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EFFECT OF PERMEATION ENHANCERS ON PERMEATION KINETICS OF MIDAZOLAM, IN- VITRO CHARACTERIZATION

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ABSTRACT

Buccal drug delivery system has seen a veritable explosion in the past decades. In the present scenario, very few buccal patches are commercially available. Midazolam being an anti-psychotic drug requires chronic administration. Since the drug has an extensive first pass metabolism and is available in the invasive intramuscular form, an attempt was made to develop buccal drug delivery system for patient compliance. In this study, flux and permeation enhancement trials of midazolam was carried out using modified Franz diffusion cells through porcine buccal mucosa. The permeability co-efficient, flux, and enhancement ratio of drug with hyaluronidase was found to be 31.256cm/hr. 156.28 µg/cm ^{2}/hr and 2 respectively. Currently, in this article chitosan and its derivatives viz partially O- and N-Acetylated chitosan and N-Acetylated chitosan were used to study the suitability for the buccal drug delivery systems and midazolam was used as a model drug.

Keywords: Midazolam, Buccal permeation studies, Hyaluronidase. Chitosan

INTRODUCTION

Midazolam being an anti psychotic drug needs chronic administration and due to extensive first pass metabolism, the buccal drug delivery offers an advantage over a non-invasive system of permeation[1]. Currently very few patches are available commercially in the market, thus an attempt is made to formulate buccal patches. Midazolam is one of the useful antipsychotic drugs useful in the treatment of psychosis (5 mg / day) IV. It has a pKa of 6.1, log P – 3.7, oral absorption- IV, $T_{1/2}$-3 h. and Protein binding-95% [2].

MATERIALS AND METHODS

Midazolam was gift sample from Sun pharmaceuticals, Gujarat, Hyaluronidase was obtained from Charles Pharma Ltd. Bombay, Urea, Oleic acid, Sodium tauroglycholate, Dimethyl sulphoxide (DMSO) and Sodium Lauryl Sulpahte (SLS) were obtained from S.d.fine chemicals, Bombay. All the carriers and solvents used were of analytical or pharmacopoeial grade.

The solubility studies

The solubility of drugs in phosphate buffer solution of varying pH was determined at 37° ± 0.2°. An excess amount of drug was added into 20 screw-cap tubes containing 10 of phosphate buffer (pH 6.6). Tubes were constantly agitated under 150 rpm speed at 37° ± 0.2° for 24 h using a water bath. After 24 h, the solution was passed through a 0.2µ filter, and the amount of drug solubilized was estimated by measuring the absorbance at 245 nm using a UV spectrophotometer (Shimadzu 1601, Kyoto, Japan). The standard curve for drug was established in phosphate buffer (pH 6.6) and from the slope of the straight line the solubility of drug was calculated.

Preparation of n-acetyl chitosan films

The 1g of chitosan was dispersed in 50 of 10 % acetic acid and stirred until complete solubility was achieved. To the above solution ethanol was added and stirred. Gelation was induced with 1.59 mmol of acetic anhydride. The viscous gels obtained were casted to films in a mold by solvent evaporation method in a drier at 45° and stored in a desiccator until further use.

Preparation of n-o acetyl chitosan films

Chitosan (1g) was dispersed in 50 of 10% acetic acid and stirred until complete solubility was achieved. To the above solution 15.87 mmol of acetic anhydride was added, stirred and casted to films by solvent evaporation method in a drier at 45° and stored in a desiccator until further use.

Moisture content

The film was weighed and kept in desiccator containing calcium chloride at 40° and dried for at least 24 h. Then the film was weighed again and again until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight [3].

Moisture uptake

A weighed film kept in a desiccator at 40° for 24 h was taken out and exposed to two different relative humidities of 75 % RH (saturated solution of sodium chloride) and 93 % RH (saturated solution of ammonium hydrogen phosphate) in two different desiccators respectively, at room temperature and then the weights were measured periodically to constant weights [4].

Flatness

The longitudinal strips were cut out from the prepared medicated film. The length of each strip was measured. Then the variation in lengths due to the non-uniformity in flatness was measured, by measuring constriction of strips and a zero percent constriction was considered to be equal to a hundred percent flatness.

$$\text{Constriction (\%)} = \frac{L1-L2}{L2} \times 100 \quad \text{----------(1)}$$

Where L1 is initial length of each strip and L2 is final length of each strip.

Determination of mucoadhesive strength

To the mounting disk porcine buccal mucosa (freshly procured from slaughterhouse) was tied and the petridish was filled with buffer solution of pH 6.0. The polymer films were made to adhere to the disk with the help of glue. After taring the weight in the balance, the jack was raised upward until the disk rested on the mucosa. After 2 min, the jack was slowly lowered at a speed of 2mm/10 sec. The Precisa software was adjusted to graphic view and the recording was started. At a particular weight the disk got detached and the graph fell down to zero. The sample of the graph is shown in the Fig. 4. The procedure is repeated 5 times and the graph obtained was used to interpret the detaching force [5,6].

In-vitro permeation through porcine buccal mucosa

In - vitro permeation studies of drug through the porcine buccal mucosa was performed using Franz type diffusion cells at 37° ± 0.2° Porcine buccal mucosa was obtained from a local slaughterhouse and used within 3 h of slaughter. The tissue was stored in buffer at 4° upon collection. The epithelium was separated from underlying connective tissues with surgical scissors and clamped between donor and receiver chambers of the Franz-type diffusion cell. The temperature was maintained at 37° ± 0.2° using a jacket surrounding the receiver chamber that was stirred with a magnetic bead. After the buccal membrane was equilibrated with Krebs buffer solution between both the chambers, the receiver chamber was filled with fresh buffer solution (pH 6.6) and the donor chamber was charged with a known volume of drug solution (4.5 mg/). Aliquots (1) were collected at a preset time and filtered through a 0.2µ filter and the amount of drug permeated through the buccal mucosa was then determined by measuring the absorbance using a UV spectrophotometer after extracting the drug from the solution. The diffusion medium of the same volume (1), which was prewarmed at 37°, was then replaced into the diffusion cell. The experiments were performed in triplicate (n = 3) and mean value was used to calculate the permeability coefficient. Permeability coefficient (P) was calculated from the slope of graph of percentage of drug transported v/s time and shown in equation No.2-4 [4, 7 & 8.]

Extraction procedure

The sample of permeation studies was extracted with 50 µl of 25% ammonia solution in a glass tube with Teflon lined screw cap. The solution was briefly mixed, and 5ml of 1-chlorobutane was added. The tube was capped tightly. After vertical agitation for 2 min and centrifugation at 5000 rpm for 10 min, the upper organic phase was transferred to a clean conical tube and evaporated and reconstituted in 0.1N HCl and analysed spectrophotometrically.

RESULTS AND DISCUSSION

Buccal absorption study

The results revealed that midazolam could penetrate through the oral cavity. It was found that about 45.90% of the drug was absorbed through the buccal membrane in 30 min. The drug exhibited a rapid absorption during the initial 4 min followed by a uniform rate of absorption.

The partition coefficient

The partition coefficient of midazolam found to be 1.316. The varying pH range buffer, in the order of decreasing alkalinity depicts increasing ionization of drugs.

The Permeation studies

The permeability studies of midazolam in a modified Franz diffusion cell through the buccal mucosa showed that the permeability co-efficient (P) and flux of midazolam was 14.32 m/hr and 71.604µg/cm^2/hr respectively. The enhancement ratios of drug with different enhancers have been studied using modified Franz diffusion cell through porcine buccal mucosa. The permeability co-efficient (P), flux and enhancement ratio of the drug with DMSO (4%) was found to be 15.52cm / hr 77.60µg/cm^2/hr and 1.08 the permeability co-efficient, flux and enhancement ratio of drug with SLS was found to be 19.98 cm / hr. 99.94 µg/cm2/hr and 1.395. The permeability co-efficient, flux and enhancement ratio of drug with urea was found to be 28.7469cm /hr. 143.73µg/cm^2/hr and 2.007. The permeability co-efficient, flux, and enhancement ratio of drug with STG was found to be 27.16cm / h. 135.83µg/cm^2/h and 1.89. The permeability co-efficient, flux and enhancement ratio of drug with oleic acid was found to be 17.01cm / hr. 85.05µg/cm^2/hr and 1.187. The permeability co-efficient, flux and enhancement ratio of drug with IPM was found to be 15.36 cm /hr. 76.82 µg/cm^2/hr and 1.072. The permeability co-efficient flux, and enhancement ratio of drug with disodium EDTA was found to be 24.19 cm / hr. 120.99 µg/cm^2/hr and 1.689. The permeability co-efficient flux, and enhancement ratio of drug with hyaluronidase was found to be 31.256cm / hr. 156.28 µg/cm^2/hr and 2.182 the overall permeability co-efficient of midazolam, indicates that the enhancement ratio of drug with hyaluronidase was highest among the other enhancers employed. The hyaluronidase disrupts the lipid organization, making the drug permeate, the essential action increases the drug diffusion co-efficient, hence the accelerant molecule jumps into the bilayer, rotating, vibrating and translocating, forming micro cavities and hence increasing the free volume available for drug diffusion. A slight increase in free volume fraction as enhancer molecule congregates and dramatically increases the diffusion coefficient. From the above experiments it was concluded that Hyaluronidase at a concentration of 4% v/v shows good flux and enhancement ratio, hence it can be used as a good permeation enhancer.

Table 1. Formulation design of Midazolam Films containing drug 5 mg of drug with plasticizerdibutyl phthalate (30%) and 1000 units of permeation enhancer (Hyaluronidase).

Formulation	Polymeric blend	Drug mg/cm2	Ratio (w/w)	SolventSystem
MCH1	Chitosan	5 mg	1%	2%acetic acid
MCH2	Chitosan		2%	2%acetic acid
MCH3	Chitosan		3%	2%acetic acid
MCH4	N-Acetylated Chitosan		1%	2%acetic acid
MCH5	N-Acetylated Chitosan		2%	2%acetic acid
MCH6	N-Acetylated Chitosan		3%	2%acetic acid
MCH7	N & O-Acetylated Chitosan		1%	2%acetic acid
MCH8	N & O-Acetylated Chitosan		2%	2%acetic acid
MCH9	N & O-Acetylated Chitosan		3%	2%acetic acid
MCH10	N -Acetylated ChitosanPVP		1:1	2%acetic acid
MCH11	N & O-Acetylated Chitosan:PVP		1:2	2%acetic acid

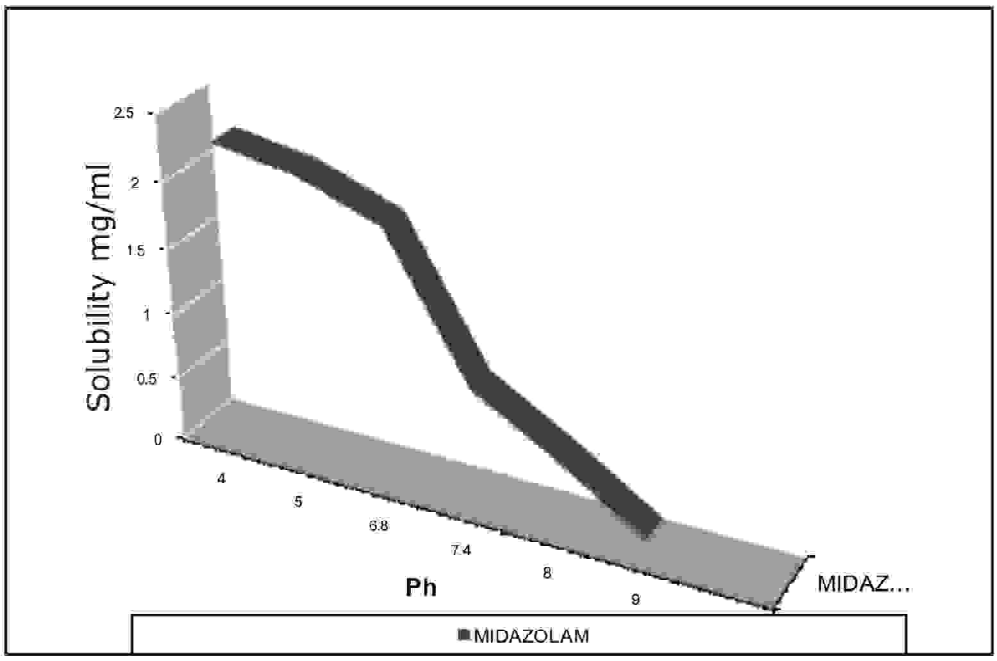

Figure1: Solubility profile of drugs at different pH conditions

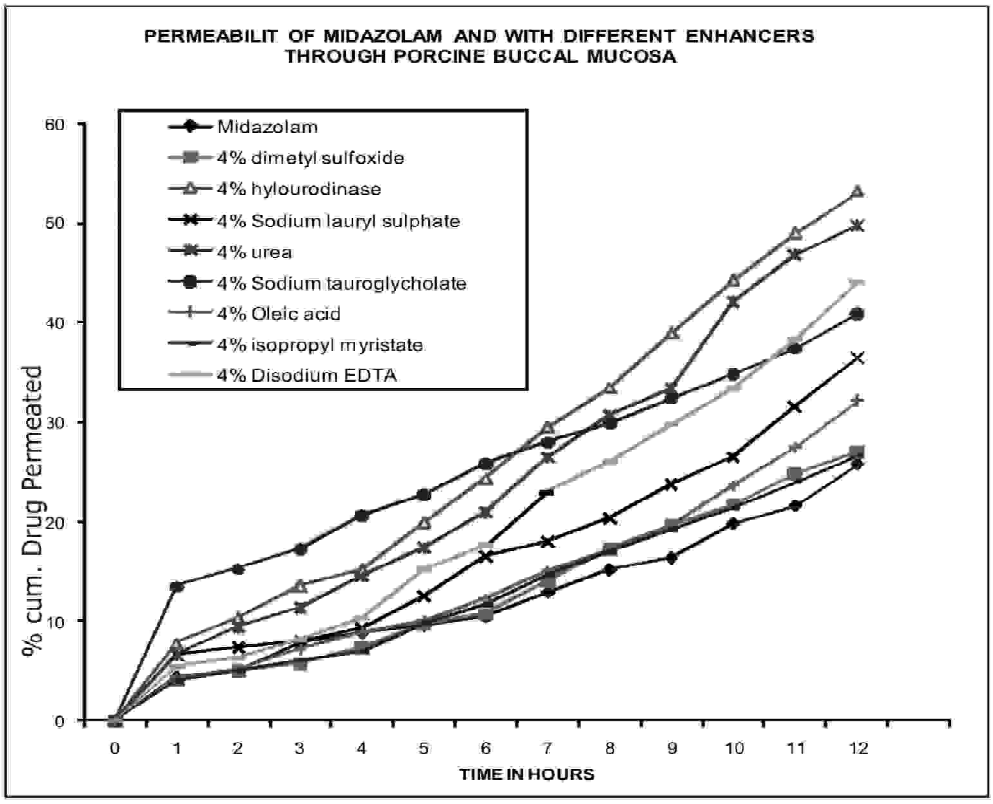

Figure 2: Prefomulation screening of midazolam with different enhancers

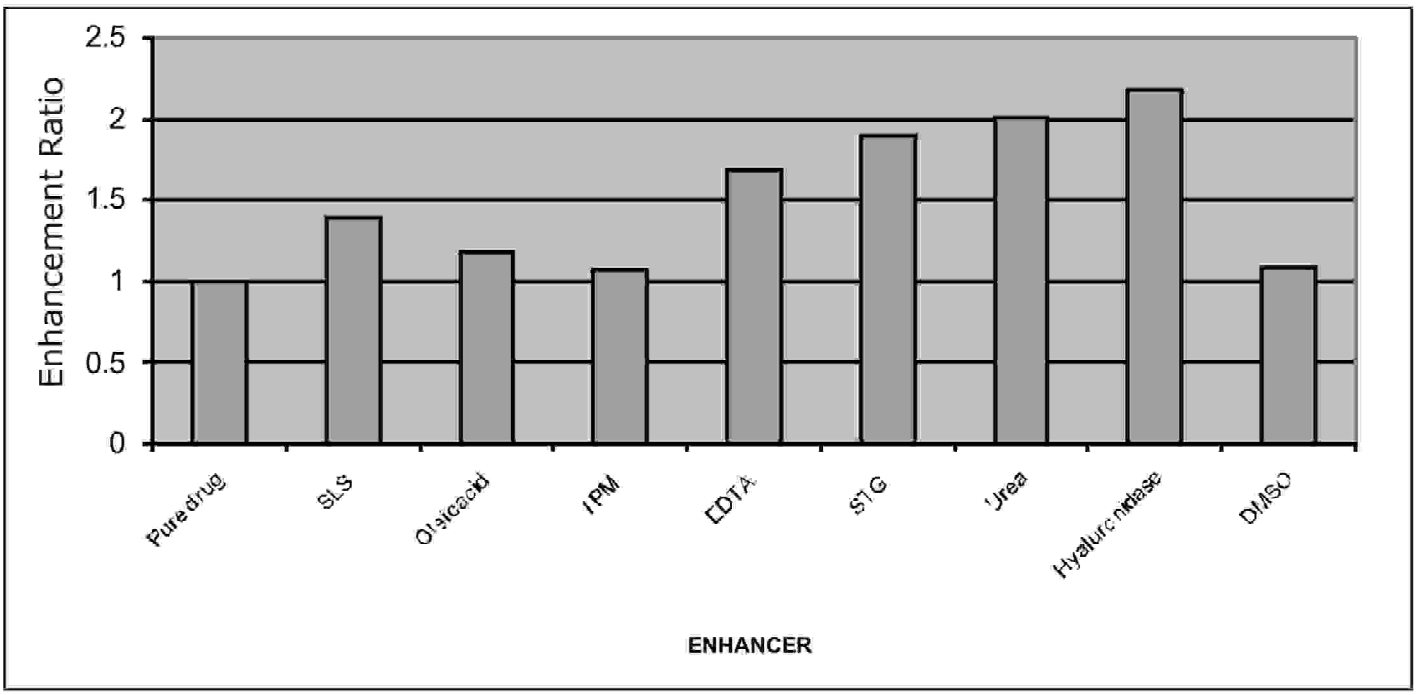

Figure 3: Effect of permeation enhancers Enhancement ratio midazolam

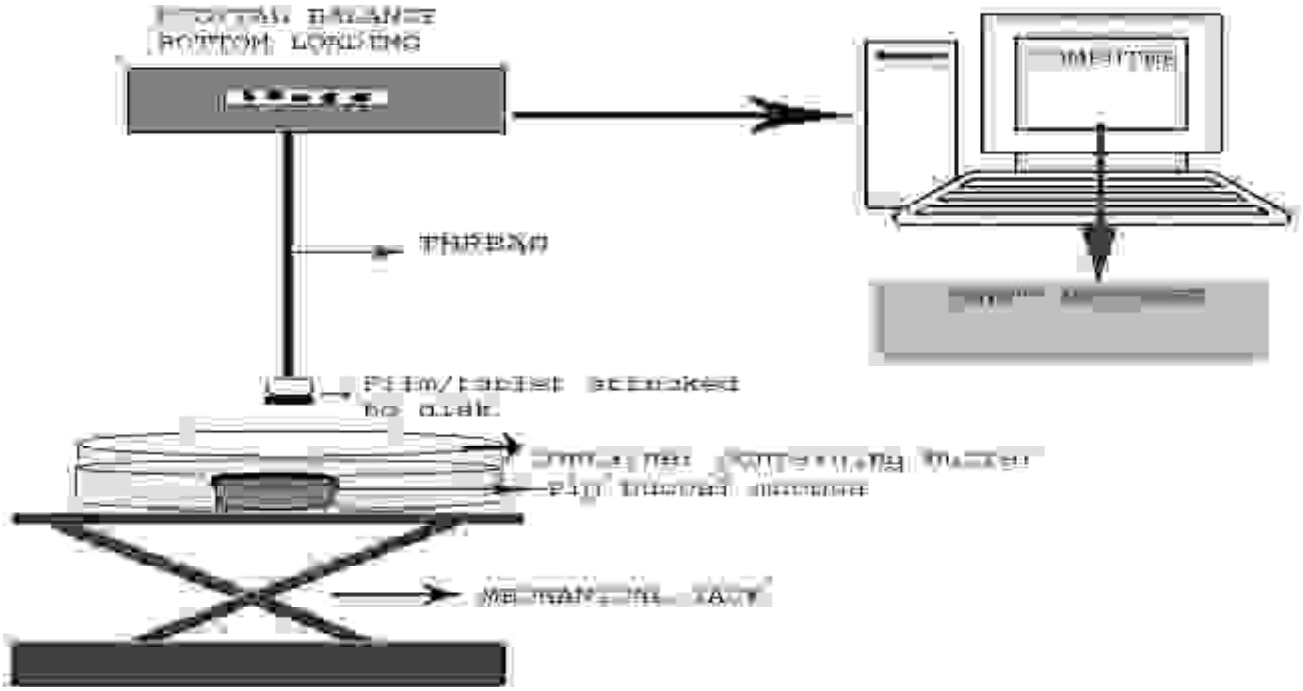

Figure4: Schematic diagram of newly fabricated mucoadhesive strength determining apparatus

Table 2: Study of physical Characterization of Midazolam polymeric films. (n=5)

Sl. no	Formulation code	% Increase by weight Moiture uptake	Thickness (n=5) (mm)	Weight variation (n=5)(mg)	Percentage of elongation (n=5)	Tensile Strength (n=5) (gm/cm^2)	Drug content (gm/cm2) $1cm^2$patch (n=3)
1	MCH1	2.876	0.22	22	100%	13.44	99.21%
2	MCH2	3.649	0.22	22	100%	13.24	99.25%
3	MCH3	3.246	0.24	25	100%	14.23	98.36%
4	MCH4	3.369	0.23	21	100%	14.22	97.52%
5	MCH5	3.215	0.23	23	100%	13.56	96.21%
6	MCH6	3.215	0.24	22	100%	13.54	99.10%
7	MCH7	3.456	0.25	23	100%	13.54	99.23%
8	MCH8	2.549	0.25	23	100%	13.54	99.25%
9	MCH9	3.215	0.23	22	100%	13.54	996.21%
10	MCH10	3.219	0.22	21	100%	13.22	96.25%
11	MCH11	3.125	0.23	23	100%	14.25	97.25%

Table 3: Study of moisture uptake (in wt%) of different formulations

Sl.No	Formulation	Relative humidity 75%	Relative humidity 93%
1	MCH1	8.215	13.564
2	MCH2	8.213	12.259
3	MCH3	8.256	13.569
4	MCH4	8.254	14.365
5	MCH5	9.213	14.25
6	MCH6	9.214	14.456
7	MCH7	9.240	14.231
8	MCH8	8.698	14.254
9	MCH9	8.985	14.254
10	MCH10	9.245	14.124
11	MCH11	9.365	14.021

Table 4: Study of mucoadhesive and duration of mucoadhesive of different formulations

Sl.No	Formulation	Mucoadhesive strength (mg.cm-2)	Duration of mucoadhesion (H)
1	MCH1	250 ±0.1	20.2 ±0.1
2	MCH2	250 ±0.1	21.2 ±0.2
3	MCH3	250 ±0.1	21.0 ±0.2
4	MCH4	350 ±0.1	21.6 ±0.2
5	MCH5	350 ±0.1	21.6 ±0.1
6	MCH6	350 ±0.1	21.8 ±0.1
7	MCH7	280 ±0.1	21.5 ±0.1
8	MCH8	280 ±0.2	21.7 ±0.1
9	MCH9	280 ±0.2	21.5 ±0.2
10	MCH10	180 ±0.2	16.2 ±0.1
11	MCH11	180 ±0.2	16.2 ±0.1

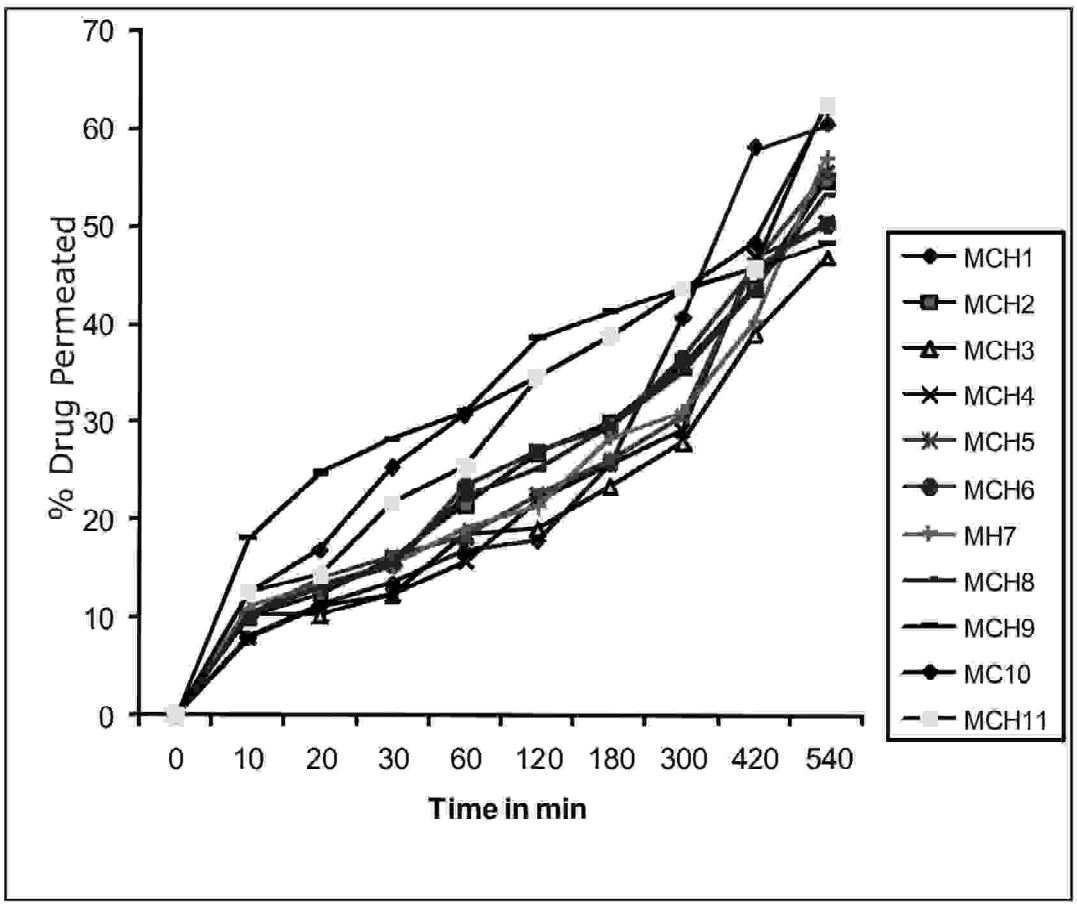

Figure 5: Profile of In-vitro permeation of midazolam from cast films through buccal mucosa

The permeability, Flux and Permeation enhancement ratio equations

Permeability coefficient (P): which is the velocity of drug passage though the membrane in.cm.h-1. The permeability coefficient was calculated from the slope of graph of percentage of drug transported v/s time as

$$P = \text{Slope} \times Vd/S \quad \text{--------(2)}$$

Vd =Volume of donor solution

S = surface area of tissue

Flux (J): Flux is defined as the amount of material flowing though a unit cross sectional barrier in unit time. It is calculated (µg/cm ^{2}/h) by,

$$\text{Flux (j)} = P \times CD \text{ -------- (3)}$$

CD -Concentration of donor solution

P- Permeability

Enhancement ratio:

Enhancement ratio was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. It is calculated by,

$$\text{Enhancement ratio} = \frac{\text{Permeability coefficient of drug with enhancer}}{\text{Permeability coefficient of drug alone}} \quad \text{----(4)}$$

In vivo bioadhesion studies

In-vitro bioadhesion characteristics of the formulated films were tested in newly fabricated apparatus and the results show that acetylated chitosan has got good bioadhesion.

The Invitro permeation of pathes

The diffusion data of most of formulation fitted well in to Higuchi release kinetics. The data fitment of the release profile was done in korsmeyer peppas model indicating values of diffusion co-efficient obtained ranging from 0.37-0.74. The mechanism of drug release in these cases were known to follow anomalous transport mechanism i.e. the drug was released by initial swelling and follows anomalous transport.

In this study most of the formulation follows the Higuchi square root release kinetic (Kh 13.50 to 16.89) and (R^2 0.981 to 0.997). The formulation shows linearity on Q versus square root of time plots, confirming square root kinetics. The release rate increased with increment of PVP, the combination at higher PVP amounts the initial release rate increased gradually, which slowed down. Normally the patches follow Higuchi's kinetics, where the permeability rate slows down as time proceeds. The drug molecule from the surface of the patch dissolves fast and hence the initial rates are high. In this system, the patch was put in the parallel combination with buccal mucosa, the buccal mucosa being rate controller. The rate decreases further in the later hours.

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