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RESEARCH ARTICLE

FORMULATION AND DEVELOPMENT OF THERMO-REVERSIBLE MUCOADHESIVE INTRANASAL *IN SITU* HYDROGEL BY USING A COMBINATION OF POLYMERS

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The prolonged residence of drug formulation in the nasal cavity is of utmost importance for intranasal drug delivery. To improve the nasal retention time of Metoclopramide hydrochloride (MCP HCl), it has been formulated as in situ mucoadhesive gel by using blend of Poloxamer 407, Poloxamer 188 and carbopol 934P. The objective of this work was to improve the nasal bioavailability of antiemetic drug, MCP HCl by increasing its nasal retention time as well as by means of nasal permeation. Increase in the concentration of mucoadhesive agent enhanced the mucoadhesive force significantly. *In vitro* release of MCP HCl from the mucoadhesive system in simulated nasal fluid was influenced significantly by the properties and concentrations of carbopol 934P and showed enhanced bioavailability through its longer nasal residence time and ability to sustain the release of the drug. The *in vitro* tests performed for mucoadhesive strength and drug diffusion showed that nasal in situ gelling formulations prepared were having good mucoadhesive strength with nearly 100% drug diffusion. The formulations were evaluated for physiochemical parameter, gelation temperature, viscosity, gel strength, content uniformity mucoadhesive force, FTIR and DSC. So, this study points to the potential of mucoadhesive *in situ* nasal gel in terms of ease of administration, accuracy of dosing, prolonged nasal residence and improved nasal bioavailability.

Key words: Nasal drug delivery, Poloxamer 407, Poloxamer 188, Metoclopramide HCl.

INTRODUCTION

Migraine, the most common cause of headache, afflicts approximately 15% of women and 6% of men. A useful definition of migraine is a benign and recurring syndrome of headache, nausea, vomiting, and/or other symptoms of neurologic dysfunction in varying admixtures. Migraine can often be recognized by its activators (red wine, menses, hunger, lack of sleep, glare, estrogen, worry, perfumes, let-down periods) and its deactivators (sleep, pregnancy, exhilaration, triptans). Conventional therapies for the treatment of migraine are given by oral, parenteral or in form of nasal drops/sprays. These formulations require frequent administration due to nasal mucociliary clearance (Kasper *et al* 2005).

The nasal epithelium is a highly permeable monolayer, the sub mucosa is highly vascularized with large and fenestrated capillaries facilitating rapid absorption. Moreover, direct systemic absorption avoids hepatic first-class metabolism, gut wall metabolism and destruction in gastrointestinal tract. Owing to these merits, various nasal drug delivery systems are available for user-friendly noninvasive painless application (Patel *et al* 2010).

Metoclopramide Hydrochloride is chemically 4-amino-5-chloro-*N*-[2-(diethylamino) ethyl]-2-methoxybenzamide hydrochloride. It is available as white or almost white, crystalline powder or crystals, which is very soluble in water, freely soluble in alcohol, sparingly soluble in methylene chloride. This antiemetic, chemically related to the procainamide, acts predominantly as a dopamine antagonist. It also has 5-HT4 agonist properties (Satoskar *et al* 2008; BP 2009). However, the oral bioavailability of MCP HCl is highly variable showing values between 32 and 98% due to extensive pre-systemic metabolism. Oral forms of MCP HCl often get vomited out before systemic absorption, compelling parenteral or rectal administration where both methods result in low patient compliance. Among various novel drug delivery systems (Tripathi *et al* 2011; Choksey *et al* 2011; Talegaonkar *et al* 2011; Khan *et al* 2012); considering properties of MCP HCl, the intranasal delivery seems to be an attractive alternative. The objective of present research work was to develop temperature dependent nasal *in situ* gel of MCP HCl.

MATERIALS

Poloxamer 407 and Poloxamer 188 (gifted from Sun Pharmaceuticals Ltd, Baroda, India), Benzalkonium chloride, sodium chloride (S.D. Fine-chemicals Ltd., Mumbai). Dihydrogen potassium orthophosphate (Glaxo, India) and sodium hydroxide (E. Merck, India) were purchased. Double distilled water was used. All other chemicals used were of analytical reagent grade. Cellophane membrane (Sigma-Aldrich), Digital pH meter (Systronics, MK-VI. Naroda-Ahmadabad. India) and Brook Field viscometer (Tokimec Co., Japan) were also used.

METHODS

Preformulation studies

Determination of λ_{max} of MCP HCl

A stock solution of 100 µg/ml of MCP HCl was prepared by dissolving 10 mg in 100 ml of deionized distilled water. The resulting solution was scanned between 200 nm to 400 nm using double beam UV-visible spectrophotometer.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to evaluate the thermal behavior of pure drug and physical mixture of the drug and excipients. Five-ten milligrams of samples were weighed and sealed in standard aluminum pans and then scanned over a temperature range from 50 to 300°C at a heating rate of 10°C/min.

Preparation and optimization of thermoreversible Pluronic (PF127/PF68) gels

The plain and drug loaded Pluronic gels were prepared by cold method (Schmolka, 1972). for drug loaded Pluronic gels, 10% of drug was stirred with sufficient quantity of distilled deionized water while for plain gels, only sufficient quantity of deionized distilled water without drug was kept overnight at 4°C in refrigerator. To this solution, required amounts of PF 127/PF 68 were added. All other excipients such as benzalkonium chloride (0.001% *w/v*) and sodium chloride 0.9% (*w/v*) were added with continuous stirring. The dispersions were then stored in a refrigerator until clear solution was obtained and finally volume was adjusted. Optimization of plain and drug loaded PF127/PF68 gels were done by varying concentration of PF127/PF68 and evaluating them for gelation temperature. Optimized concentration of PF127/PF68 was used for further study of effect of mucoadhesive polymer on gelation temperature and mucoadhesive strength. The concentration of mucoadhesive polymers Carbopol 934P and Noveon AAi (Polycarbophils) were screened in the range of 0.02 to 0.08% and 0.2 to 0.8% respectively. The composition of developed gel formulations is summarized in **Table 1**.

Characterization of the prepared formulations

Gelation point

It is temperature at which the liquid phase makes a transition to gel. A gelation temperature range suitable for thermoreversible nasal gel would be 30-36°C. Gelation point was considered as the temperature where formulations would not flow when test tubes were tilted to 90° angle, as the temperature was gradually increased.

pH of the gels

The pH of each batch was measured using digital pH meter which was calibrated using buffers of pH 4 and pH 7 before the measurements.

Content uniformity

Formulations were tested for content uniformity. Vials (n=3) containing the formulation were properly shaken for 2-3 min.

Table 1. Composition of developed thermoreversible *in situ* gel for MCP HCl

Ingredients (% *w/v*)	Formulation code								
	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9
***Metoclopramide HCl**	10	10	10	10	10	10	10	10	10
PF 127	18	18	18	18	18	18	18	18	18
PF 68	03	03	03	03	03	03	03	03	03
Carbopol 934P	–	0.02	0.04	0.06	0.08	–	–	–	–
Noveon AA1	–	–	–	–	–	0.2	0.4	0.6	0.8
Benzalkonium chloride	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Deionized distilled water (*q.s.*)	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml

*Each formulation contains 10 mg/ml of Metoclopramide HCl

One milliliter of the formulation was transferred to a 100 ml volumetric flask. Fifty ml of simulated nasal fluid was added. The formed gel was completely crushed with the help of a glass rod, followed by vigorous shaking until the formed gel got completely dispersed to give a clear solution. The volume was adjusted to 100 ml with simulated tear fluid. The solution was filtered through a 0.45 mm filter membrane and the drug concentration was determined with a UV-Visible spectrophotometer at 272.4 nm (Shastri *et al* 2010).

Rheological studies

Viscosity of the prepared formulations was measured by using Brookfield LVDV-E Viscometer. The suitable spindle was lowered perpendicularly into the fixed volume of gel which was to be measured. The spindle was rotated at varying speeds and the suitable speed was selected. The temperature was increased initially above 40°C and then the viscosity was measured as the system was allowed to cool gradually (Patel *et al* 2010).

Gel strength determination

A sample of 50 g of the nasal gel was put in a 100 ml graduated cylinder and gelled in a thermostatically controlled water bath at 37°C. A weight of 35 g was placed onto the gelled solution. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel (Badgujar *et al* 2010).

Determination of mucoadhesive strength

The mucoadhesive strength was determined as per the reported method (Choi *et al* 1998). The mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosa was fixed on each of two glass slides using thread. Fifty milligrams of gel was placed on first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2 min to ensure intimate contact between them. Then weight was kept rising in second pan until slides get detached from each other. The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation. The nasal mucosa was changed for each measurement (Gaikwad, 2010).

Mucoadhesive strength (dynes/cm^2) = mg/A (1)

where:
m = weight required for detachment
g = acceleration due to gravity
A = area of mucosa exposed

In vitro release studies

Drug release from gel was tested with nasal diffusion cell, using dialysis membrane (mol. wt. 12,000-14,000) with permeation area of 2 cm^2. 20 ml of simulated nasal fluid pH 6.4 was added to the acceptor chamber. Gel containing drug equivalent to 10mg was placed in donor compartment. At predetermined time points (1 h), 1 ml sample were withdrawn from the acceptor compartment, replacing the sampled volume with SNF after each sampling for a period of 8 h. The samples were suitably diluted and measured spectrophotometrically at 272.4 nm. The concentration of drug was determined from a previously constructed calibration curve ($y = 0.039x + 0.003$, $R^2 = 0.9988$).

In vitro permeation study

Fresh nasal tissue was removed from nasal cavity of sheep obtained from local slaughter house. Tissue was inserted in the nasal diffusion cell (Franz diffusion cell) with permeation area of 0.785 cm^2. Similar way as in drug release study, gel containing drug equivalent to 10 mg was kept in donor compartment. At predetermined time point sampling was done. Blank samples (without drug) were run simultaneously throughout the experiment. Amount of drug permeated was determined by UV-spectrophotometry. Cumulative percentage drug release after 1 h (t_1) and 8 h (t_8) were calculated using the Beer-Lambert calibration curve in the linearity range of 0-20 μg/ml (Gaikwad, 2010).

Histopathological evaluation of mucosa

The histopathological evaluation of tissue incubated in phosphate buffer (pH 6.8) for 8 h after collection was compared with tissue incubated in diffusion chamber with formulation. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin and eosin. Sections were examined to detect any damage to the tissue (Mahajan and Gattani, 2009).

Statistical analysis

The data were analyzed by using two-way analysis of variance (ANOVA). $P < 0.05$ were considered statistically significant.

Stability study

Thermoreversible *in situ* nasal gel formulation containing PF127/PF68 (18/3) and Carbopol 934P 0.06% mucoadhesive polymers were tested for stability under the actual condition of storage. Gels were stored in clean, dry, airtight moisture proof bottles, kept away from light. The gel samples were withdrawn after 30 days and evaluated for gelation temperature, gel strength, mucoadhesive strength, pH and drug content. To assess long term stability of the prepared gelling systems of Metoclopramide HCl, formulations were stored at 40°C/75% relative humidity (RH) in the stability chamber for 3 months. The samples were withdrawn at different time intervals (0, 1, 3 months) and observed for physical characteristics, drug content and in vitro drug release characteristics. The results were supported by statistical analysis using student 't' test and ANOVA (significance level $p < 0.05$).

RESULTS AND DISCUSSION

MCP HCl exhibited λ_{max} at 272.4 nm. Linearity was observed in the range of 2 to 20 μg/ml with the r^2 value of 0.998. FTIR and DSC studies were carried out on pure drug as well as its combination with selected polymers and exhibited no interaction. The preliminary studies indicated that the minimum concentration in combination of PF127 with PF68 that formed gel below 35°C was 18% and 03% *w/w* respectively. The gelation temperature of different concentration of pluronics is shown in **Table 2**. The loading of MCP HCl in the different formulations was kept at 10% (*w/w*) such that 100 μl gel (the optimum volume for nasal administration) would contain 10mg which is the adult dose. Being water soluble in nature, MCP HCl might cause modification of the process of micellar association of poloxamer gels thereby increasing their $T_{sol-gel}$. The $T_{sol-gel}$-lowering effect of mucoadhesive polymers could be explained by their ability to bind to polyethylene oxide (PEO) chains present in the poloxamer molecules promoting dehydration and causing an increase in entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding. The physiological range of the nasal mucosa temperature lies between 32 and 35°C (Mygind and Dahl, 1998). Our target was to develop a nasal thermosetting gel with a phase transition temperature between 25 and 35°C. It would then behave as a liquid at the room temperature for ease of administration and accurate measurement of the dose but sets into a

Table 2. Preformulation gelation temperature study of *in situ* nasal gels

Pluronics	Conc. (% *w/v*)	Gelation temp. (◦C)	Pluronics	Conc. (% *w/v*)	Gelation temp. (◦C)
PF-127	15	>50	PF127/PF68	15/09	>50
	16	42.67±1.15		16/03	>50
	18	25.73±1.50		16/06	>50
	20	23.77±0.47		16/09	48.33±1.26
PF-68	2	>50		17/03	49.97±0.84
	4	>50		17/06	46.23±0.25
	6	>50		17/09	43.40±0.66
	8	47.23±0.87		18/03	31.30±0.62
	10	36.23±1.12		18/06	34.50±0.56
	12	26.73±1.14		18/09	36.87±0.90
PF127/PF68	15/03	>50		20/03	42.77±0.25
	15/06	>50		20/06	38.70±0.75
				20/09	35.33±0.50

gel with increased residence time at the lower limit of the nasal physiological temperature range. It is known that the normal physiological pH of nasal mucosa is 4.5-6.5; however the nasal mucosa can tolerate solutions within pH range of 3-10. pH of the all formulations were found to be within 6.5 to 7.04 that is between physiological range of pH of nasal mucosa. The percentage drug content of all the prepared gel formulation were checked and found to be in the range of 99.21-100.17%.

Mucoadhesive strength was determined in term of detachment stress *i.e.* force required to detach the formulation from mucosal surface. Results of mucoadhesion tests were found as per the given data (**Table 3**). Determination of mucoadhesive strength in terms of detachment stress showed that adhesive property increased with addition of Carbopol 934. Previously reported work with carbopol polymer indicated that it was the availability of carboxyl group that determined bioadhesion. Carbopol has very high percentage (58-68%) of carboxyl group that undergoes hydrogen bonding with sugar residues in oligosaccharide chain in mucus membrane, resulting in strengthened network between polymer and mucus membrane[15]. The stronger the mucoadhesive force is, the more it can prevent the gelled solution coming out of the nose. But if the bioadhesive force is too excessive, the gel can damage the nasal mucosal membrane.

Table 3. Evaluation parameters of formulations

Formulation Code	pH	Gelation temp (°C) ± S.D.	Drug content % ± S.D.	Gel strength (sec) ± S.D.	Bioadhesion force (dynes/cm^2) ± S.D.	Viscosity (cPs) ± S.D.
T-1	6.50±0.10	30.53±0.93	99.21±1.00	81.33±14.85	2701.13±180.08	13248±348.94
T-2	6.73±0.21	31.67±0.29	99.59±0.51	110.83±6.72	13005.42±1386.22	47014.33±267.16
T-3	6.70±0.20	32.33±0.55	100.17±0.30	115.33±2.12	13805.75±1200.50	49594.33±214.23
T-4	6.50±0.17	34.27±0.21	99.69±0.74	118.00±0.71	14145.89±242.59	50799±227.10
T-5	6.00±0.26	36.87±0.21	99.90±0.62	135.33±10.61	15226.34±227.25	56367±513.36
T-6	6.70±0.26	31.10±0.53	99.52±0.53	59.00±4.24	9804.08±916.90	8861±68.55
T-7	6.67±0.25	32.57±0.40	99.97±0.86	72.00±4.24	10604.42±693.11	14911.33±184.19

*Values expressed as mean±S.D, n=3

The gelation temperature, pH and viscosity of the various formulations are shown in **Table 3**. It can be seen from the table, that there is definite relation of gelation point with viscosity. The formulations which exhibited minimum gelation point had maximum viscosity at 37°C.

For the in situ gelling system that gels with an increase in temperature, the sol-gel transition temperature have to be significantly lower than temperature (35°C) in the nasal cavity. Aqueous solutions containing Noveon® AA-1 polycarbophil and Poloxamers (pluronics) were

prepared to evaluate the compositions suitable for in situ nasal gelling system. An ideal in situ nasal gelling system should be a free flowing liquid with low viscosity which can be conveniently dropped as a solution into the nasal cavity, where they undergo a transition into a gel at physiological condition and thus increase the nasal residence time of the delivery system and thereby enhance nasal bioavailability of the drug. The use of Noveon® AA-1 polycarbophil in situ gelling system is substantiated by transformation into stiff gels when the pH and temperature are raised. However, the concentration (0.2-0.8% *w/w*) of Noveon® AA-1 polycarbophil required to form stiff gels resulted in highly acidic solution which are not easily neutralized by the buffering action of the nasal fluid. A reduction in Noveon® AA-1 polycarbophil concentration without compromising the gelling capacity and rheological properties of the delivery system may be achieved by the addition of thermoreversible gelling polymer which is basic in nature. From the preparation procedures of Poloxamers solutions, it was found that the phase transition temperature increases with decreasing concentration.

From viscosity study on formulations showed increase in viscosity at 37°C. This indicated the temperature induced gel structure formulation of Poloxamers. The results were reported (**Table 3**), and showed that viscosity resulted at physiological temperature at 20 rpm by using suitable spindle of rotating type Brookfield viscometer.

The release profile of MCP HCl mucoadhesive nasal *in situ* gel shown in (**Figure 1**) for formulation T-1 to T-9 showed the release profile of these formulations. It showed the drug release profiles of various formulations of *in situ* gel. Pluronics formulations prepared as Plain without any bioadhesive polymers found 100% drug released within 5 h. Carbopol 934P moderately sustained the drug release to 8 h without any lag time. The rate and extent of MCP HCl release from in situ gelling system significantly decreased with an increase in carbopol concentration.

All the formulations showed dissimilar gelation characteristic depending upon polymer concentration. Formulation T-1, T-6 and T-9 showed weakest gelation while remaining found gelation satisfactorily and all formulations showed moderate to stiff gelation. The jumping release of T-1 was rapid due to incomplete (weak) gel formation as compared to other formulations having stiff gelation property. The drug release from the formulation T-2, T-3, T-4 and T-5 were at slower rate than T-1.

On the basis of results of characterization of in situ gelling systems and *in vitro* drug release study T-4 was selected as optimum formulation. Comparative permeation profile of pure drug and formulation T-4 is shown in **Figure 2**. To investigate the drug release mechanism, the release data of all formulations were analyzed using models representing zero order, first-order, Higuchi's square root of time and Korsmeyer-peppas model. The examination of coefficient of determination vales indicated that the drug release from the in situ gells followed the diffusion control mechanism (**Table 4**).

Table 4. Kinetic values obtained from different plots of the thermoreversible *in situ* gel formulations

Formulation code	Zero order plots	First order plots	Higuchi's plots	Korsmeyer-Peppas plots		Type of release
	$R^{2\,a}$	$R^{2\,a}$	$R^{2\,a}$	$R^{2\,a}$	n^{b}	
T-1	0.915	0.828	0.965	0.962	0.71	Non-Fickian
T-2	0.992	0.923	0.992	0.997	0.79	Non-Fickian
T-3	0.969	0.860	0.997	0.989	0.72	Non-Fickian
T-4	0.998	0.974	0.993	0.995	0.50	Fickian
T-5	0.815	0.734	0.892	0.908	0.41	Fickian
T-6	0.999	0.897	0.988	0.993	0.61	Non-Fickian
T-7	1.000	0.977	0.986	0.993	0.67	Non-Fickian
T-8	0.989	0.928	0.990	0.992	0.73	Non-Fickian
T-9	0.997	0.947	0.984	0.993	0.78	Non-Fickian

[a]Correlation coefficient, [b]The diffusional exponent is based on Korsmeyer-Peppas equation, $M_t/M_\infty = kt^n$

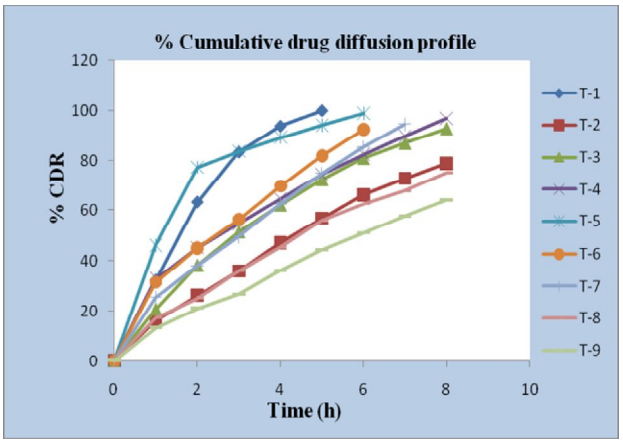

Fig. 1. Diffusion profile of formulated thermo-sensitive *in situ* nasal gel

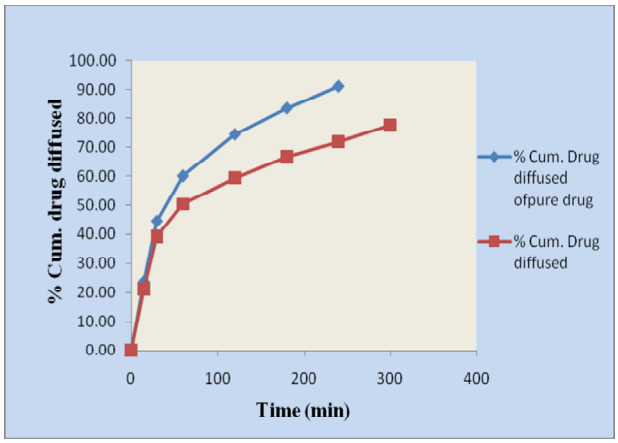

Fig. 2. Drug permeation profile of pure drug solution and T-4 formulation

Also the data were fitted to the Peppas exponential model.

$Mt/M_\infty = Kt^n$

where:
Mt/M_∞ = fraction of drug released after time t
k = the kinetic constant
n = the release exponent which characterizes the drug transport mechanism.

The n values were 0.501and 0.41 indicating that T-4 and T-5 formulations followed the fickian transport where as remaining formulations followed anomalous (non-Fickian) transport mechanism of drug release. The light micrograph was taken of nasal mucosa following diffusion study of 8 h. Examination of tissue showed no severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells (**Figure 3**).

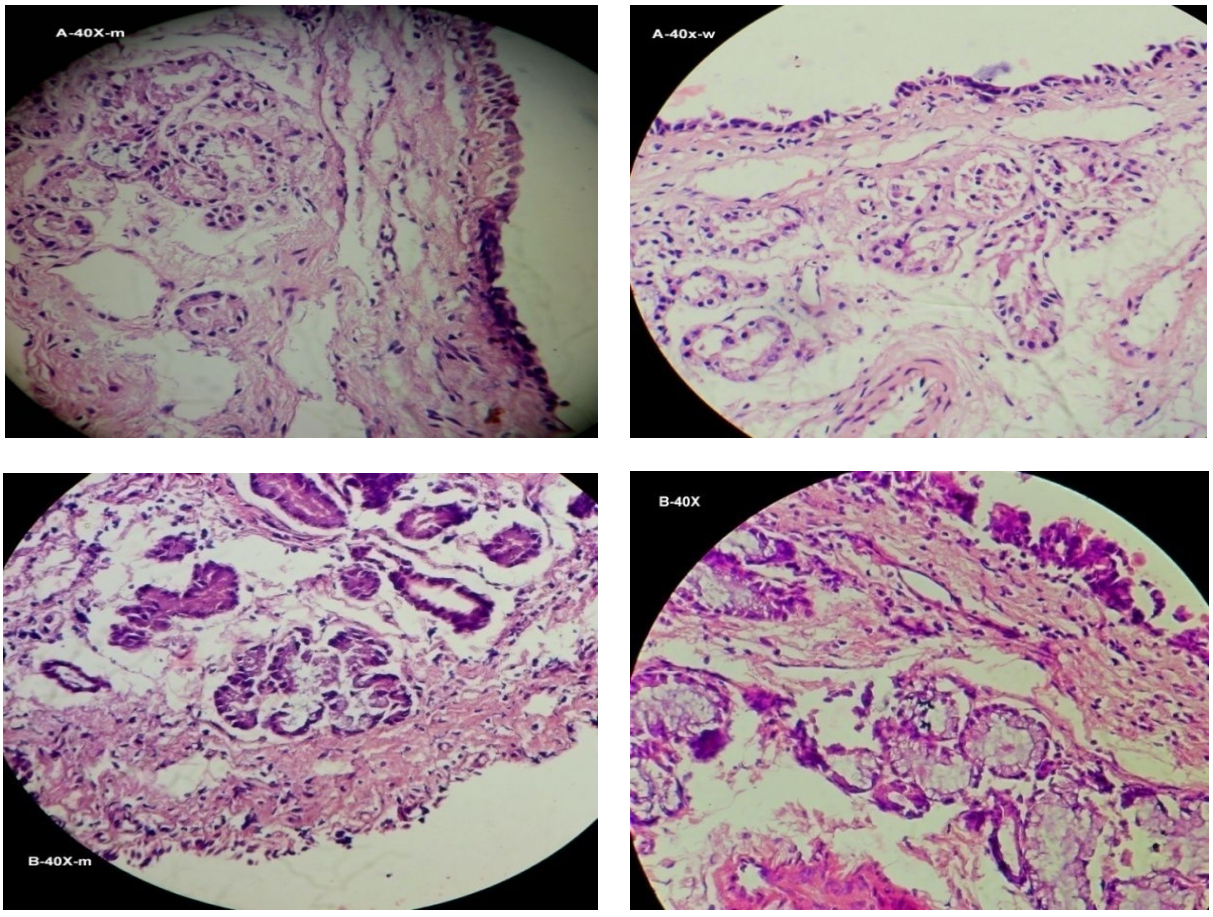

Fig. 3. Light photomicrograph of the nasal mucosa
A) Normal mucosa B) Metoclopramide HCl thermoreversible *in situ* nasal gel treated mucosa

CONCLUSION

When Poloxamers were used in combination for developing in situ nasal gel, low to moderate amounts of Carbopol 934P and Noveon AAi were used to achieve the desired gelation temperature, mucoadhesion, drug release profile and viscosity required for sustained nasal drug delivery system of MCP HCl. It was concluded that the amounts of Carbopol 934P had a significant effect on bioadhesion force, gel strength and gelation temperature of the formulated gels. Moreover, the optimized in situ nasal gel demonstrated enhanced diffusion. Nevertheless, the most prominent advantage of the in situ gel over the silent gel is that it is fluid-like prior to contact with the nasal mucosa: a feature that is warranted for convenience of administration for patients, accuracy of drug dosing and avoidance of the bitter taste of the antiemetic drug.

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REFERENCES

Badgujar SD, Manish A. Sontakke MA, Narute DR, Karmarkar RR, Tupkari SV, Barhate SD. Formulation and evaluation of sumatriptan succinate nasal in-situ gel using fulvic acid as novel permeation enhancer. *Int. J. Pharm. Res. Dev.* 2010;2(8):1-52.

British Pharmacopoeia, Vol II, British Pharmacopoeia Commission: London, 2009; 3921-3.

Choi H-G, Oh Y-K, Kim C-K. In situ gelling and mucoadhesive liquid suppository containing acetaminophen: enhanced bioavailability. *Int. J. Pharm.* 1998;165(1):23-32. [DOI: 10.1016/S0378-5173(97)00385-2]

Chouksey R, Jain AK, Pandey H, Maithil A. *In vivo* assessment of atorvastatin nanoemulsion formulation. *Bull. Pharm. Res.* 2011;1(2):10-4.

Gaikwad V. Formulation and evaluation of in-situ gel of metoprolol tartrate for nasal delivery. *J. Pharm. Res.* 2010;3(4):788-93.

Kasper DL, Braunwald E, Hauser S, Longo D, Jameson JL, Fauci AS. Harrison's Principles of Internal Medicine, 16th edition, McGraw-Hill Professional: New York, 2005; 36-76.

Khan IN, Khan MI, Mazumder K, Ajrin M, Sen N, Rashid A, Bhuiya AM. Characterization and *ex-vivo* skin permeation study of domperidone maleate transdermal patch. *Bull. Pharm. Res.* 2012;2(1):15-21.

Mahajan HS, Gattani SG. Gellan gum based microparticles of metoclopromide hydrochloride for intranasal delivery: development and evaluation. *Chem. Pharm. Bull.* 2009; 57(4):388-92. [DOI: 10.1248/cpb.57.388]

Mygind N, Dahl R. Anatomy, physiology and function of the nasal cavities in health and disease. *Adv. Drug Deliv. Rev.* 1998;29(1-2):3-12. [DOI: 10.1016/S0169-409X(97)00058-6]

Patel M, Thakkar H, Kasture PV. Preparation and evaluation of theroreversible formulations of flunarizine hydrochloride for nasal delivery. *Int. J. Pharm. Pharm. Sci.* 2010;2(4):116-20.

Satoskar RS, Bhandarkar SD, Rege N. Pharmacology and Pharmacotherapeutics, Revised 21st edition, Popular Prakashan: New Delhi, 2008; 587-8.

Schmolka IR. Artificial skin I: Preparation and properties of pluronic F-127 gels for the treatment of burns. *J. Biomed. Mater. Res.* 1972;6(6):571-582. [DOI: 10.1002/jbm.820060609]

Shastri DH, Prajapati ST, Patel LD. Thermoreversible mucoadhesive ophthalmic *in situ* hydrogel: Design and optimization using a combination of polymers. *Acta Pharm.* 2010;60(3):349-60. [DOI: 10.2478/v10007-010-0029-4]

Talegaonkar S, Tariq M, Alabood RM. Design and development of *o/w* nanoemulsion for the transdermal delivery of ondansetron. *Bull. Pharm. Res.* 2011;1(3):18-30.

Tripathi M, Radhika PR, Sivakumar T. Formulation and evaluation of glipizide hollow microballoons for floating drug delivery. *Bull. Pharm. Res.* 2011;1(1):67-74.

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