RESEARCH ARTICLE

Synthesis, Characterization, and Drug Release Study of Modified Chitosan

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ABSTRACT

This research included loading of drugs (ibuprofen, ketoprofen, and fenoprofen) on a chitosan polymer to produce drug-loading polymer by entering the chitosan in a Schiff base reaction to protect -NH2 group, the reacting of the product with drug chloride. The prepared compounds were identified spectroscopically, by using infrared spectroscopy, ^{1}H-NMR, and ^{13}C-NMR. The thermal stability of chitosan was measured by using differential scanning calorimeter (DSC) and Differential thermal analysis (DTA). The drug release of ibuprofen, ketoprofen, and fenoprofen from chitosan in hypothetical stomach fluid was studied. The drug concentration that was released in stomach fluid several times was determined by measuring the absorbance in ultraviolet (UV) spectroscopy.

Keywords: Chitosan, Drug release, Schiff base.

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INTRODUCTION

Chitosan, a linear β-1,4-D-glucosamine, is a biocompatible and nontoxic compound, mainly prepared from the deacetylation of chitin, a natural structural component present for instance in crustaceans.[1]

Natural polymers, such as, proteins (silk, collagen, and keratin), and carbohydrates (glycogen, starch, and chitosan) are widely used materials for conventional and novel dosage forms. These polymers are non-toxic, chemically inert, less expensive, biodegradable, eco-friendly, and widely available.[2] The development of new applications for chitosan and its derivative is mainly because these are a renewable source of natural biodegradable polymers.[3]

Polymers are extensively used for the delivery of an active pharmaceutical ingredient. They can form a matrix or membrane that can control the release of a drug over a prolonged period, thus, avoiding repetitive dosing. They can also be used to form nanocarriers to deliver drugs, in particular, poorly soluble drugs or biotechnology-based drugs. Both systems can protect the drug from degradation.[4] The release of the drug usually occurs by diffusion through the polymer, by disorganization of the supramolecular structure of the carrier, or by the degradation of the polymer.

Among all the polymers available to be used for drug delivery systems, biodegradable polymers are highly recommended. Indeed, one of the key points of this kind of system is the removal of the carrier after the release of the active pharmaceutical ingredients. Moreover, to avoid side effects, in particular, when the carrier is injected, the polymer must be biocompatible. For all of these reasons, natural polymers, such as, polypeptides, polysaccharides, or phospholipids are generally used as building blocks for the formulations.[5]

Chitosan has a large number of applications in the pharmaceutical dosage form. It has been widely used for drug-carrying devices in controlled drug delivery systems.[6] Its further application can be exploited by modifications of the basic structure to obtain polymers with a range of properties. It can be done by several approaches, such as, chemically, as well as, by enzymatically. Chitosan can be modified into an ester form, such as, chitosan glutamate, chitosan phthalate, and chitosan succinate. These chitosan esters have a different solubility profile.[7] It can be modified into N-trimethylene chloride, which is a quaternary derivative of chitosan and has superior aqueous solubility, intestinal permeability, as well as, higher absorption of neutral and cationic peptide analog over a wide pH range.[8]

Chitosan has better physiological activities and applications in antiviral and antibacterial fields. Among the substituted biopolymers, particularly whole worthy are the Schiff bases obtained from free amino groups of chitosan that reacted with an active carbonyl compound, such as, aldehyde or ketone.[9] The Schiff base reaction of amine groups with aldehyde groups increases the stability of the biopolymer.[10] The incorporation

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of a drug into a chitosan matrix to form a monolithic device can expand the use of this biopolymer. To date, the study of drug-loaded chitosan films was focused on the release behavior of the drug from chitosan matrix films.[11] Depending on the amount of chitosan,[12] film thickness,[13] and dissolution medium, the liberation chitosan films drugs varied from fast release to slow release. In the case of the sustained release, the drug was released from the chitosan film following zero-order or first-order kinetics.[14] Chitosan is non-toxic and easily bioabsorbable with gel-forming ability at low pH. Moreover, chitosan has antiulcer and antacid activities, that prevent drug irritation in the stomach. Also, chitosan matrix formulations appear to float and gradually swell in the acid medium. All these interesting properties of chitosan have made this natural polymer an ideal material for controlled drug release formulations.[15,16]

MATERIALS AND METHODS

Materials and Instruments

All the chemicals used have been supplied by Fluka and Aldrich. Ultraviolet spectra were recorded by Shimadzu UV-vis recorder over the range of 500–4,000 cm^{-1}. Infrared spectra were taken on a Shimadzu FT-IR-8400S Fourier transform, in the range between 250 and 4,000 cm^{-1}. ^{1}H-NMR spectra were recorded on a Bruker-NMR, ultra shield 400 MHz instrument in d^6-DMSO as a solvent. DSC Shimadzu-60 instrument at a heating rate of 10ºC.min^{-1}, under air (normal) temperature range from room temperature up to 500ºC. Thermogravimetric differential thermal analyzer (DTA) Shimadzu instrument at a heating rate of 10ºC.min^{-1}, under air (normal) temperature range from room temperature up to 500ºC.

Preparation of Chitosan's Schiff Base Compound

One-gram of chitosan was dissolved in a mixture of ethanol and acetic acid at room temperature, then 2 mL of crosslinking agent (glutaraldehyde) was added to the chitosan solution with stirring for 12 hours in a water bath at 60ºC. The reaction content was washed with ethanol and filtered off.[17] The yield of the product after drying was 86%.

Reaction of Drug with Thionyl Chloride

A 3.5 mL of thionyl chloride was added to a round-bottomed flask equipped with a condenser prepared with a cottonwood guard tube, then 1.5 grams of the drug (ibuprofen, ketoprofen, and fenoprofen) was added to thionyl chloride gradually. The reaction content (drug-chloride) was filtered and dried, then it was recrystallized by ethanol (Table 1).

Preparation of Drug Loading Chitosan

In a round-bottomed flask equipped with a condenser, prepared with a cottonwood guard tube, 1-gram of chitosan's Schiff base compound was dissolved in 5 mL of DMSO. The flask was immersed in an ice bath, then different amounts of drug-Cl were added to chitosan's Schiff base solution gradually, with stirring for 24 hours, to prepare drug loading rate 25, 35, and 50%. The reaction mixture was allowed to settle for 24 hours, then it was added to 10 mL of water containing a small amount of sodium bicarbonate. The product was filtered and washed with ethanol. The product was dried by using anhydrous calcium sulfate. To prepare the drug loading polymer matrix, drug loading polymer was heated in an oven at 120ºC for 2 hours, then it was pressed as a disc with 8 × 11 dimensions, by using a compression device firmly (10 tons).[18]

Table 1: Physical Proparties and Yield of (Drug-Cl).

Drug-Cl	*Melting point (ºC)*	*Color*	*Yield (%)*
Iboprofen-Cl	176-177	Off white	79
Ketoprofen-Cl	150-151	White crystal	65
Fenoprofen-Cl	163-164	Yellow	82

Stomach Fluid

2 grams of sodium chloride was dissolved in 400 mL of distilled water, then 8 mL of hydrochloric acid was added to the sodium chloride solution. The prepared solution was diluted to 1,000 mL.[19]

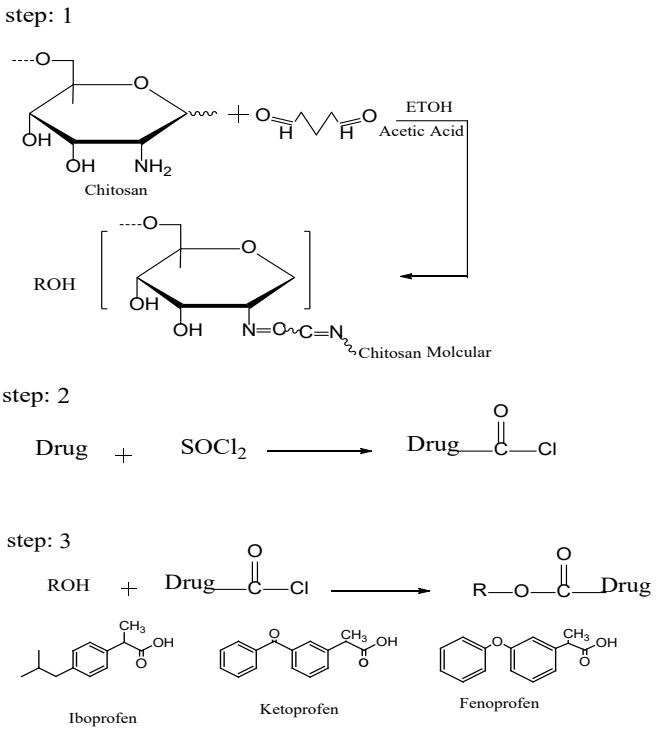
Study of Drug release

0.5-gram of the drug (ibuprofen, ketoprofen, and fenoprofen) loading chitosan powder and tablet, separately, were added to 25 mL of the hypothetical stomach fluid (pH = 1.2) in 37ºC. A periodic assay of samples was obtained by taking 1-mL of the sample. The concentration of each drug (ibuprofen, ketoprofen, and fenoprofen) was determined by using UV spectrophotometer at λ_{max} 286, 226, and 252 nm, respectively, by calibration curves.

RESULTS AND DISCUSSION

Preparation of Chitosan's Schiff Base Compound

Chitosan's Schiff base was obtained, as shown in Scheme 1, by the reaction of chitosan with glutaraldehyde as a cross-linking



Scheme 1: Synthesis of drug loading chitosan

agent, with heating at 60ºC. The infrared (IR) spectrum of chitosan's Schiff base showed C-N asymmetric vibration at 835 cm^{-1}, CH=N stretching vibration at 1,841 cm^{-1}, and absence of NH_2 asymmetric and symmetric vibrations. Figure 1 shows the thermal behavior of chitosan's Schiff base; the weight loss is 29.634% in the range of 80 to 208ºC, accompanied by an endothermic peak at 88.42ºC in the DTA curve because of volatilization of extraneous small molecules absorbed physically to the polymer. The second weight-loss step (41.39%) is between 290 and 400ºC, accompanied by two exothermic peaks at 294.2 and 355ºC in the DTA curve. The first peak is due to the water elimination, which is absorbed physically to the polymer, and the second one is due to dehydration of the saccharide ring. The third weight loss step (8.077%) is between 620 and 750ºC, with an exothermic peak at 724.9ºC in the DTA curve, due to the degradation of chitosan's Schiff base.

In the DSC curve (Figure 2), the presence of glass transition temperature appears on the reversing heat flow signal. In this curve, t_g of chitosan was 61ºC because of the presence of water, which acts as a plasticizer in chitosan and forms an intermolecular hydrogen bonding with amine and hydroxyl groups of chitosan. This peak was absent in the DSC curve of chitosan's Schiff base. This indicates the disappearance of the amine group, and the peak appeared at 102.83ºC.

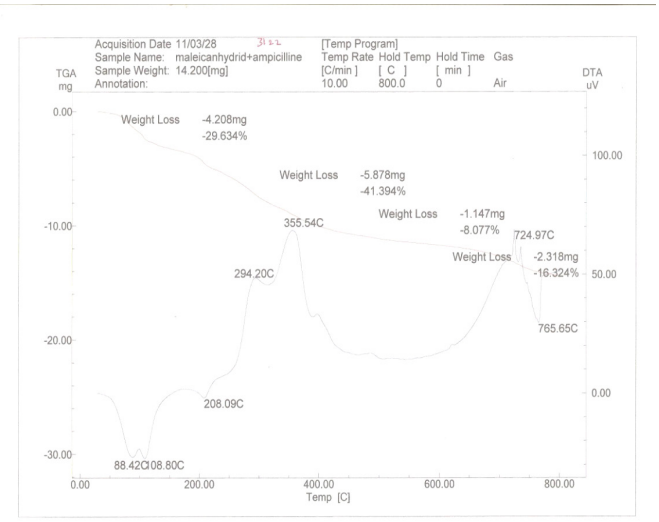


Figure 1: DTA curve of chitosan's Schiff base

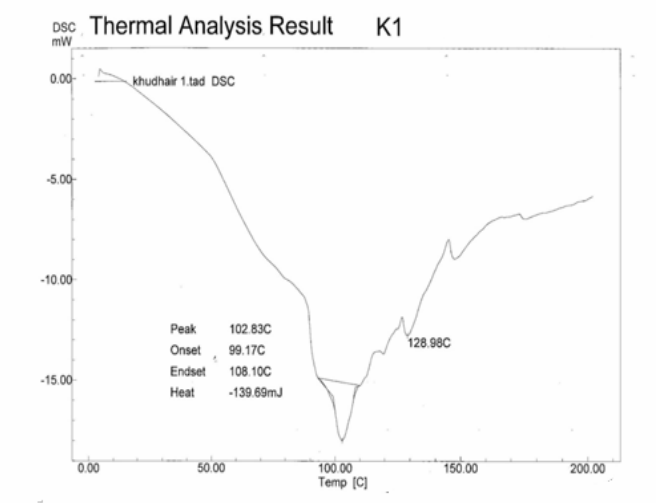


Figure 2: DSC curve of chitosan's Schiff base

Drug-Loading Chitosan

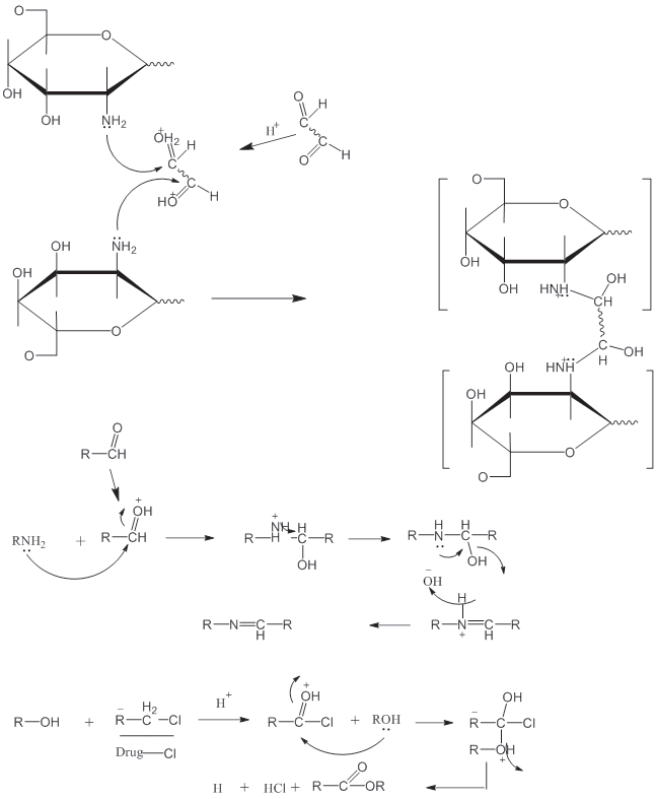
The drugs used in this study were (ibuprofen, ketoprofen, and fenoprofen), as shown in Scheme 1. These drugs were reacted with thionyl chloride to prepare drug chloride in yield 79, 65, and 82%, respectively. Drug chloride was reacted with chitosan's Schiff base to prepare drug-loading chitosan. The suggested mechanism of drug-loading chitosan was illustrated in Scheme 2.

The Fourier-transform infrared spectroscopy (FTIR) spectra of drug-loading chitosan with all three drugs (ibuprofen, ketoprofen, and fenoprofen) showed C=O stretching vibration at 1,658, 1,700, and 1,695 cm^{-1}, respectively, OH stretching vibration at 3,250, 3,248, and 3,341 cm^{-1}, respectively, OH bending vibration at 1,205, 1,220, and 1,238 cm^{-1}, respectively, and CH=N stretching vibration at 1,411, 1,502, and 1,514 cm^{-1}, respectively.

The ^{1}H-NMR (Figures 3–5) and ^{13}C-NMR (Figures 6–8) spectra of drug-loading chitosan with ibuprofen, ketoprofen, and fenoprofen, showed special peaks of chitosan and drug moieties, as shown in Tables 2 and 3, respectively.

Calibration Curve of Drugs

The stomach fluid was prepared to produce different concentrations of drugs (ibuprofen, ketoprofen, and fenoprofen);



Scheme 2: Suggested mechanism of drug-loading chitosan

Table 2: ^{1}H-NMR spectra data of drug-loading chitosan

^{1}H-NMR data ppm (δ)								
	For chitosan moiety						*For drug moiety*	
Drug-loading chitosan	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-Ar*	*Others*
Ibuprofen	3.1	3.35 m	3.7 d	4.1 T	4.5 d	5.1	7.1	2.3 d Of CH_3 2.5 m of CH
Ketoprofen	2.9	3.2	3.5	3.9	4.3	4.9	6.9	2.5 d of CH_3
Fenoprofen	3	3.3	3.6	4.2	4.4	5.2	7.3	2.7 d of CH_3

Table 3: ^{13}C-NMR spectra data of drug-loading chitosan

^{13}C-NMR data ppm (δ)						
	For chitosan moiety				*For drug moiety*	
Drug-loading chitosan	*C-OH*	*C-N*	*C-O-C=O*	*C-O-C*	*C-C*	*Others*
Ibuprofen	64.5-70	81	156-162	115-120	135-139	35 of CH_3 39 of CH
Ketoprofen	61.6-72	85	161-170	118	140-142	37 of CH_3 42 of CH
Fenoprofen	62-79	89	166-172	117-127	138-145	31 of CH_3 36 of CH

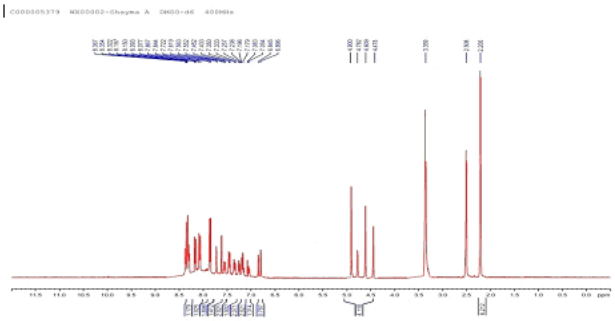


Figure 3: ^{1}H-NMR spectrum of ibuprofen

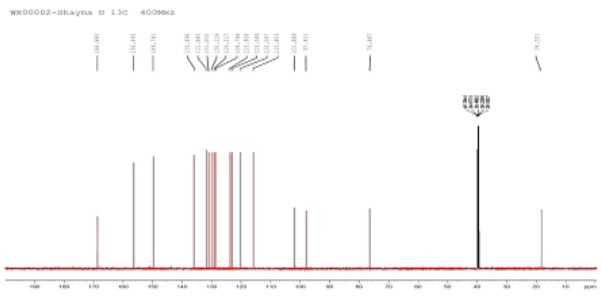


Figure 6: ^{13}C-NMR spectrum of ibuprofen

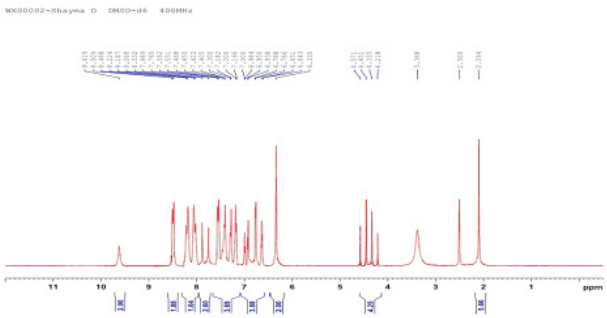


Figure 4: ^{1}H-NMR spectrum of ketoprofen

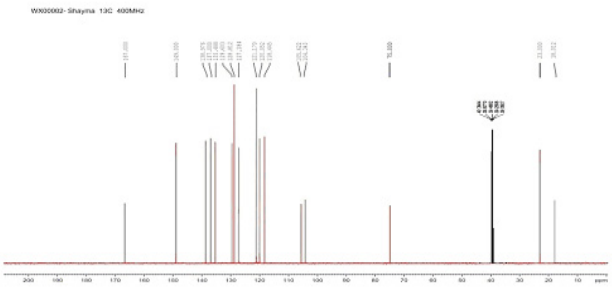


Figure 7: ^{13}C-NMR spectrum of ketoprofen

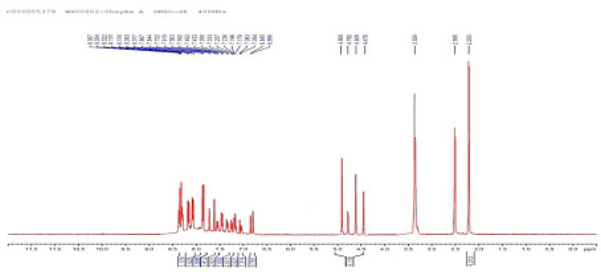


Figure 5: ^{1}H-NMR spectrum of fenoprofen

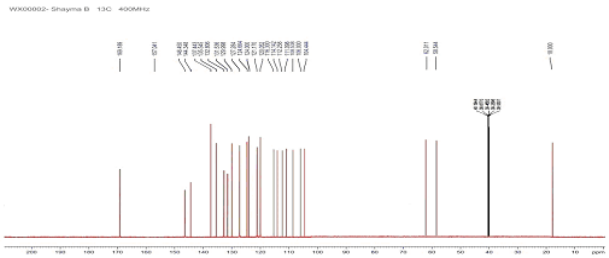


Figure 8: ^{13}C-NMR spectrum of fenoprofen

Table 4: Concentrations of released ibuprofen in stomach fluid

Time	*Powder*	*Disk*
0.5	0.19	0.07
1	0.2	0.1
1.5	0.27	0.13
2	0.36	0.18
3	0.42	0.19
4	0.52	0.21
6	0.68	0.27
8	0.81	0.31
10	0.82	0.39
12	0.85	0.46

Table 5: Concentrations of released ketoprofen in stomach fluid

Time	*Powder*	*Disk*
0.5	0.25	0.04
1	0.32	0.12
1.5	0.4	0.18
2	0.46	0.21
3	0.52	0.28
4	0.61	0.32
6	0.71	0.34
8	0.78	0.39
10	0.81	0.42
12	0.84	0.44

Table 6: Concentrations of released fenoprofen in stomach fluid

Time	*Powder*	*Disk*
0.5	0.55	0.31
1	0.61	0.39
1.5	0.7	0.44
2	0.75	0.51
3	0.82	0.58
4	0.91	0.62
6	1.04	0.7
8	1.18	0.88
10	1.22	0.96
12	1.24	0.98

the curve between concentration and absorbance was drawn to obtain the standard calibration curve of drugs ibuprofen, ketoprofen, and fenoprofen. λ_{max} of drugs was measured; they were 226 nm for ibuprofen, 286 nm for ketoprofen, and 252 nm for fenoprofen.

Study of Drug Release

The drug release of drugs-loading chitosan, as powder and tablet of each drug (ibuprofen, ketoprofen, and fenoprofen), were studied separately, by using the hypothetical stomach fluid (pH = 1.2) at 37ºC. The absorbance of each solution was measured at different times (1, 2, 3, …, 12 hours), by using a UV spectrophotometer, and determined the concentrations of released drugs in stomach fluid, as shown in Tables 4 to 6, by

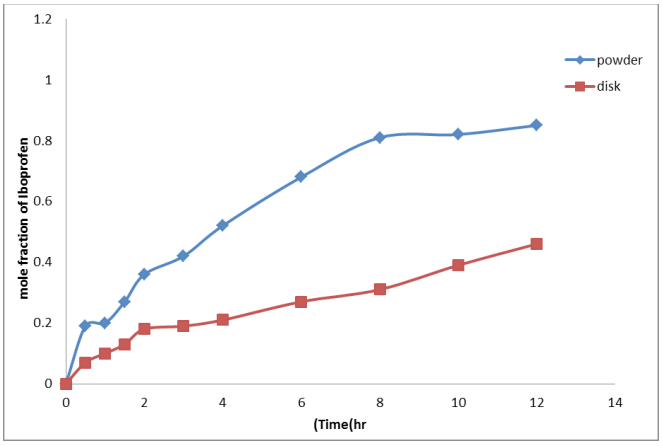


Figure 9: Release of ibuprofen from ibuprofen-loading chitosan

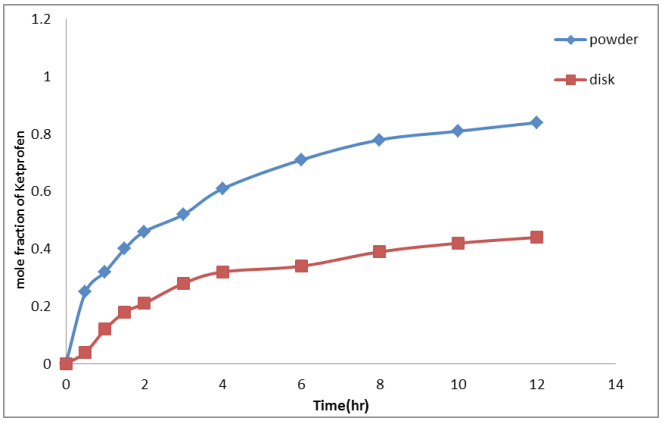


Figure 10: Release of ketoprofen from ketoprofen-loading chitosan

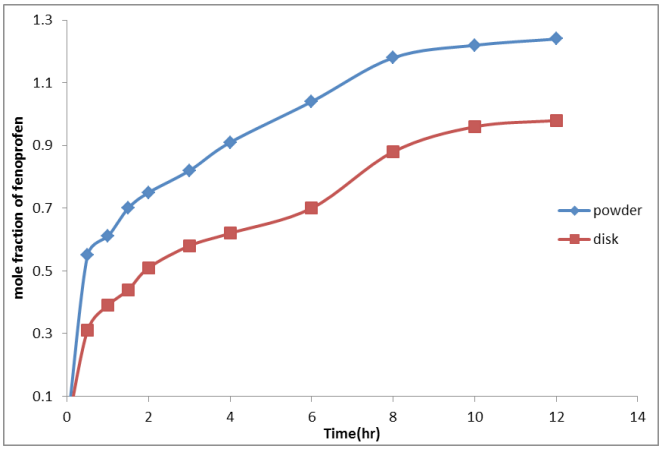


Figure 11: Release of fenoprofen from fenoprofen-loading chitosan

using the calibration curve of each drug (ibuprofen, ketoprofen, and fenoprofen), as shown in Figures 9 to 11, respectively.

CONCLUSION

From the results that obtained, when fenoprofen was used, the highest loading with chitosan was occur ; and in drug release studying it was found that the powder case was faster than the disk, as well as the fenoprofen-loading chitosan was faster released than ketoprofen-loading chitosan and ibuprofen-loading chitosan.

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