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Amaricf-Basra office@yahoo.com
abdulalwan@yahoo.com
marshbulletin@yahoo.com

Isolation and Identification of Natural Polymers from the shells of Shrimp *Metapenaeus affinis* (H.Milne Edwards) Collected from Basrah Marshes South of Iraq

Z.N. AL-Sokanee[a]*, A.A. Mahdi[b] , A.S. Abdullah[c]

[a] *Department of Chemistry-College of Science- Basrah University.*
[b] *Marine Science Center- Basrah University.*
[c] *Department of Pharmaceutics-College of Pharmacy- Basrah University.*

Abstract

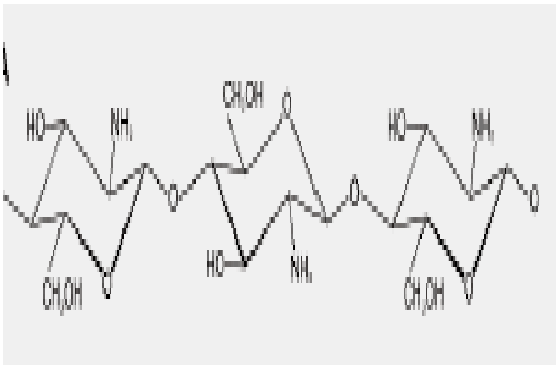
Shrimp *Metapenaeus affinis* , were collected from Basrah marshes. Chitin were isolated from shrimps shell and transformed to Chitosan by deacetylation and then cross linking with pectic acid by glutaraldehyde to formed a semi IPNs then loaded with Nalidixic acid to decrease the absorption of the drug on urinary tract and increasing the retention time of drug in the body and this modification lead to the use of the drug for infections other than urinary tract infections and decreasing the side effects of drug.

1.Introduction

The history of chitosan dates back to the nineteen century, when Rouget discussed the deacetylation form of chitosan in 1859 (Muzzarelli, 1977). during the past 30 years, a substantial amount of work has been published on this polymer and its potential use in various applications (Jaworska *et al.*, 2003). Recently, chitosan has been considered for pharmaceutical formulation and drug delivery applications (Amigi, 1996) in which attention has been focused on its absorption enhancing, controlled release and bioadhesive biodegradable properties (Dodane and Vilivalam, 1998).

*Corresponding author

Synthesised from a naturally occurring source, this polymer has been shown to be both biocompatible and biodegradable.(Berger *et al.*, 2004). Chitosan is a linear co polymer of β(1-4) linked 2-acetamido-2-deoxy- β-D-glucopyranose and 2-amino-2-deoxy- β-D-glucopyranose. It is easily obtained by deacetylation of chitin, a polysaccharide widely distributed in nature (e.g. crustaceous, insects and certain fungi) (Bugamalli *et al.*, 1998).



Structure of Chitosan

Due to the limited solubility of chitin in aqueous solutions chitosan is more suitable for industrial applications (Orient *et al.*, 1996).

The main parameters influencing the characteristics of chitosan are its molecular weight (MW)and degree of deacetylation (DD), representing the proportion of deacetylated units. These parameters are determined by the conditions set during preparation. Moreover, they can be further modified. For example the DD can be lowered by reacetylation and MW can be lowered by acidic depolymerisation (Patel and Amigi, 1996).

Chitosan is currently receiving a great deal of interest for medical and pharmaceutical applications. The main reasons for this increasing attention are certainly its interesting intrinsic properties. Indeed, chitosan is known for being biocompatible allowing its use in various medical applications such as topical ocular application, implantation or injection (Orient *et al.*, 1996). Moreover, chitosan is metabolized by certain human enzymes, especially lysozyme, and is considered as biodegradable.

In addition it has been reported that chitosan acts as a penetration enhancer by opening epithelial tight-junctions (Cervera *et al.*, 2004). Due to its positive charges at physiological pH, chitosan is also bioadhesive, chitosan also promote wound healing and has bacteriostatic effects (Amigi, 1998).

Hydrogels based on covalently cross linked chitosan can be divided into three types with respect to their nature. Chitosan cross linked with itself, hybrid polymer networks (HPN) and semi-interpenetrating polymer networks (IPN). The semi- IPN's contain a non-reacting polymer added to the chitosan solution before cross linking. This leads to the formation of cross linked chitosan network in which the non-reacting polymer is entrapped (semi-IPN). It is also possible to further crosslink. This additional polymer in order to have two entangled cross linked networks forming a full IPN, whose microstructure and properties can be quite different from its corresponding semi-IPN (Aieden *et al.*, 1997).

In this work we are isolated the chitin from shrimps shell and transformed to chitosan by deacetylation and then cross linked with pectic acid by glutaraldehyde and loaded with nalidixic acid to increase the retention of the drug for a long period and controls the release

of drug in the urinary tract and expanded the uses of drug to treat other infections by change the route of administration .

2.Materials and Methods

Shrimps shell, NaOH (BDH), pectic acid (Sigma),, HCl (Fluka), and Nilidixic acid .
I.R.spectroscopy , Pye Unicom SP3 ,
UV- visible, spectrophotometer (Shimadizu).

Isolation of chitin

The shells was granulated to fine powder, then treated at 10%HCl and stirred at room temperature for 24 hrs, washed with D-distilled water, dried and treated with 5% NaOH at 80C° for 3 hrs, washed with D-distilled water, dried and treated with H_2O_2 5%(v/v) for 5 hrs, filtered and washed with D-distilled water, dried in oven vacuum at 50C° and 0.1 mm Hg overnight, yield obtained was 60%.

Preparation of Chitosan

The chitin powder was treated with saturated solution of NaOH at 120C° for 3 hrs, repeated this process three times with freshly NaOH solution, filtered with D-distilled water and washed with 50:50 ethanol: acetone.

Preparation of Semi-IPN

Chitosan was dissolved in 0.1M acetic acid, the pectic acid dissolved in water, was added to the chitosan solution, the Glutaraldehyde was added with stirring in concentration 0.1% for 3hrs.

The feed mixture was allowed to gel for 24hrs at room temperature, followed by extensive washing with distilled water. The hydrogel was allowed to dry to a constant weight in room temperature. The same procedure was repeated with addition of proper amount of drug.

Drug content

The Semi- IPNs loaded the drug was treated with acid solution of 12 N HCl for 24 hrs (Anderson *et al.*, 1996) and the drug content was estimated in UV measurement each 1gm of matrix contain 0.5 M of drug.

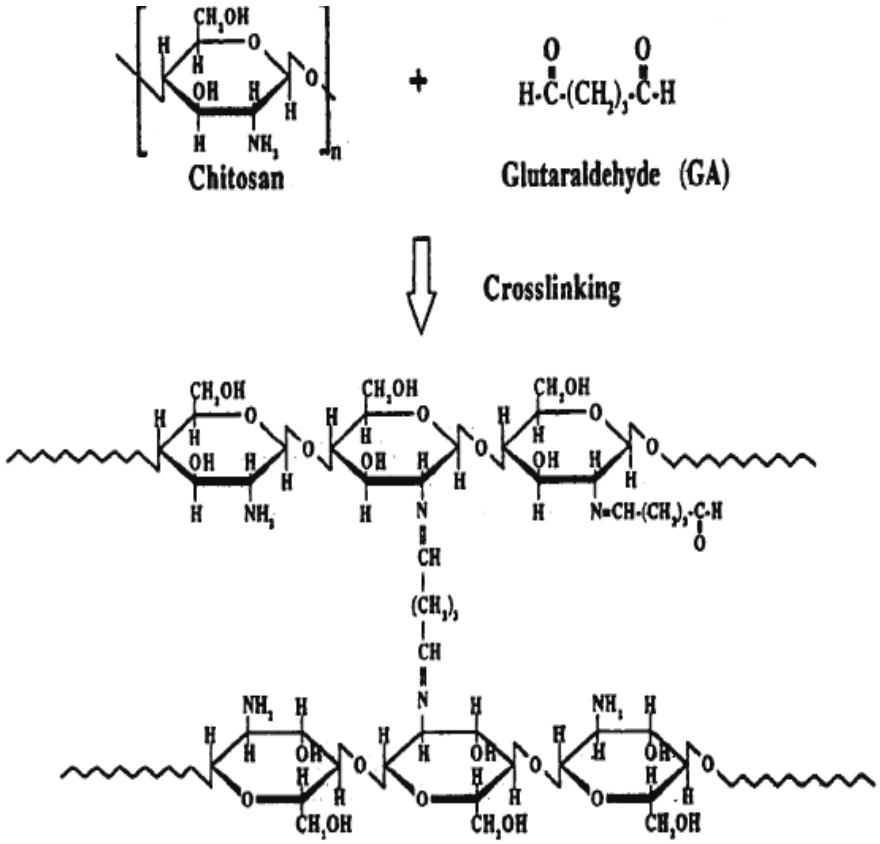
3.Results and discussion:

Chitin is the most abundant organic component of invertebrates, and have an average molecular weight of (1.5×10^5) (Zeke, 2000) . The conversion of chitin to chitosan take place in alkali aqueous media. NaOH by hydrolysis of (-NH-CO-CH_3)group to(-NH_2) of the repeating units. Chitosan is mostly in the form of primary aliphatic amino groups.

Chitosan therefore undergo typical reactions of amines, of which N- acylation and Schiff base reaction, the cross linking agent (glutaraldehyde) have the aldehyde groups reacted with amine groups of chitosan causes cross linked of polymer. Cross linkers are molecules with at least two reactive functional groups that allow the formation of bridges between polymeric chains. Dialdehydes allow direct reaction in aqueous media, under mild conditions and without the addition of auxiliary molecules such as reducers,. which is advantageous with respect to biocompatibility.

Although, a novel semi- interpenetrating networks(semi- IPNs)system was developed to provide a polymer networks as hydrogel for water uptake.

In this study the IPNs samples was pressed as a thin discs (10 mm diameter), the discs was immersed in 25 ml of Phosphate buffer solution [KH_2PO_4(2.14 g) Na_2 HPO_4(11.46g) in 1 liter of water] the solution contain sodium azide (0.03%,W/W) to prevent the growth of bacteria. (zake, 2000).



The drug concentration was determined at intervals of time from calibration curve, the drug concentration was measured at (258)nm using UV spectrophotometer. The drug release was evaluated using the following equation:

$$\text{Drug release(\%)} = \frac{\text{Amount of drug released}}{\textit{Total amount of drug loaded}} \times 100$$

The figures (1,2,3) show the drug release (Nalidixic acid) from chitosan-pectic acid IPNs hydrogel. From the drug release profile we can suggest that the drug release occurs mainly by the swelling follwoed by diffusion mechanism. The pH effect on drug release was investigated and we can see, at the pH =3 the release of the drug was increase when compared with the pH = 7.4 and pH =9 respectively. The pH sensetive swelling behavior of chitosan-pectin is lead to increase the release of drug on lower pH depened on ionization of glucose amine

residues by the hydronium ion that lead to electrostatic repulsion between like charges in adjacent polymer chains in chitosan-pectin matrix.

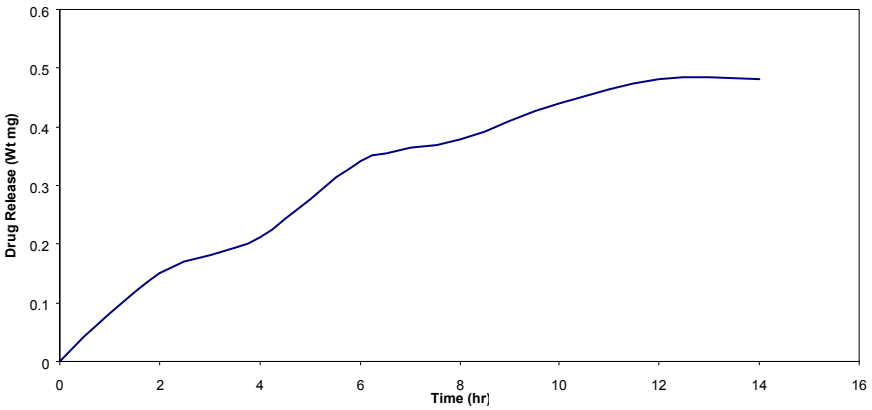


Fig.(1): The drug release (wt mg) at pH=3

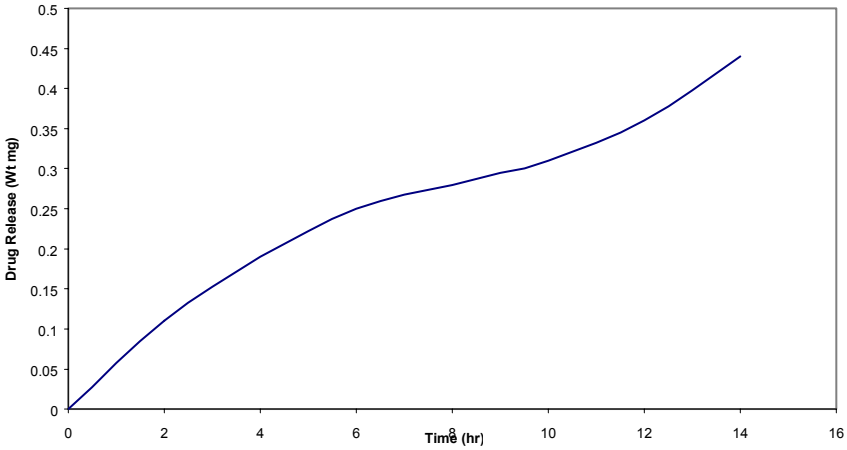


Fig.(2):Drug release (wt mg) at pH=7.4

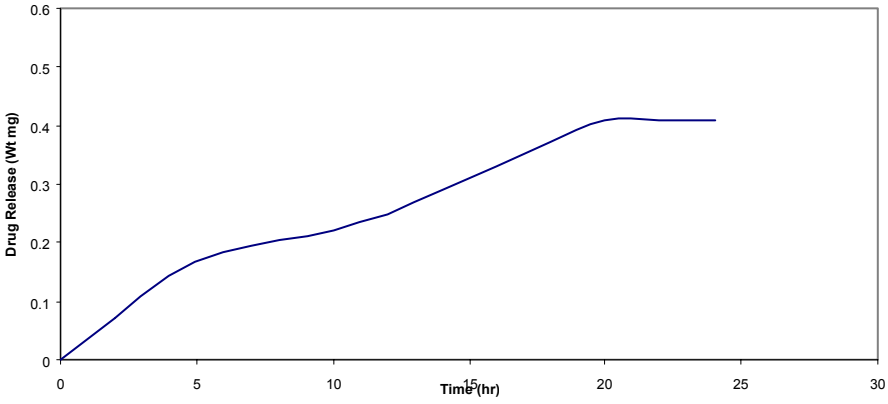


Fig.(3):Drug release (wt mg) at pH =9

This effect is important for the extensive swelling of the matrix in lower pH. The drug release at pH =3 shown the approximately 50% of the drug after 6 hrs at pH= 7.4 and pH= 9 this lead to concluded that the lower pH affect the release of drug depend on the chitosan positively charged amine unit and the drug release was completely after 14 hrs that give a good results to the drug release because the entrapped the drug on the polymer matrix decrease the passage of drug on urinary tract and lead to uses of nalidixic acid on other uses not for treatment of urinary tract infections.

A good release for the drug on the pH = 7.4 and the complete release after 14 hrs and the release of drug on pH =9 less than the other pH.

Swelling is mainly influenced by ionic interactions between chitosan chains, which depend on the crosslinking density set during the formation of the network. An increase in crosslinking density induces a decrease in swelling and pH-sensitivity, by improving the stability of the network and ,results in decreased drug release. However, in ionically crosslinked hydrogels the crosslinking density is further modified by external conditions after administration, mainly by the pH of the application medium. If the pH decreases, the charge density of the crosslinker and therefore crosslinking density decrease, which leads to swelling . Moreover, swelling is favored by the protonation and repulsion of chitosan free ammonium groups. If the pH decrease is too large, dissociation of ionic linkages and dissolution of the network can occur, leading to a fast drug release. If the pH increases, the protonation of chitosan decreases and induces a decrease of the crosslinking density, no allowing swelling. If the pH becomes too high, amino groups of chitosan are neutralized and ionic crosslinking is inhibited. On the other hand, a covalently crosslinked hydrogel does not exhibit swelling in basic conditions and as the crosslinking density does not vary in a covalently crosslinked hydrogel, swelling is less pronounced but dissolution is avoided. The incorporation of an additional polymer, whose hydrophilicity is different from chitosan, allows pH and ion-sensitive swelling in acidic conditions .The additional polymer (pectin) should perturb covalent crosslinking between chitosan chains, hence, decreasing crosslinking density and making available more protonable amino groups. This pH-sensitive swelling in an acidic environment allows the preparation of controlled drug delivery systems, the release from which is modulated by the crosslinking density and the pH of the medium. The mechanism of pH-sensitive swelling involves protonation of the amino groups of chitosan when the pH decreases[12]. This protonation leads to chain repulsion, diffusion of proton and counter-ions together with water inside the gel and dissociation of secondary interactions allowing swelling. This dissociation, together with increased hydrophilicity, can explain the higher swelling degree of a semi-IPN hydrogel, containing a hydrophilic polymer. Such systems present a higher versatility than hydrogels formed by chitosan crosslinked with itself, but

their higher swelling degree can lead to dissolution of the gel, as in the case of a semi-IPN containing a pectin.

4.References

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عزل وتشخيص بوليمرات طبيعية من قشور الروبيان من اهوار البصرة جنوب العراق

زكي ناصر السكيني* ، امل عبد الجليل مهدي و آسيا سلمان عبد الله*****

**قسم الكيمياء- كلية العلوم ، جامعة البصرة*
***مركز علوم البحار ، جامعة البصرة*
****فرع الصيدلانيات- كلية الصيدلة ، جامعة البصرة*

الخلاصة

تم استخلاص مادة الكايتين من قشور الروبيان المجموع من اهوار البصرة في جنوب العراق, تم تحويل الكايتين الى الكايتوسان بعملية deacetylation وتم مشابكة الكايتوسان مع حامض البكتيك بواسطة(الكلوتيرالديهايد) لتحويله إلى بوليمرات شبيهة شبكية التداخل ثم تحميله Nalidixic acidبدواء ( ) وان الهدف من عملية حجز الدواء داخل البوليمر هو لكي يقلل كمية طرح الدواء إلى خارج الجسم وتقليل امتصاصه من قبل الجهاز البولي ولاستعماله في مجالات أوسع ولعلاج أمراض أخرى ولتقليل الأعراض الجانبية للدواء.