

## LOCALLY MADE CHITOSAN COVALENTLY LINKED TO INDOMETHACIN: A NOVEL APPROACH TO FORMULATE SUSTAINED RELEASE INDOMETHACIN CAPSULE IN BASRAH

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### ABSTRACT

The sustained release capsules are pharmaceutical dosage form used for the treatment of chronic rather than acute diseases and are useful because of its convenience and less side effects. This work describes an attempt to formulate indomethacin sustained release capsule using indomethacin covalently linked to chitosan. Chitosan was prepared by alkaline -N-deacetylation of chitin, which was isolated from the shells of local shrimps. The yield of chitosan was found to be 80%. This polymer was found to be white crystal, odorless and the IR spectroscopy of it was similar to a standard reference. Chitosan has been covalently linked to indomethacin and the ratio of drug to polymer was found to be 5:1. The chemical interaction between the drug and the polymer was proved by IR studies. In vitro release at different pH were performed and the release of indomethacin in the prepared sustained release capsule were tested and compared with conventional indomethacin capsule INDOMIN-25mg, and a commercial sustained release capsule INDO-75-SR. The release of indomethacin in our product was completed within 12 hours, while the conventional INDOMIN capsule and commercial INDO-75-SR capsule released within 2.5 and 5 hours respectively. In conclusion a cheap, locally made chitosan can be used in formulation of sustained release capsules by covalently linked to drugs containing carboxyl group (like indomethacin) or can be modified to have a carboxyl group.

### INTRODUCTION

Naturally produced polymers such as cellulose, starch, pectin and alginate represent biodegradable and toxicologically harmless raw materials of low cost and are therefore used as abundant excipient in various pharmaceutical formulations for many decades.<sup>[1]</sup> Among natural polymers with properties for chemical modifications, in particular chitin has gained considerable attention. The deacetylation of chitin, which can be isolated from insects, crustacea such as crabs and shrimps as well as fungi such as *Aspergillus niger* lead to poly (B1--4 D-glucosamine) or so called chitosan.<sup>[2-3]</sup> Because of its superior characteristics together with a very safe toxicity profile,<sup>[4]</sup> chitosan is widely used as a pharmaceutical excipient.<sup>[5]</sup> Furthermore, the presence of a primary amino group at the 2-position of each polymer subunit makes further chemical modifications feasible and the features of chitosan can be optimized according to a given task in delivery systems. Sustained release delivery system includes any drug delivery system that achieves slow release of drug over an extended period of time. If the system is successful at maintaining constant drug levels in the blood or target tissue, this is considered as a controlled-release system. Prolonged release, therefore, is considered when the system extends the duration of action over that achieved by conventional system.<sup>[6]</sup> All

sustained release products share the common goal of improving drug therapy over that achieved with their non-sustained counterparts.<sup>[7-8]</sup> Formulation of sustained release dosage forms could be achieved through, matrix dosage forms, microencapsulation and drug complex.<sup>[9-10]</sup> Covalent conjugation of drugs to polymers via hydrolytically or enzymatically cleavable covalent bonds represents one of the methods of achieving sustained drug delivery. Water insoluble polymer-drug conjugates have been studied extensively for the controlled release of herbicides, pesticides, contraceptive and anti-inflammatory agents.<sup>[11]</sup> Drugs best suited for incorporation into a sustained release product are those having relatively fairly rapid rate of absorption and excretion, those having relatively small doses, drugs that are uniformly absorbed from the gastrointestinal tract and drugs which are used in the treatment of chronic rather than acute conditions.<sup>[7]</sup> Indomethacin having a molecular formula of C<sub>19</sub>H<sub>15</sub>ClNO<sub>4</sub> with a molecular weight of 357.8 Dalton<sup>[12]</sup>, is a nonsteroidal anti-inflammatory drug having antipyretic, and analgesic properties as well as it is a potent inhibitor of prostaglandin synthesis. It has been shown to be an effective anti-inflammatory agent, appropriate for long term use in rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and patent ductus arteriosus.<sup>[13]</sup>

**In this study** we are aiming to isolate and purify chitin from shrimp obtained from Basrah local market and to modify this chitin to chitosan and then conjugate a model drug (Indomethacin) to chitosan in order to formulate a sustained release chitosan-Indomethacin capsules. The study also aims at comparing locally made sustained release indomethacin capsules with the other indomethacin capsules present in the local pharmaceutical market. (In vitro study).

## MATERIALS AND METHODS

### *Isolation and Purification of Chitin*

Shrimp (12Kg) obtained from Basra local market were boiled in tap water for 10 minutes, shells, then removed from all parts of the shrimp, washed in distilled water and exposed to direct sun light for dryness and the dried shells were milled to obtain fine particles. Then for each one part of the dry shell powder 4 parts of Hydrochloric acid 5%v/v was needed to get rid of minerals (511 ml HCl 5%v/v for each 156 gm of dry shell powder). The powder was added slowly to a large beaker containing the acid required with continuous stirring. Stirring was continued for 24 hours at room temperature. The suspension was then filtered, the precipitate was extensively washed with distilled water until the pH of the filtrate became 6. The precipitate was left to dry in an open air. Sodium hydroxide 5% w/v was used to get rid of all proteins present within the powdered shell and for each 110 gm of the powdered obtained after demineralization process 400 ml of NaOH, 5% w/v was used in a conical flask with continuous mixing for 5 hours at 95°C. The suspension was left to cool and precipitate. The precipitate was then separated by filtration, washed with distilled water until the pH of the filtrate became 6. The product allowed to dry in an open air.

### *Preparation of Chitosan*

Chitosan was prepared by alkaline deacetylation of chitin (14-16) as follow: In a round bottle, 5 g of chitin and 500 ml NaOH (40% w/v) was boiled for 5 hours at 120 °C. Reflex apparatus was connected to the neck of the bottle to keep the volume of NaOH constant and to prevent evaporation. After 5 hours the mixture was left to cool at room temperature, and then filtered. This procedure was repeated for 2 times in order

to ensure complete deacetylation of chitin. After filtration the precipitate was extensively washed with distilled water until the filtrate pH became 6. The precipitate was dried in an open air. The whole procedure was repeated ten times in order to treat 50 g of chitin.

### *Preparation of covalently linked Chitosan-Indomethacin conjugate*

To 500 mg of Indomethacin dissolved in 50 mg dimethylformamide (DMF), a 500 mg of dicyclohexyl carbodiimide (DCC) was added and the reaction mixture was incubated at room temperature under continuous stirring for 24 hours. White precipitate (N, N-dicyclohexyl urea) was formed and removed by centrifugation.<sup>[17]</sup> To the solution (activated indomethacin), a 250 mg of chitosan dissolved in 40 ml of 0.37% HCL and the mixture was incubated at room temperature under continuous stirring for 24 hours and 400 ml of 20% aqueous sodium chloride was used to precipitate the resulting conjugate. The precipitate was separated by filtration and washed with 250 ml ethanol and 250ml diethylether to remove any unreacted Indomethacin. The product was dried in an open air and stored at room temperature until use. The calculation of total amount of indomethacin present in the Chitosan-Indomethacin conjugate has been done as follows: an accurate amount (500 mg) of the conjugate powder was suspended in 500 ml of 3N NaOH and the reaction mixture was stirred at 37°C until complete release of indomethacin from the conjugate due to complete destruction of all amide bonds with chitosan (3 hours needed according to a pilot study being done to optimize the desired concentration of NaOH and the time for complete release of the drug). The suspension was then filtered and NaOH solution was added to the filtrate to produce 500 ml. The absorbance of the diluted solution was determined by spectrophotometer at  $\lambda$  max 284nm.

### *Infrared spectroscopy analysis*

Infrared spectra for indomethacin, chitosan and chitosan-indomethacin conjugate have been done by using infrared spectroscopy and all samples were scanned using KBr discs from 4000-200 cm.

### **Characterization of Indomethacin**

The melting point of pure indomethacin powder was determined by using Thomas Hoover electrical apparatus. The determination of  $\lambda$  max for indomethacin in phosphate buffer solutions pH 7.4 and 8 has been done by taking 5% w/v solutions of indomethacin in the required buffer and scanned on spectrophotometer between 350-200 nm and  $\lambda$  max was recorded.

Standard curves for indomethacin in phosphate buffers pH 7.4 and 8 were also done by preparing a stock solution of indomethacin (1mg/ml) by dissolving pure anhydrous indomethacin powder in the smallest possible amount of ethanol and bring it to 100 ml volume with phosphate buffers pH 7.4 or 8. Various amount of stock solutions were taken and diluted up to 25 ml with phosphate buffer pH 7.4 or 8 to make the final concentration 2, 5, 10, 20, 30, 40, 50 and 60 ug/ml respectively. The absorbance values were measured by spectrophotometer at 266 nm versus phosphate buffer pH 7.4 or 8 as blanks.

Standard curve of indomethacin in 3 N NaOH solution has been also done by preparing a stock solutions of indomethacin 1 mg/ml in 3 N NaOH solution and diluted with the same solution to get the required working concentrations of indomethacin (2, 5, 10, 15, 20, 25, 30, 40, 50 and 60). The absorbance values were measured by spectrophotometer at 284nm versus 3 N NaOH solution as blank.

### **In vitro release studies**

Chitosan-indomethacin conjugate powder was suspended in 10 ml of phosphate buffer pH (7.4 or 8). The suspension was placed in a dialysis membrane bag (surface area 16 cm<sup>2</sup>). The bag was closed tightly from the two ends and immersed in the receiving compartment containing 290 ml of the same buffer. Continuous stirring was applied to the receiving compartment and the temperature was kept constant at 37°C. Samples for the determination of the released drug was obtained from the receiving compartment. Each sample was 3 ml, and replaced immediately by 3 ml of the same buffer suitable to guarantee sink conditions throughout the runs. Measurement was done in triplicate. Sampling was continued for 14 hours. Spectrophotometer was used for the drug determination at  $\lambda$  max 266 nm. This procedure was used to study drug release of commercially available sustained release capsule INDO-75-SR, using the powder of one capsule (300 mg) and the commercially available immediate release indomethacin, INDOMIN-25 mg using the powder of 3 capsules (25 x 3).

### **RESULTS**

Sixty eight grams (68g) of pure chitin has been isolated from 156g of dry shells of shrimps; dry shells were obtained from 12Kg of fresh shrimp. The yield of pure chitin produced was 44%. Chitosan, 40g, white, crystal like, odorless powder has been produced out of 50g of pure chitin used with a yield of 80%. When a 500mg of indomethacin react with 250 mg of chitosan by carbodiimide coupling a 300mg of fine powder with a faint yellow color was produced. **Figure 1** shows the chemistry of activation and conjugation between chitosan and indomethacin.

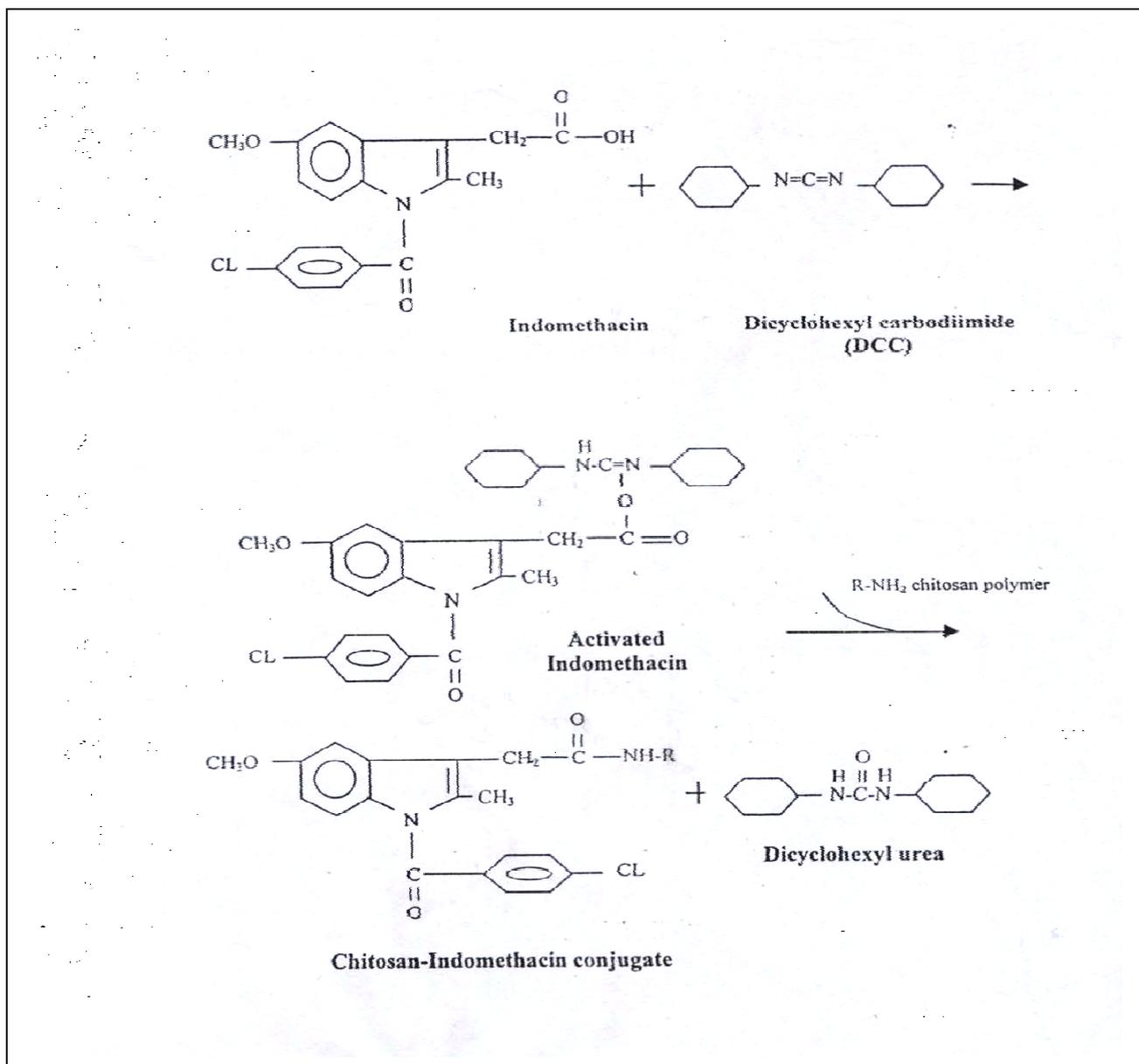
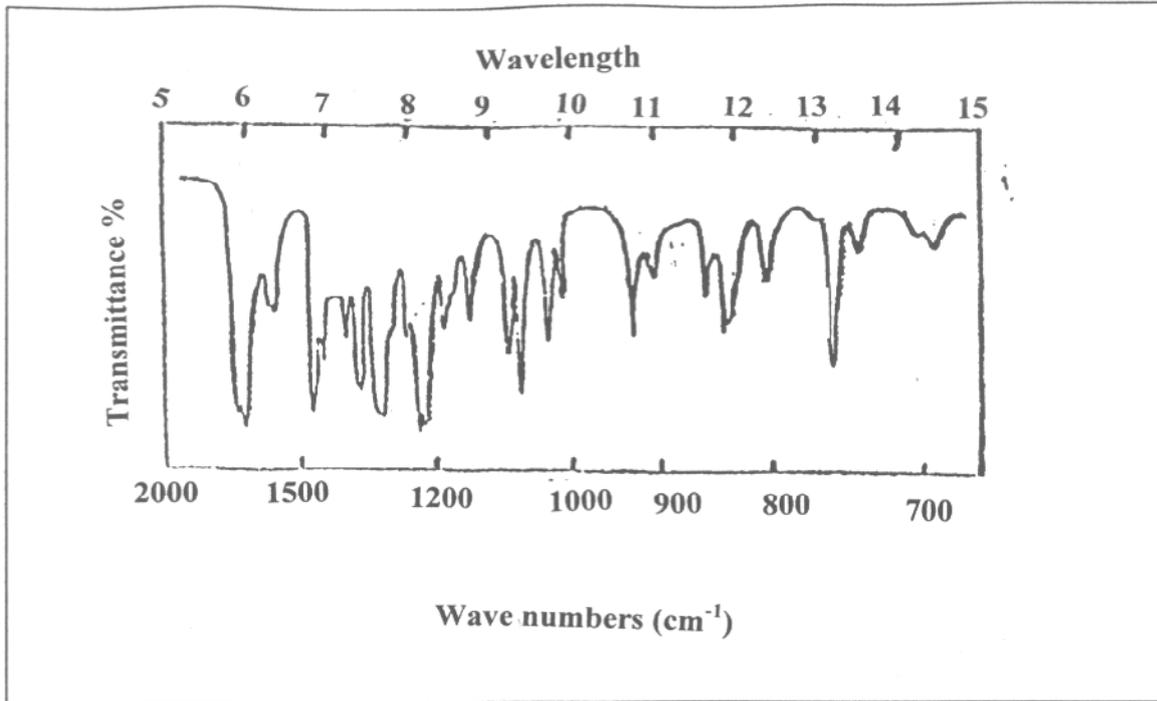


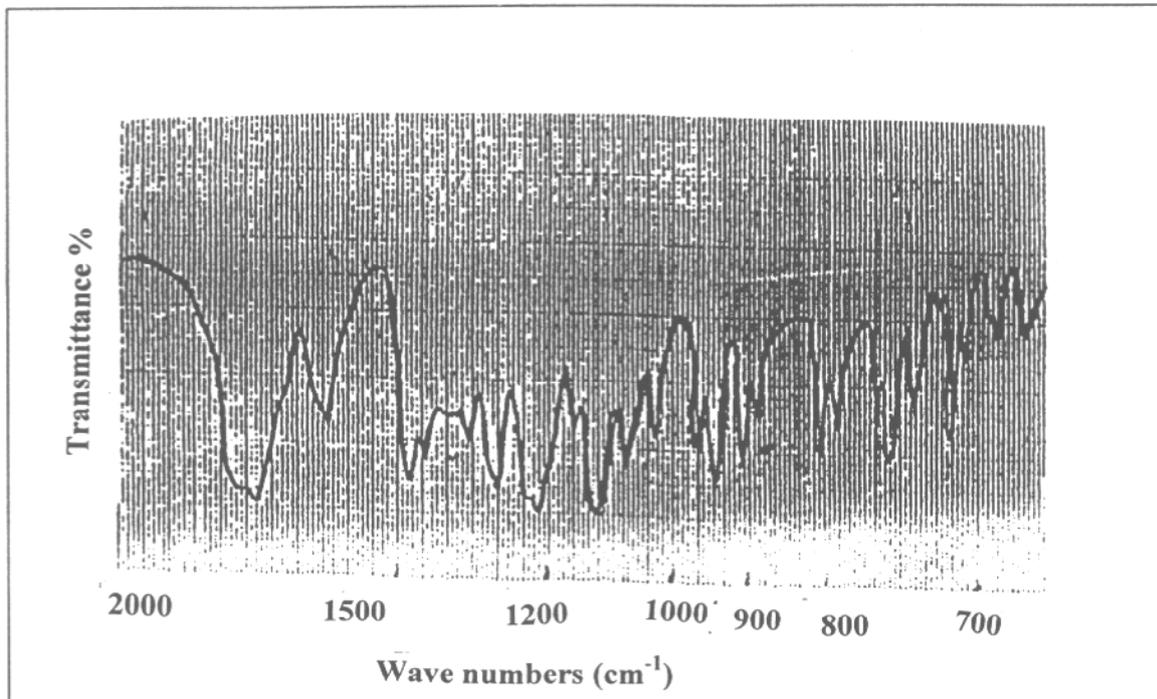
Fig 1. Mechanism of activation and conjugation between chitosan and indomethacin.

The amount of indomethacin in the chitosan-indomethacin conjugate has been calculated as percentage w/w by a spectrophotometer at 284nm and was found to be 18%. Measurements of the melting point of indomethacin powder used was performed and found to be 156-160°C. The λ max of indomethacin in phosphate buffers pH 7.4 and 8 was found to be 266 nm. The amount of indomethacin in the contents of one capsule of INDO-75-SR has been calculated using buffer solution pH 7.4 and 8 and was found to be 73.5

mg, while the amount of indomethacin present in INDOMIN has been calculated and was 25 mg using the same buffer solutions. IR spectroscopy to chitosan, indomethacin and chitosan-indomethacin conjugate has been performed and compared to a standard reference for indomethacin and chitosan in order to confirm the conjugation reaction. **Figures 2, 3 and 4** represent IR spectra for indomethacin, chitosan and chitosan-indomethacin conjugate respectively.

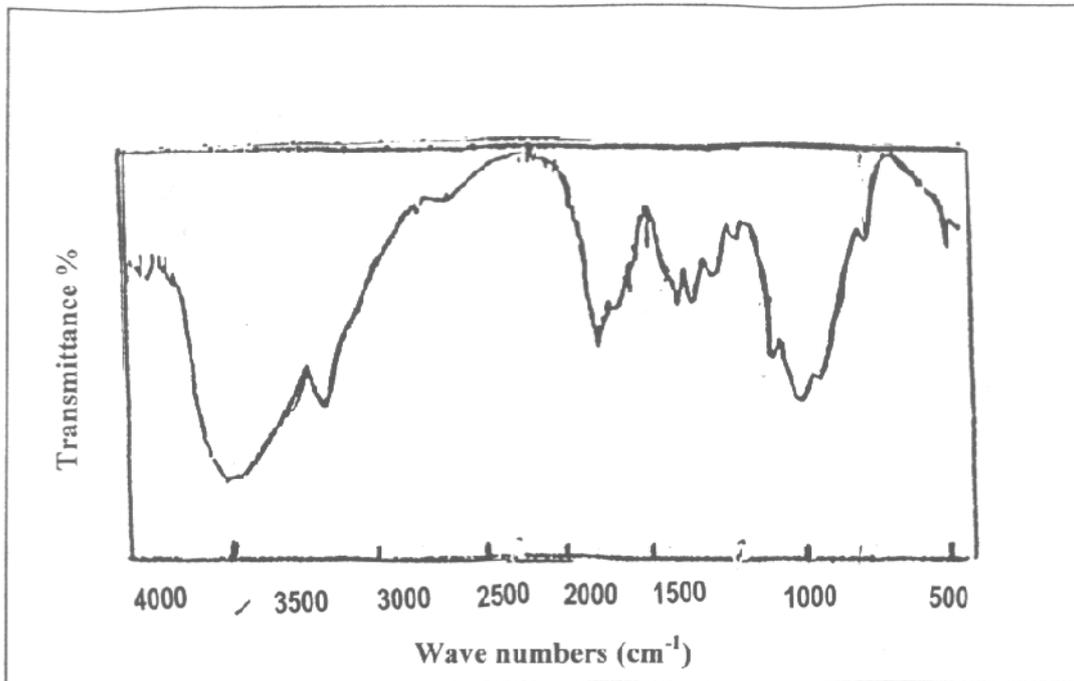


(a)

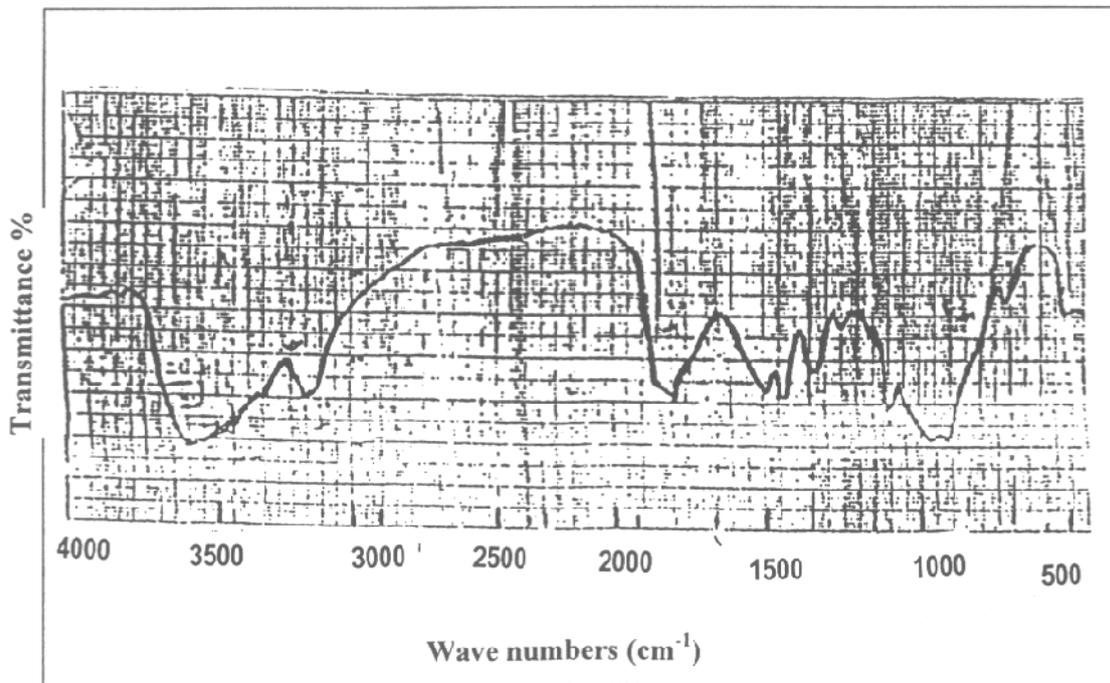


(b)

*Fig 2. Infrared spectra of a. standard reference of indomethacin (Ref 18)  
b. Indomethacin pure powder used in the study*

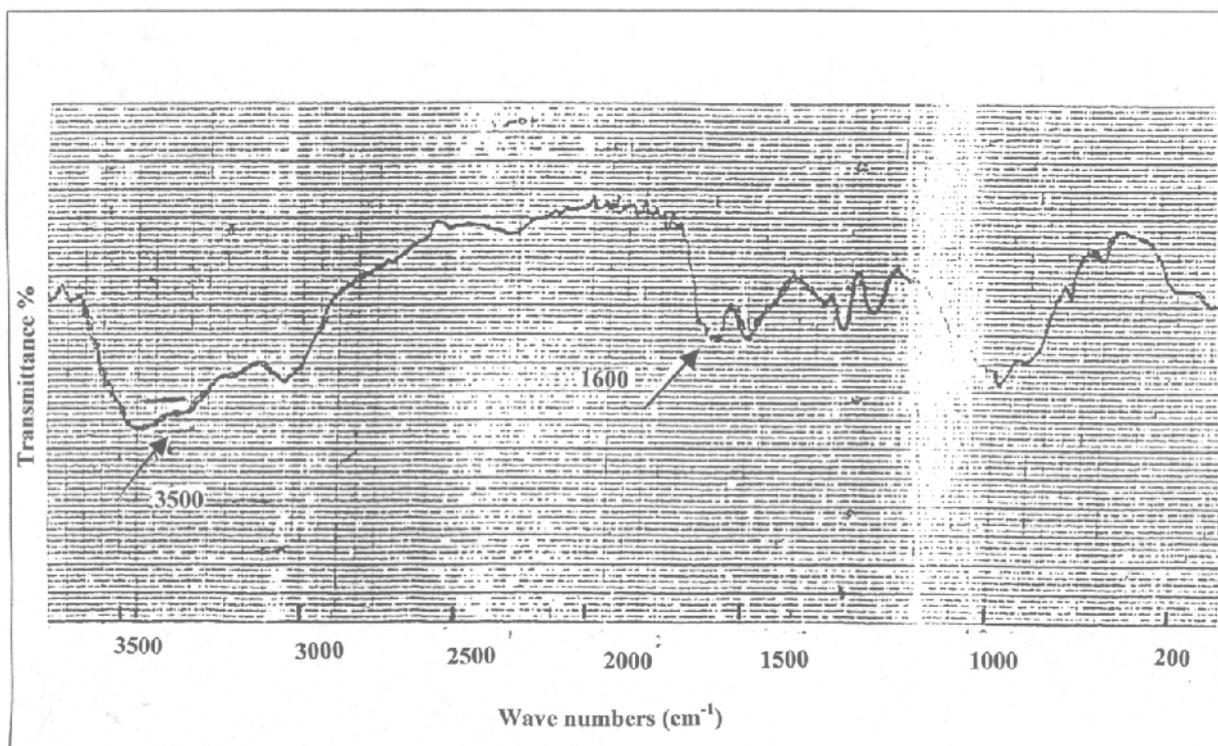


(a)



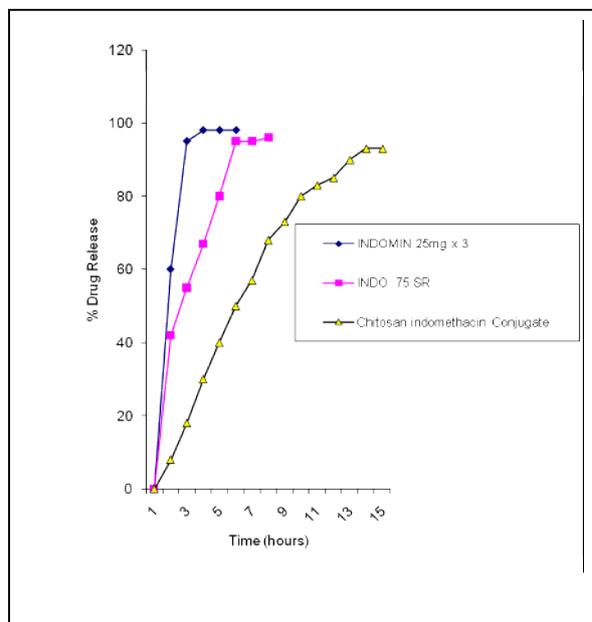
(b)

**Fig 3. Infrared spectra of a. standard reference of chitosan (Ref 19)  
b. locally prepared chitosan**



***In vitro release studies***

In vitro release of indomethacin from chitosan-indomethacin conjugate has been studied in phosphate buffers pH 7.4 and 8 and the release was completed within 13 and 12 hours respectively. The release of indomethacin from the commercial product INDO-75-SR in buffer solutions pH7.4 and 8 was completed within 6 and 5hours respectively, while the release of indomethacin from the commercial immediate release capsules INDOMIN completed within 2.5 hours in the two buffer solutions. **Figure 5 and 6** show the release profile of indomethacin in chitosan-indomethacin conjugate, INDO-75-SR and INDOMIN in phosphate buffers pH7.4 and 8 respectively.



**Fig 5. In vitro release profile of indomethacin in chitosan-indomethacin conjugate, INDO-75-SR and INDOMIN in phosphate buffer pH 7.4**

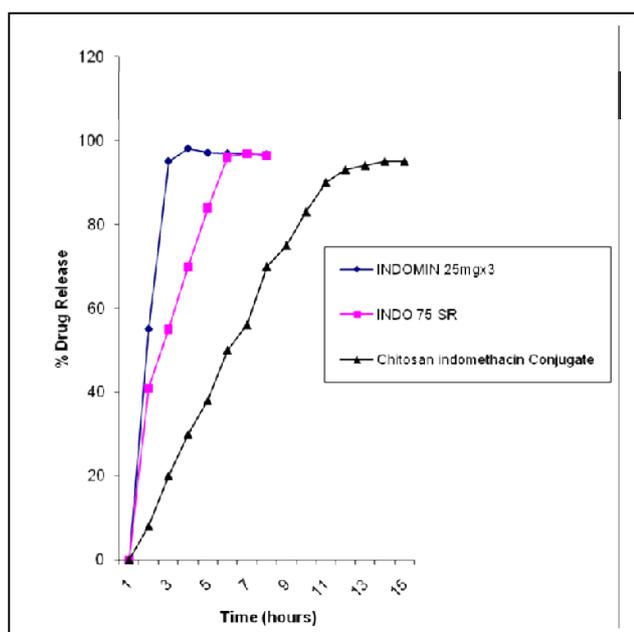


Fig 6. *In vitro* release profile of indomethacin in chitosan-indomethacin conjugate, INDO-75-SR and INDOMIN in phosphate buffer pH 8

## DISCUSSION

Chitosan is being selected in the preparation of a sustained release oral dosage forms because it is easy to be isolated and prepared with the possibility of being covalently cross-linked through its free amino group and it is relatively cheap and safe enough to be used clinically compared to other materials.<sup>[20]</sup> The only source of chitosan is the chemical modification of chitin.<sup>[21]</sup> Indomethacin is being selected as a model drug because it fulfills the criteria of being formulated as a sustained release dosage form; these are: short biological half life (4.5 hour) and its adverse effect on gastric mucosa<sup>[12]</sup> and it also contains functional carboxyl group in its chemical structure which makes indomethacin suitable for covalently linked with the free amino groups present in chitosan molecules. The conjugation of indomethacin to chitosan required activation of carboxyl groups of indomethacin molecules prior to reaction with the free amino groups present in chitosan molecules. Dicyclohexyl carbodiimide (DCC) is used for this activation and dimethyl formamide (DMF) as a solvent medium at 25°C. Pilot studies have been done to optimize the coupling reaction between chitosan and indomethacin. These studies required using fixed amount of chitosan (250 mg) with increasing amount of indomethacin in the presence of excess of DCC.

Maximum yields is achieved by using 500 mg of indomethacin with 250 mg chitosan to give 300 mg of the conjugated product which is composed of 50 mg indomethacin and 250 mg chitosan), 1: 5 coupling ratio, this ratio has been confirmed after the complete destruction of all conjugated bonds after using 3N NaOH and complete release of free indomethacin (the amount of indomethacin 18% w/w). In the characterization study of chitosan-indomethacin conjugate, IR spectrum shows absorption band assigned of amide groups at 1660  $\text{cm}^{-1}$  which indicates that chemical reaction between chitosan and indomethacin has taken place and absorption band assigned of free amino groups at 3350  $\text{cm}^{-1}$  indicates that uncoupled amino groups are still present. The conjugate is being formulated in the form of capsules (each capsule contains 75 mg of indomethacin, to comply with the amount of indomethacin present in the available commercial products). In order to get this amount of indomethacin, 416 mg of the conjugate is used. The pH of the medium used for the *in vitro* release of the capsule dosage form is 7.4 and 8 which is similar to the pH of the fluid content of small and large intestine. Studies in acidic medium having pH 1.2 to 3 which is similar to the pH of stomach contents is excluded because indomethacin is a weak acidic drug and its solubility in mediums up to pH 5 is less than 0.9%.<sup>[22]</sup> The release of indomethacin from the prepared sustained release capsule has been compared with INDOMIN and INDO-75-SR (the dose of indomethacin in the three formulations studied is 75 mg), and the time required for complete release of indomethacin from the prepared dosage form is about 12 hours while 6 hours required for the complete release of indomethacin of the commercial INDO-75-SR. This difference in the release time reflects the difference in the process of formulation. Chemical bonds in our dosage form need hydrolysis of the drug from the polymer and diffusion while in INDO-75-SR indomethacin is coated by the polymer. INDOMIN, which is the immediate release form of indomethacin, the release is relatively rapid and completed within 2.5 hours. In all these formulations there were no significant differences in the release of the drug in the two pH (7.4 and 8) and this might be

due to the similarity of solubility of the acidic drug in these two pH.

**In conclusion**, preparation of chitosan from chitin is simple, cheap and feasible. Chitosan is a suitable material that can be used as a retardant in sustained release dosage forms using different types of drugs and a reliable sustained release indomethacin capsule using a natural chitosan in the form of covalently linked indomethacin has been confirmed.

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