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Studying the Release Profile of 5-Fluorouracil Prepared by Controlled Porosity Osmotic System As A Colon Targeting

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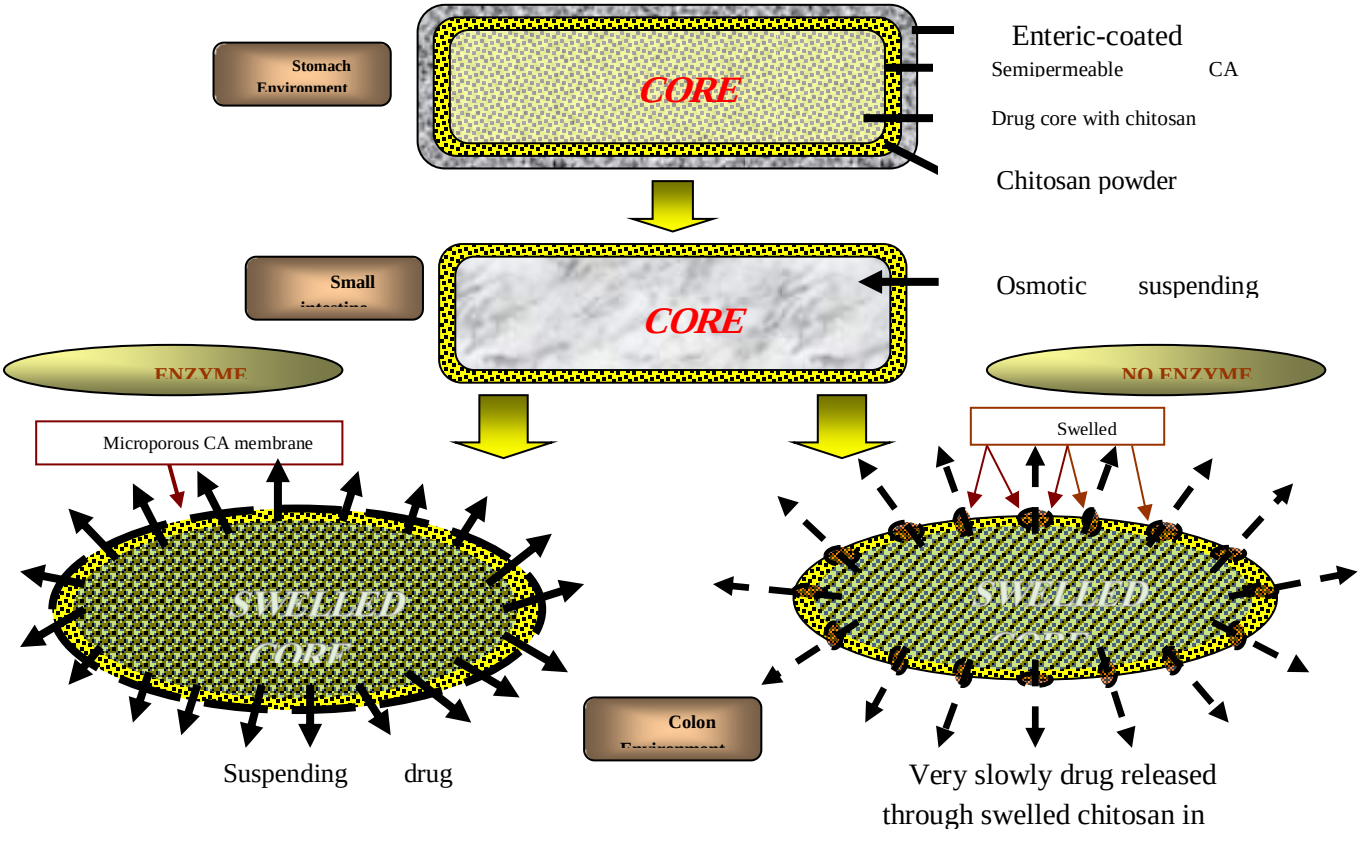
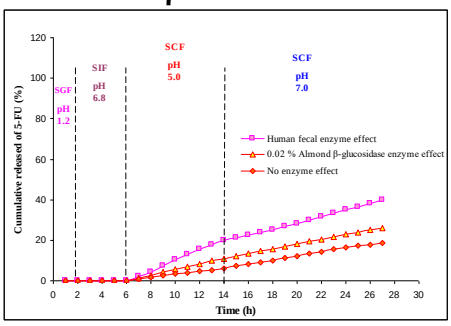
Abstract

Colon-specific drug delivery systems are designed to permit targeted drug release to the terminal ileum and proximal colon. OROS-CT is used as a once- or twice-a-day formulation for targeted delivery of drugs to the colon and can be used for local or systemic therapy. Osmotic pump tablet in form of controlled porosity osmotic pump system as a colon targeting (CPOP-CT) has advantage that used as a model in formulation of poorly soluble drugs. In this study, different formulas (F1-F7) containing 4 mg of 5-fluorouracil (5-FU) were prepared as a tablet in form of controlled porosity osmotic pump-colon targeting (CPOP-CT) system for oral administration. The main ingredients included in formulation of these formulas were citric acid, sodium chloride, low molecular weight chitosan (LMCh), medium molecular weight chitosan (MMCh), silicified microcrystalline cellulose 90 µm (ProSolv SMCC ® 90) and magnesium stearate. All the ingredients were mixed with 5-FU and compressed directly as a core of tablets. The prepared core of tablets of all formulas were double coated into inner layer containing chitosan and polyethylene glycol-400 (PEG-400) as a microporous semipermeable cellulose acetate membrane and outer layer as an enteric coating containing Eudragit®L100-55, triethyl citrate and talc. The

solubility of 5-FU in different dissolution media was studied that found of 6.21 mg/ml in 0.1 M acetate buffer saline (pH 5.0), 13.45 mg/ml in distilled water and 51.8 mg/ml in phosphate buffer saline (pH 7.0). The results indicated that the increased amount of LMCh and decreased amount of citric acid for all formulas lead to slow the release rate of 5-FU. F1, F2, F3, F4, F5, F6 and F7 containing 5- FU in simulated colonic fluid **(**SCF; pH 5 and pH 7) showed sustained release with 61%, 29%, 32%, 43%, 52%, 39% and 68%, respectively within 21 h. In addition, the release rate of 5-FU was affected by increasing or decreasing the weight gain percentages of semipermeable cellulose acetate membrane. 6% and 10% weight gains of F3 released 5-FU completely within times ($t_{100\%}$) of 46 h and 65 h, respectively which are acceptable as a colon target sustained release when compared with 14% weight gain ($t_{100\%}$ = 135 h). For all formulas, the time required to sustained released of 5-FU ($t_{100\%}$) in colon would be limited which no exceed colonic transit time (78 h). Furthermore, there was a significant ($p<0.05$) enzymic effect on the release of 5-FU for F6 within 21 h in SCF. The release percentage of 5-FU was 18% with no enzyme; but improved when used synthetic almond emulsin β-glucosidase and human fecal enzyme to 26% and 39%, respectively. These enzymes are responsible for degradation of LMCh present in semipermeable cellulose acetate membrane and produce pores to release drugs and then modulate the kinetic mechanism of poorly soluble 5-FU from zero order into first order kinetic. It has been concluded may be the poorly soluble 5-FU prepared by CPOP-CT system to become as a candidate for colon targeting therapy to colon cancer with reduced dose and duration of therapy.

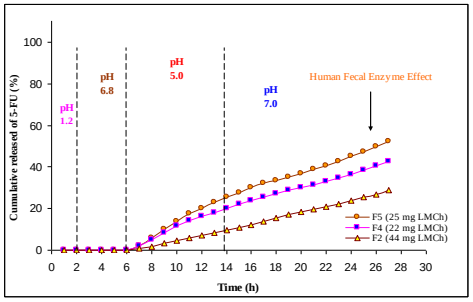
Keywords**:** Controlled Porosity Osmotic System, Osmotic Pressure, Chitosan, Enzyme, 5-FU





Before Coating

After Coating



Graphical Abstract

1. Introduction

Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (ulcerative colitis, Chron's disease, carcinomas and infections). The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability [(1)]. In recent years, a large number of solid formulations targeting the lower parts of the GI tract, especially the colon, have been reported. Liu designed a new microbially triggered colon-targeted osmotic pump (MTCT-OP) based on chitosan which consists of an osmotic core (containing drug and chitosan with organic acid as an excipient), an inner semipermeable membrane layer composed of the mixture of cellulose acetate and chitosan powder, and an outer enteric-coating layer.

Moreover, the outer enteric-coating layer of MTCT-OP remains intact in the stomach and dissolves in the small intestine, where pH is above 6, and water is imbibed into the core. During its transit through the GI tract, due to the action of organic acid in core, the chitosan begins to swell and to constantly form a flowable gel through which drug dispersed. However, the drug is still not released because the pore former chitosan in the semipermeable membrane is scarcely dissolved. When MTCT-OP reaches the colon, chitosan in the semipermeable membrane is specifically degraded by microflora of the colon and thereby results in an in situ formation of a microporous membrane. Targeting drugs to the colon by microflora-activated mechanism and controlled release procedure at a pre-programmed rate are the main characteristics of chitosan-based MTCT-OP [(2)].

Also it has been reported that chitosan in solution was degraded by rat cecal and colonic enzymes, and that its susceptibility to degradation was dependent on both its molecular weight and degree of deacetylation [(3)]. In many approaches two coatings microporous semipearmable membrane coating or enteric coating (inner layer coating) and enteric coating (outer layer coating) are included in order to provide the release in the proximal colon while minimizing total enteric coating thickness is required. The outer coating layer consists of an enteric polymer coating material which begins to dissolve at a pH 6.8-7.2 when the dosage form is in the distal small intestine. The inner layer consists of enteric polymer coating material that begins to dissolve at pH 5-6.3 and more preferably a pH between 5 to 5.5 or consists of natural polysaccharides (e.g. chitosan, pectin, amylase and etc.) that are susceptible to colonic microflora [(4,5)].

Liu et al designed a new osmotic pump tablets based on chitosan as swellable polymer (osmopolymer). The tablet contained poorly soluble budesonide drug, citric acid as osmotic agent, chitosan as osmopolymer, ProSolvSMCC ® 90 as binder and magnesium stearate as lubricant. The invention shows that the release of drug from core is increased by increasing in swellability of gelled and hydrated chitosan which resulted from increasing in amount of citric acid and consequently increase in osmotic pressure of core that pushed the drug suspension out of the system. Invitro release profile of drug in osmotic pump tablet was modified from a zero order release profile to a first order release profile after the addition of enzymes degradable chitosan to a semipermeable cellulose acetate membrane [(2).] Thickness of the membrane has a

profound effect on the drug release from osmotic systems.

The release rate from osmotic systems is inversely proportional to membrane thickness. To ensure that the coating is able to resist the pressure within the device, thickness of membrane is usually kept between 200 and 300 µm [6]. In case of monolithic osmotic tablets of nifedipine, release rates were found to decrease with the increase in membrane thickness from 85 to 340 µm [7]. There are few approaches for an oral administration of 5-FU as a colon targeting. Lamprecht et al formulated 5-FU microsphere as a colonic delivery for purpose of colon cancer treatment [8]. 5-FU was first synthesized in 1957 [9]. It was studied extensively by Heidelberger and co-workers and still today it is a major agent which is utilized in the treatment of several malignancies [10]. It remains one of the most effective chemotherapeutic agents in such conditions as colorectal cancer, even at its limited response rates (10-30%) [11]. In addition, the prepared of osmotic pump tablets in form of controlled porosity osmotic pump system as a colon targeting (CPOP-CT) has advantage that used as a model in formulation of both poorly soluble and freely soluble drugs [12]. The aim of this work is to prepare and study the release profile of 5-FU (sparingly soluble) as a new formulation of controlled porosity osmotic pump - colon targeting (CPOP-CT).

2. MATERIALS AND METHODS

2.1. Materials

5-Fluorouracil, emulsin almond β-glucosidase and talc powder were purchased from Fluka BioChemica, Germany. Low molecular weight chitosan, medium molecular weight chitosan and trietyl citrate were obtained from Sigma-Aldrich, Germany. Disodium hydrogen phosphate, sodium acetate trihydrate, pepsin 10000 E powder and magnesium stearate ordered from Reidel de Haën AG, Germany. Eudragit® L 100-55 was purchased from Degussa Rőhm GmbH, Germany. Cellulose acetate, potassium dihydrogen phosphate and potassium dihydrogen phosphate were obtained from BDH Chemicals Ltd, England. Citric acid monohydrate was obtained from LUDECO Brussels, Belgium. Glacial acetic acid, hydrochloric acid, methanol and ethanol were obtained from GCC Analytical reagent, UK. Sodium chloride and methylene chloride extra pure were purchased from Thomas Baker, Mumbai-India. Thioglycolate medium w/o dextrose powder was purchased from HiMedia Lab., Pvt-Ltd, Mumbai-India. ProSolv SMCC ® 90 was purchased from JRS PHARMA GmbH, Germany. PEG-400 was purchased from Yixing Zhouwang Chemical Co. Ltd, China. Pancreatin powder was ordered from Philip Harris Biological Ltd, Oldmixon-Weston Super.

2.2 Methods

2.2.1 Characterization of 5-Fluorouracil (5-FU)

2.2.1.1 Study calibration curve of 5-FU

λ_{max} of 5-FU was determined by scanning 16 µg/ml of 5-FU solution in different buffers by Cary100 WinUV software analysis spectrophotometer. Then the stock solution of 5-FU was prepared (10 mg/ml) in different media of 0.1 N HCl, phosphate buffer (pH 6.8 and 7.0) and acetate buffer (pH 5.0), then a serial dilutions of the drug were analyzed spectrophotometrically at its λ_{max}.

2.2.1.2 Effect of osmogents on solubility study of 5-FU

The solubility of 5-FU was investigated in various dissolution mediums. The saturated solution of 5-FU was prepared in 20 ml distilled water, acetate buffer (pH 5.0) and phosphate buffer (pH 7.0) in a closed container with mechanically shaking for about 48 h at 37± 0.5 ºC. Then, the filtered solutions were analyzed by spectrophotometer at 266 nm after suitable dilutions. The solubility of 5-FU in aqueous solution containing different concentrations of osmogents as citric acid (0.55% and 1.1%), 0.55% NaCl and a mixture of 0.55% citric acid and 0.55% NaCl was also studied at room temperature [13].

2.2.2 Preparation of buffers

2.2.2.1 Simulated gastric fluid (SGF) (pH 1.2)

It was prepared by dissolving and mixing 2.0 g of NaCl, 3.2 g of pepsin and 3.0 mL concentrated HCl in distilled water. Then diluted to 1000 ml distilled water (136 mOsm/kg) [14].

2.2.2.2 Simulated intestinal fluid (SIF) Sorensen's phosphate buffer (pH 6.8)

Phosphate buffer saline (1/15 M pH 6.8) was prepared by mixing 534 mL of the dissolved 9.08 g of potassium dihydrogen phosphate in 1 L distilled water with 499 mL of dissolved 9.469 g of disodium hydrogen in 1 L distilled water. Then prepared buffer was mixed with 0.878 g of NaCl (0.15 M) and pancreatin (10 mg/ml) to produce SIF (643 mosm/kg, osmolality) [15].

2.2.2.3 Phosphate buffer saline (PBS) (pH 7.0)

Phosphate buffer saline (pH 7.0) was prepared by mixing 413 mL of the dissolved 9.08 g of potassium dihydrogen phosphate in 1 L distilled water with 587 mL of dissolved 9.469 g of disodium hydrogen in 1 L distilled water. Then different concentrations of NaCl (0.15 M, 0.3 M and 0.5 M) were added to 1000 ml of prepared buffer to get different osmolality of 560 mOsm/kg, 790 mOsm/kg and 1127 mOsm/kg [16].

2.2.2.4 Acetate buffer (pH 5.0)

Acetate buffer (0.1 M pH 5.0) was prepared by mixing the 673 mL of dissolved 13.61 g of sodium acetate trihydrate in 1L Distilled water with 322 mL of 0.6% of glacial acetic acid and osmolality of acetate buffer was measured which equal to 306 mOsm/kg. To get different osmolality of acetate buffer saline, different concentrations of NaCl 5.85 g (0.1 M), 17.55 g (0.3 M) and 29.25 g (0.5 M) were added to 1000 ml of buffer to give osmolality of 520 mOsm/kg (12.69 atm), 875 mOsm/kg (21.35 atm) and 1261 mOsm/kg (30.77 atm), respectively [16].

2.2.2.5 Simulated colonic fluid (SCF) (pH 5.0 and pH 7.0)

It was prepared by mixing the prepared acetate buffer (pH 5) and prepared PBS (pH 7.0) with human fecal enzyme media or 0.02% of almond emulsin β-glucosidase enzyme [17,18].

2.2.3 Dissolution

5-FU drugs were studied using dissolution apparatus I (basket method). The studies were carried out under sink condition using 900 ml dissolution medium of SGF (pH 1.2) for 2 h followed by 900 ml of SIF (pH 6.8) for 4 h with stirring speed of 100 rpm and then followed by

using 500 ml of SCF (pH 5.0 and pH 7.0) for 21 h with stirring speed of 50 rpm and all at 37± 0.5 °C. Filtered samples were taken for analysis at different time intervals and replaced with the same volume of the fresh media and the samples absorbance was determined spectrophotometrically for 5-FU at their λ_{max} 266 nm [19].

2.2.4 Formulation of 5-FU osmotic tablets

2.2.4.1 Preparation of core tablets

Each ingredient was sieved through 100 mesh screen to achieve a good compaction in tabletting. The accurately weighed ingredients were mixed manually for 10 min. The resultant powder mixture was directly compressed into round tablets using 1/4 inch (6.35 mm) standard concave punches on a single punch tablet machine. The compression force was fixed at 0.5 KN. The surface morphology of the coated tablets was smooth. The weight of tablets was determined to be range from 99-100 mg. The core compositions of 5-FU are illustrated in Table 1.

2.2.4.2 Preparation of microporous semipermeable membrane coating (Inner layer)

The coating solution consists of 4.0% (w/w) total coating materials dissolved in a mixture of coating solvents like dichloromethane and methanol (95:5). 4% (w/w) of total coating materials in the coating solution composed of 2.7% of cellulose acetate, 0.3% of PEG-400 and 1% of low molecular weight chitosan (LMCh). Cellulose acetate (CA) was dissolved in dichloromethane and methanol (95:5) mixture to get a clear solution (pH 6.15). Flux regulating agent as PEG-400 was added to a clear solution in order to enhance the physical–mechanical property of cellulose acetate membrane. Finally, a pore forming agent as LMCh was added to get a suspension. The coating of tablet was performed by modified pan coater. Sufficient coating

solution was applied until desired weight gain (6%, 10% and 14% ± 0.3%) was obtained. After coating, the coated tablets were dried at 50°C in an oven for 12 h before being evaluated. The percentage weight gain and thickness of the coating membrane were measured. Total coating composition of semipermeable membrane layer consists of 50% of cellulose acetate, 25% of PEG-400 and 25% of LMCh [2,20]. The percentage weight gain of coating membrane was calculated by the following equation.

$$TWG = (W_t - W_o) / W_o \times 100$$

2.2.4.3 Preparation of enteric coating (Outer layer)

Eudragit ® L100-55 (6 g) and triethyl citrate (TEC) (0.6 g) were dissolved in 95% alcohol (92 g). Talc (1.4 g) was then suspended in the solution. The resultant mixture was coated on the surface of microporous semipermeable membrane in a pan coater maintaining an inlet air/bed temperature of 30 °C. The surface of osmotic tablet had a smooth and uniform appearance. Coated tablets were dried for 4 h at 40 °C (2).

2.2.5 Statistical analysis

Release study of 5-FU was expressed as mean + SD where n = 3 in all cases. Student (t-test) and ANOVA variance analysis were used in all cases of release that studied to investigate the significance of the differences of means at 95% confidence interval level.

Table 1: Core Compositions of 5-FU Osmotic Tablet

Ingredients (mg/tablet)	Core code						
	F1	F2	F3	F4	F5	F6	F7
5-FU	4	4	4	4	4	4	4
Low molecular weight chitosan (LMCh)	-	44	22	22	25	-	-
Medium molecular weight chitosan (MMCh)	-	-	-	-	-	22	-
NaCl	-	-	-	-	-	-	22
Citric acid	44	0	22	57	50	22	22
ProSolv SMCC ® 90	51	51	51	16	20	51	51
Magnesium stearate	1	1	1	1	1	1	1

3. RESULTS AND DISCUSSION

3.1Study calibration curves of FU

5-FU showed λ_{max} at 266 nm in different buffers (Fig.1) and its calibration curve was measured in 0.1 N HCl (pH 1.2), phosphate buffer (pH 6.8), acetate buffer (pH 5.0) and phosphate buffer (pH 7.0) that in a good agreement with Beer's-Lambert Law (Fig.2)

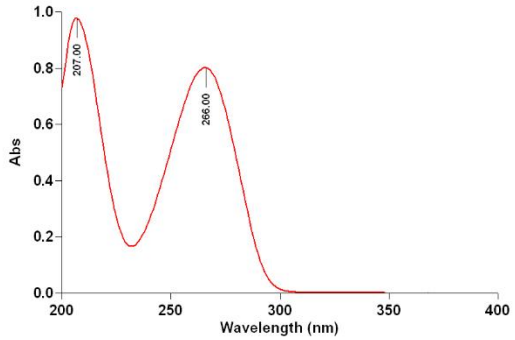


Figure 1: UV scanning of 5-FU in different buffers

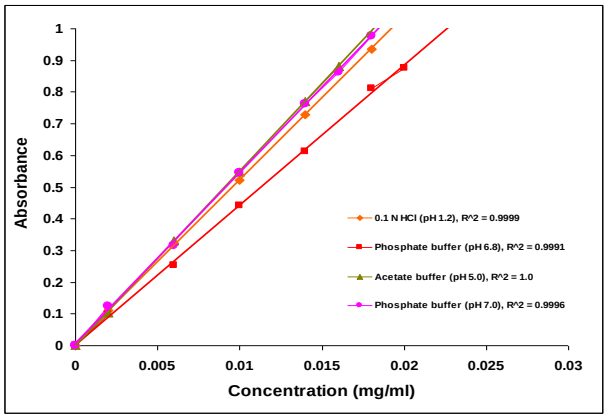


Figure 2: Calibration curves of 5-FU in different pH media

3.2. FTIR for compatibility of 5-FU with excipients

All the characteristic bands of 5-FU before and after mixing with excipients (chitosan, citric acid, ProSolv SMCC ® 90, magnesium stearate and cellulose acetate) gave an indication for compatibility of 5-FU with these excipients used in formulation of osmotic tablets (Fig 3, Fig 4).

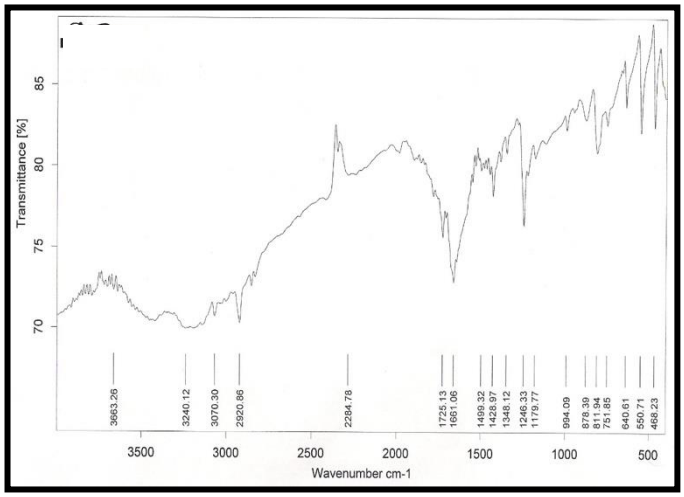


Figure 3: FTIR spectra of pure 5-FU

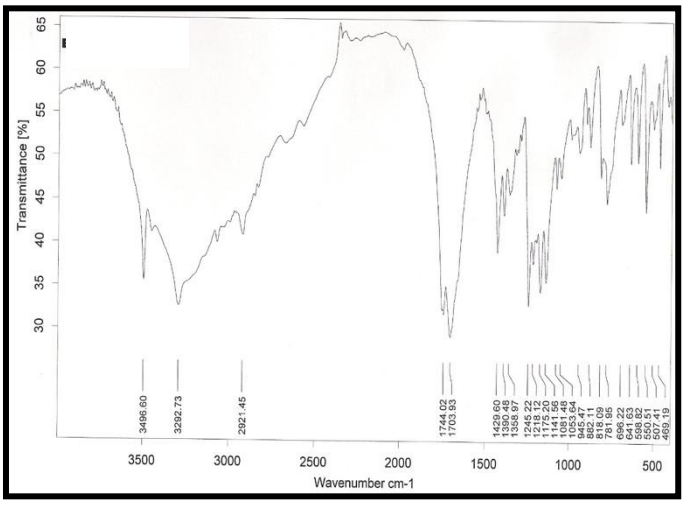


Figure 4: FTIR spectra of physical mixture (5-FU, chitosan, citric acid, ProSolv SMCC 90, magnesium stearate and cellulose acetate)

3.3Effect of osmogents on solubility of 5-FU

The kinetics of osmotic drug release is directly related to the solubility of the drug within the formulation. The solubility of 5-FU in various dissolution media was 6.21 mg/ml, 13.45 mg/ml and 51.8 mg/ml in 0.1 M acetate buffer saline (pH 5.0), distilled water and phosphate buffer saline, respectively. Osmogent as citric acid (0.55%) enhances the solubility of 5-FU into 17.54 mg/ml; while 1.1% citric acid had slightly effect on solubility of 5-FU (13.72 mg/ml). In addition, other osmogent as NaCl (0.55%) decreased the solubility of 5-FU into 9.89 mg/ml. However, combination of osmogents in same concentration (0.55% NaCl and 0.55% Citric acid) caused the increase in aqueous solubility of 5-FU into 15.13 mg/ml (Fig. 5).

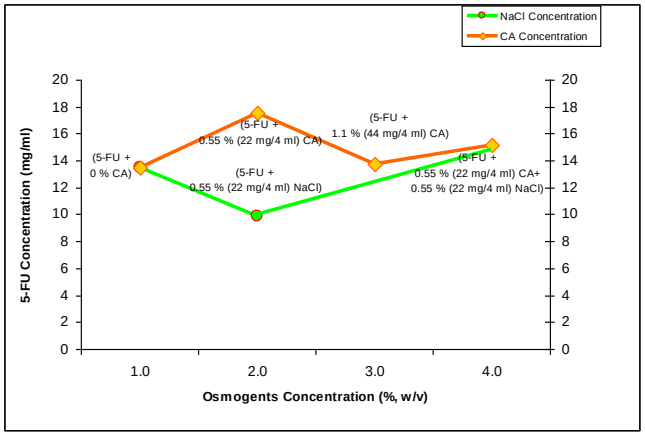


Figure 5: Effect of osmogents (citric acid (CA) and NaCl) on aqueous solubility of 5-FU

3.4Role of excipents added in formulation of 5-FU osmotic tablet

The main constituents of core of tablets include the high functionality excipients such as ProSolv SMCC ® 90 as a binder, magnesium stearate as a lubricant, chitosan as osmopolymer and citric acid as solubility modifier osmogent. The hydration and gel formation of chitosan are very much dependent on the pH of surroundings and soluble in acidic and slightly acidic pH values. The viscosity of chitosan gel increases as molecular weight and amount of chitosan increase [(21)]. In addition, inclusion of citric acid as pH-regulating excipient in the developed formulations was expected to decrease the microenvironmental pH of the core to a suitable level at which chitosan could form appropriate viscous gelling solution and hence, to enhance the osmotic pressure of core tablets [(2)].Sodium chloride was used as an osmogent instead of chitosan in F7. The tablet core was surrounded by a semipermeable membrane consisting of chitosan (pore forming agent), PEG-400 (flux enhancer) [(22)] and cellulose acetate (water insoluble polymer with acetylation degree of 54.5 to 56 %) and this membrane is a rate controlling membrane that hardly degraded by microorganism [(23)]. The pore forming agent (chitosan 25% w/w coating composition) in the membrane showed average lag time (6 ± 0.1 h) in the whole dissolution set-up. Furthermore, the semipermeable membrane was coated by enteric coat with weight gain of 6% using anionic copolymer like Eudragit® L 100-55 (pH > 5.5) [(2)]. Eudragit® L 100-55 was most stable with talc and triethyl citrate [(24)].

3.4.1 Effect of chitosan concentration on 5-FU release

Formulas F2 (44% w/w LMCh), F4 (22% w/w LMCh) and F5 (25% w/w LMCh) were selected for 5-FU to explain the influence of chitosan concentration on the drug release as illustrated in Fig. 6 The results indicated that in absence of citric acid, F2 (29% ± 1.1) which contains the highest amount of chitosan gave the slowest release of 5-FU in vitro compared to F4 (42% ± 1.3) and F5 (52% ± 1.9). The osmotic pressure of formulas (F1, F2, F3, F4, F5, F6, F7) for 5-FU were studied to support the relationship between the drug release and the osmotic pressure force that responsible for pushing the drug suspension out of the system (Table 2). A slower release profile of F2 was obtained compared to both F4 and F5 which may be related to osmotic pressure of the core which swells upon hydration of chitosan. In addition, the osmotic pressure of viscous gel formed chitosan of F2 (0.1708 atm) was less efficient than the osmotic pressure of viscous gel formed chitosan of both F4 (2.3668 atm) and F5 (2.9036 atm). The formulation of 5-FU containing the lowest amount of chitosan (F4) was demonstrated a significant cumulative release higher ($p<0.05$) (42% ± 1.3) than F2 (29% ± 1.1) and lower than F5 (52% ± 1.9) within 27 h and these results also depends on the osmotic pressure of tablets core. The higher cumulative release of 5-FU in F5 is due to the presence of citric acid and larger amount of chitosan that leads to more increase in viscosity of the core suspension which promoted more 5-FU particles exposed to dissolution . This is in agreement with the results obtained by Liu et al [2]. Under human fecal enzyme effect, $t_{25\%}$ values for the 5-FU formulations of F2, F4 and F5 were 18.28 h, 12.25 h and 10 h, respectively. In addition, SCF $t_{100\%}$ values of F2, F4 and F5 of the 5-FU were 73 h, 49 h and 40 h, respectively. F2, F4 and F5 give indication for sustained release formulation.

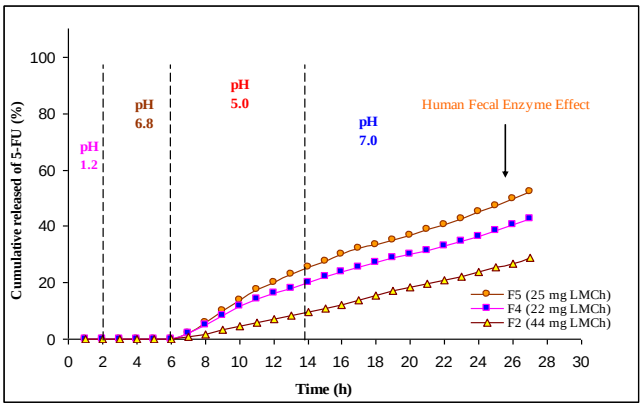


Figure 6: Effect of chitosan concentration on 5-FU release

3.4.2 Effect of citric acid concentration on 5-FU release

The organic acids which are used as excipients in both the granules and tablet matrices might be sufficiently delayed disintegration time of tablets and drug liberation until reach the colon. It was found to be very important to include citric acid in the tablet matrix when preparing colon-specific formulations (26). Drug release takes after a lag time especially once the citric acid percentage in the tablet matrix exceeds 10. Generally, it appears that the delivery rate of a drug from an osmotic pump depends to a large extent on the solubility of drug at saturation, osmotic pressure of formulation and the release pattern of controlled porosity osmotic pump system (27). The data in Figure 7 clearly demonstrate that the concentration of citric acid in the core formulation had a marked effect on 5-FU release in SCF (pH 5 and 7). The results show that the cumulative release of 5-FU for F1 (61% + 2.3) was significantly ($p<0.05$) higher than F4 (43% + 1.4), F5 (52% + 1.9) and F3 (32% + 0.6) within 27 h. These results are related to the aqueous solubility of 5-FU in F1 (13.72 mg/ml)

Table 2: pH, Osmolality and Osmotic Pressure of Formulations Containing 5-FU (n=3)

CODE	AFTER 30 MIN SHAKING						AFTER 24 H SHAKING			
	No drug			5-FU			No drug		5-FU	
	pH	Osmolality (mOsm/kg)	Osmotic pressure (atm)	pH	Osmolality (mOsm/kg)	Osmotic pressure (atm)	Osmolality (mOsm/kg)	Osmotic pressure (atm)	Osmolality (mOsm/kg)	Osmotic pressure (atm)
F1	2.45	60	1.464	2.47	63	1.5372	81	1.9764	89	2.1716
F2	7	4	0.0976	6.3	6	0.1464	5	0.122	7	0.1708
F3	3.40	13	0.3172	3.41	17	0.4148	18	0.4392	20	0.488
F4	2.78	50	1.22	2.92	61	1.4884	85	2.074	97	2.3668
F5	2.89	39	0.9516	2.91	49	1.1956	106	2.5864	119	2.9036
F6	3.31	15	0.366	3.35	19	0.4636	27	0.6588	33	0.8052
F7	2.42	173	4.2212	2.44	177	4.3188	325	7.93	331	8.0764

which are higher than the aqueous solubility of 5-FU in F3, F4, and F5 (11.76 mg/ml). This also expected since incorporation of citric acid with chitosan in core of tablets for F3, F4 and F5 resulted in gelling of chitosan that would slow down drug release [28]. $t_{25\%}$ values of 5-FU formulas (F1, F3, F4 and F5) that studied in human fecal enzyme were 8.54 h, 16.1 h, 12.25 h and 10 h, respectively but in SCF, $t_{100\%}$ values of F1, F3, F4 and F5 for 5-FU were 34 h, 64 h, 49 h and 40 h, respectively. In addition, osmotic pressure of F5 that push drug outside (2.9 atm) was significantly ($p<0.05$) higher than osmotic pressure in F4 (2.3668 atm) and F3 (0.81 atm) and this is related to difference in amounts of citric acid with chitosan. As the concentration of citric acid increased, viscosity of chitosan solution also increased, which caused the increment of osmotic pressure of core tablets [22].

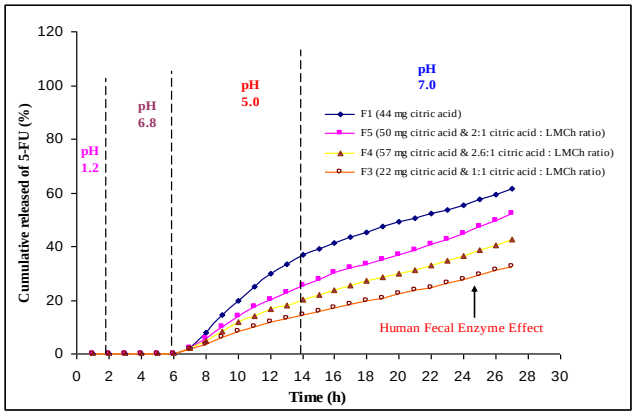


Figure 7: Effect of citric acid concentration on 5-FU release

3.4.3 Effect of molecular weight of chitosan on 5-FU release

It has been reported that the increases in the molecular weight of chitosan markedly increases the viscosity of gel formed [25]. It appears that the cumulative release drug in F6 containing MMCh (39% ± 0.7; pH 3.31) was significantly higher ($p<0.05$) than F3 containing LMCh (32% ± 0.6; pH 3.41) as shown in figure 8. In addition, a high osmotic pressure in core of F6 (0.8052 atm) in comparison with a low osmotic pressure in core of F3 (0.488 atm) (Table 2) which resulted in the faster pushing of 5-FU suspensions out of the system in F6 than as in F3. This explained the reason of higher release rate of drug from F6 than F3.

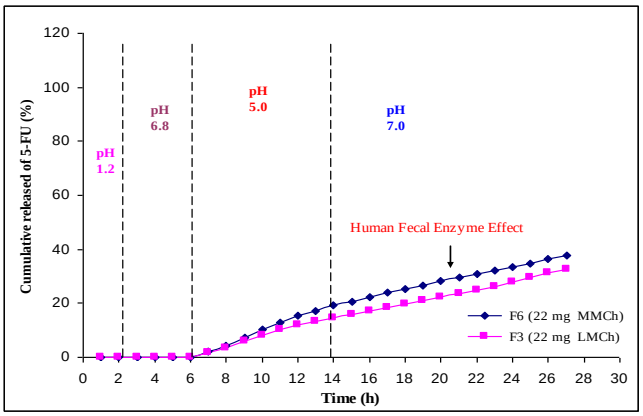


Figure 8: Effect of molecular weight of chitosan on 5-FU release

3.4.4 Effect of osmogents on 5-FU release

The effect of different concentrations of osmotically active sodium chloride in double coated pellets cores on drug release rate was studied [142]. The addition of sodium chloride increased drug release rates irrespective of the pH of the dissolution medium. Our model of osmotic tablets also was double coated to verify the same principle of pH-independent drug release profile whenever the osmogent as sodium chloride was added. The decrease in the aqueous solubility of 5-FU (9.89 mg/ml) was observed after addition of 0.55% of sodium chloride into the saturated aqueous solution of drug (Fig. 5). This result is in agreement with the report of McClelland coworkers [20]. The addition of sodium chloride alone was excluded

from the prepared formulations of osmotic tablet. In this study, a combination of sodium chloride with citric acid for F7 (1:1) compared with F1 containing citric acid alone and explained that the cumulative release of drug was significantly higher ($p<0.05$) in F7 (68% ± 1.8) than in F1 (61% ± 2.3) within 27 h (Fig. 9). This may be due to the higher solubility of 5-FU (15.13 mg/ml) in F7 compared with F1 (13.72 mg/ml) (Fig. 5). Moreover, the osmolality and/or osmotic pressure of the core for 5-FU in F7 (331 mOsm/kg and/or 8.0764 atm) was higher than in F1 (89 mOsm/kg and/or 2.1716 atm) (Table 2). The mechanism of pH-independent release profile of drugs in double coated osmotic tablet can be explained as follows; the addition of the osmotically active ingredient increased the imbibing of aqueous fluids into the tablet cores thus providing a saturated drug solution inside the core and increasing drug concentration gradients to increase drug release rates at low and high pH [(30)].

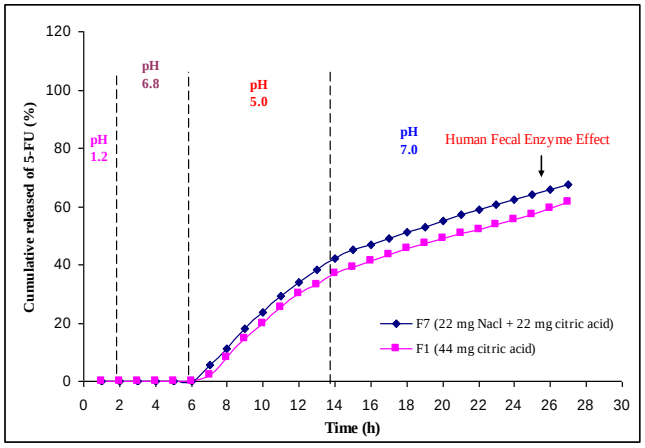


Figure 9: Effect of osmogents on 5-FU release

3.4.5 Effect of wall thickness and weight gain of semipermeable membrane on 5-FU release

The selected 5-FU osmotic tablets of F3 with different weight gain (6%, 10% and 14%) of microporous membrane were prepared to demonstrate the effect of coating thickness on drug release. Figure 10 shows that within 27 h the cumulative release of 5-FU were 45% ± 1.2, 32% ± 0.6 and 15% ± 0.5 for 6%, 10% and 14% weight gains of membrane, respectively. It was evident that drug release decreased as the weight gain of the semipermeable membrane increased. This could be as mentioned that the thickness of membrane increased, the imbibing water rate of semipermeable membrane and the liquefaction rate of the tablet core decreased correspondingly, resulting in the decrement of drug release [(2)]. In addition, more weight gain of cellulose acetate membrane would make the chitosan incorporated less accessible to bacterial degradation, resulting in the slower drug release. In all of the prepared formulas, no bursting of the systems was observed during the dissolution procedure, thus assuring that the prepared formulas can be expected to remain intact in GIT without any incidence of dose dumping. Based on the results obtained, it could be concluded that membrane thickness had a profound effect on drug release from CPOP-CT system. Under human fecal enzyme effect, $t_{10\%}$ values for 6%, 10% and 14% weight gain of the semipermeable membrane of 5-FU osmotic tablet (F3) were 4.60 h, 6.43 h and 13.49 h, respectively. There were statistically significant ($p<0.05$) difference among the cumulative release percentage of 5-FU of different weight gain of semipermeable membrane of F3. In addition, SCF $t_{100\%}$ values for 6%, 10% and 14% weight gain of the semipermeable membrane of 5-FU osmotic tablet (F3) were 46 h, 65 h and 135 h, respectively. Furthermore, a good linear significant correlation (R^2 = 0.9984, correlation coefficient = 0.999 and $p<0.05$ at 95%

confidence level for fitting a linear model) was found between 5-FU release rate of F3 (0.16 mg/h, 0.067 mg/h and 0.038 mg/h, respectively) and reverse wall thickness (83.33 cm^{-1}, 50 cm^{-1} and 37.04 cm^{-1}, respectively) of different weight gain (6%, 10% and 14%, respectively) of semipermeable membrane as well as data show that the 5-FU release rate increased when the thickness of semipermeable membrane decreased (Fig. 11).

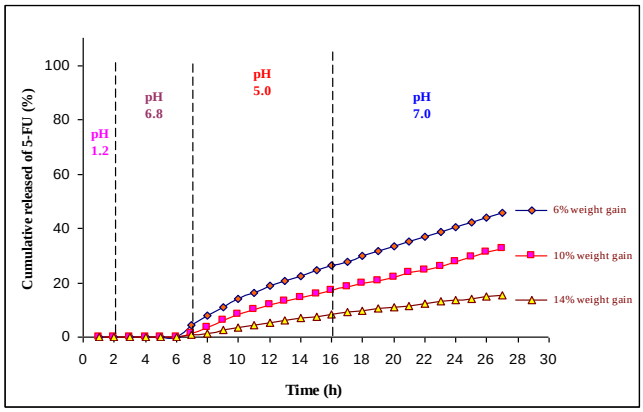


Figure 10: Effect of weight gain of semipermeable membrane on 5-FU release in F3

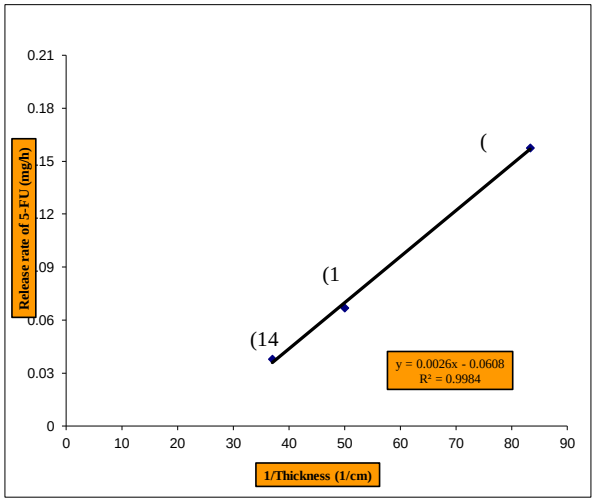


Figure 11: Relationship between 5-FU release rate from F3 and the reverse thickness of different weight gain (6%. 10% and 14%) of microporous membrane

3.5 Effect of osmotic pressure of the media on 5-FU release

The effect of osmotic pressure on the release of the selected formulas with acceptable hardness (5.68 ± 0.16 kg), friability (0.22%) and thickness (3.39 ± 0.028 mm) of F7 containing 5-FU were conducted in media of different osmotic pressure. The results showed that the drug release is highly dependent on the osmotic pressure of the media. In absence of enzyme, the data indicate that the decrease in osmotic pressure of buffer media (pH 5) (30.67 atm < 21.7atm < 12.69atm) leads to increase in release of 5-FU for F7 (7% ± 0.8 > 14% ± 0.2 > 19% ± 0.5) (Fig. 12) within 14 h. A good linear significant correlation (R^2 = 0.9999, correlation coefficient = -0.9999 and $p<0.05$ at 95% confidence level for fitting a linear model) was obtained between 5-FU release rate of F7 and external osmotic pressure as shown in Figure 13. Furthermore, osmotic pump mechanism governing drug release was proved by plotting a relationship between release rate of drug and osmotic pressure difference. A linear relationship was obtained for 5-FU (Fig. 14) (R^2 = 0.9999 and $p<0.05$ at 95% confidence level for fitting a linear model) confirming that osmotic pumping is the major mechanism of drug release from prepared formulas [31, 32].

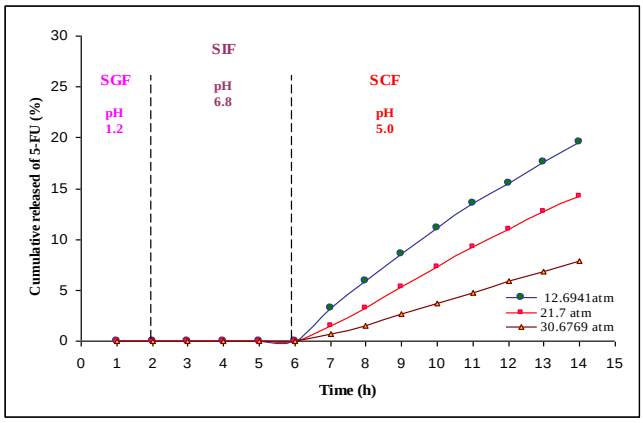


Figure 12: Effect of osmotic pressure of the media on 5-FU release of F7

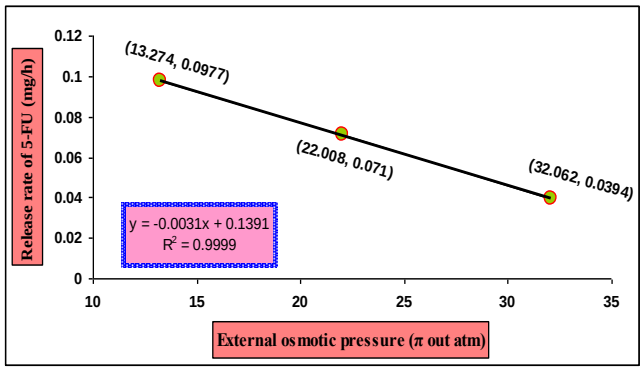


Figure 13: Relationship between 5-FU release rate of F7 and the external osmotic pressure

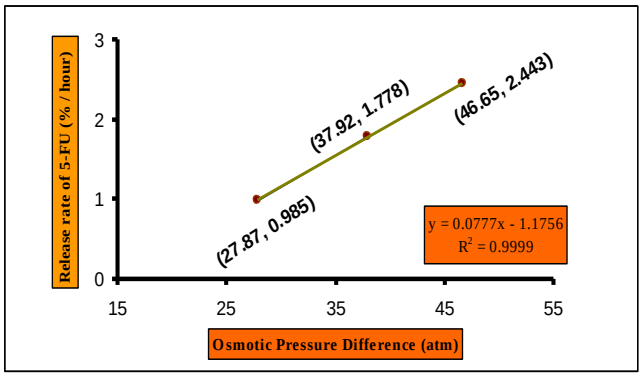


Figure 14: Effect of osmotic pressure difference across the membrane on the 5-FU release of F7

3.6 Study the kinetics of drug release

The kinetics of osmotic drug release is directly related to the solubility of drug within the core. The different release kinetics might reflect different release mechanisms. Three kinetic models of release including the zero-order equation (Eq.1), Higuchi equation (Eq.2) and first-order equation (Eq.3) were applied to process in-vitro data in order to find the equation with the best fit [35].

$$Q = k_1 t \quad (1)$$

$$Q = k_2 (t)^{0.5} \quad (2)$$

$$\ln (Q) = k_3 t \quad (3)$$

Where Q is the release percentage at time t. k_1 (% / h), k_2 (% / $h^{1/2}$) and k_3 (h^{-1}) are the rate constants of zero-order, Higuchi and first order model, respectively. Drug release data of the selected formula (F6) was fitted to various mathematical models as shown in Table 3. It was evident that poorly soluble 5-FU release of F6 was fitted well into zero order kinetic (R^2= 0.9972) compared to other model kinetics in the absence of enzyme effect. After 6 h lag time, the enzyme will be started to degrade the chitosan in semipermeable cellulose acetate membrane and leads to formation of pores in the film that caused a gradual increase in drug release and then altered the zero order kinetic model of 5-FU into first order model with best fitted (R^2= 0.9923). This is in agreement with the results of Liu et al and Verma et al [36].

Table 3: Fitting of Drug Release Data of Selected Formula of 5-FU (F6) According to Various Mathematical Models

Models	No enzyme				Human fecal enzyme			
	R^2	Intercept	Slope	k	R^2	Intercept	Slope	k
Zero order	0.9972	0.0341	0.7848	0.7848 [a]	0.9898	0.3367	2.38	2.38 [a]
First order	0.9778	3.074	-0.0754	-0.0754 [b]	0.9923	3.6942	-0.0468	-0.0468 [b]
Higuchi	0.9537	-7.3968	5.3627	5.3627 [c]	0.9864	-8.3279	9.5354	9.5354 [c]

a refers to unit of zero order kinetic model (mg ml^{-1} h^{-1}); **b** refers to unit of first order kinetic model (h^{-1}); **c** refers to unit of Higuchi model (mg ml^{-1} $h^{-1/2}$); **R^2** refers to squared regression coefficient; **k** refers to rate constants of zero-order, first-order and Higuchi model.

3.7 In vitro enzymatic degradation study

Low molecular weight of chitosan was chosen for its ease of degradation by colonic enzymes [(37)]. The degradation of low molecular weight chitosan (LMCh) (0.5% w/v) was studied by measuring of viscosity and characterized by the decrease of the specific viscosity after a given time of degradation. Figure 15 represents the specific viscosity versus degradation time of chitosan in absence and presence of 0.02% (w/v) almond emulsion β-glucosidase and human fecal enzyme under the reaction conditions. The data shows that the specific viscosity of the reaction mixture significantly decreased ($p<0.05$) over time in presence of almond emulsion β-glucosidase (29 ± 1) and human fecal enzyme (16 ± 1) compared with the control one (44.8 ± 2) without enzyme. The reduction in the specific viscosity of control is probably due to the acidic hydrolysis of the chitosan sample at pH 5.0. Enzymatic treatment resulted in a substantial loss in viscosity of the chitosan solution and indicating depolymerization that occurs by an apparently random splitting of interior glycosidic bonds, and such depolymerization took place very quickly during the initial 2 h [(38)]. The results also show that the almond emulsin β-glucosidase has chitosanolytic activity and its ability to degrade chitosan.

3.8 Effect of enzyme on the release of 5-FU

Thirty percent of the total fecal flora are bacteroides that constitute the most abundant members of the intestinal microflora of mammals and responsible for depolymerization of chitosan by producing a wide range of glycosidase enzymes [(3,39)]. Depending on this idea the human fecal flora which was prepared would be considered as the most easier method than the preparation of rat cecal flora because the preparation of rat cecal flora required continuous supplying of carbone dioxide to keep the environment anaerobic while the

human fecal flora was prepared by using anaerobic thioglycolate media which responsible for growing of anaerobic bacteria. The data shows that a core coated with 25 % (w/w) LMCh of semipermeable membrane of cellulose acetate was susceptible to degradation by almond emulsin β-glucosidase and human fecal enzymes as shown in Figure 16. Therefore the effect of these enzymes on drug release of 5-FU for osmotic tablets was studied. The selected formulas F6 that contains chitosan showed a significant ($p<0.05$) differences among of cumulative releases of 5-FU within 27 h concerning the comparison between groups containing 0.02% almond emulsin β-glucosidase (26% $\pm$ 0.2), human fecal enzymes (39% $\pm$ 0.7) and absence of enzymes (18% $\pm$ 0.8). After 6 h lag time, $t_{10\%}$ values of drug with no enzyme effect, almond emulsin β-glucosidase and human fecal enzyme were 11.16 h, 8 h and 5.29 h, respectively and showed that the mimic enzyme (almond emulsion β-glucosidase) and human fecal enzyme had significantly increasing effects on cumulative release of 5-FU about 1.39 and 2.11 times, respectively compared with the release of 5-FU from F6 in the absence of enzyme.

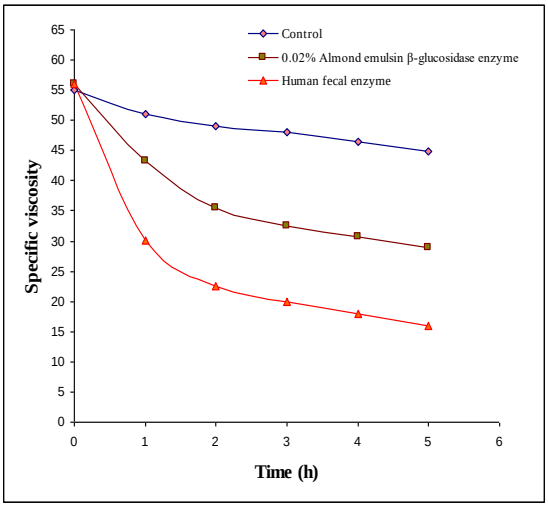


Figure 15: Specific viscosity-time profile of chitosan (0.5% w/v) degraded by enzyme at 30 ° C (mean $\pm$ SD, n = 3)

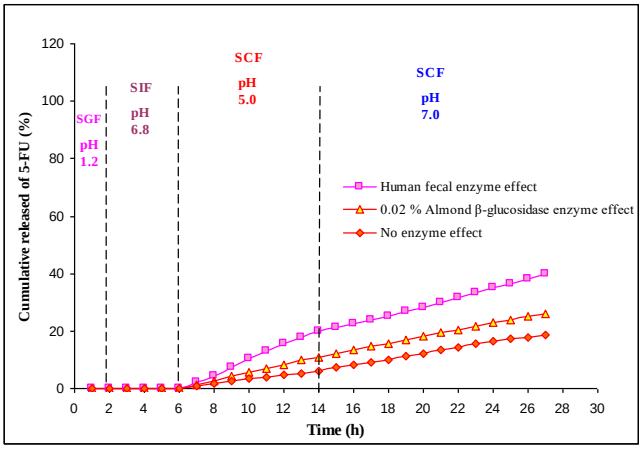


Figure 16: Effect of enzyme on release of 5-FU from F6

3.9. Conclusion

The FTIR spectrum data of 5-FU showed that there is no interaction between drug and their excipients in physical mixture and in formulations. F2 (44% w/w LMCh) which contains the highest amount of chitosan gave in vitro the slowest release of 5-FU compared with F4 and F5. In Formulas containing chitosan, the decreased amount of citric acid in core of F3 (22% w/w CA) leads to lower in release profiles of 5-FU compared with F1 (44% w/w CA), F4 (57% w/w CA) and F5 (50% w/w CA). MMCh contained in core of F6 made the formula to release 5-FU with percentage higher than the release of drug form F3 which contains LMCh. In absence of chitosan, F7 containing mixture of osmogents in ratio 1:1 of sodium chloride: citric acid has a higher release of drug than F1 containing citric acid alone. The release rate of 5-FU in F3 increased as weight gain percentage and wall thickness of semipermeable cellulose acetate membrane coated tablet decreased. The mechanism of kinetic release of 5-FU was changed due to the degradation effect of enzyme on chitosan in semipermeable cellulose acetate membrane. The specific viscosity of chitosan solution (0.5% w/v) under influence of human fecal enzyme and almond emulsin beta-glucosidase was decreased within 5 h into 16±1 and 29±1, respectively compared with control one (44.8±2).

REFERENCES

1. Leopold CS. Coated dosage forms for colon-specific drug delivery. Pharm Sci Tech Tod 1999; 2(5): 197-204.
2. Liu H, X Yang G, Nie SF, Zhou LL, Liu H, Tang R, Pan WS, Chitosan-based controlled porosity osmotic pump for colon-specific delivery system: Screening of formulation variables and in vitro investigation. Int J Pharm 2006; 24 Sep: 1-10.
3. Zhang H and Neau SH. In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents. Biomaterials 2002; 23(13): 2761-2766.
4. Chourasia, MK, Jain SK, Pharmaceutical approaches to colon targeted drug delivery systems, J Pharm Pharmaceut Sci 2003; 6(1): 33-66.
5. Kelm GR, Kondo K, Nakajima A. Pharmaceutical dosage form with multiple enteric polymer coatings for colonic delivery. US Patent 5914132 1999; 22: 1-18.
6. Santus G, Baker RW. Osmotic drug delivery: A review of the patent literature. J Control Release 1995; 35: 1–21.
7. Liu L, Khang G, Rhee JM, Lee HB. Monolithic osmotic tablet system for nifedipine delivery. J Control Release 2000; 67: 309–322.
8. Lamprecht A, Yamamoto H, Takeuchi H, Kawashima Y. Microsphere design for the colonic delivery of 5-fluorouracil. J Control Rel 2003; 90: 313–322.
9. Duschinsky R, Pleven A, Heidelberger C. The Synthesis of 5-Fluoro- Pyrimidines. J Am Chem Soc 1957; 79: 4559-4560.
10. Heidelberger C. In: Antineoplastic and Immunosuppressive Agents. Sartorelli AC, John DG, eds. Part 11, Springer, Berlin, Heidelberger, New York 1975: 193-231.

11. Malet MM, Martino R. Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): A review. Oncologist 2002; 7(4): 288-323.

12. Mandel HG. The incorporation of 5-fluorouracil into RNA and its molecular consequences. Prog Mol Subcell Biol 1969; 1: 82-135.

13. Nei S, Fan X, Peng Y, Yang X, Wang C, Pan S. In vitro and in vivo studies on the complexes of vinpocetine with hydroxypropyl-β-cyclodextrin. Arch Pharm Res 2007; 30(8): 991-1001.

14. US Pharmacopeia XXIII. Buffer Solution. US Pharmacopeial Convention. Rockville MD 1995: 2053.

15. Kohler F. Buffer solutions. In: Scientific Tables. Lentner C ed. Geigy. Basle, Ciba-Geigy Ltd 1984: 58–60.

16. Singh BN. Modified-Release Solid Formulations for Colonic Delivery. Recent Patents on Drug Delivery Formulation 2007; 1: 53-63.

17. Sekigawa F, Onda Y. Coated solid medicament from having releasability in large intestine. US patent 5217720 1993; 8: 1-10.

18. Cappuccino JG, Sherman N. Techniques for the Cultivation of Anaerobic Microorganisms. In: Microbiology a Labrotary Manual, 8th. San Francisco, USA 2008: 127-128.

19. US Pharmacopeia XXIII. 711 Dissolution. US Pharmacopeial Convention. Rockville MD 1995: 1578-1579.

20. Kanagale P, Lohray BB, Misra A, Davadra P, Kini R. Formulation and Optimization of Porous Osmotic Pump–based Controlled Release System of Oxybutynin, AAPS Pharm Sci Tech 2007; 8(3): E1-E7.

21. Kristl J, Smid- Korbar J, Struc E, Schara M, Rupprecht H. Hydrocolloids and gels of chitosan as drug carriers. Int J Pharm 1993; 99: 13- 19.

22. Nangia A, Li B, Dely A, Lodin U, Cardinal JR. Controlled release composition with semi-permeable membrane and poloxamer flux enhancer. US Patent 0048119 2005; 3: 1-7.

23. Nakatsuka SH. Cellulose acetate semipermeable membrane and process for producing the cellulose acetate semipermeable membrane. US Patent 6372136 B1 2002; 16: 1-12.

24. Petereit HU, Weisbrod W. Formulation and process considerations affecting the stability of solid dosage forms formulated with methacrylate copolymers. Euro J Pharma Biopharm 1999; 47: 15-25.

25. Säkkinen M. Ph.D. Thesis .Biopharmaceutical Evaluation of Microcrystalline Chitosan as Release- Rate- Controlling Hydrophilic Polymer in Granules for Gastro- Retentive Drug Delivery. Department of Pharmacy. Division of Biopharmaceutics and Pharmacokinetics, University of Helsinki 2003: 1-63.

26. Nykänan P. Ph.D. Thesis. Development of Multiple-Unit Oral Formulations for Colon-Specific drug Delivery Using Enteric Polymers and Organic Acids as Excipients, Department of Pharmacy. Division of Biopharmaceutics and Pharmacokinetics, University of Helsinki 2003: 1-55.

27. Anagale P, Lohray BB, Misra A, Davadra P, and Kini R. Formulation and Optimization of

Porous Osmotic Pump–based Controlled Release System of Oxybutynin, AAPS Pharm Sci Tech 2007; 8(3): 1-7.

28. Säkkinen M, Tuononen T, Jürjenson H, Veski P, Marvola M Evaluation of microcrystalline chitosan for gastro- retentive drug delivery. Eur J Pharm Sci 2003: 19: 345-353.

29. McClelland GA, Sutton SC, Engle K, Zentner GM. The solubility-modulated osmotic pump: In vitro/in vivo release of diltiazem hydrochloride. Pharm Res 1991; 8: 88–92.

30. Verma RK, Mishra B, Garg S. Osmotically controlled oral drug delivery. Drug Dev Ind Pharm 2000; 26: 695–708.

31. Jensen JL, Appel LE, Clair JH, Zentner GM. Variables that affect the mechanism of drug release from osmotic pumps coated with acrylate/methacrylate copolymer latexes. J Pharm Sci 1995; 84: 530–533.

32. Ozturk AG, Ozturk SS, Palsson BO, Wheatley TA, Dressman JB. Mechanism of release from pellets coated with an ethylcellulose-based film. J Control Rel 1990; 14: 203–213.

33. McClelland GA, Sutton SC, Engle K, Zentner GM. The solubility-modulated osmotic pump: In vitro/in vivo release of diltiazem hydrochloride. Pharm Res 1991; 8: 88–92.

34. Theeuwes F. Elementary osmotic pump. J Pharm Sci 1975; 64: 1987–1991.

35. Wu PC, Tsai MJ, Huang YB, Chang JS, Tsai YH. In vitro and in vivo evaluation of potassium chloride sustained release formulation prepared with saturated polyglycolyed glycerides matrices. Int J Pharm 2002; 243: 119-124.

36. Verma RK, Garg S. Development and evaluation of osmotically controlled oral drug delivery system of glipizide. Eur J Pharm Biopharm 2004; 57: 513-525.

37. Nunthanid J, Huanbutta K, Luangtana-anan M, Sriamornsak P, Limmatvapirat S, Puttipipatkhachorn S. Development of time-, pH-, and enzyme-controlled colonic drug delivery using spray-dried chitosan acetate and hydroxypropyl methylcellulose. Eur J Pharm Biopharm 2007; 31(5): 1-7.

38. Zambito Y, Di Colo G. Preparation and in vitro evaluation of chitosan matrices for colonic controlled drug delivery. J Pharm Pharmaceut Sci 2003; 6(2): 274- 281.

39. Cummings JH, Macfarlane GT. The Colonic Flora, Fermentation and Large Bowel Digestive Function. In: The large intestine: Physiology, Pathophysiology and Disease. Philips SF, Pemberton JH, Shorter RG eds. Raven press Ltd., New York 1991: 51-92.