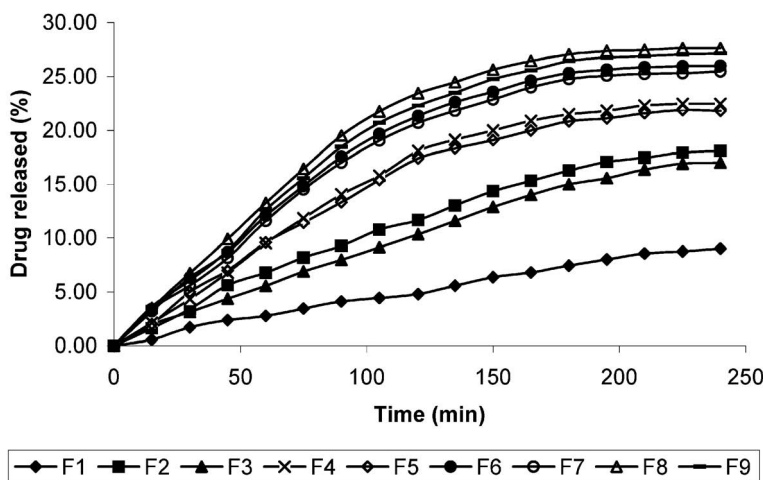


Fig. 6. *In Vitro* Permeation Studies of NMBTs in Phosphate Buffer pH 6.6, at  $37 \pm 0.2^\circ\text{C}$ .

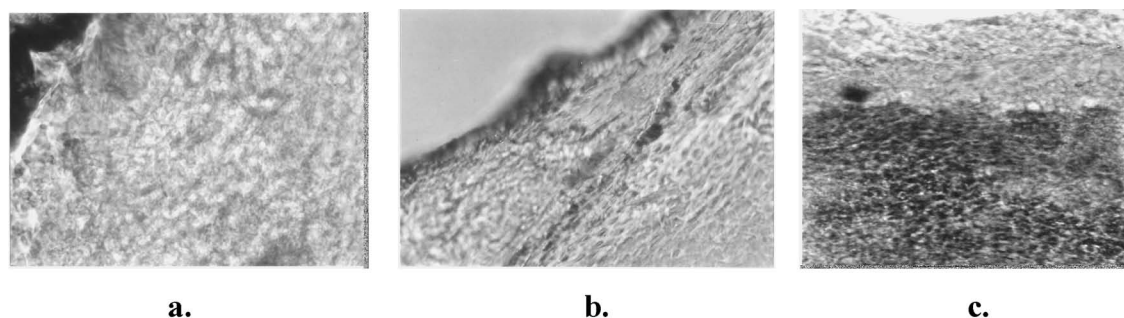


Fig. 7. Representative Light Micrographs ( $40\times 0.55\mu$  PHP magnification) of Hematoxylin and Eosin-stained Buccal Mucosa a) Control without Application of NMBT, b) after Application of NMBT (0.75% w/w STc) and c) after Application of NMBT (1.0% w/w STc)

bond strength showed that adhesive cups prepared with DPM exhibited greater adhesive strength than the other polymers studied.

The *in vitro* retention time was determined using a specially designed apparatus. Retention times increased with the increase in the amount of mucoadhesive material from polymer to MCC ratios of 1 : 3 to 1 : 1. At a polymer: MCC ratio of 3 : 1, the adhesive cups could not retain their structural integrity upon hydration after approximately 2.5 h. Thus the adhesive cups prepared at 1 : 1 polymer ratios (batches F6 and F7) were used to prepare NMBTs.

NMBTs prepared without any enhancer showed very poor permeability. After 4-h permeation, only 9.02% of oxytocin permeated through the mucosa. This may be due to the large molecular size of oxytocin. The tablet formulations using enhancers such as sodium taurocholate and sodium thioglycollate exhibited a considerable increase in the permeation rate. There was a significant increase in the permeation rate when the enhancer concentration increased from 0.25% to 0.50% w/v. A moderate increase in the permeation rate was observed from 0.50% to 0.75% w/v concentration and there was no significant increase in the permeation rate at concentration greater than 0.75% w/v.

Histologic studies on excised buccal mucosa at the end of 4-h *in vitro* permeation using formulations with increasing concentrations of permeation enhancers suggested that there are no significant changes in the histology of the buccal mucosa in the range of 0.25% to 0.75%. However, a change in the histology was observed at concentration of 1.0%, irrespective of the enhancer used. Based on the results of *in vitro* permeation and histologic studies, batch F6 will be selected for further investigations.

## CONCLUSION

Oxytocin is very potent, and only a minute quantity is required to elicit its pharmacologic effects. In the present study, the *in vitro* retention time of adhesive cups, and *in vitro* permeation studies of NMBTs confirmed that oxytocin could successfully be delivered and absorbed into the systemic circulation following application of NMBTs to the buccal mucosa. Hence this will be a promising alternative route to oxytocin injection. The technique can also be extended for other potent proteins and peptides for noninvasive drug delivery.

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