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FORMULATION AND *IN-VIVO* STUDY OF KETOPROFEN TABLETS PREPARED USING CHITOSAN INTERPOLYMER COMPLEXES

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تم دراسة تكوين المعقدات الناتجة من التفاعل بين عديدات الجزيئات وهى الكيتوزان مع كل من الجينات الصوديوم ، كربوكسى ميثيل سيليلوز الصوديوم والبكتين ، ولقد تم فحص المعقدات الناتجة باستخدام الأشعة تحت الحمراء والمسح السعرى الحرارى ، وبعد ذلك تم تحضير الأقراص المحتويه على عقار الكيتوبروفين مع عديدات الجزيئات وحدها أو الخليط الفيزيائى المكون من الكيتوزان مع كل من الجينات الصوديوم وكربوكسى ميثيل سيليلوز الصوديوم والبكتين بنسب مختلفة وهى 3:1، 1:1 ، 1:3 أو المعقدات الناتجة من التفاعل بينهما٠ كما تناول البحث أيضا دراسة الإنطلاق المعملى للعقار فى وسطين احدهما يحتوى على حمض الهيدروكلوريك ذو أس هيدروجينى 1.2 والآخر منظم الفوسفات ذو أس هيدروجينى 7.4. وأظهرت النتائج أن معدل إنطلاق العقار يتأثر بنوع المعقد الناتج من التفاعل بين عديدات الجزيئات ، التفاعل بين الكيتوزان وعديدات الجزيئات فى الخليط الفيزيائى وكذلك على الأس الهيدروجينى لوسط الذوبان. وفى النهاية تم إختيار صواغين إحداهما يتكون من المعقد الناتج من التفاعل بين الكيتوزان وكربوكسى ميثيل سيليلوز الصوديوم والأخرى تتكون من الخليط الفيزيائى منهما بنسبة 1:1 وتم دراسة التوافر الحيوى للعقاربإستخدام الأرانب. وأثبتت النتائج أن الصواغ الذى يحتوى على الخليط الفيزيائى من الكيتوزان وكربوكسى ميثيل سيليلوز الصوديوم قد أدت إلى إنخفاض ملحوظ فى نسبة العقار فى بلازما الدم للأرانب وأعطت تركيزا ممتد المفعول بالمقارنة بذلك الذى يحتوى على المعقد الناتج من التفاعل بينهما أو الأقراص الحاكمة.

The application of interpolymer complexes (IPCs) for oral controlled drug delivery systems was tested between chitosan and various anionic polymers viz sodium alginate, sodium carboxymethylcellulose and pectin. The prepared IPCs were investigated using Fourier transform infra-red spectroscopy and differential scanning calorimetry. Ketoprofen tablets were prepared using the polymers alone, physical mixtures of chitosan with sodium alginate, sodium carboxymethylcellulose or pectin in different ratios; 1:3, 1:1 and 3:1, and the corresponding IPCs. In-vitro release studies were carried out in two dissolution media; 0.1 N HCl of pH 1.2 and phosphate buffer of pH 7.4.

It was found that, chitosan - sod. carboxymethylcellulose IPC tablets showed more controlled drug release compared to that containing chitosan - sodium alginate and chitosan - pectin IPCs. The dissolution rate from tablets prepared using physical mixtures of polymers were found to be dependant on the interaction between chitosan and each of the anionic polymers in the physical mixtures, their ratios and pH of the dissolution medium. Tablets prepared using chitosan - sod. carboxymethylcellulose physical mixture 1:1 and chitosan - sod. carboxymethylcellulose IPC were selected for the in-vivo study using albino rabbits. The results showed a lower peak plasma concentration and marked controlled release effect of drug in tablets containing the physical mixture compared to that of the IPC and the control tablets.

INTRODUCTION

It has been reported that, polyions of opposite charges interact electrostatically with each other to form interpolymer complexes (IPCs). The properties of IPCs depend on various factors including; nature and position of the ionic groups, their concentrations, proportion of opposite charges and molecular weight of the macromolecules[1-3]. Other factors influencing IPCs properties include pH of the medium, temperature and order of mixing[4].

Various methods have been used to investigate interactions between polymers. The

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most commonly used techniques for characterization of IPC formation are turbidity, viscosity measurement, Fourier transform infra-red (FT-IR) and differential scanning calorimetry (DSC)[5-7].

Chitosan is a natural, non-toxic, biodegradable and biocompatible polysaccharide that has been used in the biomedical areas in the form of wound healing material and drug delivery system. It is a very promising biomaterial for drug delivery system, however, its use in oral administration is restricted by its fast dissolution in the stomach and its limited capacity as controlled drug delivery system[8]. To overcome these disadvantages, many researchers have investigated the IPC of chitosan with other anionic polymers like sodium alginate, carrageenan, hyaluronate sodium, pectin and polyacrylic acid[9&10] for controlled release formulations.

Moreover, it has been reported that, drug release from tablets containing physical mixtures of polymers will form *in-situ* polyion complexes that affords more sustained effect compared to the prepared complexes[11&12]. Also, Bhise *et al.*[13] have found that, minimum drug release was observed for naproxen from matrices containing physical mixture of chitosan and κ-carrageenan at acidic and alkaline pH and this may be due to the formation of *in-situ* polyelectrolyte complexes that could be suitable for sustained drug delivery.

Ketoprofen [2- (3-benzoylphenyl) propionic acid] is a non-steroidal anti-inflammatory drug (NSAID). It is widely used to reduce pain, inflammation and stiffness caused by several conditions such as osteoarthritis, rheumatoid arthritis or abdominal cramps associated with menstruation. Ketoprofen prepared in conventional dosage forms is rapidly and completely absorbed from the gastro-intestinal tract. After oral administration, the peak plasma concentration is attained in 1-3 hrs[14].

Ketoprofen may be a candidate for formulation in controlled release dosage forms due to its short half-life and its poor solubility in water, which affects its bioavailability[15&16]. Therefore, in order to maintain therapeutic plasma levels, modified release dosage forms may be beneficial for allowing only one daily dose of the drug with consequent improvement of patient compliance[17-19].

The aim of the present work, was to prepare and characterize the interpolymer complexes composed of chitosan with anionic polymers as sodium alginate, sodium carboxymethylcellulose and pectin. Also, studying the effect of the tested polymers on the dissolution rate of ketoprofen from the prepared tablets. In addition, to investigate the *in-vivo* performance of the drug from the selected formulae compared to the control tablets using albino rabbits.

EXPERIMENTAL

Materials

Chitosan (high molecular weight), ketoprofen and naproxen (Sigma-Aldrich Chemie, Germany), sodium alginate (BDH chemicals, Ltd, Poole, England), pectin (Winlab, U.K.), EDTA disodium salt and sodium carboxymethylcellulose (EL-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt), Diethyl ether and acetonitrile HPLC grade (Scharlau Chemie S.A., European Union). Other materials are of analytical grade.

Equipment

Single punch tablet machine (Erweka-Apparatebau, GmbH, Germany), tablet hardness tester (Erweka-Apparatebau, GmbH, Germany) and Roche friabilator (Erweka-Apparatebau, GmbH, Germany), UV-visible spectrophotometer (JASCO, V-530, Japan), six jars dissolution apparatus (DA-6D, India), Fourier transform infra-red spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), Differential scanning calorimeter (Pyris 6 DSC, Perkin Elmer, USA) and High performance liquid chromatography (HPLC) (Perkin Elmer, USA).

Preparation of interpolymer complexes

Chitosan solutions (1% w/v) in 5% v/v acetic acid solution were added to sod. alginate, sod. CMC or pectin solutions in distilled water (1% w/v). The mixtures were incubated at 37°C for 24 hrs, followed by centrifugation at 5000 rpm for 20 min. The precipitated products were separated from the solution by centrifugation, washed with distilled water, then, dried for 2 days at

40°C[9].The dried complexes were ground using a micronizing mill. Finally, the powders were passed through 200 μm sieve and stored in a desiccator until used for further investigation by FT-IR and DSC.

Fourier transform infra-red spectroscopy (FT-IR)

Infra-red spectra of chitosan, sod. alginate, sod. CMC and pectin each alone, physical mixtures of chitosan with each of the previous anionic polymers and the prepared IPCs were determined. Two-mg sample was mixed with 200 mg potassium bromide (KBr). These mixtures were ground into fine powder, then compressed into KBr disc using a hydraulic press. Each KBr disc was scanned over a wave number region of 500-4000 cm^{-1} and the resolution was 4 cm^{-1}. The characteristic bands were recorded for all samples.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry used to characterize the thermal behavior of chitosan, sod. alginate, sod. CMC and pectin individually, physical mixtures of chitosan with each of the previous anionic polymers and the prepared IPCs. Temperature calibration was performed using indium as a standard (transition point: 156.60°C). Samples were weighed (10 mg) directly in uncovered aluminum pans and scanned between 30 and 450°C at a heating rate of 10°C/min under constant purging of dry nitrogen at 30 ml/min.

Preparation of ketoprofen tablets

Tablets weighing 200 mg, each contains 50 mg ketoprofen were prepared by direct compression. The composition of each formula is shown in table 1. The interpolymer complex of chitosan with sod. alginate, sod. CMC and pectin, also, their physical mixtures in different ratios (1:3, 1:1 and 3:1), in addition to the individual polymers were used as tablet matrices. Each of the previously mentioned powders were mixed with ketoprofen, HPMC (5% w/w as a binder) and magnesium stearate (1% w/w).

Evaluation of tablets

The prepared tablets were evaluated for hardness value, thickness, friability percent, disintegration time and drug content uniformity according to USP XXVII[20].

***In-vitro* drug release**

The *in-vitro* release studies of ketoprofen from the prepared tablets were performed according to the USP XXVII[20] using dissolution apparatus I. One tablet was placed in each basket and immersed in 500 ml of dissolution medium which rotated at 50 rpm and maintained at 37±0.5°C. The dissolution medium was 0.1 N HCl of pH 1.2 or phosphate buffer of pH 7.4. Samples of 1 ml were withdrawn at specified time intervals (15, 30, 45, 60, 90, 120, 180, 240, 300 & 360 min) and the volume was compensated to the initial volume by adding fresh dissolution medium after each sampling. The samples were diluted, filtered using millipore filter (0.45 μm) and spectrophotometrically analyzed at 260 nm. The experiment was carried out in triplicate and the data of *in-vitro* release were expressed as mean ± standard deviation (±S.D).

Kinetic release study

The mechanism of ketoprofen release from the prepared tablets during dissolution study in 0.1 N HCl of pH 1.2 and in phosphate buffer of pH 7.4 was determined using Korsmeyer-Peppas equation[21].

***In-vivo* study**

Selected ketoprofen formulae

The selected ketoprofen formulae for bioavailability study were; tablets containing physical mixture of chitosan - sod. CMC 1:1 (CSC2) and chitosan - sod. CMC IPC (CSC4). They were selected on the basis of acceptable physical characteristics and drug release. They were compared to control tablet (L) which contains 50 mg ketoprofen and lactose monohydrate.

Study design

Male albino rabbits weighing 2.0-2.5 Kg were randomly selected for the bioavailability study. The animals were divided into three groups, each group includes six rabbits which received one of the tested formulae. Twelve hours before drug administration, food was withdrawn from the rabbits until 24 hrs post-dosing, while, water was available for rabbits throughout the study. The tablets were administered to rabbits using a balling gun. Blood samples (1 ml) were withdrawn from the ear vein before dosing (zero time) and at time intervals of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs

Table 1: Composition of ketoprofen tablets prepared using chitosan IPCs and physical mixtures with different anionic polymers.

Formulae	Formulae Code	Drug (mg)	The quantity of ingredients in each tablet (mg)							Total Weight of tablet
			Polymer				Interpolymer complex (IPC)			
			Chitosan	Sod. alginate	Sod. CMC	Pectin	Chitosan - sod. alginate IPC	Chitosan-sod. CMC IPC	Chitosan-pectin IPC	
Chitosan	C	50	138							200
Sod. alginate	SA	50		138						200
Sod. CMC	SC	50			138					200
Pectin	P	50				138				200
Chitosan : sod. alginate (1:3)	CSA1	50	34.5	103.5						200
Chitosan : sod. alginate (1:1)	CSA2	50	69	69						200
Chitosan : sod. alginate (3:1)	CSA3	50	103.5	34.5						200
Chitosan : sod. alginate IPC	CSA4	50					138			200
Chitosan : sod. CMC (1:3)	CSC1	50	34.5		103.5					200
Chitosan : sod. CMC (1:1)	CSC2	50	69		69					200
Chitosan : sod. CMC (3:1)	CSC3	50	103.5		34.5					200
Chitosan : sod. CMC IPC	CSC4	50						138		200
Chitosan : pectin (1:3)	CP1	50	34.5			103.5				200
Chitosan : pectin (1:1)	CP2	50	69			69				200
Chitosan : pectin (3:1)	CP3	50	103.5			34.5				200
Chitosan : pectin IPC	CP4	50							138	200

after administration. EDTA disodium salt was used as an anticoagulant. Plasma was separated by centrifugation at 5000 rpm for 10 min, frozen and stored at −20°C until used.

HPLC analysis for ketoprofen in rabbit's plasma

The plasma samples were analyzed using validated HPLC method described by Corveleyn *et al.*[22]. The mobile phase was a mixture of 0.05 M phosphate buffer of pH 7.0 and acetonitrile in ratio of 84:16 v/v, respectively. A solution of 50 µg/ml naproxen in the same mobile phase was used as an internal standard. The rabbit plasma samples were thawed at room temperature, then, for each 0.5 ml of plasma sample, 200 µl of naproxen solution in the mobile phase (50 µg/ml) was added as an internal standard, then acidified with 1 ml of 1 M phosphate buffer of pH 2.0. After that, the samples were extracted with diethyl ether (5 ml), vortexed for 1 min and centrifuged for 10 min at 5000 rpm. The upper organic phase was separated and evaporated to dryness at 40°C. The dry residue was dissolved in 0.5 ml of mobile phase and the produced solution was filtered through a millipore filter (0.22 µm) and 20 µl of the filtrate was injected into the loop of HPLC apparatus. The mobile phase used was pumped at a flow rate 1.5 ml/min, using U.V detector at 258 nm.

Pharmacokinetic parameters

The maximum plasma concentration (C_{max}) and the time required to reach maximum plasma concentration (T_{max}) after oral administration were directly determined from the plasma concentration-time curves. Also, the area under the plasma concentration-time curve from 0 to 24 hrs (AUC_{0-24}) was calculated using trapezoidal rule. All results are represented as means ± SD.

Statistical analysis

Comparison between the pharmacokinetic parameters of ketoprofen following the oral administration of three tested formulae was carried out using ANOVA test followed by Tukey Kramer test for comparison. The test was performed using instate 2-computer program (Graphpad software Inc., V2, San Diego, CA, U.S.A.).

RESULTS AND DISCUSSION

Fourier transform infra-red spectroscopy (FT-IR)

The FT-IR spectra of chitosan, sod. alginate, sod. CMC and pectin individually, physical mixtures of chitosan with each of sod. alginate, sod. CMC and pectin and their IPCs are shown in figures 1-3. The FT-IR spectrum of chitosan shows two bands at 1649 & 1587cm^{-1}. The band at 1649 cm^{-1} is due to the carbonyl stretching vibration of the secondary amide group, while that at 1587 cm^{-1} is due to N–H bending vibration of amino group, because it is obtained from partial *N*-deacetylation of chitin[23].

The FT-IR spectra of chitosan, sod. alginate, their physical mixture and the corresponding IPC are shown in figure 1. Sod. alginate spectrum showed an absorption bands at 1617 and 1418 cm^{-1} which corresponding to asymmetric and symmetric stretching vibration of carboxylate group[24&25]. Chitosan - sod. alginate IPC spectrum showed a shift of chitosan absorption bands to lower wave number at 1644 and 1570 cm^{-1}, while, the physical mixture showed only one band at 1620 cm^{-1}. This shift was due to the formation of ionic bonds between the protonated amino group of chitosan and carboxylate group of sod. alginate which confirm the formation of IPC. These results were in agreement with that obtained by Sankalia *et al.*, Abdelbary & Tadros, Moustafine *et al.* and Shu *et al.*[7,9,26&27].

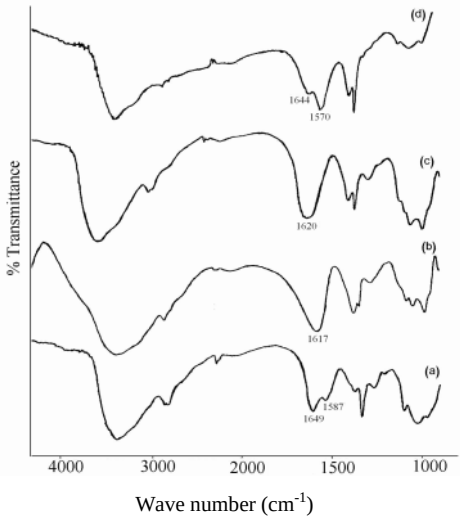

Fig. 1: FT-IR spectra of chitosan, sod. alginate, their physical mixture and IPC.
(a) Chitosan (b) Sod. alginate
(c) Physical mixture 1:1 (d) IPC 1:1

Also, the FT-IR spectrum of the chitosan - sod. CMC IPC showed a characteristic broad band at 1602 cm^{-1} which is different from that of the individual polymers or the physical mixture spectra. Furthermore, the band at 1434 cm^{-1} which was due to symmetric stretching of carboxylate group of sod. CMC was reduced and shifted to lower wave number at 1400 cm^{-1} in the IPC spectrum due to its interaction with the amino group of chitosan as shown in figure 2. Rosca *et al.*[28] stated that, the shift of the amino band to 1602 cm^{-1} is expected, since, a complex formation occurs through the electrostatic interaction of the cationic groups from chitosan with the anionic ones from sod. CMC.

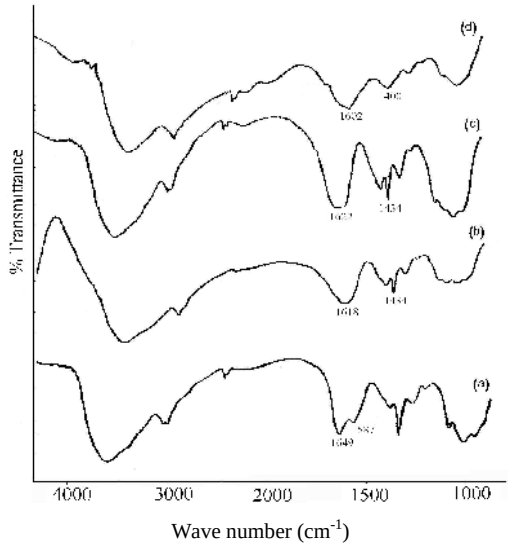

Fig. 2: FT-IR spectra of chitosan, sod. CMC, their physical mixture and IPC.
(a) Chitosan (b) Sod. CMC
(c) Physical mixture 1:1 (d) IPC 1:1

The FTIR spectrum of pectin (Fig. 3), shows two bands at 1741 and 1632 cm^{-1}, that could be related to C=O stretching of ester and carboxyl groups, respectively[29]. However, the spectrum of chitosan - pectin IPC (Fig. 3) showed a shift of pectin bands to lower wave numbers at 1730 and 1624 cm^{-1} compared to their physical mixture which indicated the evidence of interaction between the amino groups of chitosan and carboxyl groups of pectin. These results confirmed the IPC formation between chitosan and pectin and were similar to that obtained by Rashidova *et al.*[30].

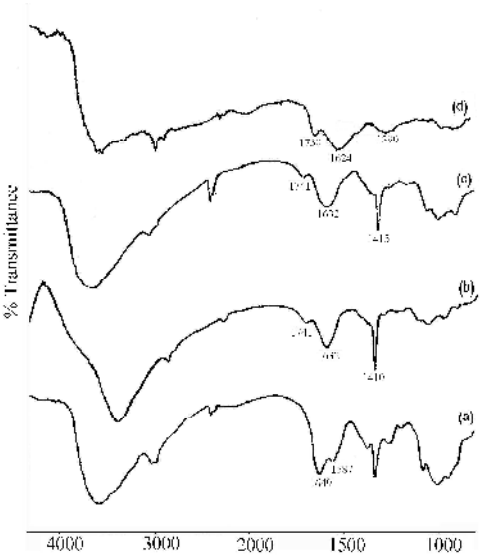

Fig. 3: FT-IR spectra of chitosan, pectin, their physical mixture and IPC.
(a) Chitosan (b) Pectin
(c) Physical mixture 1:4 (d) IPC 1:4

Differential scanning calorimetry (DSC)

Thermograms of DSC were performed in order to investigate the possible solid-state interactions between the components. The figures of thermal analysis of chitosan, sod. alginate, sod. CMC and pectin, individually, their physical mixtures and IPCs are shown in figures 4-6. The thermogram of chitosan showed a broad endothermic peak at 80.39°C, due to the polymer dehydration, followed by a second exothermic peak at 310°C. Similar results were obtained by Khalid *et al.* and Borges *et al.*[31&32]. The thermograms of sod. alginate, sod. CMC and pectin showed endothermic peaks at 89.49, 86.64 and 89.18°C, in addition to exothermic ones at 261, 310 and 238°C, respectively.

Endothermic peaks are corresponding to the loss of water associated with the hydrophilic groups of polymers which have a strong affinity for water and their hydration properties depend on their molecular structures[33&34]. While, the exothermic ones referred to the degradation of polyelectrolytes due to thermal decomposition and depolymerization reactions most probably to the partial decarboxylation of the carboxylic groups and oxidation reactions[35&36].

The DSC thermograms of chitosan, sod. alginate, chitosan - sod. alginate physical

mixture and their IPCs are shown in figure 4. It is obvious that, the thermogram of chitosan - sod. alginate physical mixture showed two exothermic peaks at 265 and 310°C that resulted from individual contribution of sod. alginate and chitosan, respectively. However, the thermogram of IPC, showed a new exothermic one at 287.5°C, which assigned to the formation of an ionic pair between the carboxylate group ($–COO^–$) of sod. alginate and ammonium group (NH_3^+) of chitosan. In addition to the disappearance of the degradation exothermic peaks of both chitosan and sod. alginate. These results were in agreement with Sankalia *et al*. and Ostrowska-Czubenko & Gierszewska-Druzyn´ska[7&37]. The same behaviour was also observed in case of chitosan - sod. CMC IPC (Fig. 5) which showed an exothermic peak at 260°C with complete disappearance of chitosan or sod. CMC exothermic peaks.

The DSC thermogram of chitosan - pectin physical mixture showed two exothermic peaks at 240 and 308°C, that correspond to pectin and chitosan, respectively (Fig. 6). However, the chitosan - pectin IPC thermogram showed a shift of the exothermic peaks to lower temperature at 213°C and 260°C which indicated the interactions between the two polymers and may be considered as a proof of their complexation. Similar results were obtained for chitosan and pectin by Ghaffari *et al.*[29].

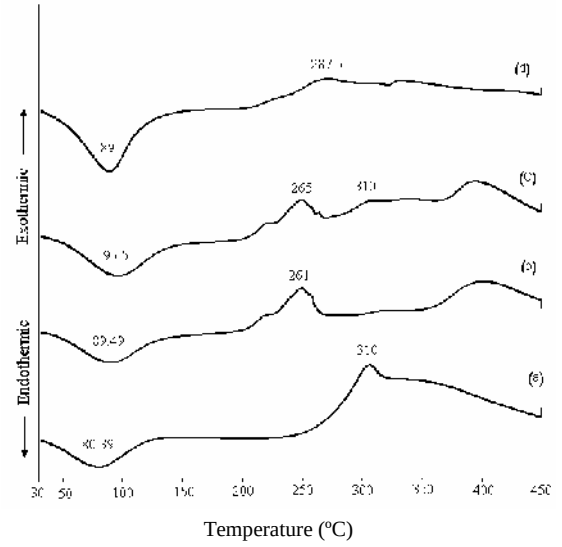

Fig. 4: DSC thermograms of chitosan, sod. alginate, their physical mixture and IPC.
(a) Chitosan (b) Sod. alginate
(c) Physical mixture 1:1 (d) IPC 1:1

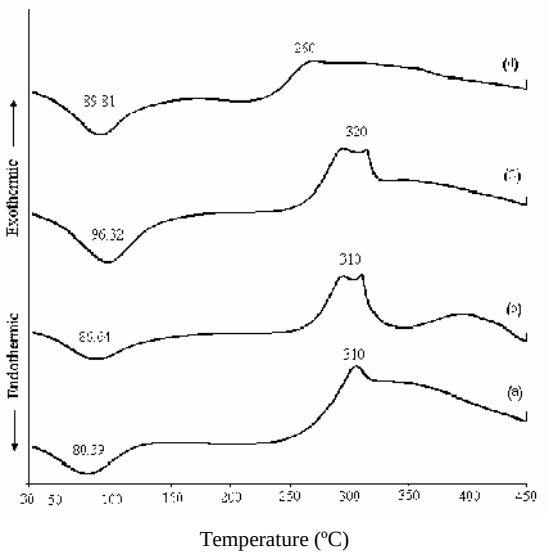

Fig. 5: DSC thermograms of chitosan, sod. CMC, their physical mixture and IPC.
(a) Chitosan (b) Sod. CMC
(c) Physical mixture 1:1 (d) IPC 1:1

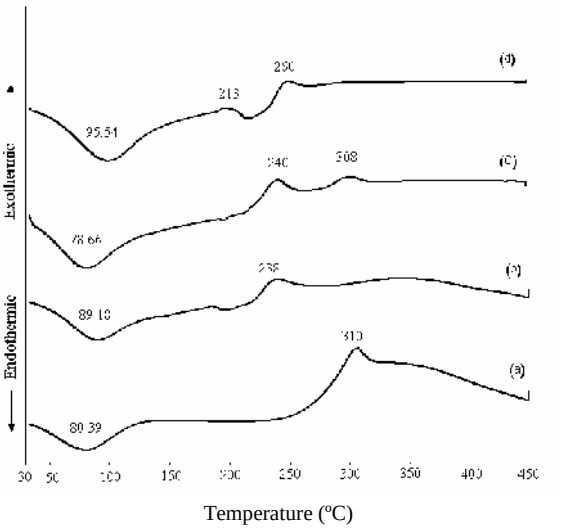

Fig. 6: DSC thermograms of chitosan, pectin their physical mixture and IPC.
(a) Chitosan (b) Pectin
(c) Physical mixture 1:4 (d) IPC 1:4

Physical properties of ketoprofen tablets

The physical properties of ketoprofen tablets are shown in table 2. The tablet formulae (except pectin tablets) showed acceptable physical properties. The drug content of the prepared tablets was within the requirements of USP XXVII[20].

***In-vitro* drug release**

The release of ketoprofen from the prepared tablets was studied using 0.1 N HCl

Table 2: Physical properties of ketoprofen tablets.

Parameter / Formulae Code	Drug content (%)	Tablet Thickness (mm)	Friability (%)	Hardness (Kg)	Disintegration time (min.)
L	99.76 ± 1.53	2.1 ± 0.07	0.85	7.25 ± 1.5	15.23 ± 0.45
C	100.91 ± 3.12	2.62 ± 0.07	0.34	7.66 ± 0.72	> 360
SA	102 ± 6.08	2.23 ± 0.47	0.21	7.75 ± 1.14	> 360
SC	91.08 ± 4.86	2.35 ± 0.02	0.59	8.75 ± 0.66	> 360
P	103.3 ± 2.2	2.36 ± 0.047	1.20	4.75 ± 0.60	25.78 ± 1.2
CSA1	97.68 ± 4.63	2.45 ± 0.03	0.49	7.83 ± 0.62	> 360
CSA2	98.55 ± 1.41	2.38 ± 0.09	0.57	7.5 ± 0.25	> 360
CSA3	98.49 ± 3.07	2.39 ± 0.04	0.58	7.8 ± 1.14	> 360
CSA4	97.69 ± 2.48	2.5 ± 0.04	0.95	5.98 ± 0.62	12.02 ± 0.03
CSC1	100.32 ± 5.18	2.37 ± 0.02	0.26	8.87 ± 0.66	129.56 ±1.2
CSC2	94.95 ± 5.46	2.4 ± 0.03	0.44	8.12 ± 0.96	> 360
CSC3	97.49 ± 2.07	2.5 ± 0.03	0.48	8.06 ± 0.96	> 360
CSC4	99.69 ± 3.58	2.48 ± 0.03	0.85	6.18 ± 0.5	> 360
CP1	99.4 ± 2.6	2.4 ± 0.03	0.95	5.56 ± 0.28	35.67 ± 1.06
CP2	97.5 ± 1.63	2.37 ± 0.06	0.61	6.16 ± 0.57	36.74 ± 1.60
CP3	100 ± 2.1	2.52 ± 0.08	0.24	6.68 ± 0.42	140.09 ± 1.06
CP4	95.7 ± 1.37	2.46 ± 0.04	0.73	6.25 ± 0.38	16.98 ± 0.08

of pH 1.2 and phosphate buffer of pH 7.4 as dissolution media. The tested polymers not interfere with the analysis of ketoprofen in the drug release studies because there were no significant peaks for the used polymers observed in the UV range from 200 to 400 nm using 0.1 N HCl of pH 1.2 and phosphate buffer of pH 7.4 as blank.

To study the effect of the tested polymers on the release of ketoprofen from matrix tablets, a control tablet using lactose has been prepared and used for comparison. The release profiles of ketoprofen from this control tablets showed that, 100% of the drug was released after 1 hr in phosphate buffer of pH 7.4, while 86.66% of the drug was released after 6 hrs in 0.1 N HCl as shown in figures (7-12). These results may be due to that, ketoprofen is a weak acidic drug (pK_a= 5.02-5.937) and its solubility is pH dependant which increases rapidly at pH values higher than the drug pK_a value. The solubility of the drug was previously determined by EL-Gibaly[38] and was found to be 0.1332 and 7.415 mg/ml in (pH 1.2) and (pH 7.4), respectively.

Chitosan - sodium alginate tablets

The release profiles of ketoprofen from chitosan - sod. alginate matrix tablets in 0.1 N HCl of pH 1.2 are shown in figure 7. It is obvious from the figure that, chitosan tablets produced minimal drug release especially during the first 3 hrs (only 5.58%). This retardation from chitosan tablets may be due to the low solubility of drug in acidic medium[39].

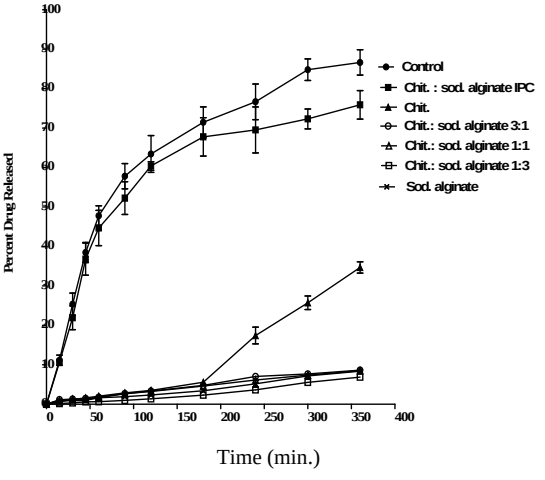

Fig. 7: *In-vitro* release profiles of ketoprofen from chitosan - sod. alginate tablets in 0.1 N HCl of pH 1.2.

In addition to the high molecular weight of chitosan used[40]. Moreover, chitosan is soluble in dilute aqueous acidic solution (pH < 6.5) and forms a gel at this acidic pH values, the viscosity of the produced gel increases as molecular weight or amount of polymer increase[41-43].

Drug diffusion through this gel layer and erosion of it may be regarded as the reason for further drug release occurring in 0.1 N HCl, and hence, the percent drug release increased uptill 34.68% after 6 hrs. On the other hand, the percent drug released from sod. alginate tablets was 9.13% after 6 hrs. This was explained by Dentini *et al.*[44] who stated that, sod. alginate is converted into an insoluble alginic acid at pH < 3 and undergo a process of association, resulting in the formation of a thick network of inter- and intramolecular hydrogen bonds which responsible for the retardation of drug release.

However, chitosan - sod. alginate IPC tablets exhibited rapid release pattern (75.9% of drug was released after 6 hrs), such results may be due to the fast disintegration of tablets. These results were in agreement with that of Satoh *et al.* and Tapia *et al.*[5&45]. While, tablets containing chitosan - sod. alginate physical mixtures showed minimal drug release compared to chitosan - sod. alginate IPC tablets where 6.88, 8.38 & 8.64% of drug were released after 6 hrs from chitosan - sod. alginate physical mixtures 1:3, 1:1 and 3:1, respectively. Gonza´lez-Rodrı´guez *et al.*[46], stated that, at acidic pH, sod. alginate is converted into the insoluble form of alginic acid which responsible for the low rate of release and it is also hindered by positively charged groups of chitosan that strongly interact with alginate and both reducing the drug release.

Moreover, Bhise *et al.*[47] found that, after compression of a mixture of naproxen sodium - loaded chitosan particles and κ-carrageenan into tablets, the interaction between oppositely charged κ-carrageenan and chitosan leads to relatively higher gel strength, which is proportional to the ability to retard the drug release at acidic pH.

The *in-vitro* release profiles of ketoprofen from chitosan - sod. alginate tablets in phosphate buffer of pH 7.4 are shown in figure 8. From the figure, it is obvious that, tablets containing chitosan-alginate IPC showed rapid release of the drug (100% of the drug was released after 1 hr) due to their disintegration as in case of 0.1 N HCl. Also, chitosan tablets showed rapid drug release where 100% of the drug was released after 2 hrs (Fig. 8). This is may be due to the weak gel forming ability and rapid disintegration characteristics of chitosan at this pH[10&48].

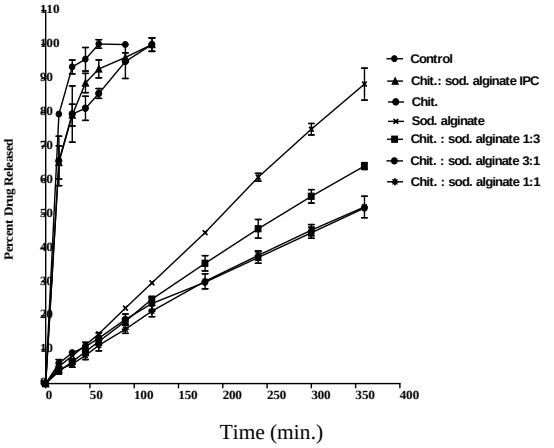

Fig. 8: *In-vitro* release profiles of ketoprofen from chitosan - sod. alginate tablets in phosphate buffer of pH 7.4.

On the other hand, the percent drug release after 6 hrs from alginate tablets in phosphate buffer (88.33%) was greater than in acidic medium. This may be due to the solubility of alginate at that pH resulting in a viscous gel as alginate tablets hydrated and swelled on contact with the aqueous medium and a gel layer was formed immediately around the tablet. Then, erosion of this gel layer occurred leading to an increase of drug release[49].

The comparison of ketoprofen release performance from tablets containing chitosan - sod. alginate physical mixtures in phosphate buffer of pH 7.4 (Fig. 8), showed that, the drug release from these tablets was significantly lower ($P < 0.05$) compared to chitosan, sodium alginate and IPC tablets. Where, 64.17, 52.1 & 51.8% of the drug were released after 6 hrs from chitosan - sod. alginate physical mixture of ratio; 1:3, 1:1 & 3:1, respectively.

These results confirmed complex formation between chitosan and sod. alginate after penetration of the dissolution medium into the tablet. Satoh *et al.*[45] stated that, tablets prepared using physical mixture of polymers

could promote IPC formation following penetration of dissolution medium into the tablet during dissolution process and this may explain why the original shape of the tablets was maintained during the dissolution process. Also, Tapia *et al.*[50], stated that, slow drug release was obtained from tablets containing chitosan - alginate physical mixture when the pH changes from 5.52 to 8.72 as the drug release from the matrix is controlled by IPC formation.

Chitosan - sodium CMC tablets

The influence of pH of dissolution medium on the release profiles of ketoprofen from chitosan - sod. CMC tablets is shown in figures 9&10. Drug release from sod. CMC tablets in 0.1 N HCl of pH 1.2 showed slow drug release pattern similar to that from sod. alginate matrix tablets, where only 16.21% of the drug was released after 6 hrs (Fig. 9). EL-Kamel *et al.*[51] stated that, the ionization of carboxylic group of sod. CMC (pK_a= 3) decreases at acidic pH, this affects polymer chain and the polymer network structure becomes tighter, therefore, the release rate is expected to be slower, in addition to the acidic nature of the drug.

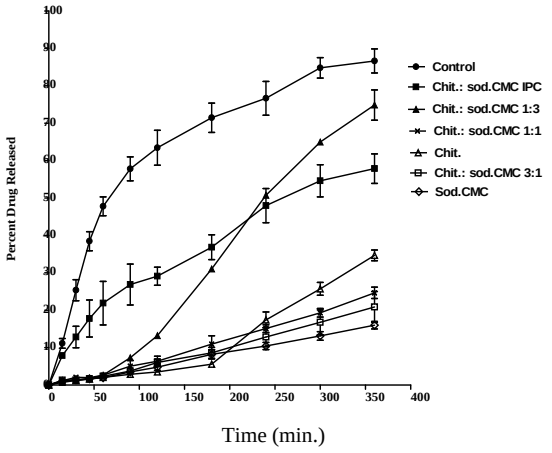

Fig. 9: *In-vitro* release profiles of ketoprofen from chitosan - sod. CMC tablets in 0.1 N HCl of pH 1.2.

From the obtained data, it is clear that, the percent drug released after 6 hrs from tablets containing chitosan - sod. CMC IPC was 57.86%. However, these percent were 20.88 & 24.66% from tablets containing chitosan - sod. CMC physical mixture of ratio 3:1 & 1:1, respectively, which were significantly lower than that from tablets containing chitosan or chitosan - sod. CMC IPC tablets (P< 0.001), moreover, no disintegration of these tablets was observed during the dissolution process, that may be due to the persistent gel layer surrounding the tablets[52]. These results were in agreement with EL-Kamel *et al.*[53], who found that, by incorporation of sod. CMC in tablets containing mixture of chitosan and sod. alginate, the release of metronidazole was retarded in acidic medium due to the ionic interaction between chitosan and sod. CMC and no disintegration of tablets occurred.

On the other hand, tablets containing chitosan-sod. CMC physical mixture of ratio 1:3 showed a different behavior where, a minimal drug release was observed in the first two hours followed by increasing the percent drug released till reached 74.86% after 6 hrs which may be due to their disintegration.

The release profiles of ketoprofen from chitosan - sod. CMC tablets in phosphate buffer of pH 7.4 are shown in figure 10. Tablets contain sod. CMC followed the same behavior as sod. alginate tablets because of solubility of sod. CMC in phosphate buffer; thus, the drug release was more rapid in the buffer than in 0.1 N HCl (81.33% was released after 6 hrs). From the obtained data, it is clear that, the percent drug released after 6 hrs from tablets containing chitosan - sod. CMC IPC was 71.23%, however, those containing different ratios of chitosan - sod. CMC physical mixtures significantly lowered (P< 0.0001) the drug release as compared to chitosan, sod. CMC or IPC tablets, where, 62.28, 64.26 and 53.37% of the drug were released after 6 hrs from chitosan - sod. CMC physical mixtures tablets of ratios; 1:1, 3:1 and 1:3 respectively.

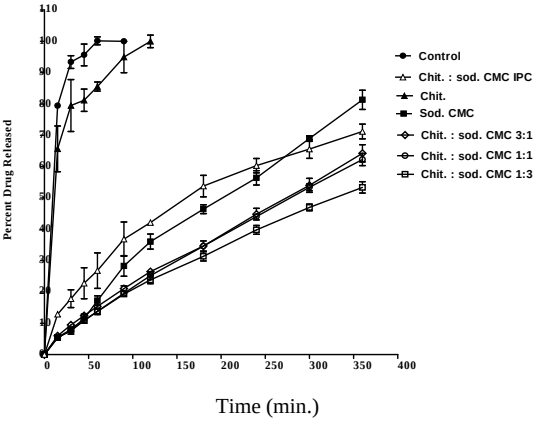

Fig. 10: *In-vitro* release profiles of ketoprofen from chitosan - sod. CMC tablets in phosphate buffer of pH 7.4.

These results agreed with that of Moustafine *et al*.[26] who found that, the release of diclofenac sodium from tablets containing chitosan - Eudragit L 100 physical mixture was much slower compared with the corresponding IPC.

Furthermore, Conti *et al*.[54], found that, the release rate of diltiazem hydrochloride is slower from tablets containing a mixture of sod. CMC and HPMC compared to that matrices containing the single polymers due to the interactions between sod. CMC and HPMC chains.

Chitosan - pectin tablets

Pectin tablets showed a higher drug release rate in acidic medium where the percent drug released was 75.6% after 6 hrs (Fig. 11), this may be due to high erosion rate of the tablets, moreover, pectin is converted to water insoluble pectinic acid in acidic medium which is expected to enhance tablet disintegration[55].

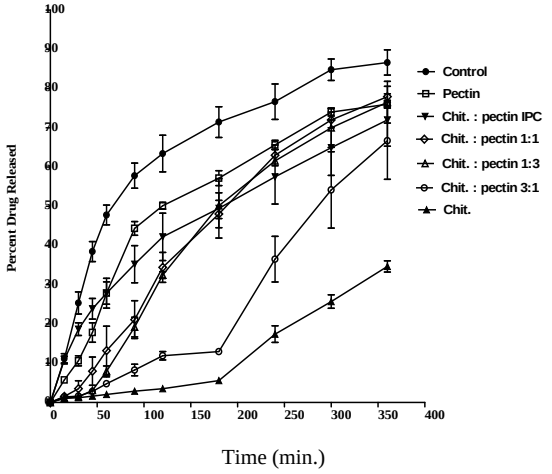

Fig. 11: *In-vitro* release profiles of ketoprofen from chitosan - pectin tablets in 0.1 N HCl of pH 1.2.

A comparison of the release profiles of ketoprofen from tablets containing chitosan - pectin physical mixtures with that containing IPC in 0.1 N HCl of pH 1.2 (Fig. 11) revealed that, both the IPC and the physical mixtures (1:3 & 1:1) showed a rapid release rate of the drug. The percent drug released after 6 hrs were 72, 76.52 and 77.96% from tablets containing chitosan - pectin IPC, physical mixtures of ratios 1:3 and 1:1, respectively. These results may be due to the erosion of these tablets followed by complete disintegration. So, each polymer remained alone and no IPC was formed. However, tablets containing chitosan - pectin physical mixture of ratio 3:1 produced slow release pattern in the first 3 hrs, then, disintegration of tablets occurred leading to increasing the percent drug released (66.7% after 6 hrs).

The dissolution profiles of ketoprofen from chitosan - pectin tablets in phosphate buffer of pH 7.4 are shown in figure 12. The percent drug released was 100% from pectin tablets after 5 hrs. It was reported that, drug release from pectin tablets depends on pectin hydrophilic character and hence, these tablets hydrated on exposure to dissolution medium, swelled and formed a gel layer. Then, drug release occurs by diffusion and erosion of this gel layer[56].

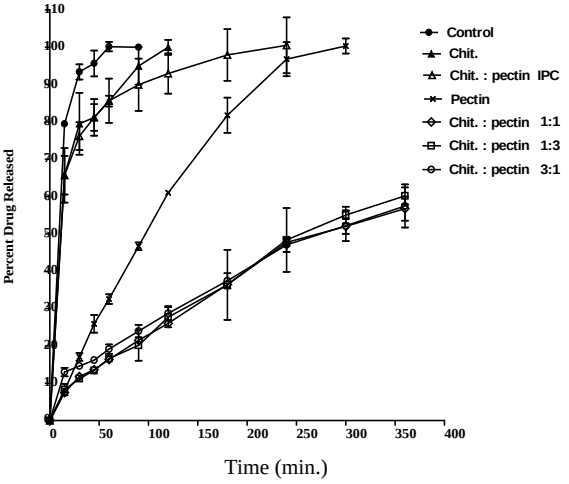

Fig. 12: *In-vitro* release profiles of ketoprofen from chitosan - pectin tablets in phosphate buffer of pH 7.4.

On the other hand, tablets containing chitosan and pectin physical mixtures of different ratios significantly ($P < 0.0001$) decreased the drug release rate compared to chitosan, pectin or IPC tablets and there was no significant difference between these ratios ($P > 0.05$). This may be attributed to the interaction between chitosan and pectin at this pH during the dissolution process. Perugini *et al*.[57] found that, fast drug release is obtained upon using pectin or chitosan alone as mucoadhesive formulations and to promote prolonged drug release in the small intestine, mixtures of chitosan and pectin have been used extensively as an IPC could develop as a consequence of ionic interactions between chitosan and pectin.

Kinetic release study

The mechanism of drug release from tablets containing swellable polymers is complicated and not completely understood. Some systems may be classified as either diffusion or erosion controlled, while the most systems exhibit a combination of these mechanisms[58]. The release data of all tested formulae were mathematically analyzed according to Korsmeyer-Peppas semi-empirical model equation. The linearity in case of Korsmeyer-Peppas semi-empirical model was evaluated by calculating the linear correlation coefficient (r^2), while, the release mechanism determined by evaluating the release exponent (n).

In 0.1 N HCl of pH 1.2, the tested tablet formulae showed correlation coefficient, r^2, between 0.919 for chitosan - sod. alginate 1:1 tablets and 0.996 for chitosan - sod. alginate IPC tablets and the n values were ranged between 0.650 for chitosan - pectin 1:3 tablets and 0.943 for chitosan - sod. CMC 3:1 tablets (i.e. $0.5 < n < 1$), indicating that, the release mechanism of ketoprofen from these matrices is an anomalous (non-Fickian) transport, which suggests that, both diffusion of the drug from the hydrated matrix and its own erosion modulate drug release[59&60].

In phosphate buffer of pH 7.4, Korsmeyer-Peppas semi-empirical model is not applied to chitosan, chitosan - sod. alginate and chitosan - sod. CMC IPCs tablets due to their rapid release. Therefore, the release exponents for these formulae could not be calculated as a result of insufficient data points, up to 60% of the drug release profiles to provide accurate values. All other formulae have ($0.5 < n < 1$) that ranged between 0.501 for chitosan - pectin 3:1 tablets and 0.927 for chitosan - sod. alginate IPC tablets indicating that, the release mechanism of ketoprofen from these matrices is an anomalous (non-Fickian) transport. So, these tablets delivered their drug content by both diffusion and erosion mechanism.

***In-vivo* study**

The mean plasma concentrations as a function of time for ketoprofen after oral administration of control tablets, chitosan - sod. CMC physical mixture in ratio of 1:1 and chitosan - sod. CMC IPC are illustrated in figure 13. From the obtained data, it could be observed that, there was a difference between the mean plasma concentrations as a function of time for ketoprofen after oral administration of the two tested formulae at all time intervals compared to the control tablet. Also, there was a noticeable difference in the C_{max} and T_{max} between the control and the tested formulae.

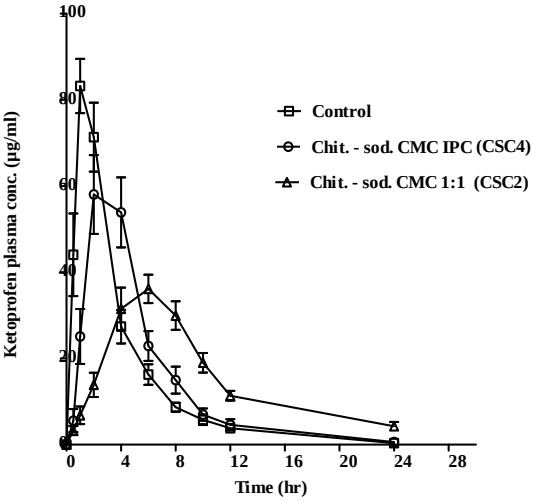

Fig. 13: Mean plasma concentration of ketoprofen after oral administration of control, chitosan - sod. CMC physical mixture 1:1 and chitosan - sod. CMC IPC tablets in rabbits.

The mean pharmacokinetic parameters of ketoprofen from different formulae represented by the value of C_{max} (µg/ml), T_{max} (hr) and AUC_{0-24} ($\mu g.hr.ml^{-1}$) are summarized in table 3. From the obtained results, it was evident that, the absorption of ketoprofen from the control tablets was rapid and reached its peak plasma concentration in 1.33±0.51 hrs, whereas, following oral administration of the tested formulae, chitosan - sod. CMC IPC (CSC4) and chitosan - sod. CMC physical mixture of ratio 1:1 (CSC2) tablets, the mean T_{max} was 2.66±0.43 and 5.66±0.81 hrs, respectively. While, the mean plasma concentrations (C_{max}) were 62.34±5.02 µg/ml for CSC4 and 36.33±3.12 µg/ml for CSC2 compared to 84.28±5.14 µg/ml for the control tablets. These results showed that, the oral absorption of CSC2 tablets leads to an increase of the mean T_{max} and lowering the mean C_{max} compared to CSC4 and control tablets and hence, indicated the delayed release behavior of CSC2 compared to CSC4 and control tablets. The mean AUC_{0-24} was found to be

341.98±17.88 μg.hr.ml^{-1} for CSC4 and 365.98±14.27 μg.hr.ml^{-1} for CSC2 compared to 336.18±10.35 μg.hr.ml^{-1} for the control tablet.

Table 3: The mean pharmacokinetic parameters of ketoprofen from control, chitosan - sod. CMC physical mixture in ratio of 1:1 and chitosan - sod. CMC IPC tablets.

Formulae / Parameter	Control (L)	Chitosan-sod. CMC 1:1 (CSC2)	Chitosan - sod. CMC IPC (CSC4)
C_{max} (μg/ml)	84.28 ± 5.14	36.33[a] ± 3.12	62.34[a] ± 5.02
T_{max} (hrs)	1.33 ± 0.51	5.66[a] ± 0.81	2.66[b] ± 0.43
AUC_{0-24} (μg.hr.ml^{-1})	336.18± 10.35	365.98[b]± 14.27	341.98[b]± 17.88

a Extremely significant from control ($p < 0.001$).
b Non significant from control ($p > 0.05$).

The statistical analysis of the pharmacokinetic parameters of ketoprofen from different formulae revealed that, there was a significant difference between CSC2 tablets and each of the control and CSC4 tablets in the C_{max} and T_{max} values ($P < 0.001$). Also, there was a significant difference between C_{max} of CSC4 and the control tablets, however, their T_{max} values were non significantly different ($P > 0.05$). In addition, there was no significant difference between CSC4, CSC2 and the control tablets in the AUC_{0-24} values which indicated that, the absorption of ketoprofen was not influenced by the *in-vivo* behavior of the prepared tablets.

These findings achieved the goal of delayed release concept from tablets prepared using chitosan - sod. CMC physical mixture in ratio of 1:1 which has been estimated in reducing high peak plasma concentration (C_{max}) and prolong the time required to reach maximum plasma concentration (T_{max}). A similar finding was reported by Vergote *et al.*[61]. They found that, the compression of pellets containing nanocrystalline or microcrystalline ketoprofen in combination with placebo wax/starch pellets resulted in sustaining the ketoprofen plasma concentrations as indicated by lowering C_{max} and prolonging T_{max}.

Conclusion

The results of the present study confirmed the formation of an IPC between chitosan and anionic polymers as sod. alginate, sod. CMC or pectin. Tablets prepared using chitosan-sod. alginate and chitosan-pectin IPCs showed rapid drug release at pH 1.2 and 7.4, however, chitosan-sod. CMC IPC tablets showed controlled drug release. On the other hand, tablets prepared using physical mixtures of chitosan with each of the previous anionic polymers showed *in-situ* IPC during dissolution process depending on the pH of the dissolution medium. So, a careful selection of IPC or polymers ratios in the physical mixture will be capable of producing systems which deliver drugs at different rates depending on the site of the dosage form within the GIT. The *in-vivo* study of tablets containing chitosan - sod. CMC physical mixture in ratio of 1:1 using rabbits showed that, these tablets were able to ensure controlled drug release for a longer period than that containing chitosan - sod. CMC IPC.

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