Investigation of Drug Polymer Interaction: Evaluation and Characterization of Diclofenac-Chitosan Co-precipitate

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ABSTRACT

Investigating the interaction between Diclofenac and Chitosan and its effect on the dissolution in water and in Phosphate buffer system was the objective of this study.

Complex formation was attempted between diclofenac and chitosan utilizing optimal condition for their simultaneous ionization in solution form. A co-precipitate was obtained upon mixing the drug and polymer solutions and the co-precipitate was characterized for drug content, differential scanning calorimetry (DSC), X-ray diffraction and infrared spectroscopy (FTIR) analysis in comparison to diclofenac sodium, diclofenac free acid and their physical mixtures with chitosan. The overall evidence of these comparisons indicated that a physical co-precipitation of chitosan and diclofenac acid was formed with no electrostatic complexation. Having diclofenac acid as a reference for comparison was the key for this conclusion, and comparing the results of the precipitate to only those of diclofenac sodium would have led to false conclusions. The no complexation between the drug and polymer could not be explained based on lack of ionization of either molecule and was explained based on the instability of the complex.

Keywords: Diclofenac, Chitosan, Complexation, co-precipitate, DSC, FTIR, Dissolution.

INTRODUCTION

Chitosan is a natural cationic polysaccharide obtained by alkaline N-deacetylation of chitin. Several interesting properties of chitosan such as film forming ability, gelation characteristics, bioadhesion properties and penetration enhancing effects which were explained by opening tight junctions of epithelial cells have been reported [(1-3)].

Moreover, chitosan is a biodegradable and biocompatible polymer and due to its promising properties it has received great attention in the pharmaceutical field as well as in food science and in cosmetic formulations. Within pharmacy it has been developed to microparticles and nanoparticles for encapsulation of drugs and biological substances[(4-6)]. Also as a dietary supplement, it is present in several over the counter products for the control of overweight and hyperlipidemia. As a nutritional supplement, chitosan has been reported to reduce fat absorption in the intestine by binding fatty acids, triglycerides and bile acids and increasing their excretion[(7)].

Whenever ionic polymers are used as excipient in pharmaceutical formulations, the release of oppositely charged drug might be strongly affected by the occurrence of charge-charge interactions, which may result in insoluble product. In some cases this occurrence is considered as a negative event to be avoided. However this polymer-drug interaction can also be exploited for controlled drug release. Water insoluble complexes between polymers and oppositely charged drugs have been described as in between the acidic pectin containing carboxylate moieties and the cationic drug Benzydamine

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hydrochloride[8], also, Carrageenan-diltiazem HCl complex was obtained by mixing aqueous solution of the drug[9]. The interaction of diclofenac and Eudragit RL and Eudragit RS (copolymers of ammoniomethacrylate) was utilized for controlled drug release[10]. The interaction was demonstrated as a reduction in the dissolved diclofenac salts in aqueous medium when added as aqueous salt solution into the solid copolymers.

The binding or interaction of oppositely charged molecules of chitosan micro/nanoparticles with drugs showed a high burst effect within half an hour and a total release of the drug within a short time[11]. This behaviour already shows that the binding properties of chitosans based on an ionic reaction may be poor and that chitosans are not the optimal excipients in formulation technology as may be seen from the literature[10]. The binding properties by ion exchange are shown by the increase in the solubility of indomethacin released from the kneaded mixture and by the solid complex of low molecular weight chitosan and indomethacin. The interaction between the amino group of chitosan and carboxyl group of indomethacin was mentioned because it was the reason for complexation[11]. Especially, the interaction of chitosans and peptides or proteins is often reported as showing a carrier and stabilizer effect[7].

Accordingly, studying the complexation between chitosan and a weakly acidic drug would provide valuable information for any possible contraindication when chitosan is used as an excipient with these drugs, or when chitosan is taken as dietary supplement and one of these drugs is taken orally concurrently. This possible complexation could be also utilized for the development of sustained release formulations. One class of the weakly acidic drugs is the non-steroidal anti-inflammatory drugs that are frequently used for the management of pain and inflammation. Diclofenac Na (DS) is a potent nonsteroidal anti-inflammatory drug (NSAID), therapeutically used in inflammatory and painful conditions of rheumatic and non-rheumatic origin.

The aim of this research work is to investigate the type of complexation between chitosan as weak base and diclofenac sodium as a salt of a weak acid in comparison to physical mixtures and diclofenac acid alone using solid state characterization methods such as DSC, TGA, FTIR and X-ray powder diffractometry as well as dissolution studies in different media.

MATERIALS AND METHODS

Materials

Low molecular weight chitosan (degree of deacetylation of 91.2 % and viscometric molecular weight for 1% solution was 38 mPas. Second) was obtained from the Jordanian Pharmaceutical Company (Naur, Jordan). Diclofenac sodium (DS) was obtained from DAR Al-DAWA Pharmaceutical Company, (Naur, Jordan). HPMC was from Koch-Light Limited (Haverhill, Suffolk, England). HCl (37%) from Sigma (Aldrich, Germany). For all experiments distilled water was used.

METHODS

Preparation of diclofenac Na (DS) and chitosan (CH) complex

The possible complex formation between DS and chitosan was studied by mixing DS aqueous solution and chitosan saturated solution in 0.05 M HCl using magnetic bar at 1500 rpm at room temperature (25°C) for 30 minutes.

The use of saturated acidic chitosan solution rather than unsaturated solution was aimed to totally consume HCl by chitosan protonation, which would allow for higher pH of chitosan solution for higher ionization of the added diclofenac in the final mixture. In addition, the formation of diclofenac free acid as a result of reaction of DS with the excess HCl will be minimal. The preventive measure of using saturated chitosan acidic solution rather than unsaturated solution was evaluated by comparing the pH of the two solutions. The pH was 1.6 and 3.9 for the unsaturated solution and saturated solution, respectively, which indicated total consumption of HCl due to the saturation of the acidic medium with the basic polymer. The addition of DS solutions at different concentrations (0.2-2 mg/ml) into the saturated chitosan solution resulted in the formation of precipitate (CH-DS). The mixtures were shaken in a thermostated water bath at 37^0C for 24 h to reach equilibrium. The resulting dispersions were filtered using Whatman paper or to obtain clear filtrate. The filtrate was assayed for free DS using A double-beam JASCO UV-visible spectrophotometer (UK), model V-530 with V-

500 Windows software at 275 nm wherein the method of analysis was validated and found to be selective for Diclofenac without interference if chitosan or other excipients (unpublished work). The amount of drug precipitated and/or co-precipitated (CH-DS) was calculated as the difference between the initial amount of drug added and the total amount of free drug at equilibrium and plotted against the initial amount added. Since the amount of diclofenac precipitated and/or co-precipitated was found to increase linearly with the increase in DS concentration added, the filtered and dried precipitate obtained from using the highest concentration of DS solutions was used for precipitate characterization.

Preparation of Diclofenac acid (DA) from Diclofenac Na (DS)

DA was prepared to serve as reference to account for the possibility of DS protonation and its precipitation as DA after its addition to the acidic chitosan solution. For this purpose, 2 g of DS were dissolved in 250 ml distilled water. The resulted solution was then treated by adding 250 ml of 2 M HCl causing the precipitation of DS as DA. The precipitate was collected using suction filtration, washed several times with water, and then left for drying under vacuum.

Solid state characterization of the precipitate

Differential scanning calorimetry (DSC) analysis

DSC curves were recorded using a differential scanning calorimeter (Mettler, Toledo DSC823e, Switzerland) configured to a Mettler® Star software system (Mettler, Toledo, Switzerland). The equipment was calibrated with indium. Powder samples were weighed into sealed aluminum pans with pierced cover. DSC curves were recorded under dry nitrogen atmosphere (80 ml/min) from 25ºC to 300ºC at heating rate of 10ºC/min.

Solid samples of the precipitate, DS, DA, physical mixture of DS and chitosan, and physical mixture of DA and chitosan were tested under the above conditions.

X-ray diffractometry

X-ray powder diffraction was recorded using Philips PW 3040/00 (Holland) with a Coα radiation. Wavelengths were: Coα1 = 1.78897 A ° and Coα2 = 1.79285 A°. Scan range was 2-45° 2θ with a scan speed of [°2θ /s]: 0.020.

Fourier transformation –infrared (FTIR) spectroscopy

FTIR spectra were recorded using an FTIR spectrometer (Shimadzu® 8400S IR spectrophotometer, Japan) for samples prepared according to the KBr disk method.

Tablet preparation, assay and dissolution

Tablet preparation

Mixtures of DS, DA or CH-DS and HPMC were mixed at 1:1 weight ratio using mortar and pestle. Two hundred milligrams of each mixture were compressed into a tablet in 1 cm die using manual tableting hydraulic press at 5000 KN.

Assay of diclofenac in the precipitate CH-DS

In order to separate the chitosan from diclofenac in the co-precipitate, fifty milligrams of the precipitate CH-DS were transferred into 100 ml volumetric flask and the volume was completed with 0.05 M HCl. The acidic medium was used to dissolve chitosan leaving the insoluble DA to precipitate. After shaking the mixture for one hour at 37 °C, the remaining solid was filtered using a micro filter paper with pore size of 0.45 μm. The filtered solid was washed several times with distilled water, transferred into 100 ml volumetric flask and the volume was completed with 0.05 M NaOH, and the mixture was stirred until clear solution was obtained. The solution was assayed for DA at 275 nm using UV spectrophotometer.

Dissolution studies

The dissolution studies were performed in triplicate in water and 0.05M phosphate buffer (pH 6.8) using USP type II dissolution apparatus at 37 °C and 75 rpm. At 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h, 5 ml samples were drawn from the dissolution medium. The samples were replaced with fresh dissolution medium and analyzed for the amount of drug released using UV Spectrophotometer at 275 nm wherein the analysis was selective and no interference of HPMC was present..

A model-independent technique was used to compare the dissolution profiles of the three products; such a model was described by Moore and Flanner (1996) [12] which is the similarity factor f2.using the following equation:

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}\left(R_t - T_t\right)^2\right]^{-0.5} \times 100\right\}$$

RESULTS AND DISCUSSION

Solid state characterization of the precipitate

Assay of diclofenac in the precipitate

The assay procedure turned out an average assay value of 92.2% ± 0.6 drug loading in the precipitate. This result indicated the presence of chitosan in the precipitate, however, it could be presented as physical co precipitate with possible precipitated diclofenac as DA and/or as a complex with diclofenac.

Differential Scanning Calorimetry (DSC) Analysis

Figure (1) presents the results of the DSC analysis for DS, DA, physical co-precipitate and the physical mixture between DA and CH.

The precipitate obtained from mixing chitosan and DS solutions was characterized in comparison to DS and its acid form DA. DA was used to check for the conversion of DS into DA as a result of its protonation in the used chitosan acidic solution. This conversion if happened would minimize the interaction of diclofenac with the protonated polymer and would cause the precipitation of diclofenac as DA as a result of low aqueous solubility of DA. As shown in Figures 1, only endothermic peaks and no distinctive exothermic peaks were obtained for the solid samples. The endothermic peaks obtained, had variable peak temperatures among the studied powders.

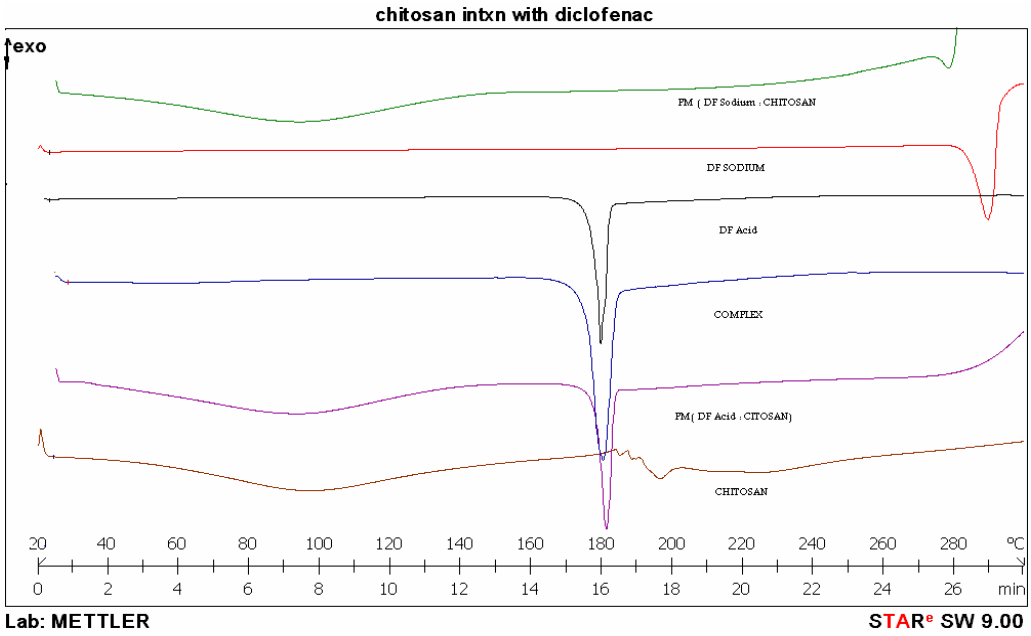


Figure (1): DSC curves (From upper to lower respectively): Physical mixture of DS and chitosan, DS, DA, co-precipitate, physical mixture of DA and chitosan.

DS and its physical mixture with chitosan showed endothermic peaks at temperatures higher than 250°C, which were higher than that of the co-precipitate (at 180.82 °C) with ΔH (526.67mJ). Accordingly, the absence of DS in the precipitate was concluded, which was a result of its high water solubility. On the other hand, DA had an endothermic peak at 182.09°C with ΔH (553.67mJ) while its physical mixture with chitosan

showed an endothermic peak at (183.04 °C) with ΔH (262.22mJ), which was different from that of the precipitate. While the difference was found to be small but still it indicated certain type of interaction between DS and CH. The difference between the endothermic peak temperature of DA and the main exothermic peak of the precipitate could be taken as slight evidence for the presence of diclofenac-chitosan complex in the precipitate by suggesting lower thermal stability for the complexed diclofenac than the free DA. This evidence was stated to be slight because there was a possibility for the formation of physical co-precipitant of protonated chitosan and protonated DA with no complexation.

X-ray diffractometry

Figure (2) shows the XRPD pattern of chitosan, DS, Co-precipitate, DA, and DA: chitosan physical mixture. Chitosan gives an amorphous x-ray diffraction pattern, The XRPD pattern of DS corresponds to that reported in literature[13].

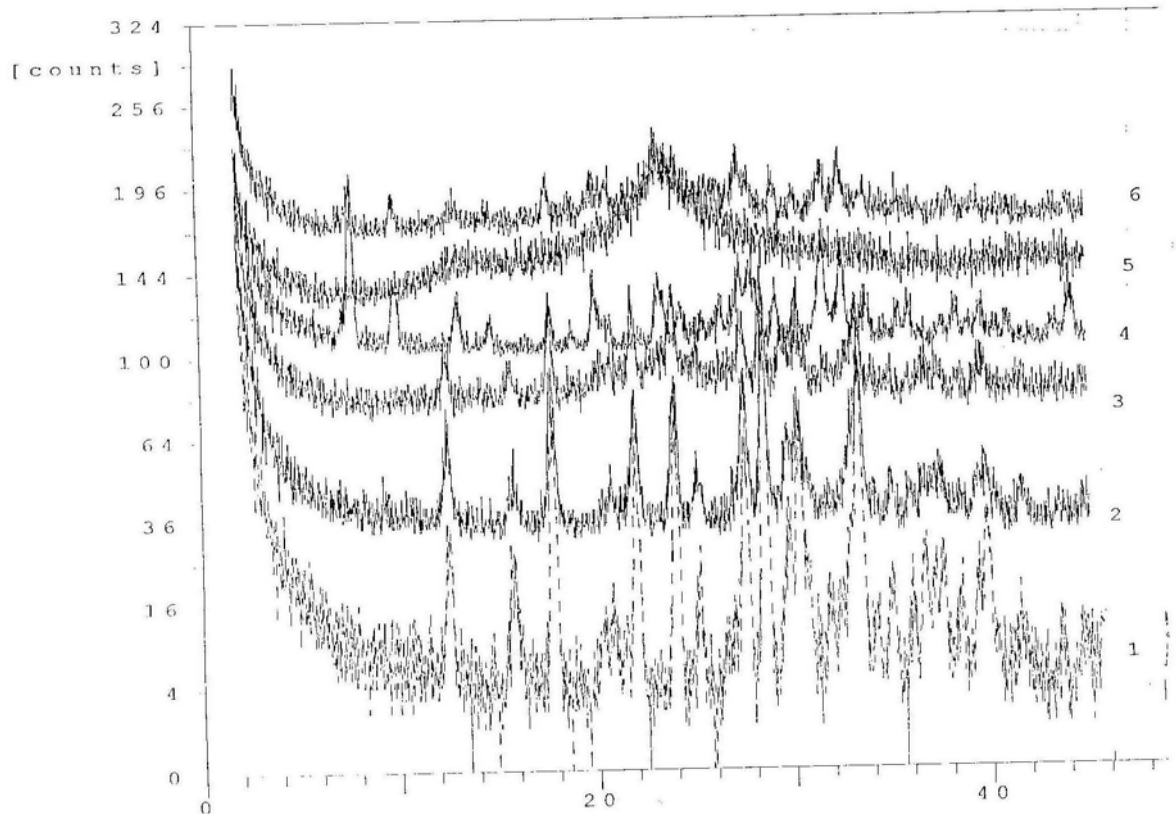


Figure (2): X-ray diffractograms of 1: DA, 2: the precipitate obtained by mixing DS and chitosan aqueous solutions, 3: physical mixture of DA and chitosan, 4: DS, 5: chitosan, and 6: physical mixture of DS and chitosan.

The XRPD pattern of the diclofenac in the coprecipitate is different from the pattern of DS before it was prepared with chitosan. The other powders showed crystalline intense peaks. DS showed clearly different peak pattern comparing to that of the precipitate which indicated the absence of DS in the precipitate and was in accordance with the DSC results. Nonguj Muangsin et al[13] reported that DS, the starting material in the preparation of a controlled-release diclofenac-containing chitosan matrix, changes in the matrix to DA which was confirmed by our XRPD in which DA and its chitosan physical mixture showed similar X-ray diffraction pattern as for the co-precipitate

Fourier Transformation–Infrared (FTIR) Spectroscopy

Figure (3) presents the Ft-IR spectra in the region 1400-3500 cm $^{-1}$ of DA, chitosan-DA physical mixture and the precipitate obtained by mixing DS and chitosan aqueous solutions.

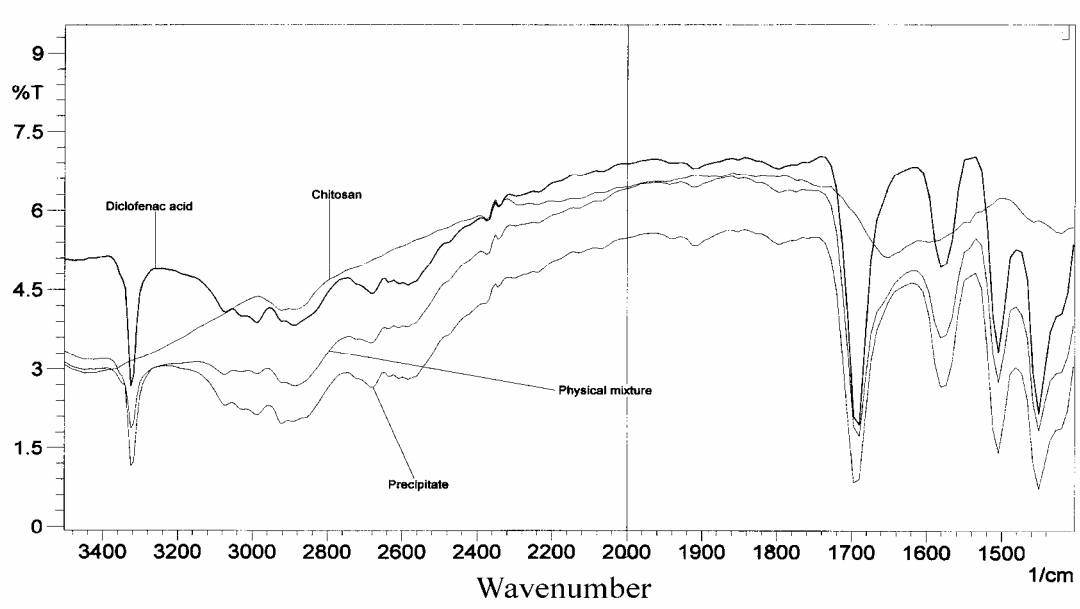


Figure (3): FTIR Absorption spectra of DA, chitosan-DA physical mixture and the precipitate obtained by mixing DS and chitosan aqueous solutions.

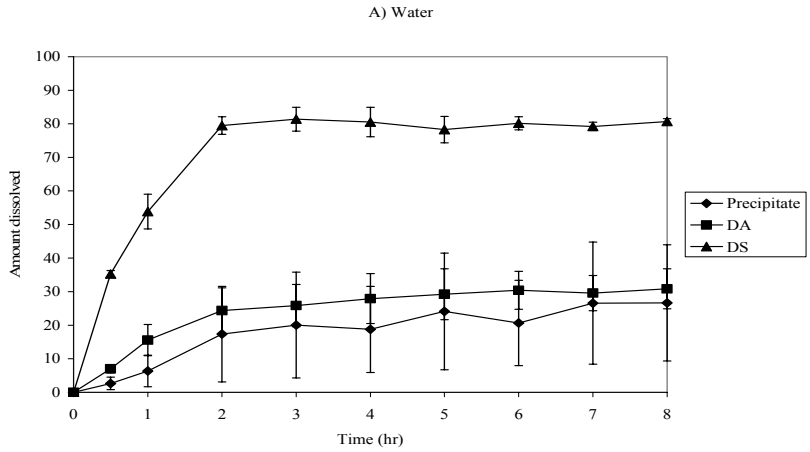
The FTIR spectrum of the co-precipitate showed similarities in its peaks with those of DA and particularly DA-chitosan physical mixture (Figure 3). The bands between 3200-3400, and 1650-1800 cm^{-1} were assigned to the hydroxyl and carbonyl group of the carboxylic group of DA, respectively. Because these two bands did not shift nor disappeared for the precipitate and no other characteristics bands appeared for the precipitate, this was taken as strong evidence for the protonation of diclofenac and no involvement of its carboxylate group in electrostatic complexation with chitosan. Accordingly, it can be concluded that using the optimal preparation procedure and conditions, the co-precipitate was a physical mixture of chitosan and DA. For optimal drug-polymer complexation, both the drug and polymer should be in ionized form for ion pairing to happen through electrostatic interaction. The formed ion pair should have high stability and low dissociation for the complex to be isolated. The pH of the filtrate separated from the precipitate was 5.2, and the pKa values of diclofenac and chitosan were 4.01[14] and 6.3[15], respectively. Accordingly, both the drug and polymer should exhibited significant ionization in the final mixture. Consequently, protonation could be excluded as a reason for the no complexation between chitosan and diclofenac. It

seems that the ionic interaction between the drug and chitosan was not the main mechanism that causes complex formation. The protonation of DS was hindered by a water layer; therefore, the possibility of an electrostatical interaction was low. In the view of this conclusion, the most probable explanation for this type of the coprecipitate formed can be the precipitation of DS was as DA that was partially bound by some electrostatic attraction and physically entrapped by chitosan molecules.

Dissolution studies

The dissolution profile of DS, DA and the co-precipitate are presented in figure (4).

A



B

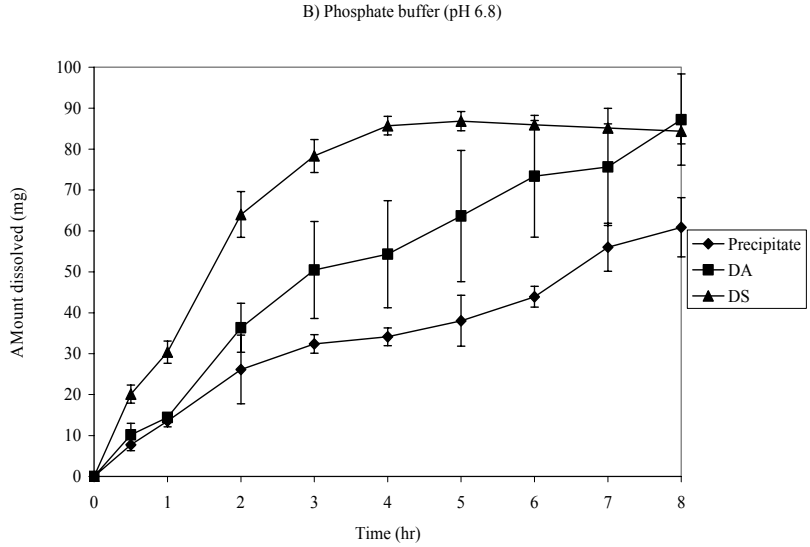


Figure (4): Dissolution profiles for HPMC matrices of DS, DA, and the precipitate obtained by mixing DS and chitosan aqueous solutions. Each data point is the mean of three determinations. A. Water B. Phosphate buffer (pH 6.8).

According to Figure A, the precipitate showed much lower dissolution rate than that of the DS phosphate buffer where the Similarity factor result was (35.4) which mean that the two dissolution profiles were not similar. Also, the precipitate showed lower dissolution profiles to those of DA in water although the results of similarity factor showed that they are similar.

The dissolution was mainly controlled by the saturation solubility of DA in water and in buffer system, and the physical co-precipitation of DA into chitosan was clear in the dissolution profile of the co-precipitate in buffer system where the complex showed much lower dissolution compared to DA.

These dissolution results confirmed the physical co-precipitation of DA and chitosan and further pointed out the importance of considering the possibility of drug protonation and physical entrapment.

CONCLUSIONS

When chitosan saturated acidic solution and DS solution were mixed a precipitate was formed. The form of the precipitated chitosan and diclofenac was investigated for all the possibilities including complexation and physical co-precipitation as a result of drug and polymer protonation. However, the overall evidence of the solid state characterization of the precipitate indicated the physical co-precipitation of the drug and polymer with no complex formation. The results could not be explained based on lack of ionization because the pH of the final mixture allows for significant diclofenac ionization. The only explanation for the results was based on the lack of diclofenac-chitosan complex stability.

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دراسة وتقييم التداخل بين معقد الكيتوسان ودواء الديكلوفيناك كراسب مشترك

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ملخـص

الهدف من الدراسة هو دراسة التفاعل بين دواء الديكلوفيناك ومعقد الكيتوسان. لهذا الهدف تم دمج محاليل من الدواء والمعقد في ظروف تضمن التأين للدواء والمعقد ونتيجة لذلك تم الحصول على راسب مشترك. تم تحليل الراسب بعدة طرق كيميائية وفيزيائية. وجد ان الراسب هو خليط فيزيائي بين الدواء والمعقد بدون أي حصول تداخل ايوني بينهما. بما ان المركبين كانا متائينين بما يكفي في ظروف التفاعل تم تفسير عدم التداخل الأيوني كنتيجة لعدم ثبات المعقد الأيوني.

الكلمـات الدالـة: دواء الديكلوفيناك، معقد الديكلوفيناك، التعقيد الأيوني.

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