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Gastrointestinal Mucoadhesive Patch System for Oral Administration of Metronidazole

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ABSTRACT

Advancement in science and technology has leds to an evolution of controlled drug delivery as one of the important facets of novel drug delivery with an aim of designing therapeutically efficient dosage forms. With this insight an attempt was made in designing an oral patch system developed with an inspiration to mimic transdermal drug delivery system. The hypothesis involved development of a compressed patch system for achieving steady therapeutic levels of a model antiprotozoal antibiotic Metronidazole. The patch system comprises of a poorly permeable layer, a mucoadhesive layer containing drug-loaded microspheres and a backing layer. The drug content of microspheres was found to be 50% with an average particle size of 100µ. Individual layers of patch system were evaluated for folding endurance, flexibility, thickness and mucoadhesion test. Finally compressed patch system was folded and encapsulated into hard gelatin capsule, then subjected for *in-vitro* dissolution test in phosphate buffer and also for *in-vitro* diffusion across cellophane membrane and rat intestine. Drug-excipient compatibility studies revealed no interaction. The stability data further assured the stability of formulations. Thus formulations seem to match mostly gastro retentive category of sustained release forms through bio-adhesion approach concluding an easier, controlled and safer means of oral administration.

Key words: Mucoadhesion, compressed patch system, gastro retentive device.

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INTRODUCTION

Historically, oral drug administration has been the predominant route for drug delivery. However, numerous drugs remain poorly available when administered by this route. Among the other reasons, this can be due to low mucosal permeability of the drug, permeability restricted to a region of the gastrointestinal tract, low or very low solubility of the compound which results in low dissolution rate in the mucosal fluids and elimination of a fraction of the drug from the alimentary canal prior to absorption and lack of stability in the gastrointestinal environment, resulting in a degradation of the compound prior to its absorption (e.g. peptides, oligonucleotides)[1].

Oral controlled release formulation is subjected to frequently changing environments during transit through gastrointestinal tract as it passes from the strongly acidic to alkaline medium in the lower part of gastrointestinal tract. The constraints involved in design of controlled release dosage form for oral delivery are, the variable absorbing surfaces over the length of gastrointestinal tract and variable gastric transit period from individual to individual etc[2] . Medications with narrow absorption window are mostly associated with improved absorption at jejunum and ileum due to their enhanced absorption properties because of large surface area or of enhanced solubility of drug in high pH[1].

Different approaches have been proposed to prolong the residence time of delivery systems in the stomach, namely,

- The use of passage-delaying excipients (for example triethanolamine myristate);
- The utilization of specially designed dosage forms such as ‘heavy pellets’ and large single-unit delivery systems;
- Bio (muco) adhesive and buoyant forms;
- Epichlorohydrin cross-linked pectin as colon specific drug delivery carriers; and multiple-unit pellet systems (MUPS) versus enteric-coated tablets.

Several strategies to enhance the efficacy of orally administered drugs are reported. Bioadhesion is an approach for increasing interaction between drugs and mucosa[3]. Mucoadhesive patches containing 10 mg were prepared with ionic polymers SCMC and chitosan revealed uniform drug release with mucoadhesion[4]. Oral patch system of fluorescein isothiocynate dextran increased the area under the curve and mean residence time[5]. Zancog and Samir have developed a novel method based on intestinal patches for oral drug delivery offering a novel approach of oral drug delivery[6]. Eiamtraarn also have reported a new gastrointestinal mucoadhesive patch system

designed for oral delivery of protein drugs[7,6]. Formulations of oral mucosal drug delivery system for the systemic delivery of bioactive materials described by Gilles suggested the conceptual and technical approaches to the design and formulation of oral mucosal drug delivery system[9].

Presently an attempt was made towards achieving an improvement in gastric-retention of a dosage form employing bioadhesion, a novel approach that offers several advantages over simple mucoadhesive particles. The proposed system includes several layers that perform different tasks including adhesion, drug encapsulation and then protection from the surroundings. The proposed design consists of three layers; The first layer an acid resistant layer to minimize the release of the drug into gastric media, second one being mucoadhesive material containing drug loaded microspheres adheres to mucosal surface and thirdly a backing layer to render the release of drug in an unidirectional way. Metronidazole was selected as the model drug as it is effective against anaerobic bacteria, which mainly resides in gastrointestinal tract.

MATERIALS AND METHODS

Materials

Bovine serum albumin and Tween-80 were purchased from Thomas Baker, Mumbai; Carbomer-940, Hydroxy Propyl Methyl Cellulose, Methanol, Liquid paraffin and Propylene Glycol from S.D. Fine Chem. Ltd, Mumbai; Pectin, Ethyl Cellulose from Loba Chemie, Mumbai; Cellulose Acetate Phthalate from Nidilase Fine Chem. Pvt. Ltd, Mumbai; N-hexane and Acetone from Merck limited, Mumbai; Diethyl Ether and Dichloromethane from Qualigen Fine Chemical, Mumbai; Castor oil (analytical grade) and Metronidazole was supplied by Knoll Pharmaceuticals Limited, Mumbai.

Preparation of microspheres

Bovine serum albumin microspheres were prepared by w/o emulsion technique, taking gluteraldehyde as the cross linking agent. Bovine serum albumin was chosen as the carrier, as it is biodegradable and non-toxic. Its use as carrier in the formulation of microspheres is reported in literature[6]. Metronidazole was used as model drug.

Initially sets of experiments were carried out to optimize quantities of bovine serum albumin, tween 80, cross-linking time and speed of rotation. The procedure briefly involves addition of drug (1%) into various concentrations (5% to 25 %) of bovine serum albumin solution and sonicated for 2 minutes. This mixture was added to 20 ml of liquid paraffin mixture containing heavy and light liquid paraffin in the ratio of 1:1. Various concentrations (0.5 to 2%) of tween – 80 were added as an emulsion stabilizer. The mixture was allowed to mix for few minutes. 0.1

ml of 25% w/v gluteraldehyde was added and allowed for cross-linking at different speed ranging from 200 to 1200 rpm. The cross linking time was varied between 1 to 6 hour. Microspheres were evaluated for drug content analysis, particle size analysis, size and shape. The optimum quantities were taken up for further experimentation. Thus obtained microspheres were washed repeatedly with n-hexane, diethyl ether and acetone, dried and stored in an airtight container.

Preparation of components of compressed patch system

Compressed patch system consists of three layers of patch. These are:

1. Acid resistant layer
2. Microspheres containing mucoadhesive layer
3. Backing layer

Concentration of polymer and plasticizer were optimized during the preparation of patches. Patches were also subjected for Folding endurance, Thickness, Flexibility and In-vitro mucoadhesion test. Folding endurance was done manually. The patch was folded repeatedly in one place until it breaks. The thickness of patches was measured by placing the patches between two microscopic slides using a micrometer (least count 0.01 mm) and subtracting the thickness of the two glass slides measured separately and previously.

Preparation of acid resistant layer

Acid resistant layer is used in order to avoid the release of drugs in gastric media. Patches were casted by solvent casting technique [12] Cellulose acetate phthalate (CAP) was dissolved in acetone and used to prepare acid resistant layer and propylene glycol was used as plasticizer. Various concentrations of cellulose acetate phthalate (1% to 5%) and propylene glycol (25%, 50%, 75%) were used to optimize the preparation of patches. The solubility of patch in phosphate buffered saline was done to check the solubility of acid resistant layer in stomach pH. Further preparations of acid resistant layer were taken up based on formula given in Table 1

Preparation of mucoadhesive layer

Mucoadhesive layer was prepared by solvent casting technique (12). Pectin and carbopol were soaked separately overnight and mixed with the help of mechanical stirrer at 500 rpm for 1 to 2 hours. The resultant mixture was added in 0.1 ml / cm^2 area in petridish, casted and kept for air-drying. Propylene glycol was used as plasticizer. Various concentration of mucoadhesive polymer mixture (1% to 3%) with the ratio of pectin: Carbopol (1:0.5, 1:1) and propylene glycol (20%, 25% and 30%) were used to optimize the preparation of mucoadhesive layer. Folding

endurance and thickness of the patch were taken into consideration for optimization and shown in Table 1.

Table 1: Formulation of different layers of patch system

Sl. No.	Polymer concentration	Ingredients	Concentration
Formula for acid resistant layer			
1.	--------	Cellulose acetate phthalate	3 %
2.		Propylene glycol	50 %
Formula for mucoadhesive layer			
3.		Pectin	1 %
4.	Pectin: Carbopol (3 %)	Carbopol	1 %
5.		Propylene glycol	25 %
Formula for backing layer			
6.		Ethyl Cellulose	1 %
7.	EC and HPMC (6 %)	Carbopol	0.5 %
8.		Castor oil	50 %

Incorporation of microspheres into mucoadhesive layer

Pectin and carbopol were soaked separately overnight and mixed with the help of mechanical stirrer at 500 rpm for 1 to 2 hours and propylene glycol was added to it. To this mixture solution required amount of microspheres equivalent to 10 mg of drug was added and mixed by means of mechanical stirrer. The resultant mixture was added in 0.1ml / cm^2 area in petridish, casted and kept for air-drying.

Modified balance method

Fresh rat intestinal mucosa was placed on the tissue holder of the apparatus in the presence of pH 7.4 buffer saline as the medium. The formulation was applied to 1.5 gm weight disc meant to be placed in contact with the mucosa. A time span at 2 minutes was allowed for the interaction. With the help of jack the weight was slowly withdrawn to detach the disk, formulation and mucosa. The amount of mucoadhesion occurred was known from the data in terms of gram recorded by the presica bottom loading balance.

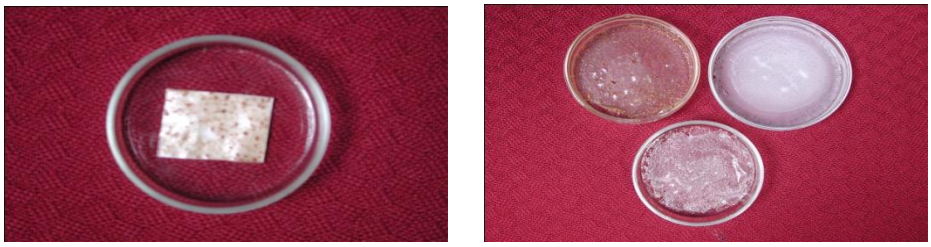
Preparation of backing layer

Patches were casted by solvent casting technique. Ethyl cellulose (EC) and hydroxy propyl methyl cellulose (HPMC) were dissolved in a mixture of methanol: dichloromethane in the ratio of 2: 1 and castor oil was used as plasticizer. The resultant mixture was added in 0.1 ml / cm^2 area in petridish, casted and kept for air-drying. Various concentrations of polymer (4%, 6%, 8%) with different ratio of ethyl cellulose: hydroxy propyl methyl cellulose (1:0.0, 1:0.5, 1:1) and castor oil (25%, 50%, 75%) were used to optimize the preparation of patches. Folding endurance, thickness and swellability of the patch in phosphate buffered saline were taken into consideration for optimization. The swellability test [14] was done by placing the prepared patch

into the phosphate buffered saline for 2 hours in a petridish. Thickness of the patch was determined before and after placing into phosphate buffered saline. Further preparation of backing layer was taken up based on formula given below in Table 2.

Preparation of compressed patch system

Acid resistant layer, mucoadhesive layer containing microspheres and backing layer were compressed together with the help of two petri plates. Figure 1 shows the patch.



(A) (B)

Figure 1: A) Components of compressed patch system B) Compressed patch system

Evaluation of microspheres

Incorporation efficiency

Incorporation efficiency of the formulation gives an estimation of amount of drug entrapped in the microspheres[10].

Drug equivalent to 10 mg were digested into 10 ml of pH 7.4 phosphate buffered saline and crushed with the help of glass rod and sonicated for 10 min. An aliquot of 5 ml was filtered through Whatmann No.1 filter paper, diluted suitably and analyzed spectrophotometrically at 321 nm against blank prepared in a similar manner using dummy microspheres.

The drug incorporation efficiency of the formulation was calculated using the formula

Efficiency = $(W_1 / W_2) \times 100$

Where W_1 – amount of drug entrapped in microspheres, W_2 – total amount of drug added

Particle size distribution of microspheres

Particle size distribution was done by optical microscopic technique. The eyepiece micrometer was calibrated. The particles were observed and measured along with an arbitrarily chosen fixed line. Totally 150 microspheres were counted and particle size was recorded.

The mean diameter of the microspheres was calculated by using the formula

D mean = ∑nd / ∑

Where n – number of microspheres observed; d – mean size range

***In vitro* release studies**

In vitro release studies for pure drug, marketed tablet and microsphere were carried out by diffusion mechanism. Initially the diffusion, studies were carried out using cellophane membrane in a modified Keshary – Chein vertical type of diffusion cell in pH 7.4 phosphate buffered saline. The receptor medium of about 100 ml was continuously stirred with a magnetic stirrer. The diffusion experiments were carried out for 12 hours. The mass transfer from donor medium to receptor medium is estimated by analyzing the aliquots withdrawn at particular intervals after suitable dilution and determined spectrophotometrically at 321 nm. and the results are tabulated in Figure 5.

Scanning electron microscopy

Scanning Electron Microscopy (SEM) is done to study the surface morphology of the microspheres using SEM model JEOL JSM-840 A. Cleaned brass specimen studs were used for mounting the samples. Wet solvent paint was applied on these brass specimen studs and while the paint was wet, the samples were placed on each stud and allowed to dry. Then the sample was put in the SEM and photographs of microspheres were taken and are shown in Figure 3.

Drug polymer interaction studies

The microspheres were tested for compatibility of the drug and polymer. This is to establish that the therapeutically active drug has not undergone any change or reaction during formulation. This was tested by carrying out infrared light absorption scanning spectroscopy studies.

Stability studies

The drug loaded microspheres were stored at different storage conditions such as room temperature, 37°C, 450C, 75% RH for a period of 4 weeks in Newtronic, Temperature / humidity control oven - QLH 2004. Physical stability was analyzed by appearance and chemical stability by the change in the drug content. The samples were withdrawn at weekly intervals and the drug content was analyzed spectrophotometrically at 321 nm. The results are given in Table 2.

***In vitro* mucoadhesive test**

Test for mucoadhesion is a measure of interaction of formulation to the mucosa, to impart a longer residence time for the formulation at the site of action or absorption. There are many reported methods to know the mucoadhesion in terms of force. The following method was adopted to test the mucoadhesion of the formulation[13].

***In vitro* evaluation of compressed patch system**

Prepared compressed patch was subjected for diffusion studies. Preliminary diffusion studies were carried out using rat intestine in a modified Keshary – Chein vertical type of diffusion cell

in phosphate buffered saline. Aliquots withdrawn at predetermined intervals and analyzed spectrophotometrically. The percentage cumulative release is reported in Figure 6.

Final dosage form

Final formulation was subjected for in-vitro dissolution testing using USP tablet dissolution apparatus Type1 The compressed patch system was folded nicely so as to encapsulate into a hard gelatin capsule containing 1.2 mg of metronidazole as loading dose and is shown in Figure 2.

Figure 2: Compressed patch system in a capsule

***In vitro* dissolution of capsule**

Dissolution test was performed for 12 hours using phosphate buffered saline 100 ml maintained at 37^0C at 100 rpm. The aliquots were withdrawn at fixed time intervals and replaced with medium and were analyzed spectrophotometrically after appropriate dilutions at 321 nm. The results are reported in Figure 4.

RESULTS AND DISCUSSION

The oral patch system offers several advantages over standard oral sustained release formulations by offering high surface area and entire mass of patch adhered to intestinal wall, this fact would be advantageous in overcoming the limited contact areas as observed in case of microspheres. Thus the patch show improved concentration gradient for the drug transport.

The patch is designed with three layers sandwiched together to form a compressed patch system. The initial one being acid resistant layer followed by mucoadhesive layer and a backing layer. The first layer being resistant to dissolve in acidic conditions of stomach blocks the drug being released into the gastric medium thus protecting the drug from acidic media. The second layer being mucoadhesive will strongly adhere to intestinal mucosa after the dissolution of acid resistant layer into intestinal medium and the third layer being protective layer minimizes the back diffusion of drug into intestine by maintaining unidirectional release of drug and also blocks the diffusion of enzymatic penetration into the patch system there by offering a greater protection for the drug. These patches are sandwiched together to formulate a compressed patch

system, which can be encapsulated into a hard gelatin capsule and the drug would be expected to release from flat surface of patch system soon after the disappearance of acid resistant layer into the GI tract.

In the preparation of microspheres as the concentration of bovine serum albumin increases the incorporation efficiency also increases but the solubility of bovine serum albumin in water reaches saturation, thus bovine serum albumin concentration was optimized as 25%. Increase in albumin concentration tends to produce a smaller microspheres and higher concentration had the added advantage of yielding a greater quantity of microspheres / batch [15.] SEM photographs of microspheres were taken and are shown in Figure 3.

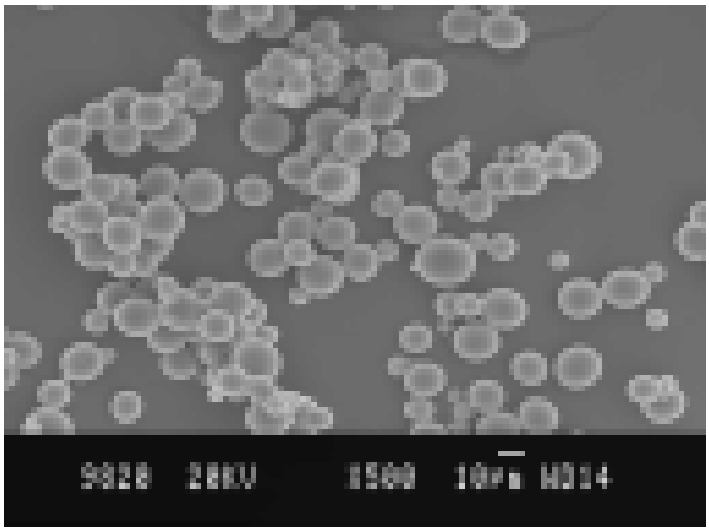


Figure 3: SEM of drug loaded microspheres (magnification – 500x)

As the concentration of tween – 80 increases the drug encapsulation efficiency also increases and at 2 % of concentration, the microspheres formed lumps. Thus tween–80 concentration was taken as 1%. Tween 80 is a non-ionic surfactant and is added as a solubilizer. Usually bovine serum albumin upon addition of gluteraldehyde shows surface deformities. Such defects are reduced by using tween 80 [16].

When cross-linking time was increased incorporation efficiency also increased. There was no change in incorporation efficiency when the time was increased from 4 hours to 6 hours, but at lower cross-linking time the incorporation efficiency was low. Thus cross-linking time was taken as 4 hours. For better engineering and the performance of microspheres, cross-linking time is a factor that should be taken in to consideration. As the cross linking time is increased a narrow size range can be obtained [17].

With increase in speed of rotation finer particles were obtained. So the speed of rotation was fixed as 1200 rpm for further studies. An increase in the speed of rotation resulted in decrease in

size of the microspheres. This will also help to increase the dissolution rate of drug loaded within the microspheres [15].

After various trials it was observed that bovine serum albumin offer good discrete free flowing microspheres at a concentration of 25%, 1% of tween – 80, 0.1 ml of gluteraldehyde 25% w/v, with cross linking time of 4 hours and 1200 rpm as the speed of rotation. The incorporation efficiency of drug in microspheres was found to be between 31.0 to 50.0 %. As the polymer concentration increases the incorporation efficiency was found to be better, this may be due to drug contact with polymer (Table 2) The cross linking time increases the incorporation efficiency up to 4 hours. Albumin microspheres prepared using gluteraldehyde exhibit grater structural strength and durability [18] and may maintain the shape and integrity of the microspheres.

Table 2: Physical stability and drug content of formulations

Formulation	Temp.	Physical Stability (%)					Drug Content (%)				
		No. of weeks					No. of Weeks				
		0	1	2	3	4	0	1	2	3	4
Metronidazole	RT	No Change in shape and appearance					99.9	98.4	98.2	98.1	97.1
microspheres	37°C	No Change in shape and appearance					99.9	98.5	98.2	97.4	97.1
	45°C	No Change in shape and appearance					99.9	98.2	97.3	97.2	97.2

Particle size distributions of the microspheres were found to be between 16.0 to 112.0 µm. Very fine particles with uniform size distribution were obtained when the speed of rotation was 1200 rpm. Albumin acts as an emulsifying agent and thus its concentration might affect the droplet size and stability [15].

During release study of microspheres, the microspheres showed initial burst release. This may be due to adsorbed drug on the surface of the microsphere. Higuchi plot is linear showing that the mechanism of the release is by diffusion. Initially, the release pattern is controlled by diffusion but gradually taken up by dissolution of drug in the medium. Albumin microsphere forms a loose texture, which may swell faster in medium, causing first a burst release and a constant release of drug diffusion from the microspheres [19.]

It can be seen from SEM of empty and drug loaded microspheres, that drug unloaded microspheres has a very smooth spherical surface, but the incorporation of the drug causes formation of slightly irregular microspheres, with changes in surface characteristics. This may be due to higher amount of cross linking time. Bovine serum albumin upon addition of

gluteraldehyde shows some surface deformities. But such defects are reduced by using non ionic surfactant stabilizer like tween 80 [15].

IR studies show that the main peaks of the metronidazole remain the same in formulation and no unaccountable extra peaks were observed. These observations indicate the intactness of the drug in the formulation thus proving that no interaction exists between drug and excipient. Stability studies revealed that there was no considerable change in the drug content and the physical stability of the formulations. Hence the formulations were found to be stable.

During casting of acid resistant layer, as the concentration of cellulose acetate phthalate increases, the flexibility of patch increases, thickness also increases solubility of patch in phosphate buffer saline decreases. Thus patch prepared by using 3% cellulose acetate phthalate was considered to be better. Similarly as the concentration of plasticizer increases, the flexibility of patch was increased, but if the plasticizer concentration increases to more than 50%, there was no change in flexibility. Thus propylene glycol concentration was fixed as 50%.During casting of mucoadhesive layer, as the polymer concentration increases, thickness of polymer solution increases but gradually pourability from the measuring cylinder decreases. Thus patches were prepared by using 3% of polymer concentration and patch prepared by using pectin: carbopol 1:1 was optimized. Similarly by increasing propylene glycol concentration, there is no change in flexibility. Thus patch prepared by using propylene glycol 25% was fixed. It is seen from in vitro mucoadhesion test of bioadhesive layer that mucoadhesion property of bioadhesive layer is 22.6 gm. During casting of backing layer, as the polymer concentration increases, thickness of patch increases and flexibility of patch increases. But desired thickness of patch was got by using 6% of polymer. Thus patch prepared by using 6% of polymer concentration was used further. As the concentration of HPMC increases, swellability of patch in phosphate buffered saline increases. Thus patch prepared by using EC: HPMC 1:0.5 was preferred. Similarly as the concentration of plasticizer increases, the flexibility of patch also increases but if the plasticizer concentration increases more than 50%, there is no change in flexibility. Thus castor oil concentration used was 50%.

In-vitro dissolution testing for final dosage form was carried out in 100ml of medium (Phosphate buffered saline pH 7.4 and the final dosage form containing 1.2mg of drug as loading dose(Figure 4)

The interesting and noticeable fact would be the plasma concentrations of metronidazole are proportional to the dose administered. It has been reported that metronidazole given orally as 250 mg, 500 mg, or 2000 mg produced peak plasma concentration of 6 μg/ml, 12 μ/ml and 40 μg/ml

respectively. This observation suggested us that loading dose given should be able to produce the same peak plasma concentration in vitro as was stated in vivo. In this regard since the volume of in vitro dissolution medium was 100 ml and in order to achieve peak plasma concentration of 12 µg/ml a 1.2 mg of loading dose is added so as to achieve 12 µg/ml, the same would be maintained by the subsequent release from the compressed patch system for a prolonged period of time.

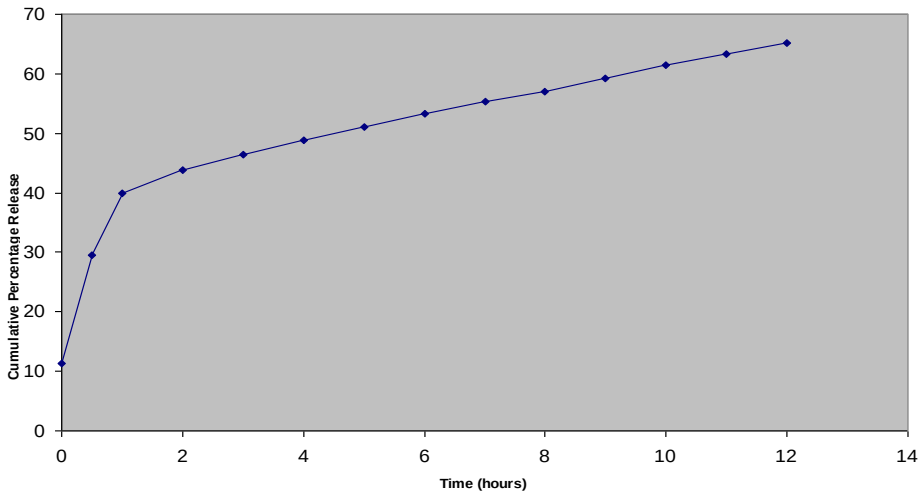


Figure 4: *In-vitro* release profile for final patch system from a capsule dosage form

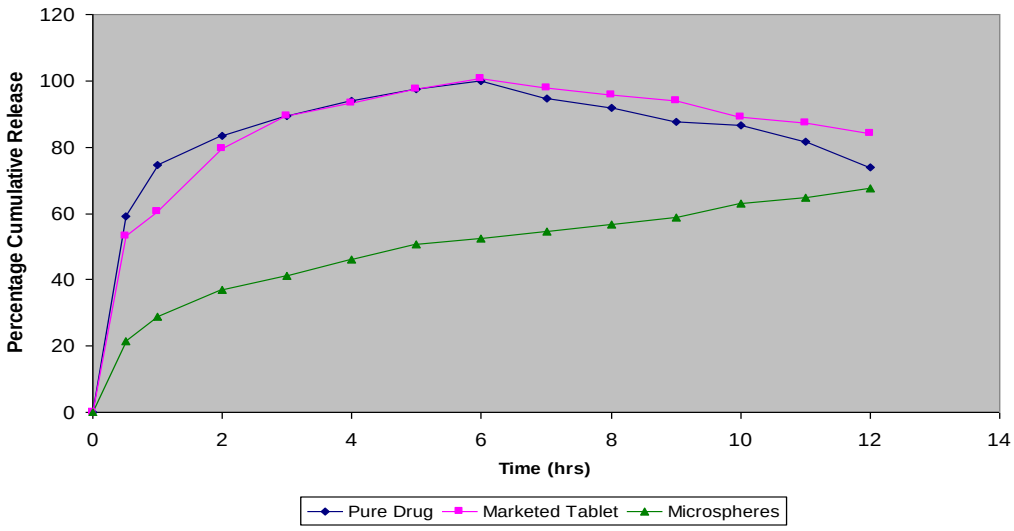


Figure 5: Comparative release profile of pure drug, marketed tablet and microspheres

The dissolution rate for the patch was found to be in a controlled manner releasing 65 % of drug up to 12 hours. Carbopol was formulated in to the drug-containing layer [20], thus they retain drug in close contact with mucosal membrane for long periods of time [21].The carbopol form gel structure in the small intestine and consequently, adhesion to the intestinal wall will prolong the residence time of metronidazole patches. Thus three-layer patch system can be applied to a

macromolecular drug to increase the retention and transit in the gastro intestinal tract. Comparative release profile of pure drug, marketed tablet and microspheres was shown in figure 5.

Preliminary diffusion studies were carried out using rat intestine in a modified Keshary – Chein vertical type of diffusion cell in phosphate buffered saline. The percentage cumulative release is reported in Figure 6.

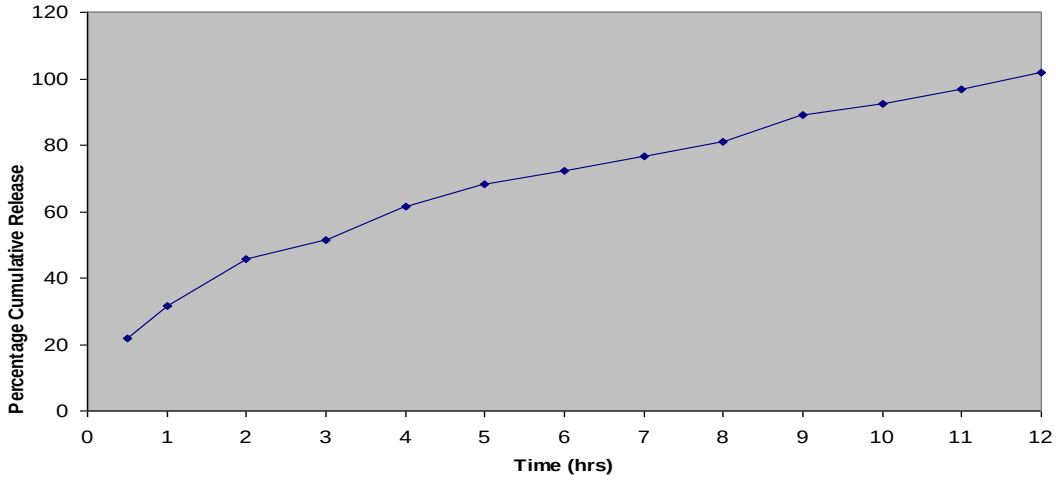


Figure 6: *In vitro* release profile of patch containing microspheres thorough rat intestine

CONCLUSION

The formulation presented offered greater advantages fulfilling all the requirements of a gastro retentive system employing bioadhesion approach. The system was also found to be suitable for the oral usage of drugs, which are susceptible to GI enzymes and those, which undergo hepatic metabolism. The formulations of such system ended up with a conclusion of its adaptability and suitability towards an efficacious oral sustained release system.

REFERENCES

1. Moes AJ. Gastric Retention System for Oral Drug Delivery. Business Briefing PharmaTech. 2003; 157-159.
2. Eytan A, Klausner, Eran Lavy, Michael Friedman, Amnon Hoffman. Expandable gastroretentive dosage forms. J Controlled Release. 2003; 90: 143-162.
3. Lavelle EC. Targeted delivery of drugs to the gastrointestinal tract. Critical review and Therapeutic Drug Carrier Systems. 2001; 18; 341-86.
4. Noha, A, Naffe, Fatima A, Ismail, Nabila A, Boraie, Lobna M, Mortada. Mucoadhesive buccal patches of miconazole nitrate: in-vitro/in-vivo performance and effect of ageing. International Journal of Pharmaceutics. 2003; 264: 1-14.

5. Sudarat Eamtrakarn, Ramprasad,YY, Shivanand P, Puthil, Yukako Yoshikawa, Nobuhito Shibata, Kanji takada. Possibility of a patch system as a new oral delivery system. Int J Pharma 2003; 250: 111-117.
6. Zancong Shen, Samir Mitragotri. Intestinal Patches for Oral Drug Delivery. Pharma Res 2002; 19(4): 391-395.
7. Eiamtrakarn S, Itoh Y, Kishimoto J, Yoshikawa Y, Shibata N, Murakami M, Takada K. Gastrointestinal mucoadhesive patch system (GI-MAPS) for oral administration of G-CSF, a model protein. Biomaterials. 2002; 23: 145-152.
8. Sudarat eamtrakarn, Ramprasad YY, Shivanand P, Puthil, Yukako Yoshikawa, Nobuhito Shibata, Kanji takada. Evaluation of gastrointestinal transit characteristic of oral patch preparation using caffeine as a model drug in human volunteers. Drug metabolism and Pharmacokinetics. 2002; 17: 284-291.
9. Gilles Ponchel. Formulation of oral mucosal drug delivery systems for the systemic delivery of bioactive materials. Advanced Drug Delivery Reviews. 1994; 13: 75-87.
10. Thanoo Chithambara B, Sunny MC, Jayakrishnan A. Cross-linked microspheres: preparation and evaluation as a matrix for the controlled release of pharmaceuticals. J Pharm Pharmacol 1992; 44: 283-286.
11. Willard Hobart H, Merritt Lyne L, Dean John A, Settle Frank A. Instrumental Methods of Analysis. 7th edition, New Delhi: CBS publishers and distributors; 1986: 287-316.
12. Kok Khaing Peh, Choy Fun Wong. Polymeric films as vehicle for buccal delivery: Swelling, mechanical and bioadhesive properties. J Pharm Pharma Sci 1999; 2: 53-61.
13. Chickering DE, Mathi Owitz E. Bioadhesive microspheres: a novel electronic balance based method to study adhesive interaction between individual microsphere and intestinal mucosa. J Controlled Release. 1995; 34: 251-261.
14. Lalla JK, Gurnancy RA, Polymer for mucosal delivery swelling and mucoadhesive evaluation. Indian Drugs. 2002; 39: 270-276.
15. Gallo JM, Hung CT, Perrier DG, Analysis of albumin microsphere preparation. Int J Pharma 1984; 22: 63-74.
16. Saleh AM, El-Khordagui LK, Robb DA, Florence AT. Effect of some drugs and additives on the cross-linking of Bovine Serum Albumin by glutaraldehyde. Int J Pharma 1989; 57: 205-210.

17. Eberth K and Merry J. A comparative study of emulsions prepared by ultrasound and by a conventional method: droplet size measurements by means of a coulter counter and microscopy. Int J Pharma 1983; 14: 349-353.
18. Sheu MT and Sokoloski TD. Entrapment of bioactive compounds within native albumin beads: III. Evaluation of parameters affecting drug release. J Parent. Sci Tech 1986; 40: 259-265.
19. Chandy T and Sharma CP. Chitosan beads and granules for oral sustained delivery of nifedipine: in vitro studies. Biomaterials. 1992; 13(13): 949-952.
20. Machida Y, Masuda H, Fujiyama N, Ito S, Iwata M, Nagai T. Preparation and phase II clinical examination of topical dosage form for treatment of carcinoma coli containing bleomycin with hydroxypropylcellulose. Chem Pharm Bull 1979; 27: 93-100.
21. Lee JW, Park JH, Robinson JR, Bioadhesive-base dosage forms; the next generation. J Pharma Sci 2000; 89: 850-866.