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# Formulation and *In-Vivo* Study of Diltiazem Hydrochloride Tablets Prepared Using Interpolymer Complexes

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***Research Article***

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## ABSTRACT

**Aim:** To investigate interpolymer complexes (IPCs) formation between carbopol and cationic polymers such as chitosan and Eudragit E for oral controlled drug delivery systems.
**Methodology:** The prepared IPCs were investigated using Fourier transform infra-red spectroscopy (FT-IR) and differential scanning calorimetry (DSC). Chitosan-carbopol and Eudragit E-carbopol IPCs loaded with diltiazem hydrochloride (DTZ HCl) with different drug:polymer ratios were also prepared. Diltiazem hydrochloride tablets were prepared using polymers alone, physical mixtures of chitosan or Eudragit E with carbopol and the corresponding drug loaded IPCs. *In-vitro* release studies were carried out in two dissolution media; 0.1 NHCl of pH 1.2 and phosphate buffer of pH 7.4.
**Results:** The dissolution rate of DTZ HCl from the prepared tablets were found to be dependant on the interaction between chitosan or Eudragit E with carbopol in the physical mixture, drug:polymer ratio and pH of the dissolution medium. Tablets prepared using chitosan – carbopol IPC, Eudragit E – carbopol IPC, and Eudragit E – carbopol physical mixture of drug:polymer ratio 1:5 were selected for the *in-vivo* study using rabbits. The results showed a lower peak plasma concentration and marked prolonged release effect of tablets containing Eudragit E – carbopol IPC and the corresponding physical mixture compared to that of commercial Altiazem tablets.
**Conclusion:** Tablets containing Eudragit E – carbopol or chitosan – carbopol physical mixtures showed prolonged drug release compared to that containing the corresponding IPCs, Furthermore, Eudragit E- carbopol matrix tablets showed slower drug release than that of chitosan – carbopol.

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*Keywords: Chitosan; Eudragit E; Carbopol; diltiazem hydrochloride; interpolymer complex; tablets.*

## 1. INTRODUCTION

It was reported that, polyions of opposite charges interact electrostatically with each other to form interpolymer complexes (IPCs). The properties of IPCs depend on various factors as nature, position of the ionic groups and molecular weight of the macromolecules (Tsuchida and Abe, 1982; Philipp et al., 1989; Koetz et al., 1996). Other factors influencing IPCs properties include pH of the solution, temperature and order of mixing (Dumitriu and Chornet, 1998).

Various methods have been used to investigate interactions between polymers. The most commonly used techniques for characterization of IPC formation are turbidity, viscosity measurement, Fourier transform infra-red (FT-IR) and Differential Scanning Calorimetry (DSC) (Moustafine et al., 2006; Sankalia et al., 2007).

Carbopol® is a cross-linked polymer of acrylic acid with a high molecular weight that forms a hydrogel in aqueous solutions depending on the degree of hydration of the carboxyl group of carbopol. It has good gel forming ability and mucoadhesive property (Singlga et al., 2000).

It was reported that, chitosan and polyacrylic acid complexes can be obtained by physical mixture of both polymers, dissolution of polymers in a solvent followed by solvent evaporation or by template polymerization (Wang et al., 1997; Dhanuja et al., 2005). This complex formation resulted in preventing dissolution and excessive swelling of the polymeric matrix in water, while its mucoadhesive properties can be improved or maintained and these are essential requirements for developing controlled drug release (Ahn et al., 2002). On the other hand, there are few reports concerned with the development of controlled drug delivery systems using Eudragit EPO-carbomer 940 interpolymer complex (Moustafine et al., 2010).

Diltiazem hydrochloride (DTZ HCl) is (+)-cis-3-(acetyloxy)-5-[2(dimethylamino) ethyl]-2,3-dihydro-2-(4-methoxyphenyl) 1,5-benzothi-azepine – 4(5H) one monohydrochloride. It is a benzodiazepine calcium ion influx inhibitor (slow channel blocker or calcium antagonist). Diltiazem hydrochloride is a coronary and peripheral vasodilator, which has been generally indicated for the treatment of angina and hypertention. Diltiazem hydrochloride is clinically used in a dose of 60 mg three times daily in case of angina pectoris which can be increased to 360 mg daily if necessary. While, in case of hypertension; an intial dose of 60 to 120 mg was taken twice daily and may be increased to 360 mg daily if necessary. The conventional tablets and capsules containing DTZ HCl are administered 3 or 4 times daily due to its short biological half-life of 3 to 5 hr (Martindale, 2007). Accordingly, the IPC phenomena plays an important role in the formulation of controlled release dosage forms of DTZ HCl (Bani-Jaber and Al-Ghazawi, 2005; Lu et al., 2007).

Therefore, the aim of the present work was to prepare chitosan – carbopol and Eud. E - carbopol IPCs loaded with diltiazem hydrochloride and characterize these IPCs by FT-IR and DSC techniques. Also, to evaluate the possibility of obtaining different prolonged drug dissolution profiles by changing the polymer matrix system (chitosan–carbopol or Eudragit E–carbopol) and the method used to include the polymers into the tablets (physical mixture or interpolymer complex). Also, we tried to explain the effect of pH of dissolution media on

drug release profiles from the matrices. Finally, to investigate the *in-vivo* performance of the drug from the selected formulae compared to commercial tablets (Altiazem®, 60 mg) using rabbits.

## 2. METHODOLOGY

## 2.1 Materials

Chitosan, Oxford laboratory, Mumbai, India. Diltiazem hydrochloride (DTZ HCl), was kindly supplied by Egyptian Int. Pharmaceutical Industries Co., (10th Ramadan city, Egypt). Carbopol® 934 was kindly supplied by Amriya Pharmaceutical Industries Co. (Alexandria, Egypt). Eudragit E®-100 (Eud. E), Röhm pharma, Darmstadt, Germany. Acetonitrile, HPLC grade (Scharlau Chemie S.A., European Union). Other materials are of analytical grade and were used as received.

## 2.2 Experimental

### 2.2.1 Determination of stoichiometric ratios between Chitosan-Carbopol and Eudragit E- Carbopol

Chitosan or Eudragit E stock solutions of concentration (1% w/v) were prepared by dispersing 1 gm of each separately in 100 ml 5% v/v acetic acid solution. The dispersions were then stirred until uniform solutions were obtained. Carbopol dispersion of concentration (0.1% w/v) was prepared by dispersing 0.1 gm in 100 ml distilled water, then stirred until a uniform dispersion was obtained. Then, different concentrations of chitosan or Eudragit E solutions (0.1-1% w/v) were added to carbopol dispersions of concentration (0.1% w/v). The mixtures were incubated at 37°C for 24 hr, followed by centrifugation at speed of 5000 rpm for 20 min. Finally, the viscosity of the supernatant solution was measured using Hakke RV3 viscometer (Berlin, Germany). The stoichiometric ratios between chitosan – carbopol or Eudragit E – carbopol mixtures were obtained when the supernatant viscosity was close to that of the solvent.

### 2.2.2 Preparation of interpolymer complexes

Mixtures of the polymer solutions which showed the lowest viscosity were used in the preparation of IPCs. The precipitated products were separated from the solution by centrifugation, washed with distilled water, then, dried under vacuum for 2 days at 40°C. 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 techniques.

### 2.2.3 Preparation of DTZ HCL – Carbopol complex

One gram of carbopol was dispersed in 100 ml distilled water, then, 0.4 ml of triethanolamine was added to the dispersion because the preliminary work showed that, this amount gave better entrapment of the drug in the complex, then, the dispersion stirred until a homogenous gel was obtained. After that, one gram of DTZ HCl was dissolved in 5 ml distilled water and the produced solution was added to carbopol gel. The mixture was further stirred using magnetic stirrer at 100 rpm for 30 min, then, kept aside for 2 hr. The precipitated product was separated by filtration with Buchner funnel, washed with distilled water and dried under

vacuum at 40℃ for 2 days. Finally, they were milled in a micronizing mill, passed through 200 µm sieve and stored in a desiccator until used.

### 2.2.4 Preparation of DTZ HCl IPCs

Different ratios of drug to polymer were used; 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6. The ratio of chitosan or Eudragit E to carbopol was kept constant at 1:1 which corresponding to the stiochiometric ratio. A constant amount (0.5 g) of DTZ HCl was dissolved in 5 ml distilled water. Chitosan or Eudragit E separately was dissolved in 25 ml 5% v/v acetic acid solution, while carbopol was dispersed in 100 ml distilled water. After that, 0.4 ml of triethanolamine was added to each dispersion with continuous stirring until homogenous gels were obtained.

The drug solution was slowly added to carbopol gels with constant stirring using magnetic stirrer at 100 rpm for 30 min. until a homogenous dispersion of the drug was obtained. Finally, chitosan or Eud. E solutions were added to drug – carbopol dispersions. The mixtures were further stirred for 30 min and kept aside for 2 hr. The precipitated products were separated by filtration with Buchner funnel and treated as mentioned before. Aliquots from the filtered solutions remaining after separation of IPCs were assayed spectrophotometrically at 236 nm to determine the amount of drug remaining in the supernatant.

### 2.2.5 Study of drug-polymer and polymer-polymer interaction

Infra-red spectra of chitosan, Eud. E and carbopol each alone, physical mixtures of Eud. E or chitosan with carbopol in a ratio of 1:1, the prepared IPCs in addition to drug – carbopol complex, drug loaded IPCs and the corresponding physical mixtures were determined using Fourier transform infrared spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA.). 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. DSC measurements were performed using differential scanning calorimeter (Pyris 6 DSC, Perkin Elmer, USA). Temperature calibration was performed using indium as a standard. Samples were weighed directly in aluminum pans (10 mg) and scanned between 30 and 450℃ at a heating rate of 10℃/min under constant purging of dry nitrogen at 30 ml/min.

### 2.2.6 Drug content of DTZ HCL in the loaded IPCs

Accurately weighed amounts of 200 mg of drug – carbopol complex or drug loaded IPCs of different ratios were dispersed in 100 ml of 0.1 NHCl of pH 1.2 and shaked for 1 hr. Aliquots were withdrawn, suitably diluted, filtered through millipore filter (0.45 µm) and the drug solutions were assayed spectrophotometrically at 236 nm using UV-visible spectrophotometer (JASCO, V-530, Japan).

### 2.2.7 Preparation of DTZ HCL tablets

Tablets weighing 360 mg, each contains 60 mg of DTZ HCl or amount of IPCs equivalent to 60 mg drug were prepared by direct compression using single punch tablet machine (Erweka-Apparatebau, GmbH, Germany). The formulae prepared are shown in table 1.

**Table 1. Composition of Diltiazem Hydrochloride Tablets**

| Formulae | Formulae Code | The quantity of ingredients in each tablet (mg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Drug | Lactose | Eud. E | Chitosan | Carbopol | Drug Carbopol complex | Eud. E-carbopol IPC loaded with drug | Chitosan-carbopol IPC loaded with drug |
| Chitosan | C | 60 | 47.4 | | 249 | | | | |
| Eud. E | E | 60 | 47.4 | 249 | | | | | |
| Drug - carbopol complex | DCC | | 106.4 | | | | 250 | | |
| Chitosan : carbopol IPC 1:3 | CCC3 | | 146.4 | | | | | | 210 |
| Chitosan : carbopol IPC 1:4 | CCC4 | | 96.4 | | | | | | 260 |
| Chitosan : carbopol IPC 1:5 | CCC5 | | 56.4 | | | | | | 300 |
| Chitosan : carbopol mix 1:3 | CCM3 | 60 | 146.4 | | 75 | 75 | | | |
| Chitosan : carbopol mix 1:4 | CCM4 | 60 | 96.4 | | 100 | 100 | | | |
| Chitosan : carbopol mix 1:5 | CCM5 | 60 | 56.4 | | 120 | 120 | | | |
| Eud. E : carbopol IPC 1:3 | ECC3 | | 124.4 | | | | | 232 | |
| Eud. E : carbopol IPC 1:4 | ECC4 | | 96.4 | | | | | 260 | |
| Eud. E : carbopol IPC 1:5 | ECC5 | | 47.4 | | | | | 309 | |
| Eud.E : carbopol mix 1:3 | ECM3 | 60 | 124.4 | 86 | | 100 | | | |
| Eud.E : carbopol mix 1:4 | ECM4 | 60 | 96.4 | 100 | | 124.5 | | | |
| Eud.E : carbopol mix 1:5 | ECM5 | 60 | 47.4 | 124.5 | | | | | |

Drug – carbopol complex or drug loaded IPCs of drug: polymer ratios (1:3, 1:4 and 1:5), their physical mixtures in the same ratios, in addition to the individual polymers were used as tablet matrices. Each of the previously mentioned powders were mixed with lactose as a diluent and magnesium stearate (1% w/w as a lubricant).

### 2.2.8 Evaluation of tablets

The prepared tablets were evaluated for hardness value, thickness, friability percent, disintegration time and drug content uniformity according to USP XXVII, 2004.

### 2.2.9 *In-vitro* drug release studies

The *in-vitro* release studies of DTZ HCl from the prepared tablets were performed according to the USP XXVII, 2004 using six jars dissolution II apparatus (DA-6D, India). The dissolution medium was 0.1 NHCl of pH 1.2 or phosphate buffer of pH 7.4. One tablet was placed in each jar containing 900 ml of dissolution medium which rotated at 100 rpm and maintained at 37 ± 0.5℃. Samples of 2 ml were withdrawn at specified time intervals (15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min), then, the volume was compensated to the original volume by adding fresh dissolution medium after each sampling. The samples were suitably diluted, filtered using millipore filter (0.45 μm) and assayed spectrophotometrically at 236 nm. The results were expressed as the mean of three determinations.

### 2.2.10 Kinetic release study

In order to characterize the drug release behavior from the polymeric systems and to understand the corresponding mechanism, the data of *in-vitro* drug release from the prepared formulae using 0.1 NHCl of pH 1.2 and phosphate buffer of pH 7.4 as dissolution media were analyzed according to Korsmeyer-Peppas semi-empirical model was applied (Korsmeyer et al., 1983):

$$M_t/M_\infty = Kt^n$$

Where,

$M_t/M_\infty$ is the fraction of drug released at time t.

K is a constant incorporating the structural and geometric characteristics of the matrix tablets.

n is the release exponent, indicative of the drug release mechanism, it is the slope of log fraction drug released versus log time.

### 2.2.11 *In-vivo* study

#### *2.2.11.1 Selected DTZ HCL formulae*

The selected DTZ HCl formulae for bioavailability study were; tablets containing chitosan – carbopol IPC 1:5 (CCC5), Eud. E – carbopol IPC 1:5 (ECC5) & Eud. E – carbopol physical mixture 1:5 (ECM5). They were selected on the basis of acceptable physical characteristics and controlled drug release. They were compared with the commercial Altiazem® tablets (60 mg).

#### *2.2.11.2 Study design*

Male albino rabbits weighing 2.0-2.5 Kg were randomly selected for the bioavailability study. The animals were divided into four groups; each group contains six rabbits which received

one of the tested formulae. Twelve hours before drug administration, food was withdrawn from the rabbits until 24 hr 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 hr 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 ℃ until used.

*2.2.11.3 HPLC analysis for DTZ HCL in rabbit's plasma*

The plasma samples were analyzed using HPLC method described by Li et al., 2003 for the determination of DTZ HCl in plasma after modification and validation. Unfortunately, when using this method, there was an interference between plasma peak and drug or internal standard peaks. So, the need to modify this method was applied by changing the mobile phase and the internal standard solution. The mobile phase used was a mixture of acetonitrile : water in ratio of 37:63 v/v containing 0.35% w/v of triethylamine, the pH of the mobile phase was adjusted with orthophosphoric acid to pH 3.0. The internal standard was a solution of 25 µg/ml verapamil hydrochloride prepared in the mobile phase as a solvent.

Then, 100 µl of verapamil hydrochloride solution (25µg/ml) as internal standard were added to each of the following calibration solutions; 50, 100, 200, 400, 600, 1000, 2000 & 3000 ng/ml of DTZ HCl in plasma. After that, 100 µl of sodium carbonate solution in distilled water (1.0 M) was added to each calibration solution and the solutions were mixed well. After mixing, 2.5 ml of hexane–chloroform–isopropanol (60:40:5) was added to the calibration solutions and vortexed for 2 min, then centrifuged for 10 min at 2000 rpm. The organic layer was isolated and evaporated to dryness at 40℃. The residue was then dissolved in 500 µl of the 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 (Perkin Elmer, USA).

The mobile phase used was pumped at a flow rate 0.95 ml/min. The U.V. detector was adjusted at 239 nm. The peak area ratio of DTZ HCl to verapamil hydrochloride was constructed against the concentration of DTZ HCl in plasma to obtain the standard calibration curve.

Accurate and precise determination of the plasma concentrations was necessary to improve the confidence in the obtained results. So, it was necessary to validate the HPLC method used in determination of DTZ HCl in plasma. Three different concentrations of DTZ HCl in plasma; 600, 1000 & 2000 ng/ml were analyzed in triplicates during the same day (intra-day precision) and on three consecutive days (inter-day precision). The percent of the measured concentrations and the relative standard deviation (RSD) values were calculated. The accuracy of an analytical method expresses the closeness between the reference value and calculated value. Accuracy was evaluated as the percent relative error (% RE) between the measured concentrations and the added concentrations (Ermer, 2001 and Shabir, 2003).

The frozen rabbit plasma was thawed at room temperature, then, for each 0.5 ml of plasma sample, 100 µl of verapamil hydrochloride solution (25µg/ml) were added as an internal standard. The procedure was then completed as mentioned before under the construction of standard calibration curve of DTZ HCl in rabbit's plasma. The DTZ HCl concentration in the rabbit plasma samples was calculated using the calibration curve, obtained after linear regression of the peak area ratio (DTZ HCl/verapamil hydrochloride) versus the DTZ HCl concentration.

*2.2.11.4 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 hr ($AUC_{0-24}$) was calculated using trapezoidal rule. All results are represented as means ± SD. The comparison between the pharmacokinetic parameters of DTZ HCl 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.).

## 3. RESULTS AND DISCUSSION

### 3.1 Stoichiometric Ratios between Chitosan-Carbopol or Eudragit E- Carbopol

Fig. (1) shows the supernatant viscosity of chitosan – carbopol and Eud. E – carbopol mixtures. From the figure, it is obvious that, the viscosity of carbopol dispersions reached a minimum value in presence of chitosan or Eudragit E concentrations of 0.1 % (w/v). This result indicates that, the stoichiometry of the interaction of chitosan – carbopol or Eud. E – carbopol mixtures is about 1:1. A further increase in the chitosan or Eud. E concentrations produces an increase in the supernatant viscosity (Rossi et al., 2003).

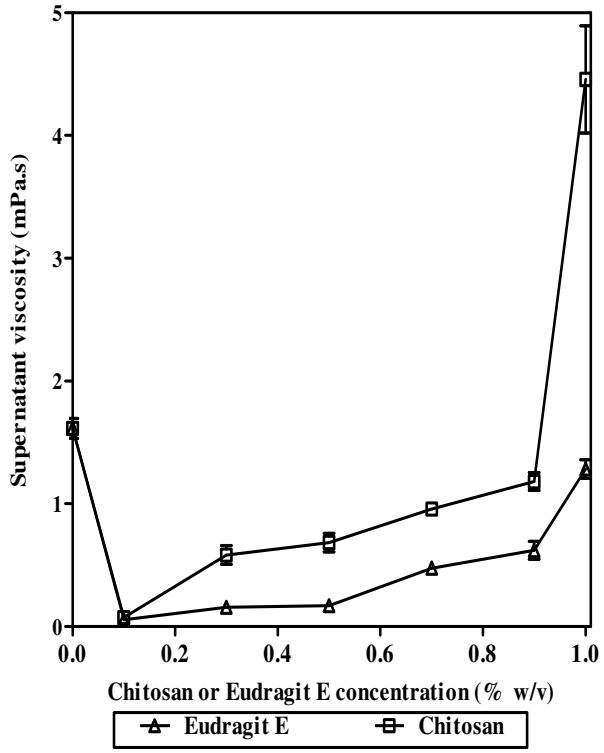


**Fig. 1. Supernatant viscosity of different concentrations of chitosan – carbopol and Eudragit E – carbopol mixtures.**

## 3.2 Study of Drug-Polymer and Polymer-Polymer Interactions

The FT-IR spectra of; chitosan, carbopol, chitosan – carbopol physical mixture and the IPC are shown in Fig. (2). The characteristic bands of chitosan itself were located at 1649 and 1587cm$^{-1}$ which corresponding to carbonyl stretching vibration of the secondary amide group and N–H bending vibration of the amino group, respectively (Sankalia et al., 2007). In addition, the carbonyl stretching vibrational band of carbopol itself appeared at 1717 cm$^{-1}$ (Ahn et al., 2002). The carbonyl absorption band of carbopol in case of chitosan – carbopol physical mixture appeared also at 1717 cm$^{-1}$, while, it was shifted to 1709 cm$^{-1}$ in case of chitosan – carbopol IPC and the two bands of chitosan were shifted to 1640 and 1562 cm$^{-1}$, respectively (Fig. 2). This shift was attributed to the formation of $-NH_3^+$ when the complex was being formed between chitosan and carbopol and this finding agreed with the results of Kao et al. (2006).

Fig. (3) Shows that, the FT-IR spectrum of Eud. E – carbopol IPC had some differences compared to the corresponding physical mixture at a ratio of 1:1. Eudragit E – carbopol physical mixture showed the characteristic bands of carboxylic group of carbopol at 1716 cm$^{-1}$ and dimethylamino group of Eud. E at 2770 and 2824 cm$^{-1}$. While, the IPC spectrum showed a shift in the absorption band assigned to – $COO^-$ to higher wave number which overlapped with the ester band of Eud. E and appeared at 1728 cm$^{-1}$. Furthermore, a broad band was observed at 2650 cm$^{-1}$, which may be assigned to the interaction of the dimethylamino groups of Eud. E with the carboxyl groups of carbopol. In addition, the bands at 2770 and 2824 cm$^{-1}$ that is characteristic to the dimethylamino group of Eud. E were significantly reduced in case of IPC, while they were still present with the same intensity in the physical mixture spectrum.

From Figs. (4 & 5), it is obvious that, the spectrum of untreated DTZ HCl showed two sharp absorption bands at 1679 and 1743 cm$^{-1}$ which corresponding to carbonyl group stretching of the lactam ring and acetate group, respectively, in addition to a strong band at 2393 cm$^{-1}$ that refers to N-H stretching of amine HCl (Mazzo et al., 1994; Mandal et al., 2009).

The FT-IR spectrum of DTZ HCl – chitosan – carbopol or DTZ HCl – Eud. E – carbopol physical mixtures, indicated the presence of the characteristic bands of the drug almost at the same wave numbers especially for the bands observed at 1679 & 2393 cm $^{-1}$, these results confirmed that, no interaction occurred between DTZ HCl and polymers on mixing (Fig. 4 & 5).

However, DTZ HCl – carbopol complex spectrum showed a marked change compared to that of untreated drug, where a complete disappearance of the band at 2393 cm $^{-1}$ was observed which confirmed the interaction between dimethylamino group of the drug and carboxylate group of carbopol. In addition, the bands corresponding to carbonyl groups stretching of the lactam ring and acetate group were shifted to 1723 & 1659 cm$^{-}$1, respectively. The same results were also obtained for DTZ HCl – chitosan – carbopol and DTZ HCl – Eud. E – carbopol IPCs as a result of interaction of the drug with carbopol (Fig. 4 & 5).

Fig. (6) shows the DSC thermograms of chitosan, carbopol, chitosan – carbopol physical mixture and the IPC. The thermogram of chitosan showed an endothermic peak at 80.39℃, in addition to an exothermic one at 310℃, referred to the decomposition of chitosan (Neto et al., 2005). The DSC thermogram of carbopol showed two endothermic peaks at 71.78 & 241 ℃ and an exothermic one at 300℃ at which the decomposition of carbopol occurred. From

the figure, it was evident that, the thermogram of the IPC was different from those of individual polymers or the physical mixture. The thermogram of the physical mixture appeared to be a superimposition of the two polymers peaks, while, the thermogram of IPC showed two endothermic peaks at 71 & 225°C with complete disappearance of chitosan exothermic peak at 310°C. This phenomenon indicated that, IPC between chitosan and carbopol has formed. Similar thermal behavior was observed in case of chitosan and carragenan by Piyakulawat et al., 2007.

The DSC thermogram of Eud. E – carbopol physical mixture showed two endothermic peaks at 64.7 & 232.44°C and an exothermic one at 340°C, while, the IPC thermogram showed two endothermic peaks; one at 71.7°C and the other one was splitted into two peaks at 224.84 and 260°C, respectively, and did not show the exothermic peak at 340°C which found in the physical mixture as shown in Fig. (7). These results confirmed the IPC formation between the two polymers.

The thermogram of DTZ HCl showed a single sharp endothermic peak at 215°C (Fig. 8 & 9). This sharp peak corresponding to the melting point of the drug and another broad one at 265.65°C which corresponding to its decomposition (Mazzo et al.,1994). The DSC thermogram of DTZ HCl – chitosan – carbopol and DTZ HCl – Eud. E – carbopol physical mixtures showed the characteristic endothermal peaks of the drug, while, the thermogram of DTZ HCl – carbopol complex and DTZ HCl - IPCs showed broad endothermic peaks only, with complete disappearance of the drug melting peak as a result of drug – carbopol interaction (Fig. 8 & 9). These results agree with that of Sultana et al., 2009, who found that, the endothermic peak of DTZ HCl in alginate microspheres was not distinctive indicating that, the drug was no longer present in the crystalline form.

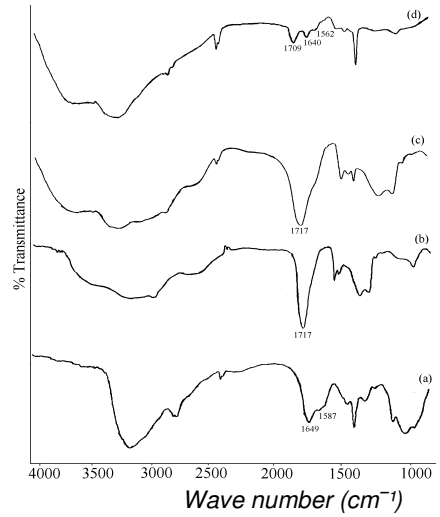


**Fig. 2. FT-IR spectra of chitosan (a), carbopol (b), their physical mixture of ratio 1:1 (c) and the corresponding IPC (d)**

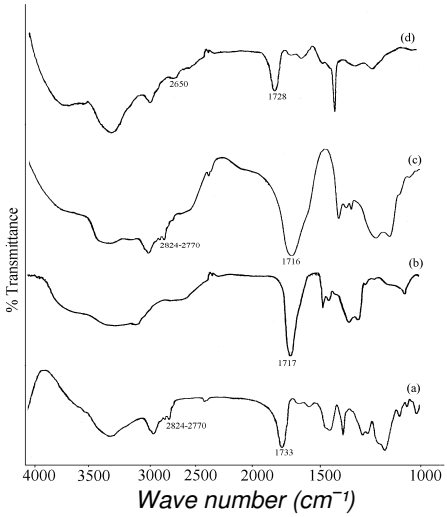


**Fig. 3. FT-IR spectra of Eud. E (a), carbopol (b), their physical mixture of ratio 1:1 (c) and the corresponding IPC (d)**

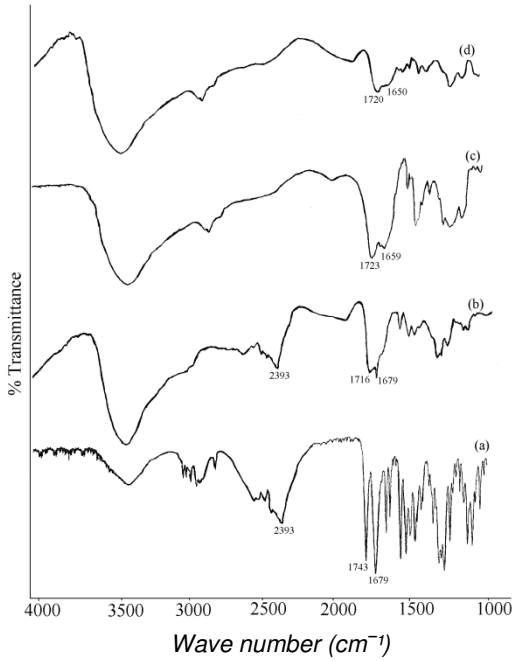


**Fig. 4. FT-IR spectra of; DTZ HCl (a), DTZ HCl - chitosan - carbopol - physical mixture (b), DTZ HCl - carbopol complex (c) and DTZ HCl - chitosan - carbopol IPC(d)**

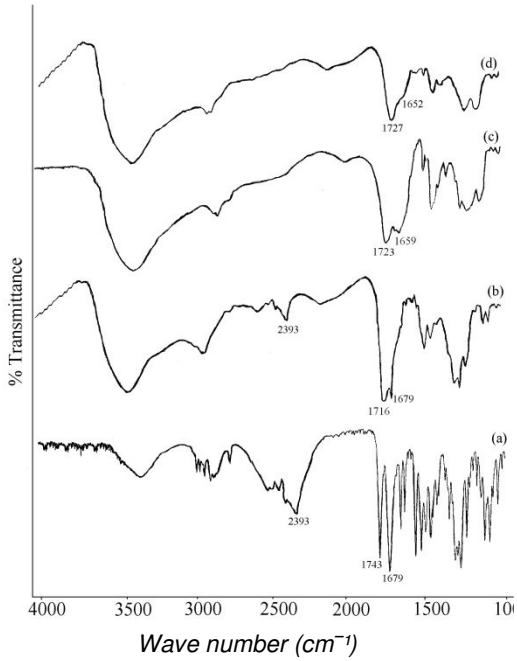


**Fig. 5. FT-IR spectra of; DTZ HCl (a), DTZ HCl – Eud. E - carbopol - physical mixture (b), DTZ HCl - carbopol complex (c) and DTZ HCl - Eud. E - carbopol IPC (d)**

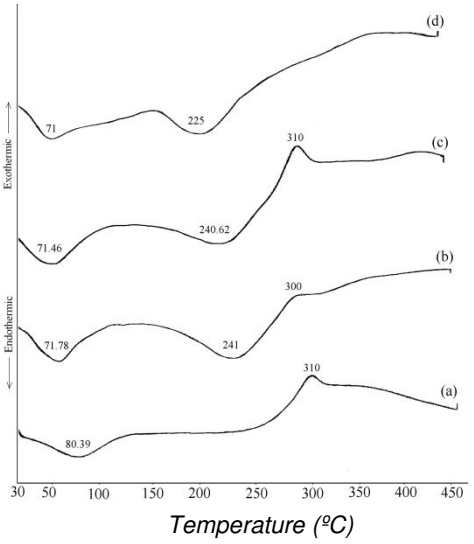


**Fig. 6. DSC thermograms of chitosan (a), carbopol (b), their physical mixture of ratio 1:1 (c) and the corresponding IPC (d)**

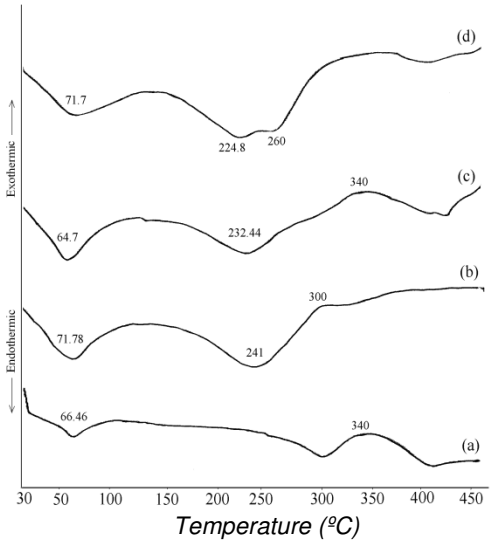


**Fig. 7. DSC thermograms of Eud. E (a), carbopol (b), their physical mixture of ratio 1:1 (c) and the corresponding IPC (d)**

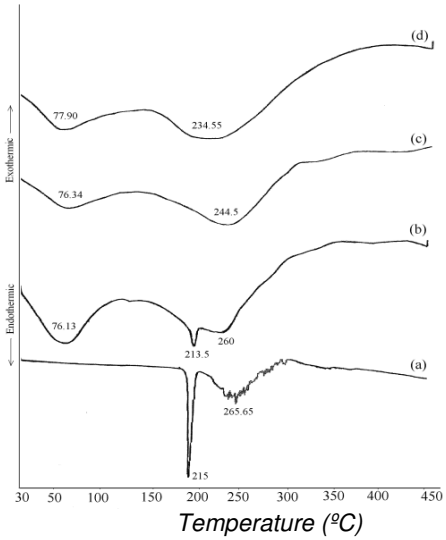


**Fig. 8. DSC thermograms of; DTZ HCl (a), DTZ HCl - chitosan - carbopol - physical mixture (b), DTZ HCl - carbopol complex (c) and DTZ HCl - chitosan - carbopol IPC (d)**

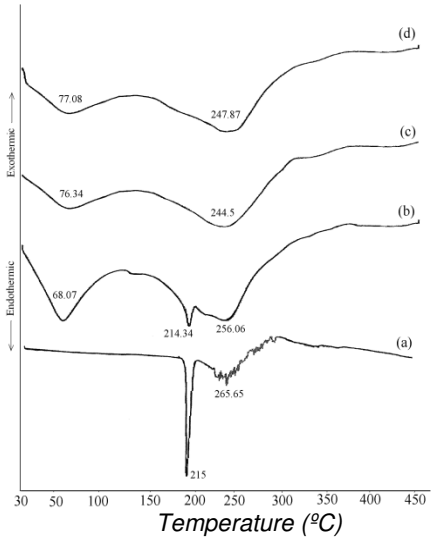


**Fig. 9. DSC thermograms of; DTZ HCl (a), DTZ HCl – Eud. E - carbopol - physical mixture (b), DTZ HCl - carbopol complex (c) and DTZ HCl - Eud. E - carbopol IPC (d)**

## 3.3 Preparation of DTZ HCL Loaded IPCs

During the preparation of DTZ HCl loaded IPCs, the addition of triethanolamine to carbopol dispersions resulted in increasing ionization of the carboxyl groups in carbopol molecules as $pK_a$ of acrylic acid is 4.25 (Maryadele, 2006), so being negatively charged and repulsion occurred between them causing expansion of molecules, resulting in gel formation (Varma et al., 2004).

When DTZ HCl solutions in water were added to carbopol gels, the transparent carbopol gels were transformed to white viscous dispersions due to the interaction between the carboxyl groups of carbopol and the dimethyl amino group of DTZ HCl. These interactions may lead to the formation of drug - carbopol complex and decreased the solubility of the drug (Kojima et al., 2008). Then, further addition of chitosan or Eud. E solution, resulting in the separation of chitosan – carbopol or Eud. E – carbopol IPCs loaded with DTZ HCl.

The percent drug remaining in the supernatant after separation of IPCs and the actual drug content of the dried IPCs loaded with the drug are shown in table 2. From the obtained data, it is clear that, the percent drug remaining in the supernatant after separation of IPCs decreased from 66.73 to 38.91% and from 69.71 to 41.01% by increasing drug: polymer ratios from 1:1 to 1:6 in case of chitosan - carbopol and Eud. E – carbopol IPCs, respectively. This may be attributed to the greater availability of active binding sites in the polymeric chains and consequently the greater degree of interaction as the quantity of carbopol increased, resulting in decreasing the free drug in the supernatant and hence, the drug loading efficiency greatly improved. On the other hand, it could be observed from table

2 that, the percent actual drug content of drug loaded IPCs decreased by increasing the drug: polymer ratios from 1:1 to 1:6 due to increasing ratio of polymers.

**Table 2. Percent drug remaining in the supernatant after separation of IPCs and the actual drug content of the dried IPCs loaded with DTZ HCl**

| Drug: poymer ratios | % Drug remaining in the supernatant after separation of IPC | | % Actual drug content of the dried IPC loaded with drug | |
|---|---|---|---|---|
| | Chitosan-carbopol IPC | Eudragit E-carbopol IPC | Chitosan-carbopol IPC | Eudragit E-carbopol IPC |
| 1:1 | 66.73±1.23 | 69.71±4.23 | 41.66±1.34 | 40.10±2.43 |
| 1:2 | 47.90±0.56 | 52.50±2.43 | 30.72±0.98 | 38.24±1.23 |
| 1:3 | 44.50±2.34 | 42.64±1.04 | 25.86±2.34 | 28.57±3.45 |
| 1:4 | 44.02±0.98 | 41.90±2.24 | 23.07±0.45 | 23.07±2.05 |
| 1:5 | 38.38±2.34 | 40.64±1.04 | 19.41±0.94 | 20.00±1.44 |
| 1:6 | 38.91±1.09 | 40.01±2.45 | 19.60±1.07 | 20.65±1.38 |

## 3.4 Physical Properties of DTZ HCL Tablets

The physical properties of DTZ HCl tablets for different formulae are shown in table 3. The tablet formulae (except Eud. E tablets) showed acceptable physical properties. The drug content of the prepared tablets was within the requirements of USP XXVII, 2004.

## 3.5 *In–Vitro* Drug Release

The release of DTZ HCl from the prepared tablets was studied in 0.1 NHCl of pH 1.2 and phosphate buffer of pH 7.4. The tested polymers did not interfere with the analysis of DTZ HCl 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. The release profiles of the prepared DTZ HCl tablets were compared with the commercial tablets (Altiazem®, 60 mg).

The release profiles of DTZ HCl from chitosan, DTZ HCl – carbopol complex, chitosan – carbopol IPCs and the corresponding physical mixtures tablets in 0.1 NHCl of pH 1.2 are shown in Fig. 10. Approximately 100% of the drug was released from chitosan tablets within 45 min. The high value of drug release from chitosan tablets coincide with the dissolution behavior of chitosan at pH 1.2 and may be attributed to the high swelling degree of these hydrogels caused by the electrostatic repulsion within the network (Torrado et al., 2004). However, the release rate of the drug from chitosan – carbopol IPCs and physical mixtures tablets was slower compared to chitosan tablets or Altiazem® tablets (100% of the drug was released within 4 hr from Altiazem tablets). From Fig. (10), it is clear that, the percent drug released from DTZ HCl – carbopol complex tablets increased by time till reaching 100% after 6 hr. However, the percent drug released after 6 hr in 0.1 NHCl from chitosan – carbopol IPCs tablets; CCC3, CCC4 and CCC5 were 95, 87.56 and 77.50 %, respectively and this percent significantly decreased ($P < 0.001$) by increasing the drug: IPC ratio from 1:3 to 1:5. These results showed that, drug – carbopol complex can be reversibly dissociated upon immersion in 0.1 NHCl.

**Table 3. Physical properties of diltiazem hydrochloride tablets**

| Formulae code | Drug content (%) | Tablet Thickness (mm) | Friability % | Hardness (Kg) | Disintegration Time (min.) |
|---|---|---|---|---|---|
| E | 95.08 ± 1.33 | 4.28 ± 0.037 | 1.24 | 4.96 ± 0.45 | 30.23 ± 0.25 |
| C | 98.56 ± 0.86 | 3.81 ± 0.067 | 0.55 | 8.04 ± 0.25 | 23.56 ± 0.14 |
| DCC | 100.23 ± 1.34 | 4.27 ± 0.05 | 0.23 | 8.25 ± 0.67 | 238.24 ± 4.2 |
| CCC3 | 98.04 ± 1.06 | 3.59 ± 0.23 | 0.91 | 6.25 ± 0.75 | 75.34 ± 0.96 |
| CCC4 | 100.87 ± 1.31 | 3.88 ± 0.04 | 0.84 | 6.75 ± 0.05 | 135.24 ± 2.3 |
| CCC5 | 101.2 ± 3.89 | 3.90 ± 0.19 | 0.75 | 7.95 ± 0.68 | > 360 |
| CCM3 | 95.99 ± 3.96 | 4.88 ± 0.05 | 0.56 | 8.76 ± 0.97 | > 360 |
| CCM4 | 97.04 ± 2.33 | 5.05 ± 0.04 | 0.31 | 8.95 ± 0.16 | > 360 |
| CCM5 | 96.66 ± 1.83 | 5.09 ± 0.02 | 0.25 | 8.95 ± 0.34 | > 360 |
| ECC3 | 98.60 ± 0.60 | 3.88 ± 0.08 | 0.72 | 6.90 ± 0.13 | 120 ± 1.2 |
| ECC4 | 96.59 ± 3.93 | 4.16 ± 0.06 | 0.65 | 6.95 ± 0.54 | > 360 |
| ECC5 | 98.76 ± 3.32 | 4.19 ± 0.04 | 0.54 | 7.23 ± 0.05 | > 360 |
| ECM3 | 100.15 ± 1.73 | 4.57 ± 0.34 | 0.60 | 8.23 ± 0.34 | 180 ± 4.5 |
| ECM4 | 96.82 ± 2.78 | 4.72 ± 0.27 | 0.45 | 8.50 ± 0.54 | > 360 |
| ECM5 | 96.42 ± 1.93 | 4.95 ± 0.39 | 0.25 | 8.50 ± 0.43 | > 360 |

To prove this result, the following experiment was carried out. At first, DTZ HCl solution (5 mg/ml, Fig. 11-a) and carbopol dispersion (5 mg/ml, Fig. 11-b) were prepared in distilled water. When they were mixed, a white coarse precipitate was formed (Fig. 11-c). This precipitate disappeared after the addition of 5 ml of 0.1 NHCl and the system appeared as a suspension (Fig. 11-d). This suspension may be caused by dissociation of DTZ HCl – carbopol complex in acidic medium (Kojima et al., 2008). As a control, carbopol dispersion (5 mg/ml) in water without DTZ HCl was prepared, followed by the addition of 0.1 NHCl, which resulted in the similar state as suspension (Fig. 11-e).

On the other hand, at pH 1.2, the majority of the amine groups in the chitosan of the IPC are in the ionized form, while, most of the carboxylic groups in the carbopol molecules are not, leading to a weak interaction between the two polymers in the IPC. As a result, dissociation of ionic interaction occurred, so, it is easier for a dissolution medium to penetrate the polymer network and hence, the dissolution degree increases at pH 1.2 (Ahn et al., 2001; Cho and Choi, 2005).

However, the drug release from tablets containing chitosan – carbopol physical mixtures in 0.1 NHCl of pH 1.2 showed a slower release compared to that containing IPCs. These results may be due to the interaction between chitosan and carbopol in the tablet matrix during the dissolution process. Similar results were obtained by Tapia et al. 2005, they prepared DTZ - HCl tablets using chitosan-carragennan physical mixture and reported that, the electrostatic attraction between the cationic amino groups of chitosan and the anionic sulfonate groups of carrageenan is the main type of interaction leading to the formation of IPC within the tablet during the dissolution in 0.1 NHCl resulting in retarding the drug release.

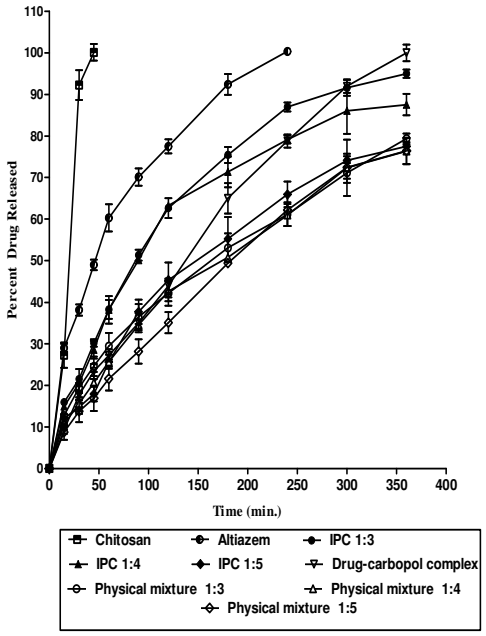


**Fig. 10. *In-vitro* release profiles of DTZ HCl from chitosan - carbopol tablets in 0.1 NHCl of pH 1.2**

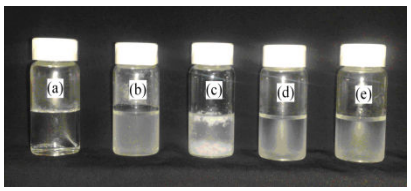


**Fig. 11. Photographs of complex formation between DTZ HCl and carbopol in distilled water**

(a) DTZ HCl solution in distilled water.
(b) Carbopol dispersion indistilled water.
(c) Mixture (1/1, v/v), of (a) and (b).
(d) Addition of 0.1 NHCl to (c).
(e) Carbopol dispersion after addition of 0.1 NHCl.

The release profiles of DTZ HCl from chitosan, Altiazem, DTZ HCl – carbopol complex, chitosan – carbopol IPCs and the corresponding physical mixtures tablets in phosphate buffer of pH 7.4 are shown in Fig. 12. Hundred percent of the drug was released within 1 hr from chitosan tablets. The rapid release of the drug from chitosan tablets may be due to their disintegration. However, Altiazem and DTZ HCl – carbopol tablets showed slower drug release compared to chitosan tablets, where, 79.10 & 72.8 % of the drug were released after 6 hr, respectively.

Moreover, it is obvious from Fig. (12) that, the release rate of the DTZ HCl from chitosan – carbopol IPCs tablets in phosphate buffer was slow compared to Altiazem tablets, where 45.46, 38.66 and 35.39 % of the loaded drug were released after 6 hr from CCC3, CCC4 and CCC5 tablets, respectively. This decrease in drug release may be due to swelling and gelling properties of these tablets compared with Altiazem tablets.

From the obtained data, it was also observed that, the percent drug released in phosphate buffer of pH 7.4 was slow compared to that in 0.1 NHCl which revealed that, the interaction between drug – carbopol and chitosan – carbopol in the IPC was maintained at that pH. Similar results were obtained by Park et al. 2008, they found that, the release of theophylline from chitosan – carbopol IPC tablets was slow in phosphate buffer compared to 0.1 NHCl. So, the extent of interaction between chitosan and carbopol in the IPCs depends on the pH of the dissolution media and hence, causing difference in the dissolution profiles (Shojaei and Li, 1997).

The percent drug released after 6 hr in phosphate buffer of pH 7.4 from chitosan – carbopol physical mixtures tablets; CCM3, CCM4 and CCM5 were 49.53, 48.96 and 48.65, respectively. Furthermore, there was no significant difference ($P>0.05$) between these

tablets. This slow release may show that, an interaction has occurred between carbopol and DTZ HCl within tablet matrix during dissolution process at pH 7.4. Similar results were obtained by Sedláková et al. 2006, who reported that, after immersion of DTZ HCl - carbopol tablets in phosphate buffer of pH 7.4, carboxylic groups of carbopol ionize and hydrate markedly, so, interaction with DTZ HCl occurred. As a result, a sparingly soluble drug-polymer complex was developed, which decreased the release of the drug as well as decreased swelling of matrices in phosphate buffer of pH 7.4 as shown in Fig. (13).

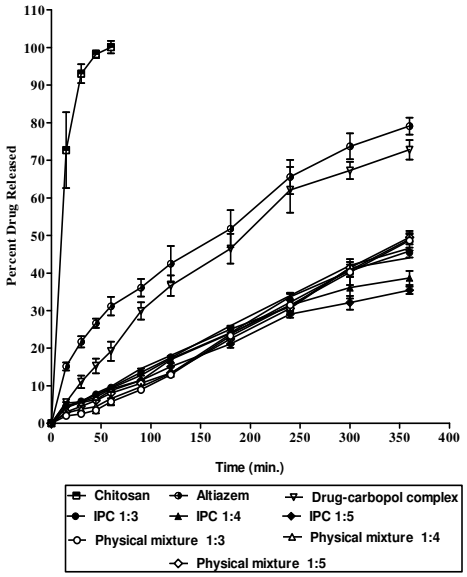


**Fig. 12. *In-vitro* release profiles of DTZ HCl from chitosan - carbopol tablets in phosphate buffer of pH 7.4**

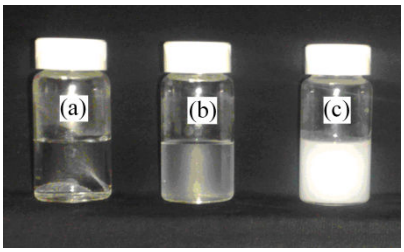


**Fig. 13. Photographs of complex formation between DTZ HCl and carbopol in phosphate buffer of pH 7.4**

(a) DTZ HCl solution in phosphate buffer.
(b) Carbopol gel in phosphate buffer.
(c) Mixture (1/1, v/v), of (a) and (b).

On the other hand, carbopol is pH-sensitive and the pH of the tablet matrix might affect the drug release rate (Bommareddy et al., 2006; Badawy and Hussain, 2007). Initially, as the release medium enters the surfaces of the tablets they become hydrated and the hydrated carbopol begin to swell forming gel as carboxylic acid groups have been deprotonated and the negative charges of the carboxylates thus formed. Then, the pH of tablet matrix started to decrease immediately due to libration of protons from hydrated carbopol molecules (Paker-Leggs and Neau, 2008). So, when the pH of chitosan - carbopol matrix was measured in phosphate buffer of pH 7.4, it was found that, it decreased from 4.6 to 4.18 by increasing the physical mixture ratio from 1:3 to 1:5. In this pH range, an interaction can occur between chitosan and carbopol during the dissolution experiment forming IPC as it is previously reported that, IPC could be formed by the electrostatic interaction between the $COO^-$ group of carbopol and $NH_3^+$ group of chitosan in the pH range 3-6 (Chavasit and Torres, 1990). This interaction may be also responsible for the slow drug release.

The comparison of the release profiles of DTZ HCl from tablets containing Eud. E – carbopol IPCs and the corresponding physical mixtures tablets in 0.1 NHCl of pH 1.2 are shown in Fig. (14). From the figure, it is clear that, the drug release from Eud. E – carbopol IPCs or physical mixtures tablets was decreased compared to Eud. E or Altiazem tablets. The percent drug released after 6 hr from ECC3, ECC4 and ECC5 were 100, 72.32 and 60.45,

respectively. These results were correlated with the disintegration of tablets, where, ECC3 tablets were disintegrated within 120 min., while, no disintegration was observed for ECC4 & ECC5 tablets during the dissolution experiment. The drug release from Eud. E – carbopol IPCs tablets in 0.1 NHCl was due to dissociation of drug – carbopol complex as in case of chitosan - carbopol IPCs tablets.

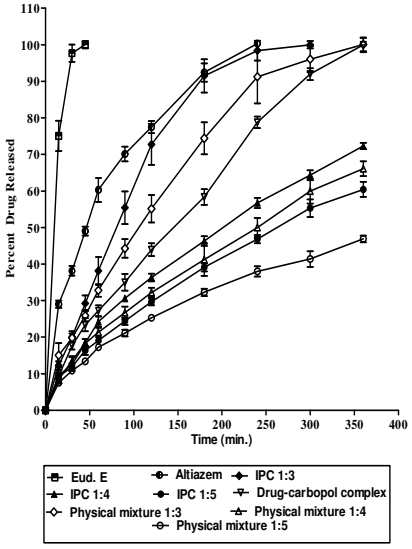


**Fig. 14. *In-vitro* release profiles of DTZ HCl from Eud. E - carbopol tablets in 0.1 NHCl of pH 1.2**

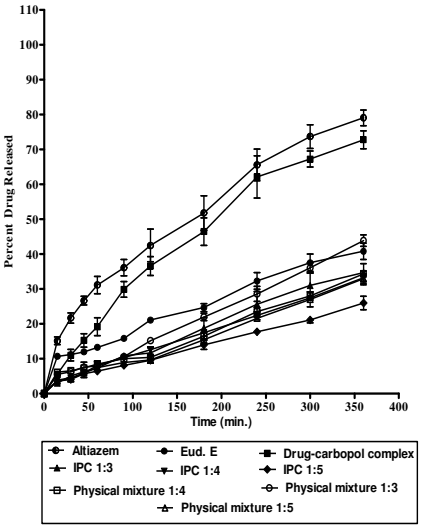


**Fig. 15. *In-vitro* release profiles of DTZ HCl from Eud. E - carbopol tablets in phosphate buffer of pH 7.4**

Regarding the drug release from Eud. E – carbopol physical mixtures tablets in 0.1 NHCl of pH 1.2, it was found that, by increasing the ratio of the physical mixture in the tablets, a decrease in the drug release rate was observed, where, 100.09, 66.09 and 46.90 % were released after 6 hr from ECM3, ECM4 and ECM5 tablets, respectively. These findings may prove that, an interpolymer cross-linking network was achieved between Eud. E and carbopol after penetration of dissolution medium into the tablets (Takayama et al., 1990).

On the other hand, it could be observed from Fig. (14) that, tablets containing Eud. E – carbopol IPCs and their physical mixtures of drug : polymer ratio of 1:4 & 1:5 were effective in decreasing the drug release rate compared to that containing drug – carbopol complex, meanwhile, they showed more retardant effect than the corresponding formulae of chitosan – carbopol.

This may be due to, tablets containing Eud. E – carbopol IPCs and physical mixtures of ratios; 1:4 & 1:5 absorbed less solvent in their matrix during dissolution and therefore, the tablets were able to maintain their integrity during the dissolution process than the corresponding chitosan – carbopol physical tablets.

The release profiles of DTZ HCl from tablets containing Eud. E – carbopol IPCs and the corresponding physical mixtures in phosphate buffer of pH 7.4 are shown in Fig. (15). From the figure, it is obvious that, tablets containing Eud. E – carbopol IPCs or their physical mixtures followed the same behaviour as chitosan – carbopol tablets, where, they

significantly lowered (P<0.001) the drug release compared to drug – carbopol complex or Altiazem tablets. These results may show that, an interaction occurred between drug – carbopol and Eud. E – carbopol after penetration of dissolution medium into the tablets. Kabanova et al. 2006, stated that, after immersion of tablets containing Eud. E – carbomer 940 physical mixture into phosphate buffer, there was a decrease in swellability due to the complexation between the polymers.

## 3.6 Kinetic Release Study

The drug release mechanism from swellable hydrogel matrices is complex. Although some processes may be classified as either diffusion or erosion controlled, many others can be governed by both. The analysis of experimental data according to Korsmeyer–Peppas and the interpretation of the corresponding release exponent values (n); leads to a better understanding of balance between these mechanisms. Korsmeyer–Peppas semi-empirical model is not applied to chitosan and Eud. E tablets due to their rapid release in 0.1 NHCl. Therefore, the correlation coefficients and the diffusional exponents for these formulae could not be calculated as a result of insufficient data points, up to 60%, on the drug release profiles to provide accurate values.

The n values for the release of DTZ HCl from tablets containing chitosan – carbopol and Eud. E – carbopol IPCs and physical mixtures in 0.1 NHCl of pH 1.2 according to different kinetic models are shown in table (4). However, other formulae showed n values ranged between 0.570 for CCM3 and 0.896 for CCC3 (i.e. 0.5< n<1) indicating that, the release mechanism of DTZ HCl from these matrices is an anomalous (non-Fickian) release, which suggests that, both diffusion of the drug in the hydrated matrix and its own erosion modulate drug release. Similar results were obtained by Ray et al. 2010, in the release of DTZ HCl from polyvinyl alcohol and polyacrylic acid microspheres.

From the obtained data in table (4), it could be observed that, drug release in phosphate buffer of pH 7.4. Korsmeyer-Peppas semi-empirical model is not applied to chitosan tablets due to their rapid release in phosphate buffer of pH 7.4, while other formulae have n values ranged from 0.566 for ECM4 to 0.985 for CCM4 (i.e. 0.5 < n < 1) indicating that, the release mechanism of DTZ HCl from these matrices is an anomalous (non-Fickian) release. So, these tablets delivered their drug content by coupled diffusion and erosion. However, Eud. E tablet formula (E) in phosphate buffer is the only one that has n value < 0.5 (i.e. 0.416) which indicates Fickian diffusion (diffusion mediated release). On the other hand, chitosan – carbopol physical mixture 1:5 tablets (CCM5) has n value equal 1 which suggests that, it follows zero order model which indicates matrix relaxation or erosion controlled release (Peppas, 1985; Siepman & Peppas, 2001).

## 3.7 *In-Vivo* Study

The chromatogram of rabbit plasma containing DTZ HCl and verapamil hydrochloride as an internal standard showed good separation and detectability of both drugs in rabbit plasma with no interference from plasma components. The retention times of DTZ HCl and verapamil hydrochloride were 7.2 and 10.3 min., respectively. Furthermore, good linearity was obtained for peak area ratio of DTZ HCl to verapamil hydrochloride and DTZ HCl concentration in range of 50-3000 ng/ml with a good correlation coefficient ($r^2$) = 0.9910. The mean equation obtained for the calibration curve was; y = 0.00042 x + 0.067, where y

represents the peak area ratio of DTZ HCl to verapamil hydrochloride and x represents the concentration of DTZ HCl in plasma (ng/ml).

The % RSD for the intra-day and inter-day precision was ranged from 1.54% to 2.95% and from 1.82-2.56%, respectively. The %RE, which is a measure of the accuracy was ranged from -0.4 to 4.24% for intra-day assay and from -3.43 to 3.65 % for inter-day assay. These results were within the acceptable values of ±5% which indicate good precision and accuracy of the assay (Bilal, 2010).

The mean plasma concentration as a function of time for DTZ HCl after oral administration of commercial tablets (Altiazem®), those containing chitosan – carbopol IPC 1:5 (CCC5), Eud. E – carbopol IPC 1:5 (ECC5) and Eud. E – carbopol physical mixture 1:5 (ECM5) are illustrated in Fig. (16). From the mentioned data, it could be observed that, there was a difference between the mean plasma concentrations as a function of time for DTZ HCl after oral administration of the three tested formulae at all time intervals compared to the commercial tablets. Also, there is a noticeable difference in the $C_{max}$ and $T_{max}$ between the commercial tablets and the tested formulae.

**Table 4. Linear correlation coefficient, $r^2$, and n values for the release of DTZ HCl from tablets containing chitosan – carbopol and Eud. E – carbopol IPCs and physical mixtures according to Korsmeyer–Peppas semi-empirical model**

| Formulae code | 0.1 N HCl of pH 1.2 | | Phosphate buffer of pH 7.4 | |
|---|---|---|---|---|
| | n value | Correlation coefficient, $r^2$ | n value | Correlation coefficient, $r^2$ |
| E | na | 0.983 | 0.416 | 0.966 |
| C | na | 0.929 | na | 0.969 |
| DCC | 0.740 | 0.990 | 0.814 | 0.993 |
| CCC3 | 0.896 | 0.979 | 0.772 | 0.971 |
| CCC4 | 0.888 | 0.965 | 0.765 | 0.977 |
| CCC5 | 0.868 | 0.967 | 0.719 | 0.976 |
| CCM3 | 0.570 | 0.998 | 0.906 | 0.937 |
| CCM4 | 0.640 | 0.996 | 0.985 | 0.947 |
| CCM5 | 0.688 | 0.988 | 1 | 0.948 |
| ECC3 | 0.886 | 0.966 | 0.856 | 0.961 |
| ECC4 | 0.745 | 0.998 | 0.714 | 0.968 |
| ECC5 | 0.599 | 0.997 | 0.647 | 0.961 |
| ECM3 | 0.852 | 0.986 | 0.624 | 0.942 |
| ECM4 | 0.655 | 0.990 | 0.566 | 0.914 |
| ECM5 | 0.619 | 0.993 | 0.576 | 0.904 |

*na, not applicable*

The mean pharmacokinetic parameters of DTZ HCl from different formulae represented by the value of $C_{max}$ (ng/ml), $T_{max}$ (hr) and AUC 0-24 (ng.hr.ml-1) are summarized in table (5). From the obtained results, it was evident that, the absorption of DTZ HCl from the sustained action commercial tablets was rapid, where the peak plasma concentration was 1631 ± 92.24 ng/ml that reached within 2.66 ± 0.34 hr, whereas, following oral administration of CCC5 tablets, the $C_{max}$ was; 1180 ± 132.7 ng/ml which lowered compared with Altiazem tablets and the mean $T_{max}$ was 3.66 ± 0.816 hr. These results were correlated with the in-vitro drug release results and may be attributed to the swelling properties and ionic interactions between chitosan and carbopol within CCC5 tablets (Torrado et al., 2004).

In case of tablets prepared using Eud. E – carbopol IPC 1:5 (ECC5) and Eud. E – carbopol physical mixture 1:5 (ECM5), the $T_{max}$ was; 5.33 ± 1.03 and 5.66 ± 0.716 hr, respectively. The mean $C_{max}$ was; 847.3 ± 29.09 and 700.7 ± 97.58 ng/ml, after administration of ECC5 & ECM5 tablets respectively. These values indicated the prolonged release effect of ECC5 & ECM5 tablets in comparison to Altiazem® and CCC5 tablets. This prolonged effect agreed with the *in-vitro* drug release results and may be attributed to the swelling capability of these tablets and hence, extended the gastric residence time (De la Torre et al., 2005).

Similar results were obtained by AL-Saidan et al., 2005, who studied the *in-vivo* evaluation of guar gum matrix tablets of DTZ HCl in human volunteers. They found that, a delayed $T_{max}$ and lower $C_{max}$ were obtained from these tablets compared to sustained release commercial tablets indicating a slow and prolonged release of DTZ HCl from tested tablets.

Moreover, EL-Kamel et al., 2003, studied the *in-vivo* performance of DTZ HCl – alginate – methylcellulose beads compared to commercial tablets (Dilzem® SR, 90 mg) in beagle dogs. They found that, the absorption from commercial tablets was faster, whereas, following oral administration of alginate - methylcellulose beads, the mean $T_{max}$ was significantly increased and the $C_{max}$ was lowered compared to the commercial tablets.

The statistical analysis of the pharmacokinetic parameters of DTZ HCl from different formulae showed that, there was a significant difference between CCC5 and the commercial tablets in the $C_{max}$ value, while, their $T_{max}$ value were not significantly different ($P>0.05$).

However, there was a significant difference between the commercial tablets and each of ECC5 & ECM5 in the $C_{max}$ and $T_{max}$ values ($P<0.001$) which indicated the prolonged effect of ECC5 & ECM5 compared to Altiazem tablets.

**Table 5. Mean pharmacokinetic parameters of DTZ HCl from different formulae**

| Parameter | Altiazem tablets | CCC5 | ECC5 | ECM5 |
|---|---|---|---|---|
| **$C_{max}$ (ng/ml)** | 1631± 92.24 | 1180[a]± 132.7 | 847.3[a] ± 29.09 | 700.7[a] ± 97.58 |
| **$T_{max}$ (hrs)** | 2.66 ± 0.34 | 3.66[b]± 0.816 | 5.33[a]± 1.03 | 5.66[a]± 0.716 |
| **$AUC_{0-24}$ ($ng.hr.ml^{-1}$)** | 8545 ± 502.9 | 9744[b]± 841.5 | 9924[b]± 368.8 | 8782[b]± 707.8 |

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

The comparison of the pharmacokinetic parameters of DTZ HCl from tablets ECC5 and CCC5 tablets showed significant difference in their $C_{max}$ and $T_{max}$ values ($P<0.05$) which indicated the sustained effect of ECC5 compared to CCC5. However, no significant difference was found between ECC5 & ECM5 in the $C_{max}$ and $T_{max}$ values ($P>0.05$). Also, no significant difference was found between the tested formulae and the commercial tablets with respect to $AUC_{0-24}$. This means that, the tested formulae and Altiazem tablets have similar extent of absorption.

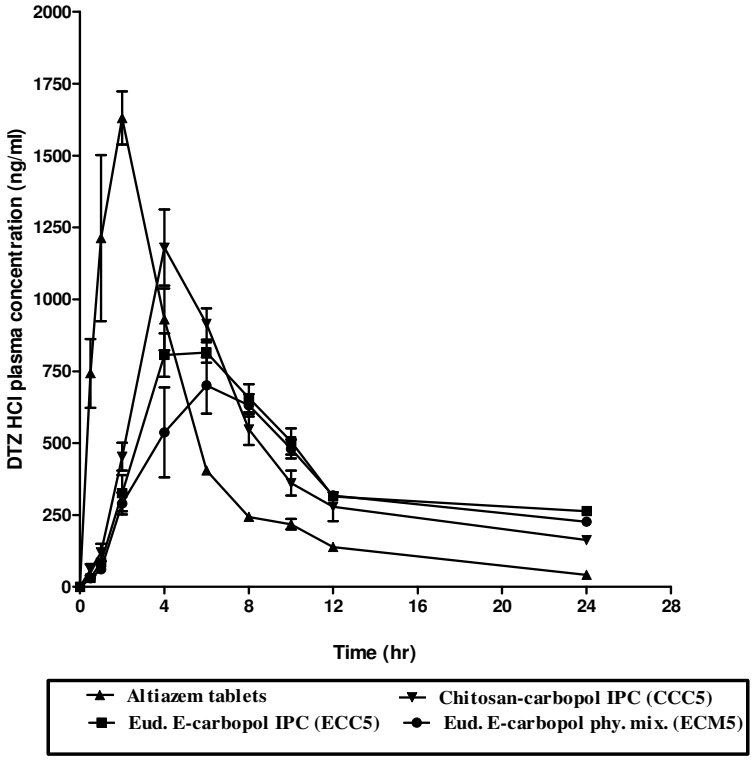


**Fig. 16. Mean plasma concentrations of DTZ HCl after oral administration of Altiazem, chitosan – carbopol IPC, Eudragit E – carbopol IPC and Eudragit E – carbopol physical mixture tablets in rabbits**

From the previous results, it is obvious that, ECC5 & ECM5 formulae were superior to Altiazem® tablets in providing oral controlled release of DTZ HCl as indicated by reducing peak plasma concentration ($C_{max}$) and prolonging the time required to reach maximum plasma concentration ($T_{max}$).

## 4. CONCLUSION

The results of the present study confirmed the formation of an IPC between each of chitosan or Eudragit E and carbopol, furthermore, complexation occurred between DTZ HCl and carbopol during the preparation of drug loaded IPCs. The *in-vitro* drug release of DTZ HCl tablets in 0.1 NHCl of pH 1.2 was higher than in phosphate buffer of pH 7.4. The Eudragit E - carbopol system is better than the chitosan - carbopol system as a prolonged drug release matrix system. Moreover, changing the mode of inclusion of the polymers either IPC or physical mixture allows us to obtain different dissolution rates. Accordingly, tablets prepared using physical mixtures showed slower drug release rate compared to that containing IPCs. The *in-vivo* study of tablets containing Eudragit E – carbopol IPC and the corresponding physical mixture showed a lower peak plasma concentration and marked prolonged release effect compared with the commercial Altiazem® tablets.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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