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A Pharmaceutical Study on Different Approaches for Itopride Hydrochloride Sustainment: *In-vitro and in-vivo* evaluation

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ABSTRACT

Recent trends indicate that dosage form drug delivery systems are especially suitable for achieving sustained or delayed release oral formulations with low risk of dose dumping, flexibility of blending to attain different release patterns as well as reproducibility. One of the approaches toward this goal was to develop and formulate extended release matrix oral tablets of itopride hydrochloride (ITO) as a highly water soluble drug and to increase its gastric retention time. Matrix tablets of (ITO) were developed using different methods such as: (wetting granulation, direct compression, and coating compression), by using different types of polymers (hydroxypropyl methyl cellulose (HPMC) K15M, hydroxypropyl cellulose (HPC) low viscosity, Eudragit® RL100, and Carnauba wax). The prepared tablets were evaluated according to various physicochemical characteristics such as: weight and thickness variation, drug content, hardness, friability, and in vitro drug release. Tablet weight variation ranged from 395 ± 0.34 to 409 ± 1.78 mg, thickness ranged from 3.16 ± 1.37 to 3.74 mm, drug content ranged from 94.5 ± 1.01% to 106.2 ± 0.08%, friability ranged from 0.04 ± 0.69 t o 0.84 ± 0.45, and hardness ranged from 5.25 ± 0.25 to 8.80 ± 0.4 Kg/cm^2. Results indicated that drug release depended upon method of preparation and polymer type. Furthermore, in vivo testing of the optimum sustained release tablet formulation (F23) using coating compression by HPMC as coat was performed in human subjects, and determined and compared to that of a commercial oral tablet (Ganaton®) as a reference formulation. The obtained the maximum plasma concentration (C_{max}) and the area under the curve from zero to infinity ($AUC_{(0-\infty)}$) values were higher following formulated tablet administration than after Ganaton® administration. The percentage relative bioavailability of ITO from the selected formula in human volunteers was found to be 243% compared to Ganaton®.

Keywords: sustained release, wet granulation, direct compression, coating compression, gastric retention time, Itopride Hydrochloride, bioavailability.

INTRODUCTION

Sustained release delivery systems have added advantages over immediate release dosage forms. These include reduction of dosing frequency by administering the drug once or twice a day (1). Since the frequency of drug administration is reduced, patient compliance can be improved; gastrointestinal side effects are reduced (2-3). In addition, sustained release dosage forms (SRFS) cause less fluctuation of plasma drug levels and leads to more uniform drug effect and lesser total dose (4). On the other hand, sustained release dosage forms have some disadvantages which include: dosage form increase quantity of drug release can cause dumping of drug which in turn leads to toxicity, reduced potential for accurate dose adjustment, need for additional patient education: (as not crush or chew the dosage unit),cost of manufacture , and stability problems (5). Hydrophilic polymer matrices are widely used for formulating SRFS (6). In fact, a matrix is defined as a well-mixed composite of one or more drugs with gelling agent i.e. hydrophilic polymers (7). Numerous oral SRFS as membrane controlled system, matrices with water soluble/insoluble polymers or waxes, and osmotic systems have been developed (8). Various drug delivery techniques have been developed to sustain the release of drugs, such as triple-layered tablets and osmotic pumps

with laser drilled holes. The latter technologies are intricate and relatively expensive to manufacture. Thus, there remains an interest in developing other formulations that allow for sustained release of drugs using readily available, inexpensive excipients (9). There are easily applied techniques such as: wet granulation (10), direct compression (11), and coating compression (12).

Prokinetic drugs acts by promoting gastric motility increasing gastric emptying, preventing the retention and reflux of gastric contents and thus providing symptomatic relief (13). Itopride (ITO) is a novel prokinetic agent. This drug was first developed and marketed in Japan 1995 (14). ITO has anticholinesterase (AchE) activity as well as dopamine D2 receptor antagonistic activity and is being used for the symptomatic treatment of various gastrointestinal motility disorders (15). Following oral administration, ITO is rapidly and extensively absorbed and peak serum concentrations are achieved within 35 minutes (16). ITO is metabolized in the liver via N-oxidation to inactive metabolites by the enzyme flavin-containing monooxygenase (FMO) (17). It is excreted mainly by the kidneys as metabolites and unchanged drug (17-18). Gastroesophageal reflux disease (GERD) is one of the most prevalent upper gastrointestinal disorders in clinical practice. GERD is a chronic disease with relapsing symptoms, and lifelong treatment is required in 25% to 50% of patients (19). For decades, GERD has been approached as a spectrum of diseases. Removing non-erosive reflux disease, erosive esophageal, and Barrett's esophagus will dramatically shift the focus from esophageal mucosal injury to mechanisms leading to symptom generation in each group and foster specific therapeutic modalities that benefit each individual group of patients (20). The usual daily dosage of ITO for adults is 50 mg orally in 3 divided doses before each meal (18). ITO is a water-soluble compound hence various release rate retardant polymers were used in earlier works to sustain the release of the drug like hydroxypropyl methylcellulose (HPMC K4M) and (HPMC K100M) (21). Sustained release oral dosage forms were designed in two ways, sustained dosage form with burst release and without burst release. (22).

The current study deals with screening different methods for preparation of ITO as sustained release oral tablet dosage form using several polymers. The prepared tablets were characterized according to hardness, drug content, friability, and drug release and to estimate the pharmacokinetics of ITO in healthy human volunteers following oral administration of the optimum tablet formulation in comparison to commercial Ganaton® (50 mg) oral tablet.

EXPERIMENTAL SECTION

Itopride HCL (ITO) was kindly supplied by Mash Premiere for Pharmaceutical Industry, Egypt. Hydroxypropyl methylcellulose K15M (HPMC) and hydroxypropyl cellulose (HPC) low viscosity EF Pharma were obtained from Colorcon, England and EPICO Pharmaceutical Company, Egypt respectively. Eudragit RL 100 was a kind gift from Rohm GmbH and Co. KG, Germany. Carnauba wax (CW) was procured from Koster Keunen Inc., USA. Calcium hydrogen orthophosphate ($CaHPO_4$), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), calcium chloride ($CaCl_2$), methylene chloride, methanol, and ethanol were obtained from Adwic, Egypt. Acetonitrile (HPLC grade) was purchased from Merck Co, Hohenbrunn, Germany. All other reagents and chemicals were of analytical grade.

Preparation of ITO sustained release oral tablets by wetting granulation:
Different tablet formulations were prepared by wet granulation technique. All the powders were passed through sieve ASTM (American Society of Testing and Materials) 80 mm mesh. A hundred mg of ITO and different amounts of $CaHPO_4$ (as diluent) and of HPMC were mixed thoroughly with the aid of a sufficient amount of granulating agent (ethanol). After enough cohesiveness was obtained, the mass was sieved through sieve 22/44 mesh. The granules were dried at 40°C in a hot-air oven (Heraus, Germany) for 12 h and thereafter kept in a dessicator over $CaCl_2$ for 12 h at room temperature. Once dry, the granules were evaluated for drug content in order to calculate the practical amount of granules compressed in the form of tablets. The tablets were compressed using a single-punch tablet compression machine (Cadmach, Ahmedabad, India) in tablets using (11 mm diameter, biconvex punches). The composition of different tablet formulations prepared by wet granulation technique is listed in Table (1).

Preparation of ITO sustained release tablets by direct compression:
Tablet ingredients were passed through sieve 20 mesh, then the accurately weighed ingredients were mixed together in a porcelain mortar for 5 min. The blend was then compressed using a single-punch tablet compression machine using (11 mm diameter, biconvex punches) as mentioned in Table (1).

Preparation of ITO sustained release tablets by coating compression:
The inner core tablets were prepared using direct compression method. The powder mixtures of ITO and $CaHPO_4$ (and either HPC, HPMC, Eudragir® RL100, polymers or CW, if necessary) were blended for 10 min in a porcelain mortar and 200 mg of the resultant powder blend of $CaHPO_4$ and different polymers was compressed using a single-

punch tablet compression machine with 9 mm diameter biconvex punches. Then, the core tablets were press-coated with 200 mg of mixed blend of $CaHPO_4$ and different polymers as given in table (2). A hundred mg of barrier layer material (polymers with/without diluent) as shown in table (2) was weighed and transferred into a 9 mm die then the core tablet was placed manually at the center. The remaining 1100 mg of the barrier layer material was added into the die and compressed using a single-punch tablet compression machine.

Table (1): Composition of different tablet formulations from F1 to F14

Ingredients per tablet (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
HPMC	20	30	40	50	20	30	40	50	-	-	-	-	-	-
HPC	-	-	-	-	-	-	-	-	20	40	50	-	-	-
EudRL100	-	-	-	-	-	-	-	-	-	-	-	20	40	50
ITO	100	100	100	100	100	100	100	100	100	100	100	100	100	100
$CaHPO_4$	280	270	260	250	280	270	260	250	280	260	250	280	260	250
Method of preparation	Wetting granulation by HPMC				Direct compression by HPMC				Direct compression by HPC			Direct compression by Eud[1]		

Eud[1] = EudragitRL100

Table (2): Composition of different tablet formulations from F15 to F34 using coating compression method

Formula	Core (200mg)						Coat (200mg)				
	ITO	$CaHPO_4$	HPC	HPMC	CW[2]	Eud[1]	$CaHPO_4$	HPC	HPMC	CW[2]	Eud[1]
F15	100	100	..	..	..	..	..	200	..	..	..
F16		50	50	..	..	..	50	150	..	..	..
F17		100	..	..	..	..	50	150	..	..	..
F18		100	"	..	..	..	100	100	..	..	..
F19		"	100	..	..	..	100	100	..	..	..
F20		100	„	..	..	..	..	..	200	..	..
F21		50	..	50	..	..	50	..	150	..	..
F22		100	..	..	..	..	50	..	150	..	..
F23		100	..	..	..	..	100	..	100	..	..
F24		..	..	100	..	..	100	..	100	..	..
F25		100	..	..	..	..	..	..	..	200	..
F26		50	..	..	50	..	50	..	..	150	..
F27		100	..	..	..	..	50	..	..	150	..
F28		100	..	..	..	..	100	..	..	100	..
F29		..	..	..	100	..	100	..	..	100	..
F30		100	..	..	..	..	..	..	..	..	200
F31		50	..	..	..	50	50	..	..	..	150
F32		100	..	..	..	..	50	..	..	..	150
F33		100	..	..	..	..	100	..	..	..	100
F34		..	..	..	..	100	100	..	..	..	100

Eud[1] = EudragitRL100, CW[2]=Carnauba wax

Evaluation of tablet formulations:

1. Thickness:

The thickness of the tablets was determined using a micrometer (CLM1–15QM, Mitutoyo, Kawasaki, Japan). The thickness of five tablets from each batch was evaluated and the results were expressed as average values ± standard deviation (SD).

2. Weight variation:

Ten tablets of each formulation were weighed using an electronic balance (Digital balance, Sartorius GmbH, Gottingen, Germany) and the average weight of tablets of each formulation ± SD was calculated.

3. Drug content:

The tablet formulations were tested for their ITO content. Six tablets of each formulation were finely powdered and quantities of the powder equivalent to 100 mg of ITO were accurately weighed (400 mg of powder), transferred to a 100 ml volumetric flask containing 50 ml of methylene chloride and shaken with intermittent sonication (Model 275 T, Crest Ultrasonics Corp., Trenton, USA) to ensure complete solubility of the drug. The solution was suitably diluted and measured spectrophotometrically at λ_{max} 260 nm (23-24) with reference to a previously constructed calibration curve in methylene chloride (R^2 = 0.999, n=3). The excipients used in the tablet formulations did not interfere under these conditions. Each determination was done in triplicate and the mean drug content ± SD was deduced.

4. Hardness :
For each formulation, the hardness of six tablets was measured using Hardness Tester (Fujiwara, Seisukusho Corporation, Japan) and the mean hardness ± SD of the six tablets was assessed.

5. Friability:
For each formulation, the friability of six tablets was determined using a Friabilator (H. Jurgens and Co. GmbH and Co., D2800, Bermen, Germany). Tablets were weighed accurately, placed in a Friabilator and after the given number of rotations (100 rotations/4 min) the loose dust was removed from the tablets and the tablets were reweighed. The difference in tablet weight is the friability of the tablet which indicated the ability of the tablets to withstand this type of wear.

6. In vitro drug release studies:
The release of ITO from the prepared tablets was carried out using a USP basket-type dissolution apparatus (Apparatus 1, PharmaTest, USA) at a rotation speed of 50 rpm, and a temperature of 37 ± 0.5°C. Simulation of gastrointestinal transit conditions was achieved by using different dissolution media. Thus, drug release studies were initially conducted in 900 ml simulated gastric fluid without pepsin (SGF, pH 1.2) for the first 2 h as the average gastric emptying time is about 2 h. Then, SGF (pH 1.2) was replaced with 900 ml of enzyme-free simulated intestinal fluid (SIF) using phosphate buffer at pH 6.8 and drug release study was continued for another 10 h to simulate the colonic conditions. Samples of 5 ml were withdrawn at the following time intervals: 0.5, 1 and 2 h in case of SGF (pH 1.2) and at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 h at (pH 6.8), filtered through 0.45 µm membrane (Nunc, New Delhi, India) and analyzed spectrophotometrically at λ_{max} 260 nm with reference to previously constructed calibration curves of SGF buffer (pH 1.2) (R^2 = 0.999, n=3) and SIF (pH 6.8) (R^2 = 0.999, n=3). The withdrawn samples were replaced with the respective fresh dissolution media. All dissolution runs were performed in triplicate and the average cumulative percent of drug released at different sampling times was calculated.

7. In-vivo study and evaluation of ITO HCL using human volunteers:
The evaluation of ITO HCL blood concentration following oral administration of the optimum formulated oral tablet (F23) in comparison to the Ganaton® 50 mg oral tablet (Abbott, USA) as a reference standard was carried out in healthy human volunteers. In addition, the pharmacokinetic parameters including; the maximum plasma concentration (C_{max}), time to maximum plasma concentration (Tmax), the area under the curve from zero to infinity (AUC (0– ∞), the mean residence time (MRT) following administration of either the optimum tablet formulation or Ganaton® 50 mg tablet were estimated. Six healthy male adult volunteers, aged between 27 and 40 years, participated in this study. The study was approved by the ethical committee of Faculty of Pharmacy; Cairo University with approval number [PI (296)]. Prior to the study, the volunteers were informed with the procedure and purpose of the study. They were also informed with the drug method of administration, sampling time intervals, and possible drug side effects. Using a cross-over design the six volunteers were divided into two groups (A and B), each group contained three volunteers. All the subjects were prohibited from taking any other medicines and from smoking for 1 week before participating in the study till the end of it. All subjects were requested to fast for at least 10 hours before the experiment.

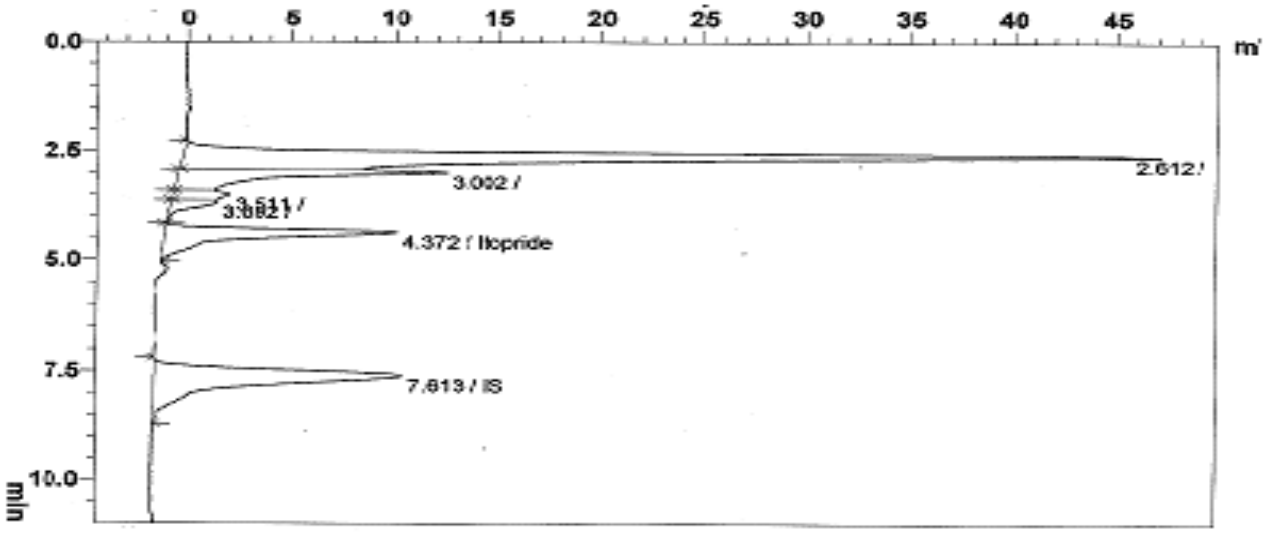
On the first week of study, each volunteer of group (A) received one oral tablet of the optimum formulation (F23) containing 100 mg ITO with 200 ml of water, while each volunteer of group (B), received two tablets of Ganaton® (50 mg) with 200 ml of water. After two weeks as washing period, each volunteer of group (A) received two tablets of Ganaton® (50 mg), and each volunteers of group (B) received one oral tablet of the optimum formulation containing 100 mg ITO. No food was allowed for 4 hours after tablet administration. The volunteers were supervised by a physician who was responsible for their safety and collection of blood samples during the trial. Venous blood samples (5 ml) were collected into heparinized tubes before tablet administration the following time intervals: 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h after administration of each treatment. The plasma was obtained by centrifugation the blood samples at 2000 ×g (Centurion Scientific LTD, West Sussex, UK) for 10 min. The plasma was frozen at -20˚C and analyzed for ITO content by high performance liquid chromatography method (HPLC) (25).

Pharmacokinetic analysis:
The pharmacokinetic parameters following tablet administration of either the optimum tablet formulation or Ganaton® (50 mg) were estimated for each subject from the constructed curve of plasma concentration of ITO versus time. (MRT) and ($AUC_{(0-\infty)}$), were calculated using the trapezoidal rule method for each treatment and compared.

Samples analysis:
The quantitative determination of ITO in plasma was performed adopting HPLC method, using a Shimadzu LC-20A pump, SIL-20 A autosampler, a SPD 20A UV/VIS detector and a µBondapak C-18 column (250 mm x 4.6 mm

ID; particle size 5 µm) (Waters, USA). The mobile phase consisted of a mixture of KH_2PO_4 buffer (0.05 M, pH 4.6) and acetonitrile (75:25 v/v). The mobile phase was filtered through a 0.45 µm membrane filter and was then degassed by ultrasonication before usage. Analysis was run at a flow rate of 1 ml/min and the detection wavelength was set at 260 nm. Frozen serum samples were thawed at ambient temperature (25°C) for at least 60 min, followed by adding 100 µl of moxifloxacin hydrochloride as an internal standard (IS) (100 µg/ml in methanol) and 4 ml of diethyl ether to 1 ml thawed plasma sample. The mixture was then mixed for 2 min by using a vortex mixer (XH-2000-I, USA.) and centrifuged at 3000 rpm for 10 min. After centrifugation, the upper organic layer was separated and the solvent was evaporated in a vacuum oven to dryness. The residue was reconstituted in 400 µl of mobile phase and 20 µl of drug solution was injected into the column. The retention times were approximately 4.5 and 8.4 min for ITO and IS respectively as shown in the chromatogram in Fig. (1).

FIG (10): Chromatogram of itopride at 4.372 and 7.613 for ITO and IS respectively.



Validation:
The peak area ratios of ITO to that of moxifloxacin hydrochloride as IS were determined. The regression equation was set up by spiking drug-free plasma with varying amounts of ITO (0.05 to 3 µg/mL) and fixed quantity of IS, and treating the plasma as described above. The peak area ratio of ITO to IS was obtained.

RESULTS AND DISCUSSION

Drug content and physical evaluation:
The components of the tablets formulated as sustained release dosage forms (SRFS) prepared by different techniques: wetting granulation, direct compression, and coating compression, and by varying the percentage of HPMC, HPC, and Eudragit® RL100, were shown in table (1). Physical parameters of the random chosen tablets from each batch such as weight variation, thickness, hardness, friability, and drug content were assessed as shown in table (3). In all tablet formulations, Thickness of the tablets ranged from 3.16 ± 1.37 (F12) to 3.74 ± 1.32 mm (F1). Weight variation of tablets ranged from 395 ± 0.34 (F28) to 409 ± 1.78 mg (F18). drug content ranged from 94.5 ± 0.82 (F30) to 106.2 ± 0.08% (F6). Tablet hardness ranged from 5.25 ± 0.25 (F1) to 8.80 ± 0.4 Kg/cm^2 (F23), which indicated good mechanical strength Tablet friability ranged from 0.04 ± 0.69 (F6) to 0.84 ± 0.45 (F3). The tablets friability was found to be less than 1% which indicated that tablets had good mechanical resistance.

In-vitro dissolution study of tablets in pH 1.2 and pH 6.8 media:
Four batches of tablets formulated were prepared by wetting granulation method using 20, 30, 40, and 50% HPMC and were coded F1, F2, F3, and F4 respectively. Fig. (1), and table (4) illustrate the effect of HPMC concentration and the method of preparation (wetting granulation) utilized on the cumulative percentage drug release of ITO from tablets prepared using HPMC polymer. Results showed that as the concentration of HPMC increased in tablets prepared using (wetting granulation) method, the rate of drug release decreased relatively, where the time required for 50% of drug to be released (t_{50}%) was found to be 0.47± 0.03, 1.46± 0.9, 1.574± 0.1, and 1.75 ±.014 hr for F1, F2, F3, and F4 respectively, The mean cumulative percentages drug released from tablets containing 20, 30, 40, and 50% HPMC in SGF (PH 1.2) after 2 hr were 80.80.69±0.13%, 62.25±0.21%, 59.97±0.16% and 55.21±0.23% respectively and in SIF (PH 6.8) after 5 hr were 101.15±0.33.%, 99.36±.0.63%, 101.80±.0.12% and 100.86 ±0.87.%

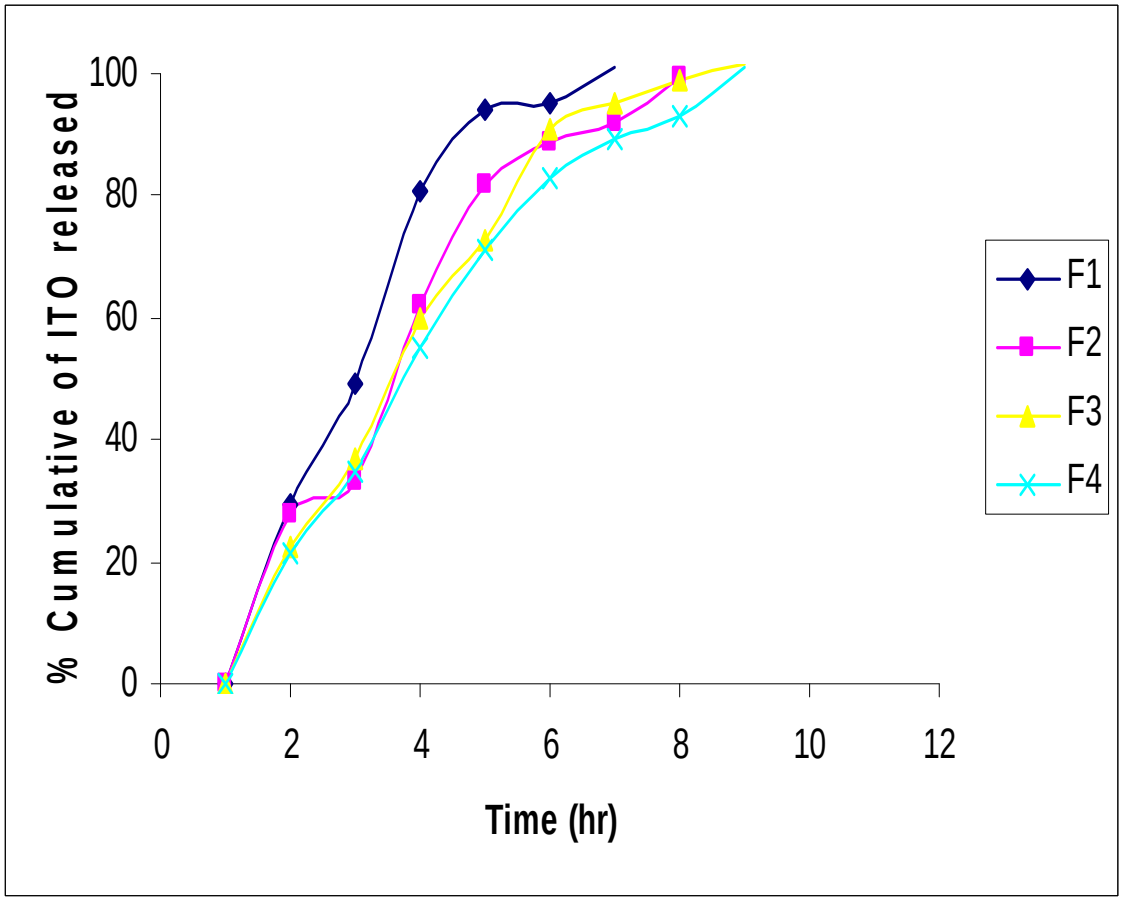
respectively. Results showed that drug release from tablets F1, F2, F3, and F4 was relatively fast and followed diffusion order of release. This could be attributed to the method utilized in tablet preparation (wetting granulation). Thus, wetting granulation method cannot be considered a good method for preparation of sustained release delivery systems for ITO.

Table (3) Physical study of different tablet formulations

F	Average Diameter (mm)±SD	Average Weight (mg) ±SD	Average thickness (mm) ±SD	Average hardness (Kg) ±SD	Average Friability (%)±SD	Average Drug content (%)±SD
F1	11	402 ± 1.82	3.74 ± 1.32	5.25 ± 0.25	0.74 ± 1.93	101.20 ± 0.09
F2	11	400 ± 3.82	3.44 ± 0.95	6.00 ± 1.28	0.69 ± 1.26	100.4 ± 0.13
F3	11	398 ± 2.02	3.36 ± 1.92	6.70 ± 1.05	0.84 ± 0.45	103.4 ± 0.14
F4	11	406 ± 0.75	3.50 ± 1.22	7.05 ± 0.85	0.82 ± 1.15	102.12 ± 0.01
F5	11	405 ± 2.20	3.28 ± 1.67	6.10 ± 0.20	0.10 ± 0.93	103.3 ± 0.55
F6	11	402 ± 2.63	3.24 ± 2.25	7.25 ± 1.94	0.04 ± 0.69	106.2 ± 0.08
F7	11	408 ± 2.93	3.52 ± 1.77	8.00 ± 0.92	0.06 ± 1.05	100.6 ± 0.02
F8	11	400 ± 1.73	3.20 ± 2.04	8.20 ± 1.76	0.20 ± 2.27	104.5 ± 0.44
F9	11	403 ± 2.58	3.38 ± 2.44	7.06 ± 0.37	0.11 ± 0.92	102.2 ± 0.21
F10	11	407 ± 1.15	3.64 ± 1.13	7.70 ± 1.61	0.22 ± 1.15	100.1 ± 0.6
F11	11	400 ± 1.23	3.30 ± 0.63	8.25 ± 0.79	0.31 ± 2.78	99.52 ± 0.06
F12	11	401 ± 0.66	3.16 ± 1.37	8.20 ± 0.31	0.39 ± 1.32	105.7 ± 0.6
F13	11	405 ± 1.19	3.54 ± 2.01	8.45 ± 0.98	0.38 ± 1.60	100.2 ± 0.1
F14	11	402 ± 1.05	3.32 ± 1.38	6.00 ± 2.34	0.8 ± .06	100.4 ± 0.5
F15	11	406 ± 2.30	3.40 ± 1.06	7.00 ± 1.59	o.3 ± 1.10	94.5 ± 1.01
F16	11	401 ± 3.01	3.49 ± 2.49	7.08 ± 2.91	o.49 ± 2.03	94.82 ± 1.31
F17	11	407 ± 3.42	3.58 ± 3.85	7.50 ± 1.84	0.44 ± 1.72	100.4 ± 0.21
F18	11	409 ± 1.78	3.40 ± 2.90	7.60 ± 0.73	0.12 ± 1,2	99.6 ± 0.7
F19	11	401 ± 3.04	3.48 ± 1.67	7.92 ± 0.41	0.33 ± 0.46	95.1 ± 2.01
F20	11	403 ± 2.35	3.38 ± 1.84	8.50 ± 1.55	0.10 ± 0.96	95.46 ± 1.38
F21	11	407 ± 0.5	3.62 ± 1.02	7.50 ± 2.2	042 ± 0.98	94.88 ± 2.1
F22	11	397 ± 1.01	3.54 ± 0.8	8.50 ± 0.41	0.65 ± 1.22	96.62 ± 0,6
F23	11	401 ± 0.24	3.44 ±0.12	8.80 ± 0.4	0.52 ± 0.33	99.8 ± 0.55
F24	11	402 ± 1,22	3.38 ± 1.32	7.00 ± 0.54	0.11 ± 1.12	95.91 ± 1.52
F25	11	401 ± 2.24	3.24 ± 1.44	7.50. ± 2.11	0.16 ± 2.02	95.25 ± 1.11
F26	11	400 ± 0.62	3.58 ± 0.43	7.00 ± 0.78	0.48 ± 0.65	96.32 ± 0.12
F27	11	396 ± 1.44	3.48 ± 0.66	6.00 ± 1.01	0.24 ± 1.24	94.42 ± 1.24
F28	11	395 ± 0.34	3.52 ± 1.24	6.50 ± 0.98	0.34 ± 0.22	98.46 ± 0.34
F29	11	401 ± 0.72	3.38 ± 0.44	7.00 ± 0.86	0.16 ± 0.68	96.86 ± 1.52
F30	11	400 ± 0.65	3.62 ± 0.12	6.50 ± 2.04	0.11 ± 1.32	94.5 ± 0.82
F31	11	408 ± 1.42	3.54 ± 0.84	7.07 ± 1.02	0.12 ± 0.48	96.65 ± 1.22
F32	11	403 ± 0.94	3.36 ± 0.42	6.00 ± 0.88	0.44 ± 0.62	94.65 ± 0.76
F33	11	396 ± 1.22	3.42 ± 1.58	6.50 ± 0.66	0.22 ± 1.66	95.52 ± 1.02
F34	11	404 ± 0.98	3.56 ± 0.44	6.60 ± 1.22	0.34 ± 86	99.24 ± 0.88

Four batches of tablets formulated were prepared by direct compression method using 20, 30, 40, and 50% HPMC and were coded F5, F6, F7, and F8 respectively. Fig. 2 and table (7) illustrate the effect of HPMC concentration on the release of drug from tablets prepared by direct compression method. HPMC was used to prepare ITO SRFS tablets (F5, F6, F7, and F8 respectively). The prepared tablets showed a significant decrease in drug release compared to those prepared by wet granulation ($p < 0.05$). The mean cumulative percentages drug release from F5, F6, F7, and F8 in SGF (PH 1.2) after 2 were 66.46±0.42 %, 55.72±0.13 %, 39.13±0.42 % and 32.65±0.43 % respectively while in SIF (PH 6.8) after 2 were 100.34±0.54 % (5 hr), 98.07±0.17% (10 hr), 99.61±0.27 % (12 hr) and 100.64±0.21 % (12 hr) respectively. Results obtained showed that as the concentration of HPMC increased in the tablets, the rate of drug release decreased significantly where the mean t_{50} were found to be 1.26±0.34, 1.97±0.12, 3.05±0.82, and 3.57±0.65 for F5, F6, F7 and F8 respectively ($p < 0.05$). It could be concluded that the direct compression method of tablet preparation by HPMC was the optimum method to sustain the release of ITO, especially F7 and F8 which contained 40 and 50% of HPMC. This could be attributed to the nature of HPMC polymer, which formed a thick gel layer around the tablet when coming in contact with the dissolution media regulating ITO release from the tablets (26).

FIG (1) Release rate of tablets prepared using wetting granulation method by HPMC



Three batches of tablets formulated were prepared by direct compression method using 20, 30, and 50% HPC and were coded F9, F10, F11, respectively, and three batches of tablets formulated were prepared by direct compression method using 20, 30, and 50% Eudragit® RL 100 and were coded F12, F13, F14, respectively. Figs. 3 and 4 and table (4) showed the effect of increasing the concentrations of polymers HPC and Eudragit® RL 100 and method of preparation (direct compression) on release rate of the drug from tablets. Results showed that as the concentrations of both HPC and Eudragit® RL 100 increased from 20 to 50% of tablet weight, the rate of drug release decreased relatively, where t_{50} were found to be 0. 0.17 ± 0.76 hr, 0.94 ± 1.11 hr, 1.14 ± 0.02 hr, 0.35 ± 0,6 hr, 0.89 ± 0.7 hr, and 1.42±0.55 hr for F9, F10, F11, F12, F13, and F14 respectively. The prepared tablets showed no a significant difference decrease in drug release compared to those prepared by direct compression using HPMC ($p < 0.05$). The mean cumulative percentages drug release in SGF (PH 1.2) after 2 hr were 98.68±0.36 %, 82.72±0.95%, and 72.56±0.54 %, and in SIF (PH 6.8) were 101.7±0.84 % (1 hr), 100.14 ±0.19% (2 hr), and 99.16 ±0.58% (4 hr) for F9, F10, and F11 respectively. The mean cumulative percentages drug release from tablets were prepared by direct compression method using 20, 30, and 50% Eudragit® RL 100 and were coded F12, F13, F14, respectively. in SGF (PH 1.2) after 2 hr 86.2±0.47%, 63.12±0.44%, and 59.5±o.96% and in SIF (PH 6.8) after 6 hr were 103.9±0.21% (6), 99.77±0.65% (6 hr), and 99.2 ±0.76% (6 hr) for F12, F13, and F14 respectively.

Formulations F9, F10, F11 F12, F13, and F14 failed to generate sustained release of drug up to 12 hr and ITO was completely released at less than 10 hr. This could be attributed to the HPC and Eudragit® RL 100 polymers. From the results obtained, it could be concluded that the method of direct compression using HPC and Eudragit® RL 100 could not be considered the optimum method to sustain the release of ITO.

FIG (2) Release rate of tablet prepared using direct compression method by HPMC

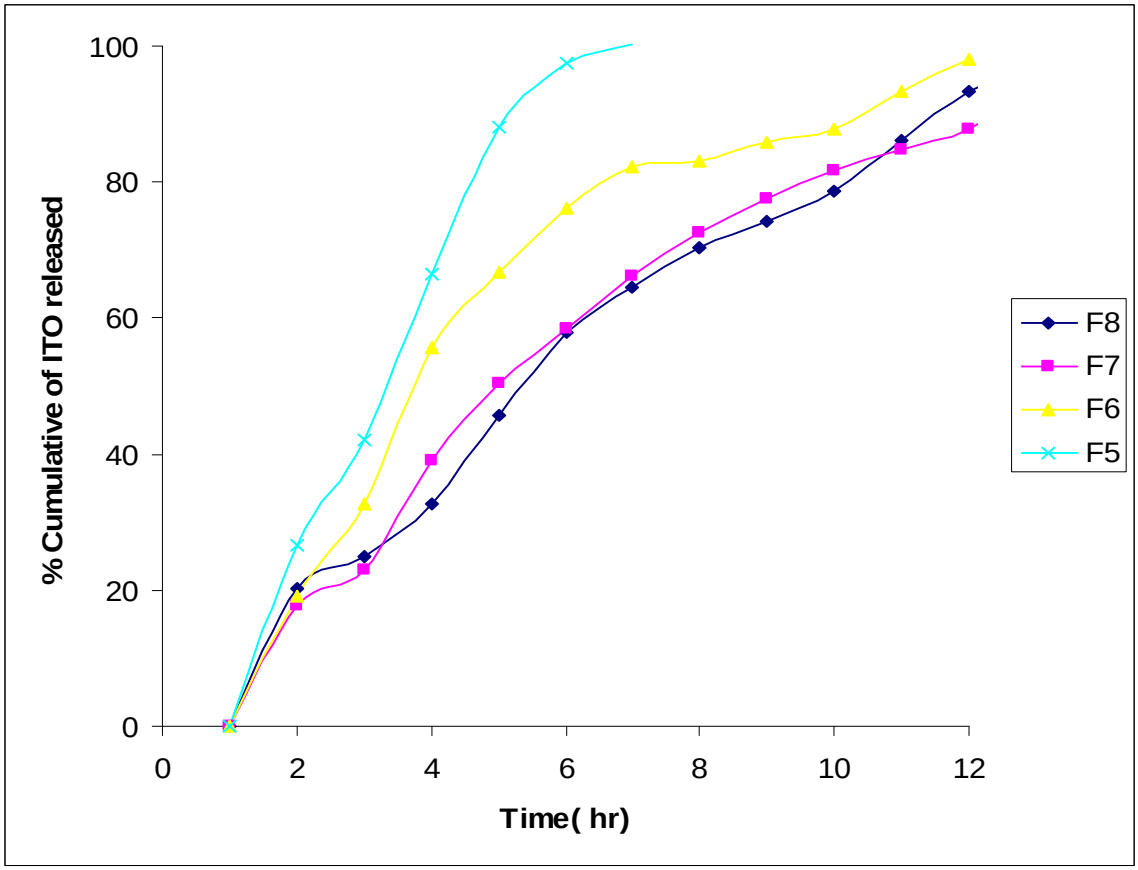


FIG (3) Release rate of tablet prepared using direct compression method by HPC

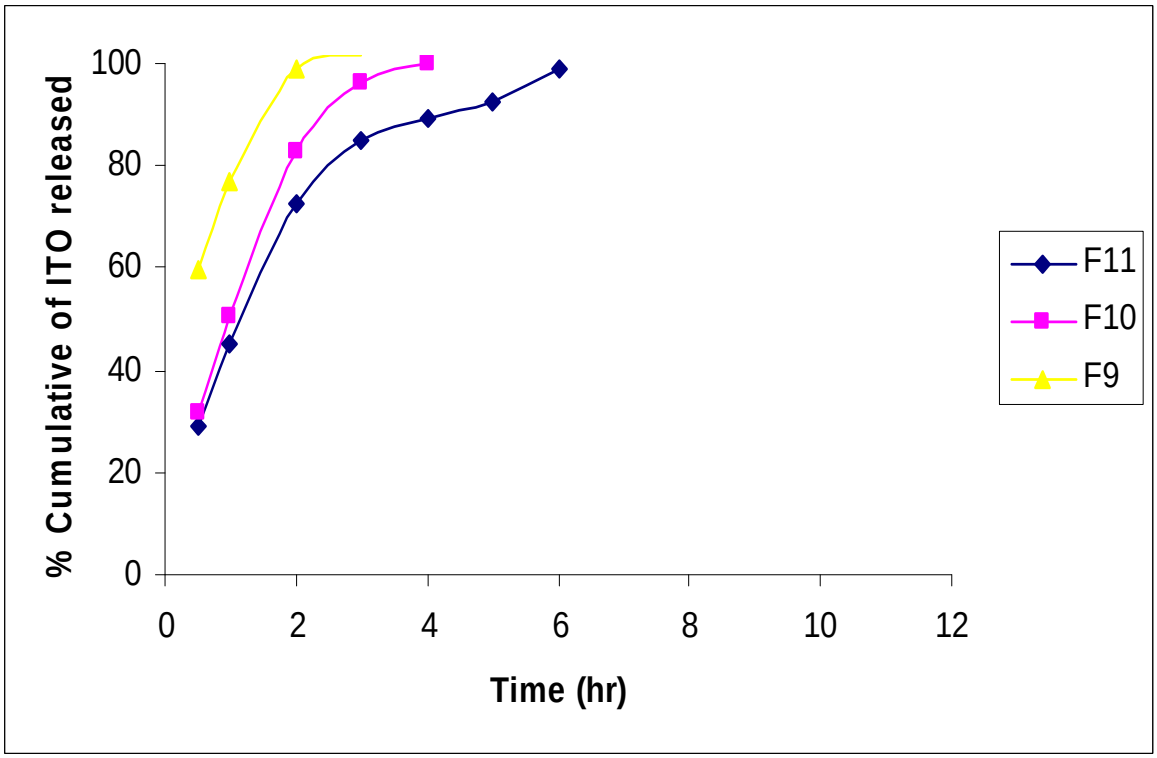


FIG (4) Release rate of tablet prepared using direct compression method by Eudragit® RL100:

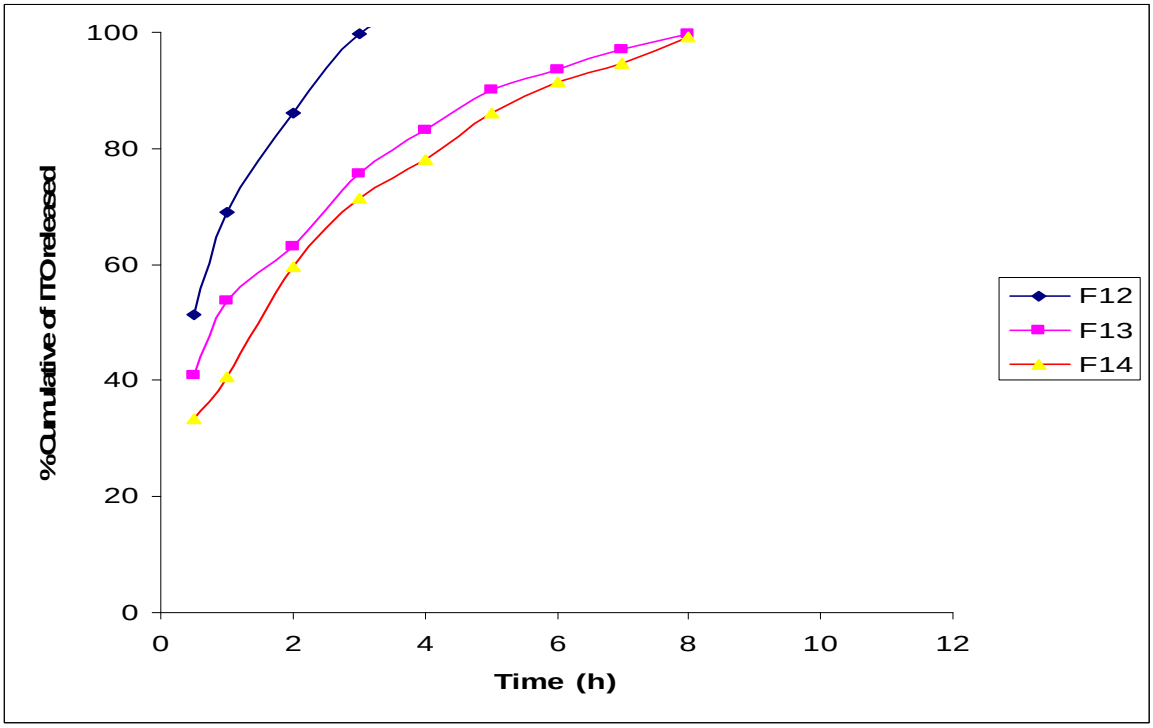
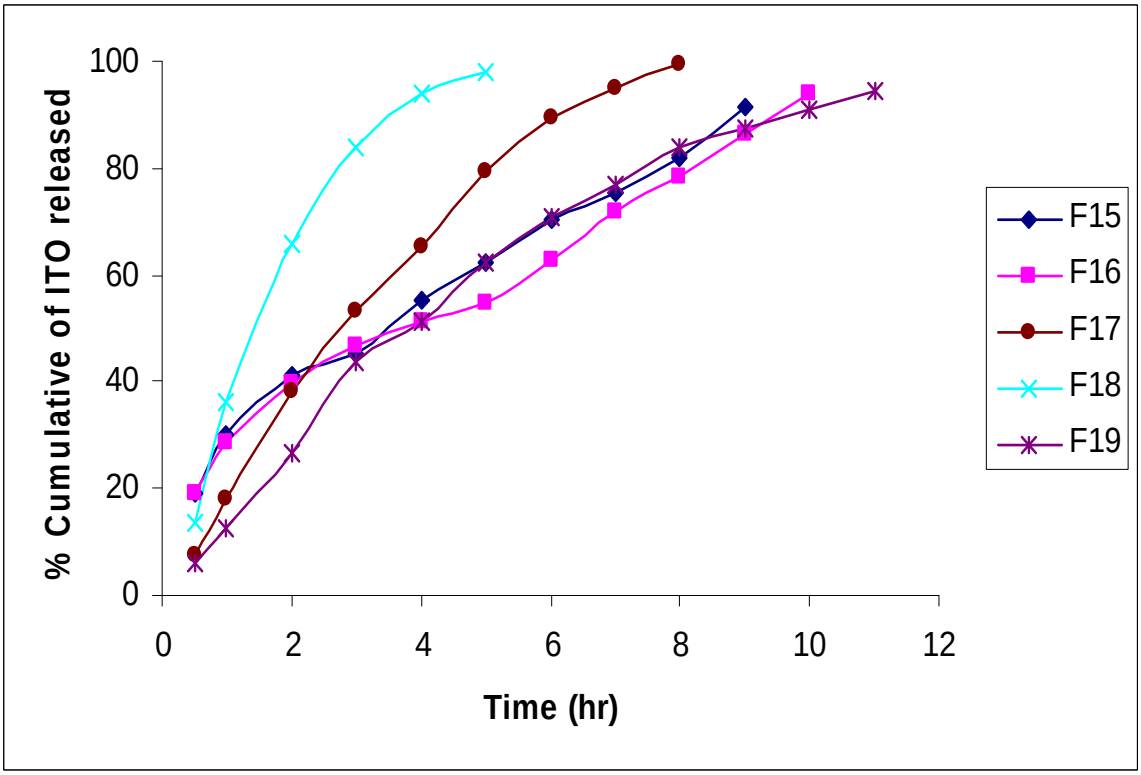


FIG (5) Release rate of tablet prepared using coating compression method by HPC

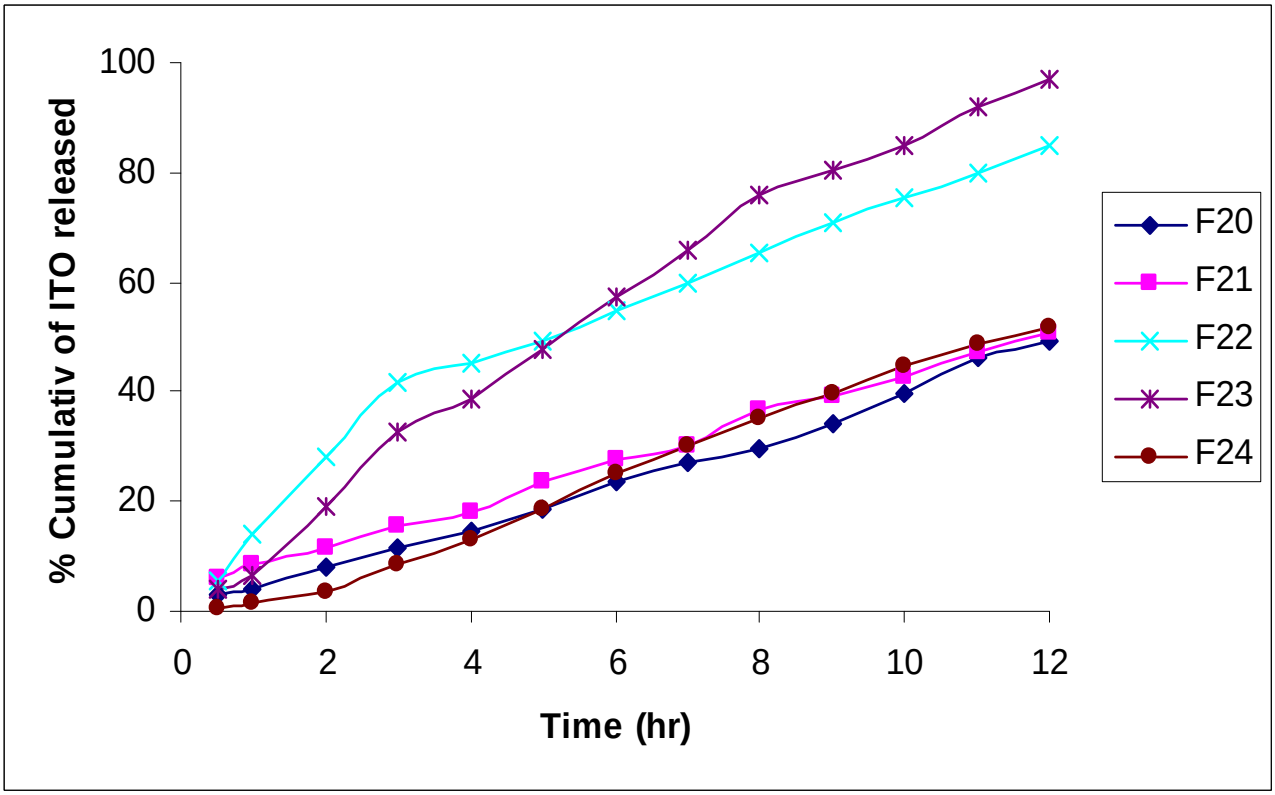


Five batches of coated tablets formulated were prepared by coating compression method using HPC polymer and were coded F15, F16, F17, F18, and F19 respectively. Fig (5) and tables (4), showed effect of increasing HPC and diluent concentrations in core and coat on release rate of drug, at constant concentration of ITO in core of coated tablet. The cumulative percentages of drug release from F15, F16, F17, F18, and F19 respectively in SGF (PH 1.2) after 2 were (pH 1.2) were 41.21±0.51%, 39.8±0.99%, 38.31±0.38%, 66.03±0.61%, and26.85±0.29%, and in SIF (PH 6.8) after 10 hr were 91.5±0.55%, 94.11±0.13 %, 99, 45±0.44%, 98.06±1.4%, and 94.35±0.66% respectively and t_{50} were 3.18±0.35 hr, 3.73±0.42 hr, 2.7±0.81 hr, and t_{50} was 1.55±0.14 hr, and was 3.73±0.23 hr respectively

for F15, F16, F17, F18 and F19 respectively. Results that showed complete drug release occurred during 11 hr and this could be attributed to the hydrophilic nature of HPC as polymer (27).

Five batches of coated tablets formulated were prepared by coating compression method using HPMC polymer were coded F20, F21, F22, F33, and F24 respectively. Fig (6) tables (4), showed the effect of the presence of HPMC in different concentrations as a diluent in the core and the coat. The cumulative percentages of drug release from F20, F21, F22, F33, and F24 in SGF (PH 1.2) after 2 were (pH 1.2) were 7.84±0.11%, 11.61±0.43%, 28.93±0.34 18.92±0.23%, and 3.63±0.51% and in SIF (PH 6.8) were 10 hr (49±0.87%), (50.92±0.54%), (85.17±0.22%), (97.15±0.49%), and (51.94±0.33%) and t_{50} were 13.38±0.35 hr, 12,05±0.86 hr, 4.84±0.69 hr, 5.30±0.14 hr, 12.02± 0.22hr. for F20, F21, F22, F23, and F24 respectively. Results showed that complete drug release from tablet formulations F20, F21, F22, F23, and F24 occurred during 12 hr, this could be attributed to properties of HPMC as polymer witch have properties hydrophilic and using coating compression method (28).

FIG (6) Release rate of tablet prepared using coating compression method by HPMC



Five batches of coated tablets formulated were prepared by coating compression method using carnauba wax and were coded F25, F26, F27, F28, and F29 respectively, Fig (7) table (4) showed effect of increasing carnauba wax and diluent concentrations in core and coat, at constant concentration of drug in core. The cumulative percentages of drug release from F25, F26, F27, F28, and F29 in SGF (PH 1.2) after 2 hr were 20.17 ±0.9%, 2.47± 0.39%, 2.32± 0.62%, 28.59± 0.8%, and 21.33± 0.95% respectively and in SIF (PH 6.8) after 10 hr were 83.96±0.76 %, 17.69± 0.52%, 35.61±0.49 %, 96.09± 0.43%, and 79.3±0.31% respectively and t_{50} were 6.14±0.71 hr, , 47.72±0.34 hr, 31.38±0.18 hr, 6.209±0.38 hr, and 5.96±0.27 hr respectively for F25, F26, F27, F28, and F29 respectively. Thus showed the slower drug release than targeted drug release at all the time points and the drug was not completely released from the tablets through 12 hr. This can be attributed to hydrophobic properties of carnauba wax (29).

Five batches of coated tablets formulated were prepared by coating compression method using Eudragit® RL 100 and were coded F30, F31, F32, F33, and F34 respectively, Fig (8) table (4) showed effect of increasing Eudragit® RL 100 and diluent concentrations in core and coat at constant concentration of ITO in core. The cumulative percentages drug release from F30, F31, F32, F33, and F34 in SGF (PH 1.2) after 2 hr were 16.67 ±0.54 %, 5.37± 0.69 %, 32.22± 0.77%, 8.85± 0.25%, and 2.76± 0.97%, and in SIF (PH 6.8) after 10 hr were 85.22± 0.13%, 36.87±32 %, 74.57±0.88 %, 51.6±0.61 %, and 45.9±0.49 %, respectively, and t_{50} were 3.96±0.19 hr, 15.24±0.37 hr, 4.99±0.74 hr, 9.97±0.45 hr, and 16.67±0.81 hr respectively, for F30, F31, F32, F33, and F34 respectively Thus showed the slower drug release than targeted drug release at all the time points and the drug was not completely released from the tablets during 12 h. This could be attributed to the hydrophilic properties of Eudragit® RL 100 (30).

FIG (7) Release rate of tablet prepared using direct compression method by carnauba wax

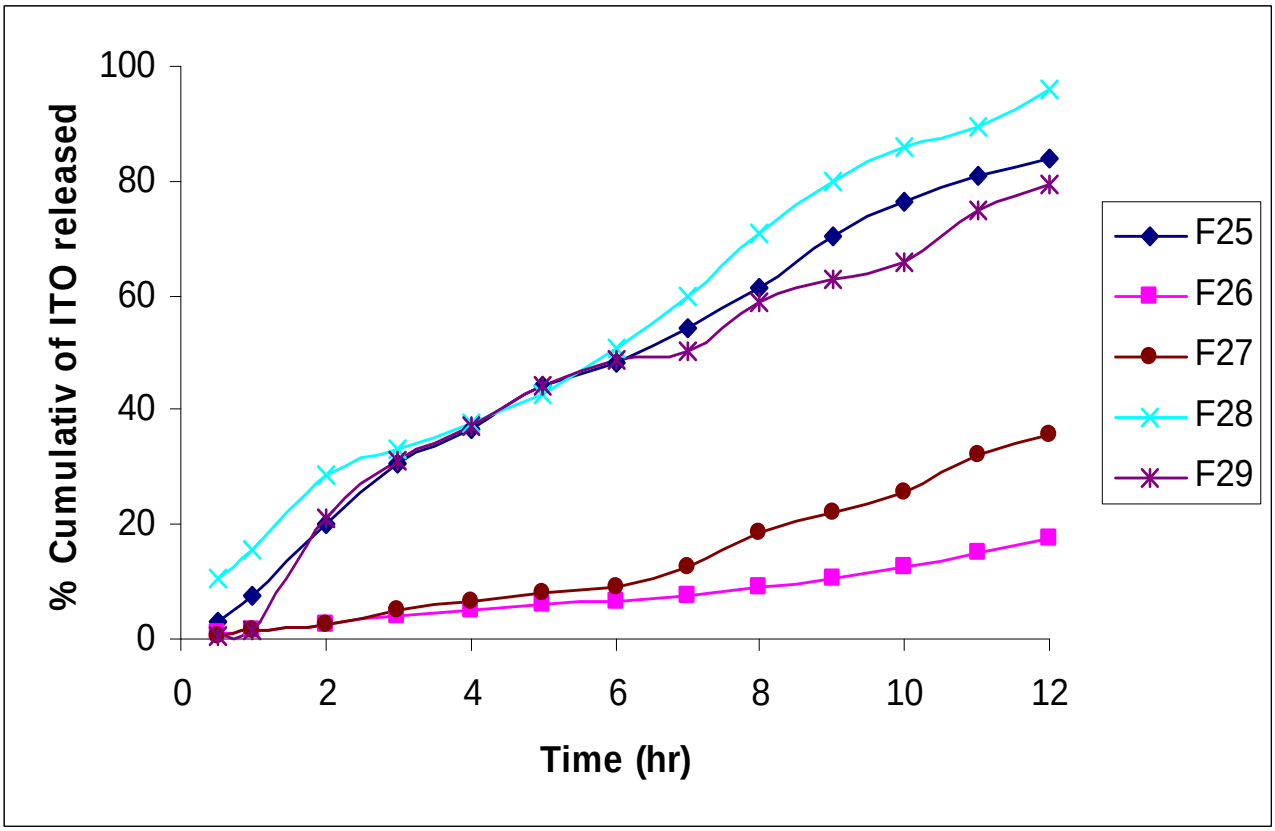


FIG (8) Release rate of tablet prepared using coating compression method by Eudragit® RL100

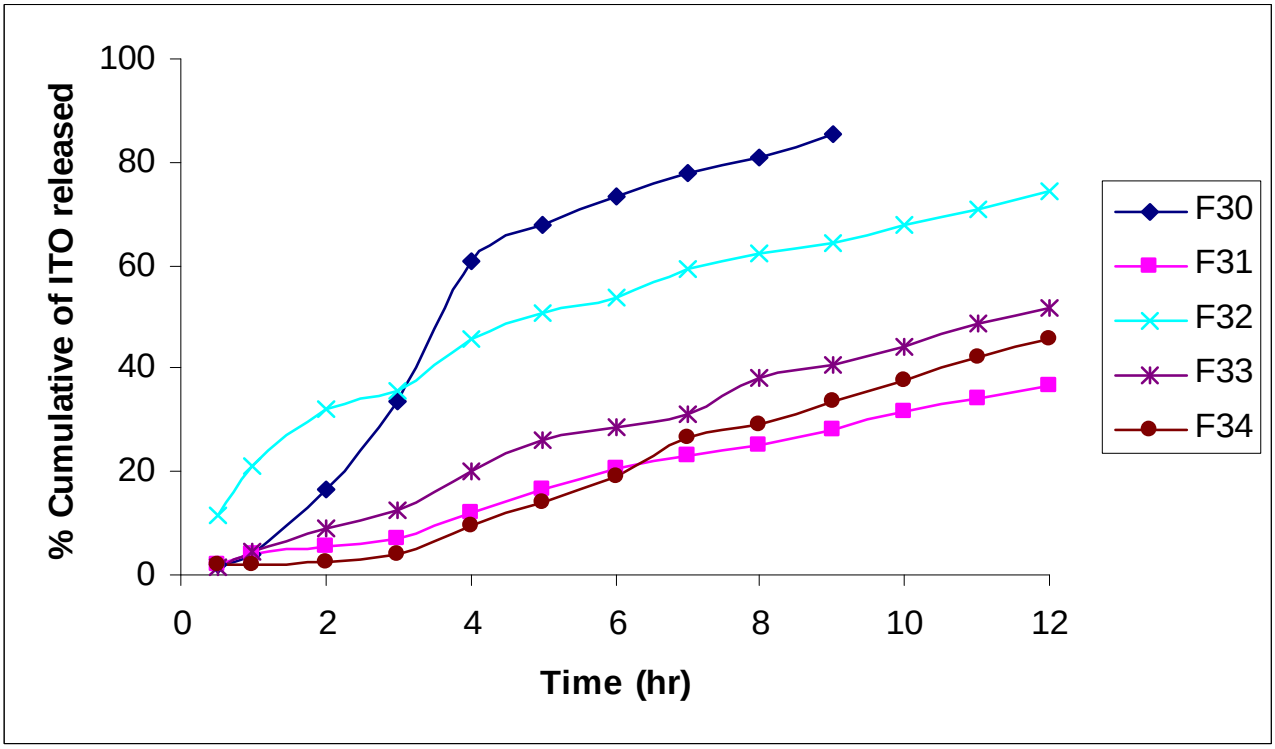
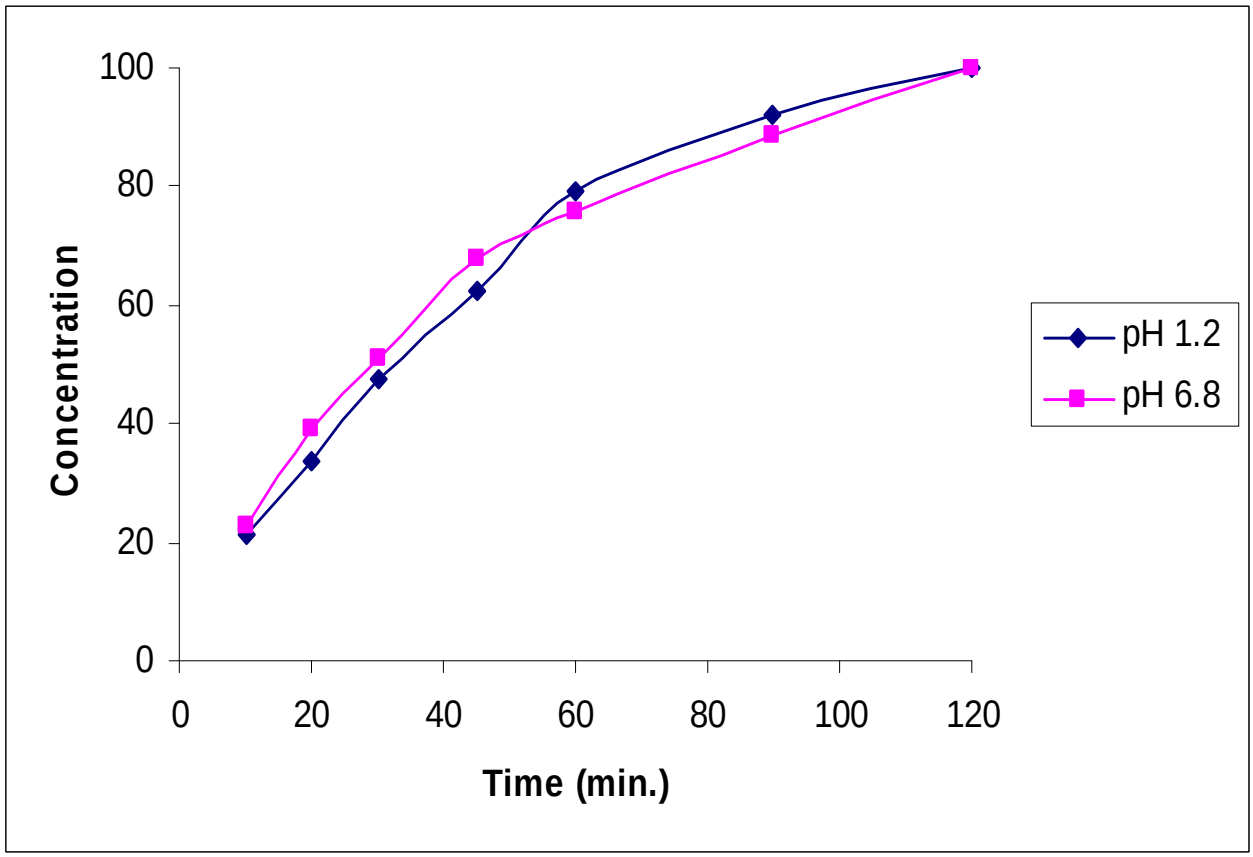


Fig (9) illustrates the release profile of Ganaton® Tablet (50 mg) used reference as market; at pH 1.2 and pH 6.8 through 120 minutes. Results showed that there were no differece between release of drug in pH 1.2 and in 6.8 buffers. From this result it could be conduced the nature of medium used in dissolution study has no effect on release of the drug from the Ganaton® Tablets.

FIG (9) Release rate of Ganaton® 50mg tablet (reference standard) at pH 1.2 and pH 6.8



Summary of the drug release parameters (Table 7) revealed that the value of the kinetic constant, k, showed a declining trend with an increase in the level of each polymer and its ratio to ITO, construing an appreciable change in the release rate with a change in the polymer composition and its ratio. The values of $t_{50\%}$ were found to vary from 0.16 to 47 hr from varies all formulation. Nearly 50 % of the drug remained captive in the hydrophilic matrix for up to 47 hours in case of (F26) with HPMC polymer, which may lead to appreciable diminution in the extent of drug absorption. Table (7) reveals that the overall rate of drug release tended to decrease with an increase in concentration of polymer and its ratio (31)**.**

From results obtained from different characteristic tests performed in the study, it was found that the formula, F23 witch was prepared by HPMC using coating compression technique, was considered the optimum formulation as it was capable of forming a gel structure, when coming in contact with dissolution medium and in terms of friability (0.52 ± 0.33%), drug content (99.8 ± 0.55%) and hardness (8.80 ± 0.4 kg). In addition, through the dissolution and kinetic study, F23 was the most appropriate in controlling ITO release from tablets as the cumulative percentage released was 97.15% of the drug during from 0.5 and 12 hours, as the t_{50} was (5.303 h), K=(8.061 hr^{-1}), r^2= 0.99076 and the release of ITO from it followed zero order kinetics.

The same reason was at F15, 16, 71, 18, and 19 due to properties of HPC hydrophilic. While in F12, 13, 14 the fast release due to the high solubility properties of drug and, the reason is at F25, 26, 27, 28, and 29 to decease of the release rate due to properties of carnauba wax which it as hydrophobic, as the same at reason is at F 30, 31, 32, 33, and 34 to decease of the release rate due to properties of Eudragit® RL 100 witch it as hydrophobic. The influence of polymer levels seems to be vital in regulating the drug release. Drug release rate of all the formulations portray an initial burst release of the drug, characteristic of most hydrophilic matrices (32).

In-vivo study and evaluation of ITO using human volunteers:

Pharmacokinetic study:

A good linear relationship (R^2 = 0.98) was observed. However, the inter day and intra day variation was found to be less than 2.8% (coefficient of variation) indicating high precision of the HPLC method. There was a high recovery (97.8 to 99.5%) of ITO indicating that the HPLC method was highly accurate.

The mean plasma level profile of ITO obtained following the application of sustained release tablet (F23) containing 100 mg drug and from orally administered two commercial tablets of Ganaton® (50 mg) (Abbott, USA) to healthy human volunteers was compared in Figure (11).

Table (4): Drug release parameters of various formulations

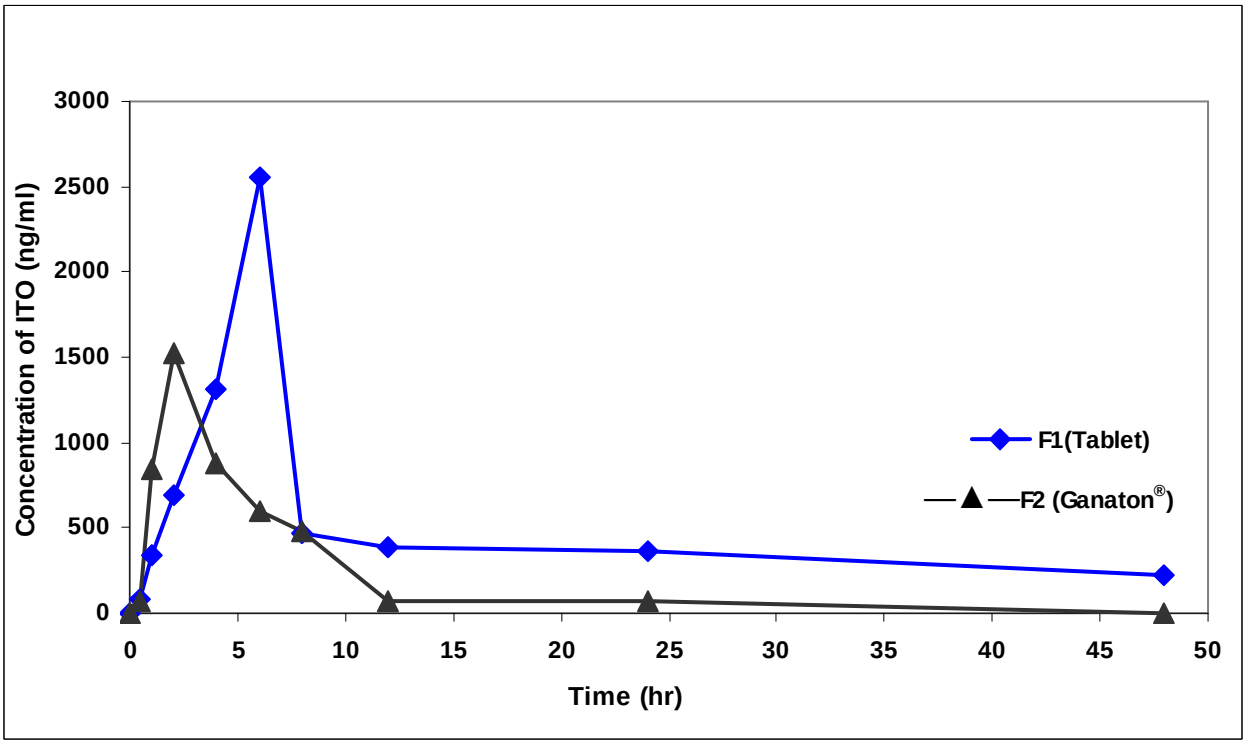
Formula	r^2			Release order	r	K (hr^{-1})	t_{50} (hr)
	Zero	First	Diffusion				
F1	0.8380	0.7553	0.9256	Diffusion	0.9621	48.9112	0.4733
F2	0.9014	0.8277	0.9611	Diffusion	0.98035	38.606	1.4642
F3	0.93034	0.7889	0.98286	Diffusion	0.991397	36.703	1.574
F4	0.93334	0.8108	0.986212	Diffusion	0.993082	34.32	1.7591
F5	0.9247	0.8456	0.9865	Diffusion	0.98849	41.9075	1.2609
F6	0.8934	0.6857	0.9664	Diffusion	0.98304	31.936	1.975
F7	0.9747	0.805	0.99565	Diffusion	0.9978	25.35	3.0506
F8	0.9868	0.8727	0.97786	Zero	0.9934	26.2	3.576
F9	0.8794	0.8492	0.9413	Diffusion	0.97018	75.55	0.16879
F10	0.8973	0.8293	0.95933	Diffusion	0.9795	49.963	0.9358
F11	0.8511	0.7581	0.9388	Diffusion	0.9689	44.995	1.1356
F12	0.9527	0.869	0.9751	Diffusion	0.9875	66.825	0.3523
F13	0.9527	0.8548	0.9919	Diffusion	0.9959	52.541	0.8877
F14	0.9538	0.8478	0.9925	Diffusion	0.9963	43.984	1.419
F15	0.9757	0.8924	0.9892	Diffusion	0.9946	28.2814	3.1803
F16	0.9485	0.7545	0.9889	Diffusion	0.9943	27.559	3.734
F17	0.9871	0.7841	0.9937	Diffusion	0.9988	17.075	2.7007
F18	0.9003	0.7463	0.9682	Diffusion	0.9839	30.4163	1.549
F19	0.9871	0.7468	0.9929	Diffusion	0.9964	12.695	3.7312
F20	0.9934	0.9071	0.9538	Zero	0.9968	4.603	13.382
F21	0.9943	0.9344	0.9604	Zero	0.9972	8.566	12.056
F22	0.9239	0.7077	0.9891	Diffusion	0.99003	12.589	4.839
F23	0.99076	0.7823	0.9827	Zero	0.9954	8.061	5.303
F24	0.9702	0.8257	0.8992	Zero	0.98497	1.5801	12.002
F25	0.9738	0.7484	0.9945	Diffusion	0.9973	7.534	6.143
F26	0.99178	0.9187	0.9862	Zero	0.9959	1.574	47.718
F27	0.9826	0.9173	0.9651	Zero	0.9913	1.298	31.381
F28	0.9657	0.899	0.9875	Diffusion	0.9942	16.59	6.2097
F29	0.94745	0.6031	0.9777	Diffusion	0.9888	2,604	5.9566
F30	0.96384	0.746	0.9454	Zero	0.9818	5.534	3.9642
F31	0.95896	0.8902	0.8882	Zero	0.9793	3.622	15.243
F32	0.9529	0.7757	0.9885	Diffusion	0.99423	19.289	4.9941
F33	0.9912	0.7958	0.9625	Zero	0.9956	3.826	9.974
F34	0.9797	0.922	0.9106	Zero	0.9895	2.479	16.67

A summary of the pharmacokinetic parameters derived from the study data was listed in Table (5). Following oral administration of the reference product, the C_{max} was (1518.4 ± 307.7) achieved after 2.0 hr of oral dosing. After oral administration of (F23), C_{max} was (2553.3 ± 630.4) achieved 6.0 h after dosing. The formula F23 spent longer times to reach the maximum drug concentration in the systemic circulation as T_{max} was (6 hr). The mean value of C_{max}, AUC_{0-10} and $AUC_{0-\infty}$, were significantly higher ($p < 0.05$) for drug administered from oral (F23) than reference oral tablet (Ganaton®) demonstrating improved bioavailability of ITO from tested formulation as show in table (5) and the mean value of MRT.($p > 0.05$) The F23 showed relative bioavailability of 243% with respect to Ganaton® tablets. The enhancement of the relative bioavailability of ITO from oral route is a direct result of the elimination of the hepatic first pass metabolism on oral delivery of the ITO. Moreover, through the study in-vivo performed on human volunteers formula (23) showed significant results, as it gave the sustainability of the longest of the tablet trade and stimulate the clinical use and applicable.

Table (5): Pharmacokinetic parameters of ITO in healthy human volunteers after oral administration of selected formula tablet (F23) and Ganaton® tablets

Pharmacokinetic parameters	Ganaton® (Reference)	F23 (SRF) Tablet
T_{max} (hr)	2 ± 0.01	6 ± 0.04
C_{max} (ng/mL)	1518.4 ± 307.7	2553.3 ± 630.4
MRT (hr)	8.76 ± 0.56	48.60 ± 42.4
AUC_{0-48} (ng h/mL)	9351.0 ± 454.6	22755.8 ± 6977.9
$AUC_{0-\infty}$ (ng h/mL)	9475.7 ± 453.5	40926.9 ± 32432.0
Relative Bioavailability (%)	----------	243%

FIG (11) In-vivo study (Pharmacokinetic parameters) of ITO after oral administration of selected formula tablet (F23), and Ganaton®:



F1= F23 (Tablet) SR Formulated
F2=Ganaton® (Reference)

CONCLUSION

New ITO sustained release tablet formulation containing 100 mg ITO prepared with satisfactory physicochemical characterizations using coating compression technique. The release patterns can be controlled and sustained by changing the polymer type and concentration. The bioavailability of optimum formulation administered to healthy volunteers via oral route was significantly higher than commercial Ganaton® tablet ($p < o.5$). The present study indicates a good potential of the prepared sustained release tablet containing ITO for systemic delivery with added advantages of prolonging drug action and increasing patient compliance. This study confirmed the potential of the above sustained release oral dosage forms as a promising candidate for sustained release delivery of ITO.

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REFERENCES

[1] Lida E, Kalantzi1, Evangelos, Karavas, Efthimios X, Koutris, Dimitrios N. Bikiaris Aristotle University of Thessaloniki, GR-541 24 Thessaloniki, Greece. Recent Patents on Drug Delivery & Formulation, 3: 49-63, **2009**
[2] Netal A, Amrita B, Madhu M. Development of Microsponges for Topical Delivery of Mupirocin *AAPS PharmSciTech:* 10 (2): 402- 409, **2009**.
[3] Ramsin B, MD, Andrea M, Trescot MD, Sukdeb D, MD, Buenaventura R. Pain Physician: Opioid Special Issue: 11:S105-S120 • ISSN 1533. *Int J Cur Sci Res*;-3159, **2008**
[4] Natasha S, Dilip, MK, Gupta, Mahaveer, Khinchi.Int A Comprehensive Review on Floating Drug Delivery System *J of Research in Pharma. and Bio* Sc I Vol. 2 (2) Apr – Jun **2011**
[5] Saraf S, Paliwal S, Saraf S Sphingosomes a novel appoach to vesicular drug delivery *Intl J of Cur, Sci Res*, 1(2): 63 – 68, **2011**.

[6] Vidyadhara S, Rama Rao P and Prasad JA. Formulation and evaluation of propranolol hydrochloride oral controlled release matrix tablets. *Indian J Pharm Sci*. **2004**; 66(2):188-192.
[7] Jantzen G, Robinson J. Sustained and controlled-release drug delivery systems, in Banker GS; Rhodes CT (Eds.) Modern Pharmaceutics, Third Edition, Revised and Expanded, Drugs and The Pharmaceutical Sciences, vol 72. Marcel Dekker, Inc., New
[8] Shalin A, Modi P, Gaikwad1 V, Bankar, S. Pawar P. sustained release drug delivery system, *Int. J Pharm Research and Development*, **2011**.
[9] Altaf AS, Friend D, MASR X. Sustained-Release Technology in Rathbone MJ, Hadgraft J, Robert MS, Modified Release Drug Delivery Technology Marce Dekker Inc., New York, York, 575-609. 1995. **2003**.
[10] Sandip B, Tiwari T, Krishna M, Raveendra P, Mehta, Pasula B. Chowdary, Controlled release formulation of tramadol hydrochloride using hydrophilic and hydrophobic matrix system *PharmSciTech*, 4 (3). **2003**
[11] Giovanna C, Marzia C, Francesca M, Natascia M, Paola M. Sustained-release matrix tablets of metformin hydrochloride in combination with triacetyl-b-cyclodextrin *European Journal of Pharmaceutics and Biopharmaceutics*, 68 :303–309 **2008**.
[12] Nissara A, Nairy H, Mohd G, *Pak. J. Pharm. Sci*., 23, .3:259-265 July **2010**
[13] Mcquaid KR, Dyspepsia, In: Mark Feldman et al (eds.), Sleisenger & Fordtran's Gastrointestinal and liver disease pathophysiology/ diagnosis/ Management, 7th edition. 102-18. **2002**.
[14] Hokuriko S. Co. Ltd. Data on file. Revised. (2nd version of new form). April **2001**.
[15] Iwanga Y, Kemura T. Miyashita N. et al., Characterisation of acetylcholinesterase inhibition by itopride. *Jpn J Pharmacol*, 66: 317-22. **1994**.
[16] Nakajima M, Uematsu T, Nakajima S, Nagata O, Yamaguchi T. Phase 1 study of HSR-803. *Jpn Pharmacol Ther*, 21(11): 4157-73. **1993**
[17] Mushiroda T.; Douya R.; Takahara E.; Nagata O., The involvement of flavin containing monooxygenase but not CYP3A4 in metabolism of itopride hydrochloride, a gastrokinetic agent: comparison with cisapride and mosapride citrate. *Drug Metab Dispos*,; 28: 1231-37. **2000**.
[18] Banka NH. Role of prokinetics in dyspepsia. *Gastroenterol Today*, 7: 14. **2003**.
[19] Louis H, Deviere *J. Gastrointest Endoscopy Clin N Am*., 13: 191-200. **2003**
[20] Fass R, *Gastroenterol Clin N Am.*, 31: S1-S10. **2002**.
[21] Bhupendra G.Prajapati, Niklesh Patel, Hitesh K. Patel *Journal of Science & Technology* (5), 5, Sept **2010**
[22] Lee TW, Robinson JR, Remington: The science and practice of pharmacy; Gennaro, Ed.; Lippincott Williams and Wilkins: Baltimore, (2):903-929. **2000**
[23] Mukesh C, Sharma S. *J. Iran. Chem. Res*. 4 : 25-31. (**2011**)
[24] GUPTA K. R, JOSHI R, CHAWLA B, and Wadodkare S. G.,*Journal of Chemistry*, 7(S1), S49-S54).**2010**.
[25] Patel H, Suhagia N, Patel. M, Patel J. R. *Chromatographia*, 65, June (No. 11/12). **2007**
[26] Mario G, Gabriele G. Mathematical Modeling and Controlled Drug Delivery: Matrix Systems *Current Drug Delivery*, 2: 1 97-116. **2005**
[27] Tuncer C, aykar, Gamze S, Go¨kcen Birlik M, Mater. E. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Weinheim, 291: 1044–1051. **2006**.
[28] Harris S, Jaeria,T, Hamid A, Merchant P. *J.. Pharm. Sci*., , Vol.19 (2), 119-124. (29). **2006**
[29] Khwaldia K. *Journal of Food Biochemistry* 34, (5), 998–1013. (30) October **2010**
[30] Yulu W, Rajesh N, Robert D, Pfeiffer *J. Supercritical Fluids* 28 :85–99 (**2004**).
[31] José L. Arias Molecules Novel Strategies to Improve the Anticancer Action of 5-Fluorouracil by Using Drug Delivery Systems **2008**, 13, 2340-2369.
[32] Chopra G, Patil V, and Motwani S. Release modulating hydrophilic matrix systems of losartan potassium: Optimization of formulation using statistical experimental design, *Eur. J. Pharm. Biopharm*. 66:73–82. (**2007**)