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In vitro and *in vivo* evaluation of a novel mucoadhesive buccal oxytocin tablet prepared with *Dillenia indica* fruit mucilage

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Novel mucoadhesive buccal tablets (NMBT) of oxytocin were prepared as core in cup fashion to release the drug unidirectionally towards the buccal mucosa. Adhesive cups were prepared with a mucilage isolated from edible *Dillenia indica* fruits (DIM). Shear, tensile and peel strengths of prepared adhesive cups were estimated on freshly excised bovine buccal mucosa. Core tablets were formulated with oxytocin using permeation enhancers viz. sodium taurocholate and sodium thioglycollate. *In vitro* permeability studies of NMBT were conducted in a Franz diffusion cell containing 50 mL of phosphate buffer pH 6.6 at 37 ± 0.2 °C through excised bovine buccal mucosa. *In vivo* studies on anaesthetized New Zealand albino male rabbits were conducted and drug levels in plasma were estimated at 220 nm by reverse phase HPLC using BDS Hypersil C_8 column using acetonitrile and 0.05 M potassium dihydrogen orthophosphate buffer pH 6.6 (20 : 80 v/v) as mobile phase, at a flow rate of 1.25 mL/min. Optimized formulation showed C_{max}, T_{max}, $t_{1/2}$ and AUC_{total}, 688 pg/mL, 2 h, 0.079 h, and 1999.72 h · pg/mL respectively. The NMBT containing 0.75% w/w sodium taurocholate showed 27% bioavailability without damaging the buccal mucosa suggesting its suitability as an alternative to non-invasive administration of oxytocin.

1. Introduction

In the field of pharmaceutical technology there is an urgent need for non-parenteral routes of administration of potentially active peptides and proteins, which are being developed currently by genetic engineering and biotechnology (Siddiqui and Chien 1987; Lee 1988; Silvia et al. 2005).

Oxytocin is clinically used for the induction and augmentation of labor, mid trimester abortion, and the cessation or prevention of postpartum hemorrhage. It is estimated that 30% of all deliveries in the United States use oxytocin to induce and augment labor (Cunningham et al. 2001). Among various transmucosal routes like nasal, rectal, vaginal, ocular, pulmonary and buccal evaluated for peptide/protein absorption, nasal and buccal routes have shown considerable absorption potential (Murakami et al. 1984; Yamamoto et al. 1989; Okada 1991; Su 1991; Wearly 1991). But the nasal route has distinct limitations such as the interference with the mucociliary activity by the penetration enhancers added to nasal formulations or irreversible peptide/protein degradation caused by enzymes (proteases and peptidases) present in the nasal mucosa (Hirai et al. 1981; Harris 1986). On the other hand, the buccal mucosa has several advantages (deVries and Bodde 1991) such as rich vascularity, moderate permeability, suitability for both local and systemic drug delivery, reduced enzymatic activity compared to other tissues such as intestinal mucosa and avoidance of first pass hepatic metabolism. Buccal mucosa is less sensitive than the nasal mucosa, less prone to irritation or damage despite being regularly exposed to a multitude of different foreign compounds (Senel et al. 1998). The accessibility of the buccal cavity makes application of drugs easy and acceptable to the patient, 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 reduction in concentration of drug as well as retention time of the dosage forms at the site of application (Weatherell et al. 1996). However, the structural integrity of the buccal mucosa also decreases the permeability for macromolecular drugs (Senel and Hincal 2001).

In the present investigation, the main objectives were to prevent the salivary scavenging effect by designing mucoadhesive dosage forms that deliver the drug unidirectionally; to improve the rate of permeation of oxytocin by the inclusion of safe and effective permeation enhancers like sodium taurocholate STc, or sodium thioglycollate STg. Mucoadhesive material extracted from *Dillenia indica* fruits was used in the formulation because of its edibility, biodegradability and biocompatibility.

2. Investigations, results and discussion

It has been proved that many oligo- and polysaccharides possess mucoadhesive properties (Harding 2003). Aqueous extracts of *Dillenia indica* fruits comprise arabinogalactan. Arabinogalactan consists of arabinose, galactose, xylose,

Table 1: Characterization of isolated mucoadhesive agent

Mucoadhesive agent	pH	Swellability (Vol./g) n = 3
DIM	6.83	5.83 ± 0.05
HPMC	7.39	7.43 ± 0.08
CP	2.87	17.90 ± 0.05

DIM – *Dillenia indica* mucilage; HPMC – Hydroxy propyl methylcellulose; CP – Carbopol 934p

uronic acid, and glucose in the molar ratio of 37.1 : 55.8 : 3.0 : 4.1 : trace (Kato and Nevins 1984). As carbohydrates being the predominant substances in *Dillenia indica* fruits, an attempt has been made to isolate the material by a hot aqueous extraction process followed by organic solvent precipitation. The yield was found to be 3.2% w/v to the initial weight of *Dillenia indica* fruits. The physical parameters were characterized with special emphasis to evaluate its mucoadhesive properties.

The pH value of a 1% w/v solution of *Dillenia indica* mucilage (DIM) was found to be 6.8 which is almost nearer to the buccal pH (6.6) suggesting its non-irritability and bio-compatibility with buccal mucosa (Table 1). The swollen volume of 1 g of DIM (5.83 ± 0.05 cm^3) was slightly less than that of HPMC (7.43 ± 0.08 cm^3) but far less than CP (17.90 ± 0.05) suggesting its moderate swellability (Table 1). Swelling is one of the primary characteristics for a polymer to be bioadhesive. However, excessive swelling due to over hydration always leads to formation of a slippery surface. Hence retention of dosage forms at the site of application is practically not possible. Further excessive swelling is not suitable for solid adhesive dosage forms because of loss of mechanical strength and structural integrity (Parodi et al. 1999).

Microcrystalline cellulose was selected as diluent both in the preparation of adhesive cups as well as of core tablets. MCC is extensively used as diluent in tablet formulation prepared by the wet granulation technique. It is also one of the most used filler-binders in direct compression method. Its popularity in direct compression is due to its excellent binding properties when used as a dry binder (Zhang et al. 2003). It has a high dilution potential in direct compression formulations.

Shear, peel and tensile strengths of prepared adhesive cups were measured to evaluate their bio/mucoadhesiveness (Fig. 1). Freshly excised bovine buccal mucosa was used as the substrate in order to assess the *in vitro* mucoadhesive strengths of adhesive cups. Force of adhesion (N) and bond strengths (N/m^2) showed that adhesive cups prepared with DIM exhibited higher adhesive strength than the other polymers studied (Table 2). This may be due to the presence of numerous carboxyl and hydroxyl groups of carbohydrates present in DIM, which may adopt a more favorable macromolecular conformation and accessibility of its hydrogen-binding sites that is essential for bio-adhesion (Nafee et al. 2004). HPMC and CP were reported to form weaker bondage with mucus, which may be due to a decrease in available hydrogen binding sites or an unfavor-

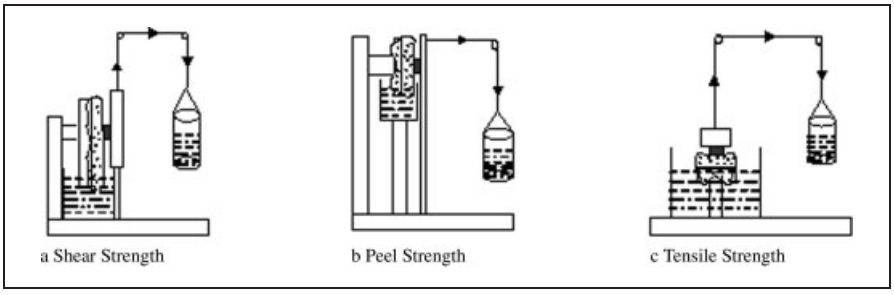

Fig. 1:
Schematic diagram of a) shear, b) peel and c) tensile strength measurement

Table 2: In vitro shear, peel and tensile strengths of adhesive cups

Formulations	Shear strength			Peel strength			Tensile strength		
	Weight (g) (n = 3)	Force of adhesion (N)	Bond strength (N/m^2)	Weight (g) (n = 3)	Force of adhesion (N)	Bond strength (N/m^2)	Weight (g) (n = 3)	Force of adhesion (N)	Bond strength (N/m^2)
DIM : MCC = 1 : 3	2.58 ± 0.11	0.0253	2798.766	2.97 ± 0.15	0.0291	3221.835	3.31 ± 0.05	0.0325	3590.665
DIM : MCC = 1 : 1	3.58 ± 0.05	0.0351	3883.559	3.86 ± 0.11	0.0379	4187.301	4.37 ± 0.11	0.0429	4740.545
DIM : MCC = 3 : 1	3.85 ± 0.11	0.0378	4176.453	4.27 ± 0.10	0.0419	4632.066	4.57 ± 0.10	0.0448	4957.504
HPMC : MCC = 1 : 3	2.40 ± 0.10	0.0235	2603.503	2.53 ± 0.11	0.0248	2744.526	2.70 ± 0.10	0.0265	2928.941
HPMC : MCC = 1 : 1	3.23 ± 0.11	0.0317	3503.881	3.33 ± 0.11	0.0327	3612.361	3.76 ± 0.05	0.0369	4078.822
HPMC : MCC = 3 : 1	3.56 ± 0.15	0.0349	3861.863	3.66 ± 0.15	0.0359	3970.342	3.93 ± 0.05	0.0386	4263.236
CP : MCC = 1 : 3	2.46 ± 0.10	0.0241	2668.591	2.56 ± 0.05	0.0251	2777.070	2.83 ± 0.11	0.0278	3069.964
CP : MCC = 1 : 1	3.33 ± 0.05	0.0327	3612.361	3.40 ± 0.15	0.0334	3688.296	3.96 ± 0.05	0.0388	4295.780
CP : MCC = 3 : 1	3.50 ± 0.15	0.0343	3796.775	3.63 ± 0.15	0.0356	3937.799	4.06 ± 0.05	0.0398	4404.260

DIM – *Dillenia indica* mucilage; HPMC – Hydroxy propyl methylcellulose; CP – Carbopol 934p

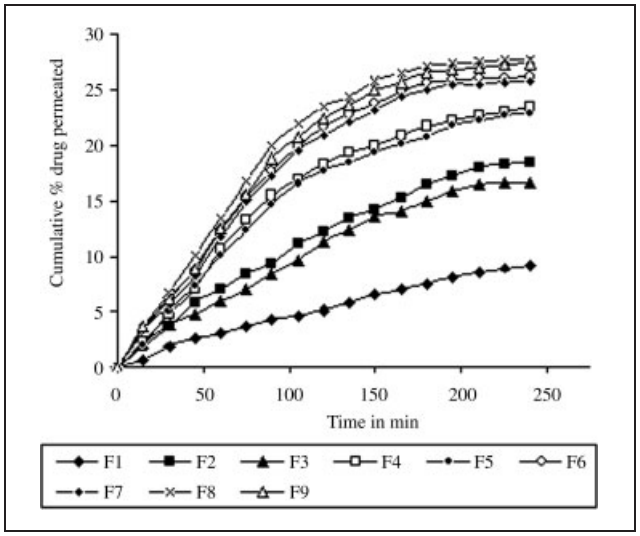

Fig. 2: In vitro permeation studies of NMBT in phosphate buffer pH 6.6 at 37 ± 0.2 °C

able contamination for entanglement to occur (Datta and Bandyopadhyay 2006).

In vitro permeation profiles of NMBT are represented in Fig. 2. The NMBT prepared without inclusion of any enhancer showed very poor permeability. After 4 h only 9.13% oxytocin permeated through the mucosa. This may be due to the big molecular size of oxytocin. The tablet formulations using enhancers such as STc, STg exhibited considerable increase in permeation rate. There was significant increase in permeation rate as enhancer's concentration increased from 0.25% to 0.50% w/v, but moderate increase in permeation rate from 0.50% to 0.75% w/v concentration and there was no significant increase in permeation rate over 0.75% w/v concentration. Batches F6 (0.75% STc) and F7 (0.75% STg) exhibited 26.19% and 25.80% permeability of oxytocin after 4 h permeation study, respectively. At 1.0% w/v enhancer's concentration, batches F8 and F9 exhibited only 27.74% and 27.38% permeation of oxytocin.

Histological studies on excised rabbit buccal mucosa at the end of four hours of *in vitro* permeation studies using the 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% w/v. However, a change in histology was observed at a concentration of 1.0% w/v of permeation enhances irrespective of the enhancer used. Based on the above *in vitro* permeation studies and histological observations, batch F6 was selected for *in vivo* absorption studies.

In vivo studies were conducted on New Zealand albino male rabbits for both intravenous bolus injection and NMBT (F6). The pharmacokinetic parameters of NMBT such as maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), total area under the concentration curve (AUC_{total}), mean residence time (MRT), volume of distribution (V_d), clearance (CL) were 688 ng/mL, 2 h, 1999.72 h · ng/mL, 2.061 h, 697.53 mL, 16001,62 ng · h · (ng/mL) respectively (Table 3). The biological half-life of oxytocin in NMBT was found to be 0.079 h (4.74 min). After intravenous bolus injection, pharmacokinetic parameters were C_{max} 4500 ng/mL; AUC_{total} 732.37 h · ng/mL; MRT 0.138017 h; CL 4369,38 h · (ng/mL) and $t_{1/2}$ 0.075 h (4.50 min). NMBT exhibited 27.31% bioavailability. Since the rabbit buccal mucosa is partially keratinized in contrast to human buccal mucosa (non keratinized), presumable large bioavailability values should result after buccal administration in humans. Moreover, the surface area available for permeation of oxytocin from the NMBT is only 6.1544 mm^2. The extent of permeation may also be presumed to increase by increasing the surface area (diameter of the core tablet).

At the end of *in vivo* experiment, the retrieved NMBT were analyzed for oxytocin content. Only 41.26% oxytocin was present in the retrieved NMBT. The remaining 31.43% oxytocin might be present in the buccal mucosa and may act as reservoir allowing slower diffusion to systemic circulation.

3. Experimental

3.1. Materials

Oxytocin was obtained as a gift sample from Hemmo Pharma, India. *Dillenia indica* L. (Dilleniaceae) fruits were purchased from local vendors. Hydroxy propyl methyl cellulose (HPMC), carbopol 934p (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. All other reagents and chemicals used were of analytical grade. Special punches and dies were designed and fabricated by Sigma engineering corporation, Sonarpur, India. New Zealand, white male rabbits (approximately 1.5–1.8 kg body weight) were purchased from Reeta Ghosh, Kolkata, India.

3.2. Methods

3.2.1. Extraction of mucilage from edible Dillenia indica fruits

Dillenia indica fruits (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 on a water-bath for 4 h. The thick viscous solution was then strained through muslin cloth. The filtrate was diluted with three volumes of double distilled water and kept

Table 3: Comparison of kinetic results of NMBT with intravenous bolus injection

Parameters	Intravenous	Buccal	Unit
C_{max}	4500 ± 115.36**	688 ± 12.48**	pg/mL
T_{max}	0	2 ± 0.11*	h
$AUC_{0\text{-}t}$	686.59 ± 21.15**	1999.72 ± 201.34**	h · pg/mL
$AUC_{t\text{-}\infty}$	45.77 ± 9.47	0.27 ± 0.008	h · pg/mL
AUC_{total}	732.36 ± 30.62**	1999.99± 201.34*	h · pg/mL
$AUMC_{0\text{-}t}$	80.06 ± 4.17	4121.45 ± 216.47	h^2 · pg/mL
$AUMC_{t\text{-}\infty}$	21.01 ± 3.18	0.49 ± 0.12	h^2 · pg/mL
$AUMC_{total}$	101.07 ± 7.35	4121.94 ± 216.46	h^2 · pg/mL
MRT	0.138 ± 0.07**	2.061 ± 0.08**	h
V_d	697.53 ± 24.17	697.53 ± 21.46	mL
$T_{1/2}$	0.075 ± 0.005**	0.079 ± 0.004**	h
CL	4369.38 ± 203.4	16001.62 ± 426.81	pg · h · (pg/mL)

t = 15 min for intravenous bolus injection and t = 300 min for buccal administration. * $p < 0.05$ – significant, ** $p < 0.01$ – very significant (students t-test)

undisturbed overnight in a refrigerator. In 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. Precipitate was washed thrice with acetone and dried at 50 ± 1 °C. Isolated *Dillenia indica* mucilage (DIM) was powdered in a mechanical grinder and passed through #80 mesh sieve (Rao et al. 1998).

3.2.2. Characterization of isolated mucoadhesive agent

pH of 1% w/v aqueous solution of DIM was measured using a Toshniwal pH meter (Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer). Swellability studies of DIM were conducted by keeping 1 g DIM with 20 mL of water in a measuring cylinder for 24 h (Park and Robinson 1987). The swollen volume was noted. All the above parameters were measured for commercially available polymers like hydroxyl propyl methyl cellulose (HPMC) and carbopol 934p (CP) and compared (Table 1).

3.2.3. Formulation of novel mucoadhesive buccal tablet (NMBT)

3.2.3.1. Preparation of adhesive cups

Granules for adhesive cups were prepared by mixing the respective mucoadhesive agent (DIM, HPMC or CP) with microcrystalline cellulose at 1 : 3, 1 : 1 and 3 : 1 ratios by the wet granulation method using the respective mucoadhesive solution as granulating solution and then passed through sieve #18. Granules were dried in a tray drier at 50 ± 1 °C for 6 h, passed through sieve #22 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 of 4.4 mm diameter and a projected upper punch of 2.8 mm inner diameter and 4.4 mm outer diameter as shown in Fig. 3a schematically.

3.2.3.2. Preparation of core tablets

Core tablets of oxytocin were formulated with different penetration enhancers such as sodium taurocholate (STc), sodium thioglycollate (STg) (Table 4) and compressed by direct compression using 2.8 mm flat punches (Fig. 3b).

3.2.3.3. Preparation of novel mucoadhesive buccal tablets (NMBT)

NMBT were prepared by inserting manually core tablets into respective adhesive cups and finally compressed in a 10 station mini press using 4.5 mm flat punches (Fig. 3c).

3.2.4. Measurement of in vitro mucoadhesive strengths of adhesive cups

3.2.4.1. Shear strength

The shear strength of adhesive cups was calculated by measuring the force required to detach the adhesive cup parallelly from the freshly excised bovine buccal mucosa as represented in Fig. 1a. The backside of the mucoadhesive cup was fixed to a movable plastic strip with the synthetic adhesive. The other side the cup was pressed over the excised bovine buccal mucosa for 30 s applying constant pressure. After 5 min, the weight required to detach the novel adhesive cup parallelly from the mucosa was recorded (Smart et al. 1984; James and Gilman 1970).
The force of adhesion and the bond strength were calculated as

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

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

and represented in Table 4.

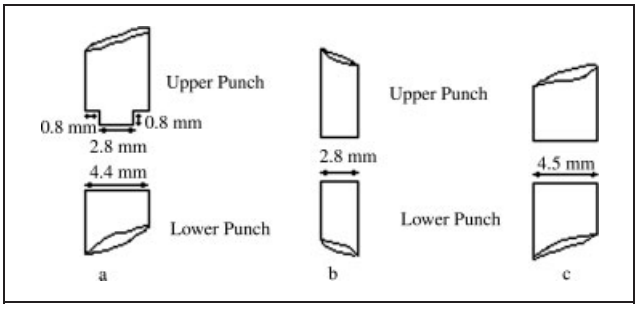

Fig. 3: Specially designed punches for the preparation of a) cups, b) core tablets, c) NMBT

Table 4: 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

3.2.4.2. Peel strength

The experiment was conducted similarly to determine the detachment force required to detach the adhesive cup tangentially from freshly excised bovine buccal mucosa as represented schematically in Fig. 1b, was calculated and represented in Table 2 (Lee et al. 2000).

3.2.4.3. Tensile strength

This method is based on the measurement of tensile strength i.e. the strength required to detach the adhesive cup perpendicularly from freshly excised bovine buccal mucosa as shown schematically in Fig. 2c (Smart 1998) Results are shown in Table 2.

3.2.5. Evaluation of NMBT of oxytocin

3.2.5.1. *In vitro* permeation study

In vitro permeation studies of NMBT 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. 4) (Frantz 1990). Immediately after purchasing the bovine buccal membrane from the local slaughterhouse, it was placed in ice-cold 0.05 M phosphate buffer. The connective tissue of the membrane was carefully removed using fine point forceps and surgical scissors to adjust an uniform thickness of 1.0 mm measured by means of an absolute digimatic caliper (Miyutoyo Corporation, Japan). Then the mucosa was mounted in the Franz diffusion cell. Samples were collected at several time intervals up to 4 h and were analyzed by a Jasco UV 1575 HPLC detector using BDS Hypersil C_8 column ($250 \text{ mm} \times 4.6 \text{ mm} \times 5\mu\text{m}$) with a mobile phase of acetonitrile and 0.05 M potassium dihydrogen orthophosphate buffer, pH 6.6 (20 : 80, v/v) at a flow rate of 1.25 mL/min. The detection wavelength was 220 nm. Chromatographic peaks were automatically integrated and recorded by Data Apex Chromatographic Station for Windows 1.7 data module (Data Apex; Prague, Czech Republic) (Fig. 2).

3.2.5.2. Histological study

This experiment was conducted to examine any potential alteration in the histology of the buccal mucosa that may have arisen due to the application of the NMBT. At the end of the four hours *in vitro* permeation study, NMBT was carefully removed from the buccal mucosa using a forceps. Then the mucosa was sectioned to get the outermost epithelium layer of about 0.2 mm thickness using a Rotary Microtome. Another unused rabbit buccal mucosa was also taken as control and was similarly sectioned. Those two epithelium layers were then immediately fixed in 10% formalin solution. The tissue was then

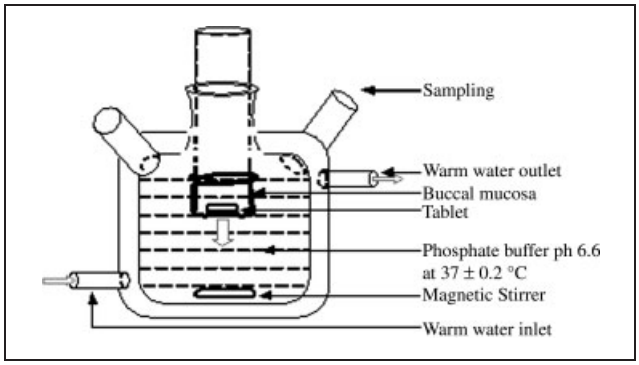

Fig. 4: Schematic diagram of Franz diffusion cell for permeation study of NMBT. (Permeation media – 50 ml of phosphate buffer pH 6.6 at 37 ± 0.2 °C, magnetic stirrer speed – 50 rpm)

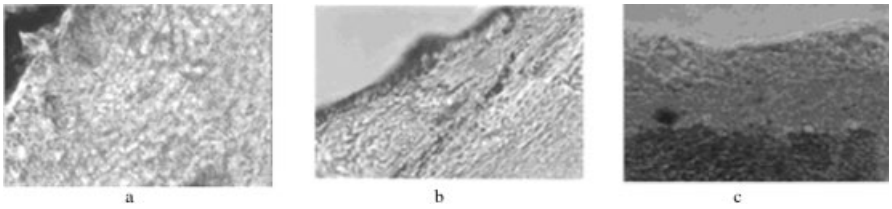

Fig. 5:
Representative light micrographs (40×0.55 μ PHP magnification) of hematoxyline and eosin stained buccal mucosa a) control- without application of NMBT b) after application of NMBT (0.75% w/w STc) and c) after application of NMBT (1.0% w/w STc)

sectioned, stained with eosin and hematoxylin and examined by Olympus CKX41 microscope, Olympus Optical Co. Ltd., Tokyo, Japan. The photographs were taken with an Olympus-SC 35 camera (Fig. 5).

3.2.5.3. *In vivo* studies

The novel mucoadhesive buccal tablets were evaluated for its potential to deliver oxytocin to the systemic circulation. Nine New Zealand albino male rabbits were kept in laboratory atmosphere for twelve days before the experiment and provided with standard diet *ad libitum*. The rabbits were kept in fasting condition for 24 h before the experiment commenced. The rabbits were grouped into three (Group I, II & III), each group containing three rabbits. Group I was used as control. Group II received an intravenous bolus injection of oxytocin. Group III was administered NMBT.
A single dose of oxytocin (1.5 IU/kg rabbit) in normal saline was administrated intravenously to compare the pharmacokinetic parameters and to calculate the bioavailability of oxytocin from NMBT. No anesthesia was used for the intravenous study (Li et al. 1997). Oxytocin was injected through a cannulated marginal ear vein. 2 mL of blood samples each time were collected in 3 mL heparinized glass tubes before intravenous injection and then at 1, 3, 5, 7, 9, 11, 13 and 15 min intervals. Immediately after collection, blood samples were kept on ice and then centrifuged at 3000 rpm for 10 min. Retrieved plasma was stored at −20 °C until the time of analysis.
Before the application of NMBT, each rabbit was lightly anaesthetized by intramuscular injection of a mixture of xylazine (3 mg/kg) and ketamine (35 mg/kg). Following induction of anaesthesia, a catheter was fixed into the central artery for blood sample collection. One NMBT was placed on the mucosa by pressing the tablet for 30 s. About 2 mL blood sample was collected prior to the application of NMBT and then at 10, 20, 30, 60, 90, 120, 150, 180, 240 and 300 min intervals in 3 mL heparinized glass tubes. The blood samples were kept on ice and centrifuged at 3000 rpm for 10 min immediately after collection to separate the plasma and stored at −20 °C until analysis. Immediately after each blood sample collection, the catheter was flushed with 0.2 mL of a 10% (v/v) heparin/normal saline solution to prevent blood clotting inside the catheter. Every 20 min rabbits received intramuscularly one-third of the initial dose of xylazine and ketamine to maintain a light plane of anesthesia.
Before the analysis by HPLC, oxytocin was separated by the liquid phase organic solvent extraction method. The supernatant (plasma) after centrifugation was mixed with acetone and petroleum ether at ratios, plasma : acetone : petroleum ether = 1 : 2 : 3 v/v/v. Petroleum ether was used to remove the lipids. After the ether phase was removed, the acetone phase that contained the oxytocin was evaporated to dryness. The residue was dissolved in 500 μl of mobile phase and analyzed by HPLC as described in above.
At the end of the 5 h experiment, the rabbits were euthanized by injection of an overdose of 5% w/v pentobarbital sodium solution (3 mL) into a marginal ear vein. Retrieved NMBT were assayed for residual oxytocin. The NMBT were placed into 5 mL of PBS and oxytocin was extracted by mixing the solution at 500 rpm for 2 h. Three unused NMBT, which had the same lot number as the retrieved NMBT, were treated similarly to quantitate the extraction efficiency of this procedure. After 2 h extraction, each solution was centrifuged at 3000 rpm for 15 min and was filtered. The filtrate was then analyzed for oxytocin by HPLC as described above (3.2.5.1). Comparing the peak area obtained for the unused NMBT, the amount of oxytocin that was remained in each NMBT, was then calculated.

3.2.5.4. Pharmacokinetic analysis

Peak plasma concentration (C_{max}), time to reach peak plasma concentration (t_{max}), area under the (concentration-time) curve (AUC), area under first movement curve (AUMC), mean residence time (MRT), elimination half-life ($t_{1/2}$), volume of distribution (V_d), total body clearance (CL), were calculated following Wagner-Nelson two compartment method by Kinetica 4.4, PK/PD Analysis, Thermoelectron Corporation. All the parameters were calculated for i.v. bolus injection of oxytocin by one compartment i.v. bolus method and tabulated in Table 3.

Fraction of dose absorbed (F) was calculated by the equation:

$$F = \frac{\text{Dose}_{\text{i.v.}} \times (\text{buccal})\text{AUC}_{0-\infty}}{\text{Dose}_{\text{buccal}} \times (\text{i.v.})\text{AUC}_{0-\infty}} \quad (3)$$

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