Colon Targeting of Theophylline from Enzyme-Dependent Release Tablet Formulations- *in vitro* Evaluation

Safaa S. El Gamal, Viviane F. Naggar, Magda S. Sokar*

Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

*Corresponding author:

Tel.: +203-48-71317; Fax: +203-48-7327

E-mail address: magdasokar@yahoo.com (Magda S. Sokar)

Abstract

The major challenges in targeting drug to various parts of the colon include control of drug release according to its environment & transit time.

The aim of the present study was to develop colon-targeted single unit theophylline (TH) formulations using natural biodegradable polymers, employed for the treatment of asthma, a diurnal rhythm disease to increase its specific systemic absorption in the colon.

The drug tablet core (Tc) containing TH, Dextran c & Avicel (2:1:2) was prepared. Tc was compressed coated with pectin in different core : coat ratios; or with ehylcellulose alone or its mixture with pectin. or with HPMC and/or chitosan in addition to pectin.

The drug release studies were carried out in 0.1 N HCl and at pH 6.8. on a selected formulation (F2). Swelling studies were performed. F2 rate of release was very appropriate in acid medium and 0.8% in the following 3 hours at pH 6.8. After 5 hours, a gradual and almost complete release occurred in absence of pectinase enzyme & showed an increase in the rate of TH released in the presence of 1mg/ml pectinase enzyme. Colon arrival time of F2 was measured in dogs and a healthy volunteer

Keywords: biodegradable polymers, diurnal rhythm, compressed-coated, pectin chitosan & pectinase

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Introduction

The colon specific drug delivery system (CSDDS) should be capable of protecting the drug in route to the colon i.e. drug release and absorption should not occur in the stomach as well as in small intestine, and only released, absorbed once the system reaches the colon (1).

Colon targeting can be very helpful for many pharmaco-therapies, including the treatment of inflammatory bowel diseases, such as Crohn's Disease (CD) and Ulcerative Colitis (UC) (2). Specific systemic absorption in the colonic region also offers interesting possibilities for the treatment of diseases susceptible to diurnal rhythm such as arthritis, inflammation or asthma (3-4). In this context, colon

targeting is of special value in achieving the desired aim of the prevention and therapy of nocturnal asthma where a delay in drug absorption is required (5)

For colon targeting, the drug may be either embedded within a polymeric matrix or drug-loaded tablets or pellets are coated with a polymeric coat (5) or film (6).

Site-specific targeting of drugs to the colon has been attempted by several approaches (7). Of these, is the utilization of enzymes produced by the bacteria residing exclusively in the colon for obtaining site specific delivery to the colon using polysaccharides which degrade by the colonic bacteria (8-9). This strategy seems more promising due to the abrupt increase of bacterial population in the colon and the associated enzyme activity which is independent of gastrointestinal transit time (10). Furthermore, systems exploiting this unique feature of the colon will also achieve better site-specific initial drug release (11).

Among such polymers, pectins, hydrophilic polysaccharides derived from plant cell walls mainly consisting of partially methoxylated poly α-(1-4)-D-galactouronic acids (12), have attracted an increasing attention due to their low cost, wide availability, variety of types & flexibility in use and since most of the synthetic polymers are immunogenic (12-13).

The potential of pectin as a carrier for colonic drug delivery has been demonstrated previously (3,14). Pectin is highly soluble in water, a fact that puts hurdles in the development of colon targeted drug delivery systems. If used alone, it swells when it comes in contact with aqueous fluids of GIT and causes the release of the entrapped drug through diffusion (3).

This problem can be manipulated by using a coat of considerable thickness applied by compression (3,15) or by combination of pectin with ethyl cellulose (EC) taking the advantage of the latter's insolubility over the whole pH range of gastrointestinal tract (15).

Incorporation of HPMC into a pectin matrix was shown to protect the core up to 6 hours in a ratio of 1:4 (16). Also, the potential of polyelectrolyte complex formation between pectin and chitosan to decrease pectin solubility was also tested (3). Films composed of pectin: chitosan: HPMC were tried as well (8).

It was reported that TH absorption from all parts of GIT is rapid and almost complete (17-18). This is probably due to its good partition properties, being to a great part in the unionized form at all the pHs of the GIT (19-20). TH is a weakly acidic compound, pk_a = 8.6 and is also very weakly basic, pk_a = 2.5 as shown from its chemical structure (Figure 1) (21).

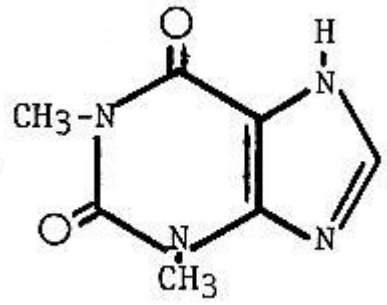


Figure 1: Chemical structure of Theophylline

The effect of food on TH absorption can be cancelled through administration of colon targeted drug delivery. Also, nausea and gastric irritation symptoms that accompany the oral administration of TH and its salts no longer exist (22-23).

Purpose

The aim of this study is to develop and evaluate single-unit sustained release formulations of TH intended for colon targeting using an inexpensive, highly safe, abundantly available, biocompatible &

biodegradable polysaccharide from natural source such as pectin, due to its ability to act as a specific substrate for the colonic microflora, showing minimal release of TH in the upper GIT & controlled release in the colon. The prepared tablet formulations are employed for the treatment of asthma, which is a disease susceptible to diurnal rhythm to increase its specific systemic absorption in the colon. TH was used since it is well absorbed in the large intestine in humans and its both anti-asthma activity & pharmacokinetic properties make it an interesting candidate for such kind of modified-release preparations.

Materials and Methods

Materials

Anhydrous theophylline powder (TH), Boehringer Ingelheim KG, Ingelheim , Germany, Dextran grade C (Dc) of molecular weight 60,000 - 90,000; Biochemical, BDH Chemical Ltd., Poole, England, Microcrystalline cellulose of chromatographic grade, (Cellulose D. Avicel-mikrokristallin (MCC), Riedel-De Haenag Seelee-Hannover). High methoxypectin (degree of methoxylation, "DM" 70%), Unipectine UHM73C, Degussa Texturant Systems, France, SAS, Chitosan (CS), $C_{30,}$ 85.87% degree of deacetylation, Saniver Ltd, Hong Kong**.,** Ethylcellulose (EC), ethoxy content ranges from 48 – 49.5%, premium type fine particles, BDH Chemicals Ltd., Poole, England, Hydroxypropylmethylcellulose (HPMC 4000) (Methocel K4M), BDH Chemical Ltd., Poole, England, Pectinase**,** from Aspergillus niger 1.10 u/mg, Fluka AG, Germany and Barium sulphate ($BaSO_4$), Sanochemia Diagnostics Deutschland GmbH, Stresemannallee 4C, D-41460 Neuss (Germany).

All other chemicals were of analytical or pharmaceutical grade.

Methods

Preparation of theophylline tablet core and compression coated tablets

All powders were sieved and fractions corresponding to particle size range 71µm-1.25mm were used. The tablet core was prepared by direct compression of geometrically mixed powders of the drug with excipients by means of 6 mm round flat faced punches. Before compression, a content uniformity test was done on a suitable number of samples accurately weighed taken from the powder mix to contain the equivalent of 500 mg TH. The composition of the tablet core is given in Table 1.

Table 1: Composition of theophylline tablet core

Ingredient	Quantity (mg)
Theophylline	**50**
Dextran C	**25**
Microcrystalline cellulose	**50**

The tablet core was submitted to quality control tests before it was compressed coated with other different excipients, alone or in mixtures (Table 2) using 12 mm concave faced punch. The compression coated tablets were also submitted also to quality control tests

Table 2: Composition of coats for different theophylline coated tablet formulations

Formulation	Ingredient (mg)			
	Pectin	Ethyl cellulose (EC)	Chitosan (CS)	HPMC 4000
F1	375	-	-	-
F2	500	-	-	-
F3	625	-	-	-
F4	-	375	-	-
F5	188	187	-	-
F6	225	150	-	-
F7	150	225	-	-
F8	341	-	34	-
F9	225	-	75	75
F10	281	-	-	94

In vitro release studies

Studies were carried out on 2 tablets (50 mg TH each) using USP dissolution apparatus I (Six station dissolution apparatus, Hanson Research Corp., Northridge California, USA), at 37± 0.5°C.The dissolution medium, stirred at 50 r.p.m., consisted of 730 ml 0.1N HCl for 2 hours, followed by a buffer of pH 6.8 adjusted by the addition of 270 ml of 0.2M tri sodium phosphate (5,24) for 25 hours. Samples (5ml) were withdrawn at predetermined time intervals, compensated with fresh dissolution medium and assayed spectrophotometrically at 271nm in 0.1N HCl and 272nm in buffer (pH 6.8) (UV visible spectrophotometer, model UV-1601 PC (Shimadzu, Japan). The ability of some selected compression coated tablet formulations of TH to release the drug in the physiological environment of the colon was assessed by performing the release in phosphate buffer solution at pH 6.8 containing 1mg/ml pectinase enzyme added since the 6^{th} hour.

In all drug assay methods mentioned, no interference occurred due to any tablet excipients or enzyme.

Swelling studies

The selected tablet formulations were subjected to swelling studies at 37± 0.1^0C. The weight of the tablet was determined (W_1). Each tablet was placed separately in a 25 ml beaker containing 7.3 ml 0.1N HCl for 2 hours, then 2.7 ml 0.2M tri sodium phosphate (to adjust pH of the medium to 6.8) were added. Tablets were removed at different time intervals (2, 5, 8, 12, 24 and 28 hours), wiped with filter paper and reweighed (W_2). The swelling index was calculated as follows:

$$\text{Swelling index} = W_2\text{-}W_1/W_1 \quad \text{.....Equation (1)}$$

Measurement of Gastrointestinal transit time

Animal experiments

Experiments were carried out in compliance with the regulations of the Committee for animal experiments, Alexandria University. Four adult male dogs (1.5-2 years), weighing between 12 and 20 Kg were fasted for 24 hours prior to the experiment. Three tablets of the selected formulation were administered orally to each dog.

The dogs were killed at the designated time (1, 2, 4, 5 and 6 hours) following administration, an incision was made along GIT and the tablets were photographed using a digital camera (Canon, IXUS 86015, China) after determining their location in the gastrointestinal tract at each time interval. The time of arrival to the colon was also detected.

X-ray photographing

The experiment was performed on a healthy non-smoker male subject (21 years old, body weight 75 kg, height 165 cm) who was not on any medication. The study followed the ethics for treatment of human volunteers according to the guidelines of the Ethical Committee of Alexandria University Hospital. After assurance that the subject understood the aim of participation in the study, he signed an informed consent.

The selected tablet formulation was dipped several times in a concentrated aqueous suspension of barium sulphate, and left to dry at room temperature for 48 hours.

After an overnight fast (10-12 hours) to standardize the conditions of gastrointestinal motility, the subject swallowed the tablet with 50 ml of water. He remained lying in supine position during most of the study and X-ray scanning to the abdominal region was carried out at different time intervals (0, 1, 3.5 , 5, 6 and 7 hours) to follow the location of the tablet.

Differential scanning calorimetry (DSC)

Thermal analysis by DSC (using Differential Scanning Calorimeter, Perkin Elmer DSC 6 thermal analyzer, supported with PYRIS software version 3, 8, USA) was carried out on TH, pectin, physical mixture (PM) of TH with the excipients of the tablet core and PM of the tablet core components with pectin.

Samples (20 mg) were weighed in flat bottom aluminum pans. Temperature calibration was made using indium as a standard. An empty pan, sealed in the same way as the sample, was used as reference. All samples were run at a rate of 10^{o}C/min, from 40^{o}C to 400^{o}C, in an atmosphere of nitrogen.

IR analyses

Samples were mixed with KBr and compressed into discs using a hydraulic press under pressure of about 10^{5} N (Perkin Elmer spectrum RXIFT-IR system (Perkin Elmer instruments, USA). The spectra were recorded over a range of 4000-500 cm^{-1}.

Effect of ageing

The selected tablet formulation was stored in airtight containers, at room temperature in a desiccator for 3, 6 and 12 months. Tablets were assessed for any change in physical properties and drug release. The release study was done using the same conditions mentioned above.

Statistical data analyses

Statistical data analyses were performed on the selected formulation using the Student t-test with $p \leq 0.05$ as the minimal level of significance. Calculations were done using the online calculation programme at *http://www.physics.csbsju.edu/stats/t-test_bulk_form.html* (25).

Most of the experiments were done in triplicates. SD values were calculated.

RESULTS AND DISCUSSION

Release studies

The release of TH from 2 tablet cores, each containing 50 mg TH (Table 1) was tried for 2 hours in 0.1 N HCl and at pH 6.8 for the rest of the dissolution run. A high retardation of release was observed, the drug being nearly completely released by 24 hours. Moreover, these tablets did not undergo any appreciable disintegration even after 3 hours. This indicates a mutual contribution from the three tablet components to the release, as the drug release was preliminarily tested from tablets containing TH & Dc alone and from other tablets containing TH & Avicel alone; the tablets in both cases gave a rather fast release.

Avicel having a large cohesive force, inner frictional coefficient, being adhesive and representing 40% of tablet weight might take a plastic deformation during compression. This results in an increase in the contact area between the different components with the formation of bonding leading to a compactly packed state (26) . Moreover, the non-spherical particles of Avicel encourage plastic deformation during compression (26-27).

Although Avicel is a hydrophilic excipient, the uptake of water does not take place through swelling (28), but rather water enters through void spaces between the particles (26). Such void spaces may become unavailable in the compact tablet combination of the three substances. So the penetration of water during release will not be sufficient because the work of dispersion of particles caused by water must overcome the binding work of the particles (29-30).

In addition, the coiled conformation of the dextran molecule (31-32) may also aid in the entrapment of the drug between its chains and those of Avicel. Hydrophobic bonding may thus occur in such a way that the polar groups of the drug may be quite shielded from the dissolution medium. Dextran C being of low molecular weight possesses a great number of free terminal hydroxyl groups available to interact with either the drug or another excipient in the formulation during the release experiment.

Results of release from compressed coated tablets are shown in Figures 2- 4. Concerning tablets coated with pectin at core : coat ratios (1:3;F1, 1:4;F2 and 1:5;F3), a slight release of the drug was detected at 5 hours with all tested core/coat ratios (Figure 2). However, it was observed that the rate of release decreased by increasing the coat thickness. A similar observation on the effect of core/coat ratio was previously reported (33). The higher pectin concentration offers an adequate gel layer for blocking the available pores.

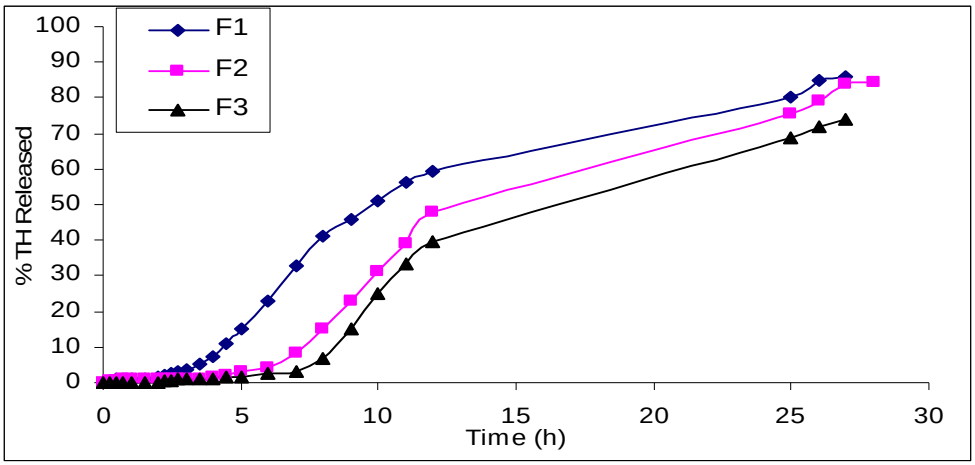


Figure 2: Release profiles of TH from different compressed tablets coated with pectin at various core:coat ratios; F1 (1:3), F2 (1:4) and F3 (1:5) in 0.1 N HCl (2 h) and pH 6.8 (26 h) at 50 rpm and 37 ± 0.1°C

The release cannot only be decreased by manipulating the proportion of core to coat but also by incorporation of a hydrophobic polymer like ethyl cellulose (EC). F4 coated with EC alone showed the least drug release among all formulations at any pH (only 24.4% by 27 hours) (Figure 3). On the other hand, the coats made of pectin and EC combinations, showed different release patterns (Figure 3). The EC can thus mask the inherent solubility of pectin and provide a coat relatively impermeable to the drug (15). The intention of combining pectin with EC was to take advantage of the latter's insolubility over the whole pH range (15). The addition of water soluble polymer such as pectin to an insoluble base can lead to the formation of aqueous pores in the coat due to the polymer dissolving and leaching out in the surrounding medium (34) . This would lead to a gradual increase in the drug release rate as the pectin dissolves. If the pectin does not dissolve, drug may diffuse through EC or pectin and the release rate will

then depend on the solubility of the drug in each polymer (35). It was observed that a relative increase in release occurred on increasing pectin concentration in the coat relatively to EC as in F5 and F6 in which the curves were nearly superimposed (Figure 3).

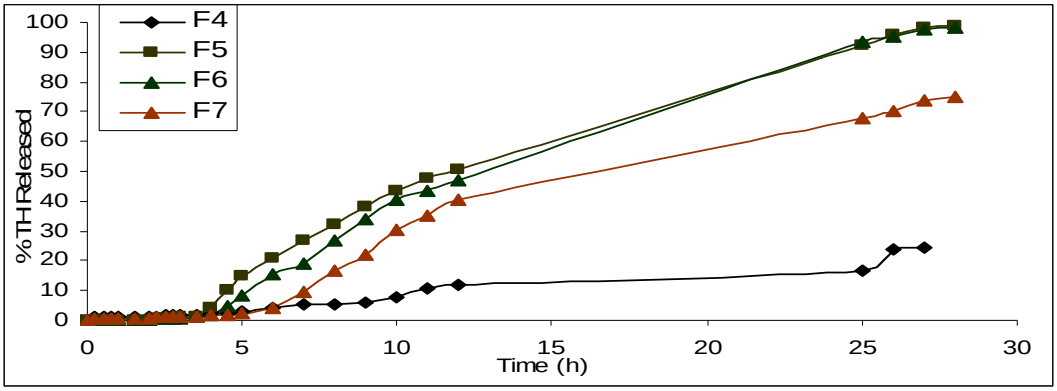


Figure 3: Release profiles of TH from different compressed tablets coated with pectin and EC in different ratios; F4 (0:1), F5 (1:1), F6 (3:2) and F7 (2:3) in 0.1 N HCl (2 h) and pH 6.8 (26 h) at 50 rpm and 37 ± 0.1°C (core/coat; 1:3)

Chitosan itself, has been shown to be of value in colonic delivery (33). However, being cationic, chitosan easily dissolves under acidic conditions and therefore addition of a polymer such as pectin would protect it from the harsh environment of the stomach. An interpolymer complex between the basic chitosan and the acidic pectin would be of value in restricting drug release in the upper GIT and allowing its release in the colon (33). Chitosan was shown to act as cross-linking agent for concentrated pectin solution (36). It was previously reported (37) that a maximum interaction between pectin and CS occurred at a weight ratio of 10 pectin/1 CS. In the present study, the release of TH in case of such mixed polymer coat in F8 (Figure 4) relative to pectin alone at the same core: coat ratio (1:3) in F1 (Figure 2), was found to be substantially retarded (35.2% by 28 hours).

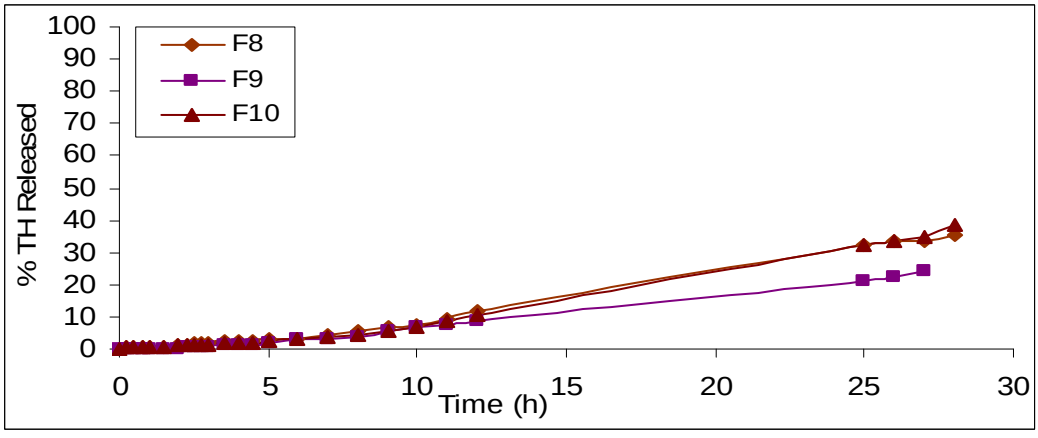


Figure 4: Release profiles of TH from different compressed tablets coated with a mixture of pectin, CS and HPMC in different ratios; F8 (10:1:0), F9 (3:1:1) and F10 (3:0:1) in 0.1 N HCl (2 h) and pH 6.8 (26 h) at 50 rpm and 37 ± 0.1°C (core/coat; 1:3)

It was observed that a coat mixture of pectin and HPMC 4000 in a ratio 3:1 (F10) gave a similar pattern to that of F8 previously mentioned (Figure 4). Moreover, the viscosity grade of HPMC was found

to be important for the performance of pectin matrix. When a fast hydrating type of HPMC was used, an intact gel layer was ensured which prevented disintegration (16) .

Using combinations of pectin, CS and HPMC 4000 in the coat matrix remarkably retarded the release, (only 24% release after 27 hours, for F9) as shown in Figure 4. It was reported that such combinations were insoluble and swelled to different extents giving rise to coats with various permeabilities (8).

The compressed coated TH tablet formulations using different coats showed no drug release in the stomach, minimal release in small intestine, not exceeding 15%, followed by increase in drug release at 6 hours corresponding to the colon arrival time.

Release from F1 was fast due to lower pectin concentration with a substantial amount of drug being released before 5 hours (15%). F3 showed minimal drug release before 5 hours (1.7%) due to the highest pectin concentration; however the tablet was very big (750 mg) and therefore difficult to swallow. F4 (coated with EC alone) and F9 gave a very slow release (24% by 27 hours). F5 and F6 released drug before 5 hours (14.9% and 8.1% respectively). So all these formulations were excluded as they could not serve the purpose of colon targeting. The rest of formulations were subjected to further release studies in the presence of pectinase.

The protection of TH from early release was followed by an acceleration in drug liberation when the tablets were exposed to the enzyme (33). The presence of pectinolytic enzymes simply accelerates pore formation by attacking pectin, therefore enhancing drug release (35).

The addition of the pectinolytic enzyme to F2 (Figure 5) resulted in increase of the release rate (48.6% by 11 hours compared to 39.3% in the absence of the enzyme). Nearly at the end of the release run (26 hours), the slight decrease in the release rate compared to F2 in the absence of the enzyme may be due to competition of hydroxyl groups from the released carbohydrate monomers, with the drug for the binding sites of water, hence decreasing the amount of TH dissolved. Such an observation was previously reported between TH and sucrose (38), and between paracetamol and some sugars (39) .

Addition of pectinase to F7 (Figure 5) caused a sudden increase in rate of drug release after 12 hours. As previously mentioned, the dissolving pectin will leave channels through which the drug can diffuse. Pectin in the mixed coat is available for enzymatic attack and channels to the core caused by dissolving and degrading pectin will be formed quicker (35).

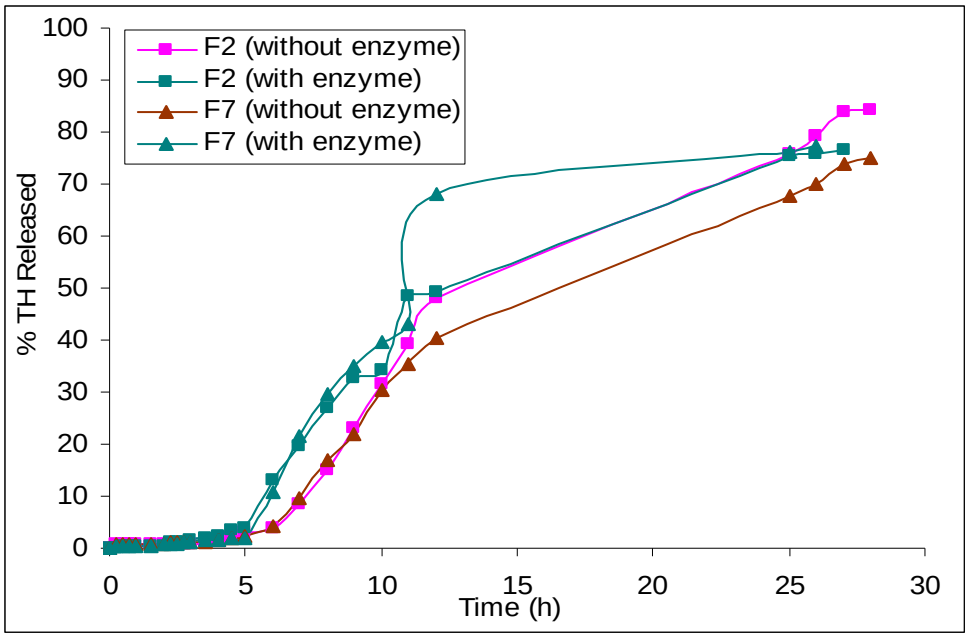


Figure 5: Release profiles of TH from F2 and F7 in 0.1 N HCl (2 h) and pH 6.8 (26 h) at 50 rpm and 37 ± 0.1°C in presence and absence of pectinase enzyme (1 mg/ml)

In case of F8, pectinase caused an increase in release (Figure 6) but still was considered low (39.3% after 28 hours). However, the addition of the enzyme to F10 (Figure 6) caused an obvious increase in drug release reaching 63.3% after 25 hours. This suggests that the formed bond between CS and pectin) (36-37) was much stronger than that between pectin and non-ionic HPMC, if any.

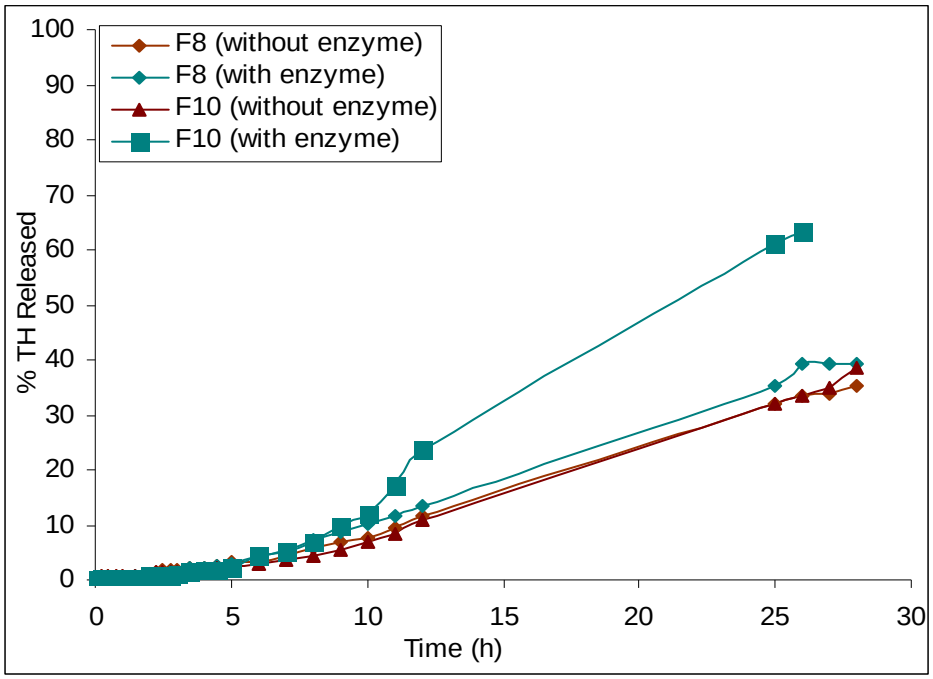


Figure 6: Release profiles of TH from F8 and F10 in 0.1 N HCl (2 h) and pH 6.8 (26 h) at 50 rpm and 37 ± 0.1°C in presence and absence of pectinase enzyme (1 mg/ml)

Elucidation of the release mechanism (for the selected formulations) can be obtained from calculation of the order kinetics. The release of drug entrapped in hydrogels occurs only after penetration of water in the matrix causing swelling of the polymer whose hydrating chains slowly relax. Water dissolves the drug which diffuses along the aqueous pathways. However, no single mathematical modeling of drug release from swellable polymeric systems can successfully predict all the experimental observations (40) as the pH of the medium and composition of hydrogels affect the release.

The determination of correlation coefficient (r) in case of formulations, F2 & F7, gave values nearly similar for both First order and Higuchi equations (41), indicating that more than one mechanism was operating at the same time (Table 3). On the other hand, complex formation in F8 & F10 which involves an interaction either ionic or through hydrogen bonding, did not yield realistic correlation coefficient values upon application of either First or Higuchi equations.

Table 3: Correlation coefficients for release patterns of TH from different coated tablet formulations according to First order and Higuchi plots

Formulation	Correlation coefficient (r)	
	First order	Higuchi
F2	-0.9783	0.9273
F7	-0.9850	0.9338

A numerical solution technique was required. Therefore, the generalized empirical power law

$$Mt/M\infty=kt^n \quad \text{Equation 2} \quad (42)$$

Where Mt and M∞ are drug release at time t and equilibrium respectively, was applied in such cases.

This equation was also tried for F2 which is considered a promising formulation (based on its release rate at the two pHs & after the addition of the enzyme, as well as its total weight is suitable to get a tablet of convenient size for swallowing). Values of n, the diffusion exponent, characteristic of the release mechanism as well as of k, the constant characteristic of the drug polymer system, are shown in Table 4.

Table 4: Kinetic parameters for the release data of TH from some selected coated tablet formulations

Formulation	n	Log K	Mechanism of release (41)
F2	1.330	-0.186	Case II diffusion
F8	1.022 ≈ 1	-0.114	Non-Fickian or anomalous diffusion
F10	0.9502 ≈ 1	-0.092	Non-Fickian or anomalous diffusion

The n values are either equal or higher than 1 (anomalous or case II diffusion respectively). This means that the rate of polymer/chain relaxation and drug diffusion are almost comparable (anomalous diffusion). On the other hand, diffusion of the dissolved drug in case II mechanism is very rapid compared to the relaxation process of the polymer (41). Therefore, drug release depends on two simultaneous rate processes, migration of water into the matrix and drug diffusion through a continuously swelling hydrogel.

In the first order release, the limiting step in the liberation of the drug depends on water penetration rate and diffusion of the dissolved drug out of the tablet through the gel layer. On the other hand, according to Higuchi model, the penetration of the medium occurs through the available pores in the tablet (43).

It is worth to say that upon application of Student t-test at $P \leq 0.05$, the difference between the drug release from TH tablet core and the coated tablet formulation (F2) was significant.

Swelling studies

Swelling index of the selected TH tablet formulations (F2, F7, F8 and F10) is shown in Figure 7.

It was reported previously that the pattern of water uptake does not entirely depend on porosity and that the mechanism of water uptake depends mainly on the type of base material (44). However, in all formulations, at early times, the presence of available pores would allow penetration of the medium to different extents.

In case of F2 with coat consisting entirely of pectin, an initial phase corresponded with water penetration through available pores. It was followed by a second phase in which pectin started to get ionized at pH 6.8 resulting in gel formation and partial blocking of pores, leading to plateau formation (45). Meanwhile, ionization of carboxyl groups caused repulsion between the chains, with consequent thinning of the gel layer, so permitting more easy diffusion of water inside the matrix. On the other hand, F7 exhibited surface erosion as visually observed, leading to hindering of swelling.

In case of F8, cross-linking occurring between CS and pectin at pH 6.8 would overcome matrix erosion with subsequent increase in water uptake. By time, when bond forming cross-linking weakens, erosion starts to occur accompanied with decrease in swelling (46). For F10, containing pectin and HPMC, gel formed is of such strength as to prevent erosion (12), while permitting water penetration. At the end, the dissolved drug can cause osmotically driven water penetration.

F2 was considered the formulation of choice, since it gave the highest swelling index from the beginning of the experiment until 9 hours and at the same time, its rate of release was very appropriate. In acid medium, it was almost negligible (<1%) and increased gradually (0.8%-2.9%) in the following 3 hours. After 5 hours, a gradual increase took place and almost complete drug release occurred in the absence of the enzyme. This may be advantageous in case of patients who suffer from deficiency in the bacterial flora; for example as a result of antibiotic therapy.

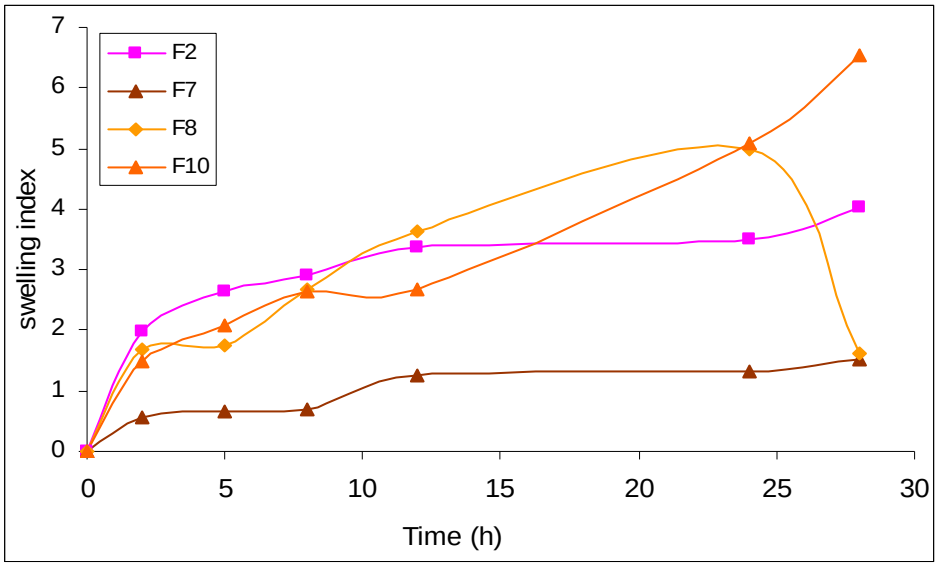


Figure 7: Swelling index profiles of F2, F7, F8 and F10 at 37 ± 0.1°C in 0.1 N HCl (2 h), then at pH 6.8

MEASUREMENT OF GASTROINTESTINAL TRANSIT TIME

Animal experiments

Results of gastrointestinal transit of F2 after oral administration of three tablets to each dog are shown in Table 5 and Figure 8.

After 2 hours, the three tablets given to dogs appeared intact in the stomach, surrounded by a gel layer. Tablets then passed into the small intestine and remained there intact for 5 hours. Unfortunately, the

dog which was to be killed at 6 hours, defecated just before killing. The tablets appeared intact in the stool. This means that the colon arrival time was 6 hours.

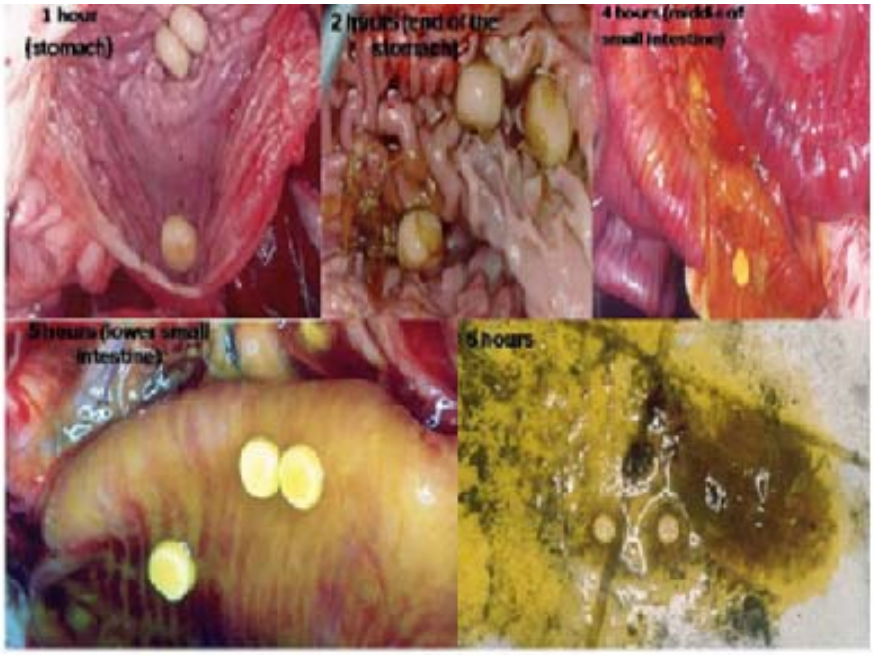


Figure 8: Photographs of F2 at 1, 2, 4, 5 and 6 hours after killing the dogs

Table 5: Gastrointestinal transit time for F2 after oral administration of three tablets to each dog

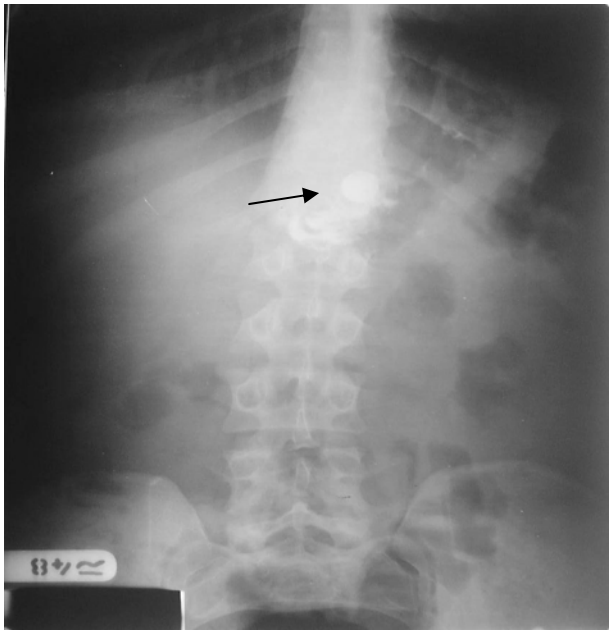
Time (h)	Gastrointestinal transit
1	Stomach
2	End of the stomach
4	Middle of small intestine
5	Lower small intestine (near the colon)

* By 6 h the dog defecated and the tablets appeared intact in the stool
* Colon arrival time was 6 hours

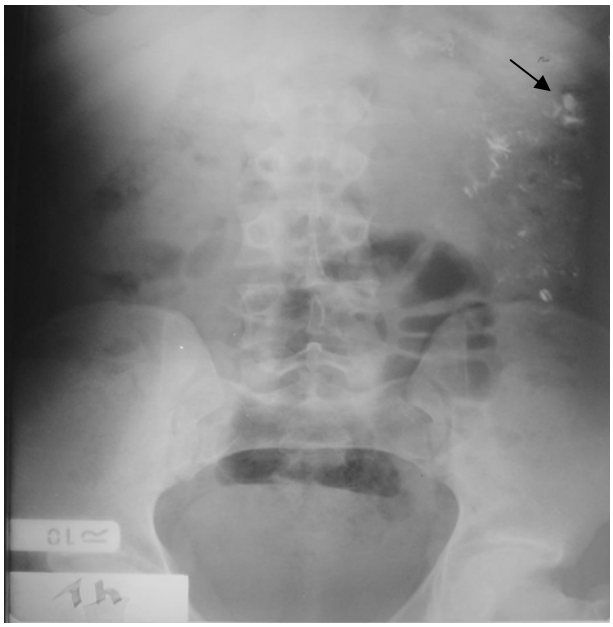
X-ray photography

The gastrointestinal transit times of F2 for the subject submitted to x-ray photography are shown in Figure 9.

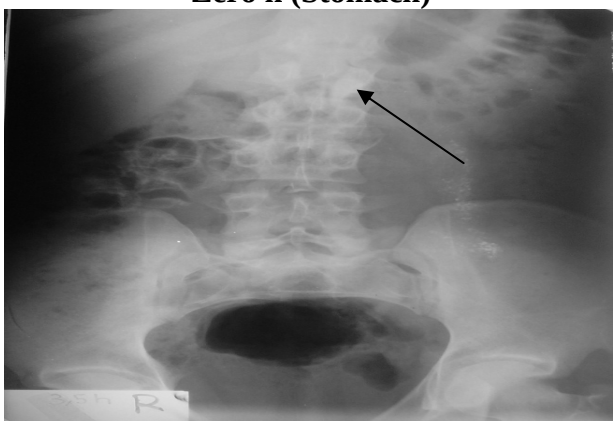
The tablet was administered on empty stomach and, being of relatively big size, will be emptied in an erratic manner depending on its arrival time in the stomach. The small intestinal transit time (SITT) of the coated tablet was determined as the time difference between the exit of the tablet from the stomach and its arrival to the cecum (47). The arrival time to the cecum was found to be 7 hours.



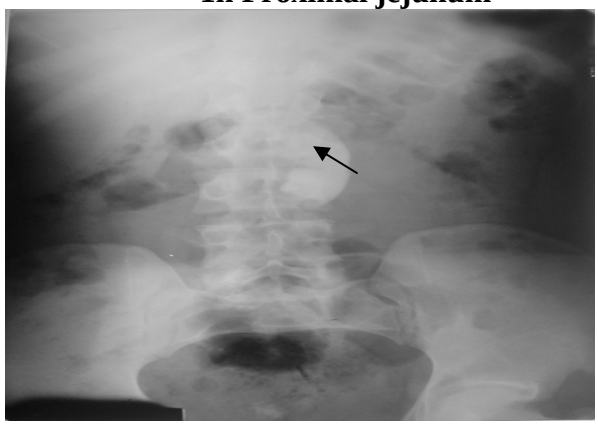
Zero h (Stomach)



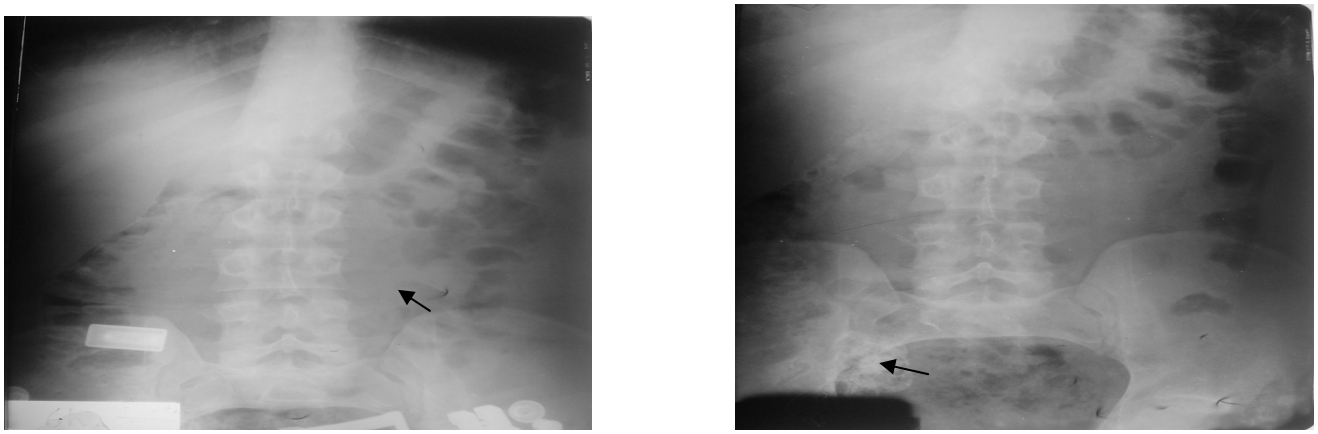
1h Proximal jejunum



3,5h (Descending jejunum)



5h (proximal ileum)



6h (Terminal ileum) **7h (Ileocecal junction)**

Figure 9: X-ray photographing of F2 at zero, 1, 3.5, 5, 6 and 7 hours

Differential Scanning Calorimetry (DSC)

DSC thermogram of TH showed a sharp endothermic peak at 272.205°C. A small rather broad endotherm at 307.180°C also appeared in the thermogram (Figure 10).

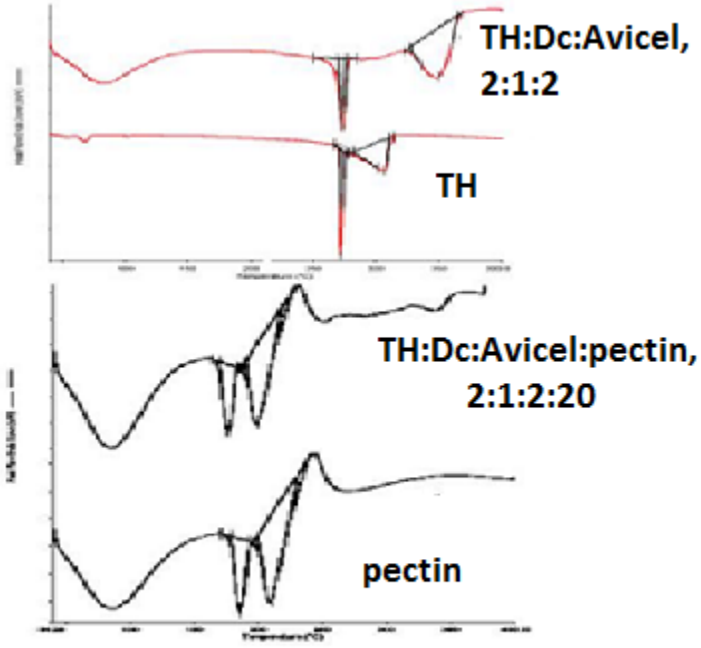


Figure 10: DSC thermograms of theophylline, PM of TH, Dc & Avicel (2:1:2), pectin & PM of TH, Dc, Avicel and pectin (2:1:2:20)

On testing a physical mixture of TH and Dextran C (2:1) and a physical mixture of TH and Avicel (1:1), the main sharp endothermic peak of the drug was still present with no change in its position. However, the broad endothermic peak of the drug at 307.180°C disappeared (such thermograms are not shown).

In the physical mixture of the tablet core components, the sharp peak of the drug was still present, while the broad one disappeared (Figure 10).

Pectin thermogram (Figure 10) revealed some characteristic peaks. A physical mixture of tablet core components and pectin showed the disappearance of the drug peaks (Figure 10). The high proportion of pectin in the mixture would presume some modification of drug peak height due to dilution. Yet, the complete disappearance of the peak of the drug suggested not only amorphization but also formation of a kind of bonding between pectin and TH. This latter suggestion needs further confirmation by IR analysis.

IR analyses

The characteristic IR absorption bands for TH are C=O stretch at 1706.5 and 1672.2 cm^{-1} (21) (Figure 11).

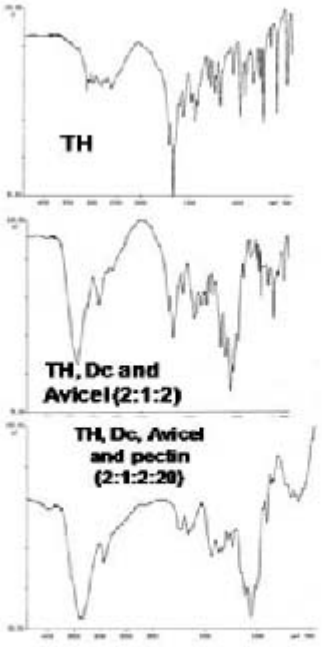


Figure 11: IR spectra of TH, PM of TH, Dc and Avicel (2:1:2) and PM of TH, Dc, Avicel and pectin (2:1:2:20)

IR spectra obtained from physical mixture of TH and Dextran C showed that the characteristic peak due to C=O group of TH is not superimposed by any peak of Dextran C (not shown in the figures). The possibility of hydrogen bond formation involving C=O group of the drug is eliminated as there is no change in either the shape or the place of C=O band of TH.

Concerning TH and Avicel 1:1 physical mixture (not shown in figures), bands of C=O group of TH at 1716.2 and 1666.2 cm^{-1} were not affected by the presence of Avicel. Moreover, the hydroxyl absorption band of Avicel at about 3347.4 cm^{-1} was rather unchanged.

IR spectrum of physical mixture of tablet core components (Figure 11), revealed no change in either position or shape of C=O band of TH. Hydroxyl bands of Dc were like those of physical mixture between TH and Dc, while hydroxyl bands of Avicel were present in the physical mixture of the tablet core ingredients with nearly no change in either position or shape.

Nevertheless, when pectin was added to physical mixture of the tablet core components (Figure 11), C=O group band of the drug was appreciably altered probably due to hydrogen bond formation involving C=O and any electron accepting atom from pectin.

Effect of ageing

After storage, the formulations were observed for any physical change. No change in release of TH from F2 occurred after 3 and 6 months, while release was increased after 1 year (Figure 12). This phenomenon can be explained by the enhancement of polymer chain flexibility followed by shrinkage of the polymer embedding the drug in the matrix, therefore increasing the penetration of the aqueous dissolution medium into the porous skeletal structure of the tablet during release. F2 nearly gave 100% complete release after ageing.

Student t-test showed no significant difference at $P>0.05$ after 3, 6 and 12 months, compared to the fresh formulation. This indicates that the release mechanism remained unchanged.

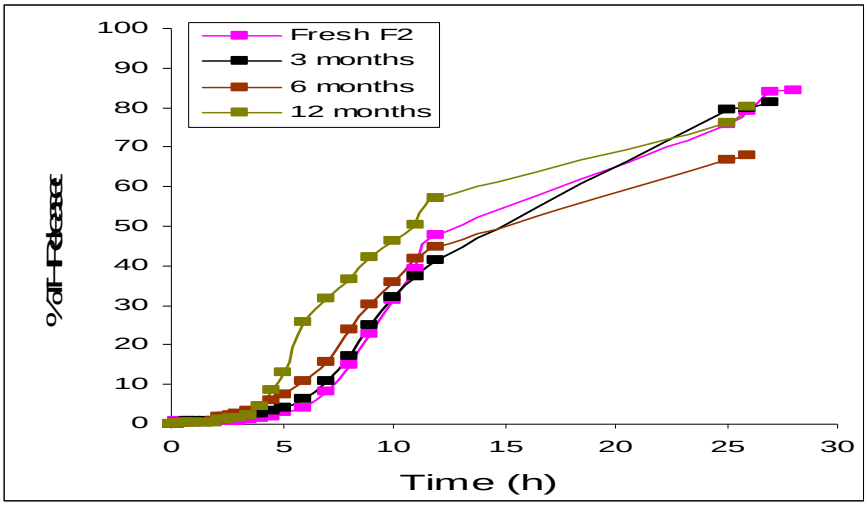


Figure 12: Release profiles of TH from F2 in 0.1 N HCl (2 h) and pH 6.8 (25 h) before and after ageing at 50 rpm and 37 ± 0.1°C

CONCLUSION

A selected formulation (F2) consisting of the core (TH, Dc, and Avicel) and a pectin coat of suitable thickness was shown to retard the drug release successfully under conditions mimicking mouth-to-colon transit. This formulation gave minimal drug release for approximately 5 hours, corresponding to small intestine transit time followed by a sustained and almost complete release in the colon even in absence of bacterial enzyme. Such gradual release profile would be advantageous in preventing occurrence of nocturnal asthma.

All excipients used are abundantly available and safe. The most popular oral dosage form is the directly compressed tablet because of its ease of fabrication involving only blending and compaction. Technique of preparation is also cheap.

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