Chitosan-Amine Oxide : A New Gelling System, Characterization and *in Vitro* Evaluations

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Homogeneous chitosan gel in amine oxide was prepared, which was almost transparent but slightly brownish. The resulting gel was expected to release the drugs at a sustained rate and the drug-release experiments were carried out using ampicillin trihydrate as a model drug. Chitosan-amine oxide gel is a novel approach for sustained dosages.

Chitosan [α-(1-4) 2-amino-2-deoxy-β-D-glucan] is a unique polysaccharide derived from chitin. Several attempts have been made to use this biopolymer in biomedical field[1]. The development of improved methods of drug delivery has received a lot of attention in the last two decades[2]. A few procedures have been published for making chitin gels[3,4], but detailed knowledge about chitosan gels is limited. There have been a few attempts to make hydrogels, such as thermoreversible chitosan-oxalate gels[5,6] and gels of chitosan-naphthalene sulphonic acid derivative[7]. Our approach has been to search for a non-toxic solvent which could directly give a gel at a certain temperature and whose degradation products are non-toxic and safe. The present paper contains preliminary results regarding synthesis, and *in vitro* evaluations of chitosan-amine oxide gel.

A highly purified prawn shell was obtained from Central Institute for Fisheries Technology, Kochi, India. N-methyl morpholine-N-oxide (NMMNO), known as amine oxide (Amines and Plasticizers, Mumbai, India) and ampicillin trihydrate (Pure Pharma, Indore, India) were used without further purification.

Chitin flakes (0.95 g) obtained by standard procedure[8] were deacetylated using 10 ml of 50% aqueous NaOH at 110° for 1 h. The upper layer was decanted and the flakes were washed with water until neutral. This procedure was repeated twice with the same amount of aqueous NaOH. The resulting chitosan flakes were washed with water and dried at 60° for a week. The experimental result of chemical analysis for chitosan agreed well with calculated one.

A mixture of 200 mg chitosan and 4 g amine oxide were kept for 48 h at room temperature and then heated to 120° for about 45 min. The resulting chitosan gel was then allowed to cool to ambient temperature. The almost transparent and brownish gel obtained was insoluble in water and in common organic solvents and is supposed to be highly crosslinked. Fifty milligrams of ampicillin trihydrate was dissolved in 2 ml phosphate buffer of pH 7.4, added to a mixture of chitosan and amine oxide and then heated with stirring until the gel formation was completed. This method of preparation ensures homogeneous distribution of the drug in the matrix gel[9].

Elemental analysis was carried out with Heraeus Carlo Ebra 1108 elemental analyser. FTIR spectra were recorded on Shimadzu 8000 spectrophotometer. Swelling tests was conducted in a buffer solution of pH 7.4 at room temperature. The degree of the swelling at time t was calculated by the following expression.

$$(W_t-W_o)/W_o \times 100\%$$

where W_t was W_o are the weights of samples at time t and in the dry state, respectively.

Before performing an *in vivo* experiment, the same

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experiment was carried out under an *in vitro* condition. Hence, for the study of *in vitro* drug release the drug loaded polymer was placed in 100 ml phosphate buffer of pH 7.4 without stirring. The release of the drug was determined by taking out an aliquot of 0.1 ml at suitable time intervals and measuring the absorbance after appropriate dilution at the λ_{max} of the drug. Concentrations of the released drug were then determined by comparing the absorbance with standard curves prepared for the pure drug in the buffer.

The solubility of chitosan was tested in many solvents and it was found to swell in amine oxide (NMMNO), but soluble in hexafluoroisopropanol and hexafluoroacetone. It is also slightly soluble in dilute acids but not capable of forming gels[10]. The degree of swelling of the amine oxide gel in a pH 7.4 buffer solution at room temperature is shown in Fig. 1. The degree of swelling of the gel began to decline after the maxima was attained, which may indicate the dissolution of the gel exceeds the swelling. This was confirmed by UV spectroscopy.

Figure 2 shows the FTIR spectra of (a) chitosan and (b) chitosan gel. The peaks at 1651 and 1550 cm^{-1} in the spectrum of chitosan [Fig. 2 (a)] can be assigned to the amino groups[11]. There is a significant new peak at 1661 cm^{-1} in the spectrum (b) which can be attributed to the formation of N = O due to the reaction between amino groups of chitosan and amine oxide. The peaks at 1074 and 1079 cm^{-1} in the spectra (a) and (b) are due to C-O stretching vibration in chitosan and gel, respectively.

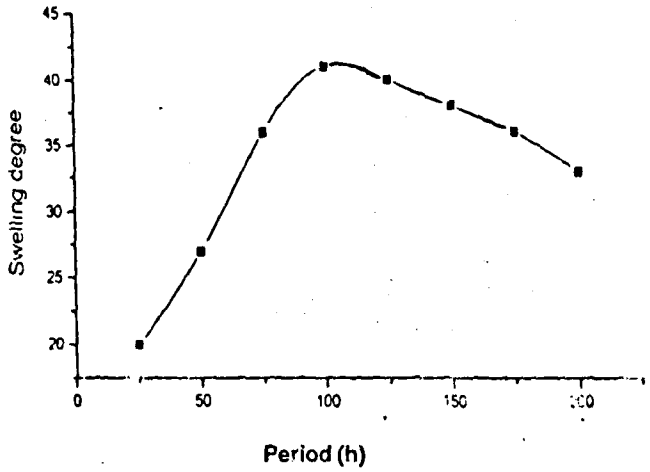

Fig. 1 : Swelling of chitosan gel in pH 7.4 at room temperature

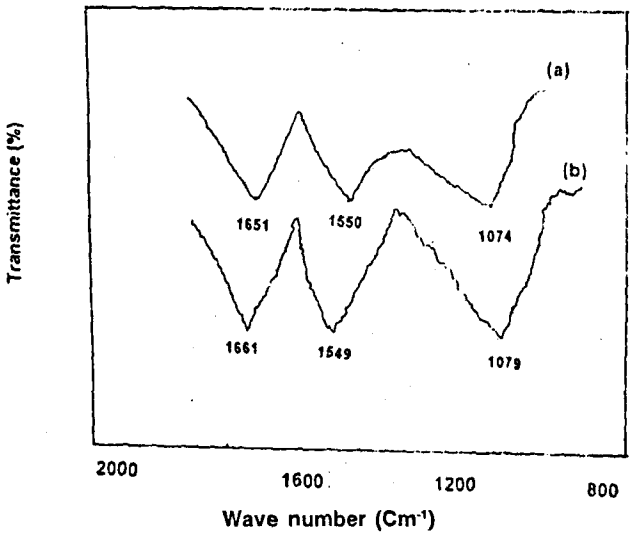

Fig. 2 : FTIR spectra of (A) chitosan and (b) chitosan gel

Further studies are in progress to establish complete data in support of gel structure.

The cumulative release from of the drug loaded matrix (1 g) containing approximately 11.5 mg of the drug is shown in Fig. 3. The cumulative amount of the drug released from the chitosan-amine oxide gel increased linearly with time and the drug was released from the gel at almost a constant rate for 3 days. Chitosan - amine oxide gel appears to possess the requirements to serve as a medium for the release of ampicillin trihydrate.

Chitosan is a microporous material. When a microporous polymer is below its glass transition temperature, the thermal movements of the chain segments are restricted in such a way that pores result from irregularities in molecular packing[12]. Because of the relatively bulky chitosan chains, pores are large enough to let small molecules and ions pass through.

During the adsorption of materials in solution on porous adsorbents, there are essentially three consecutive stages involved[12], transport of the adsorbate to the external surface of the adsorbent and then into the pores of the adsorbent and adsorption of the solute on the internal surface of the adsorbent. This last stage is relatively rapid and, if the stirring is made sufficiently fast, the adsorption rate will be controlled by the rate of diffusion of the solute into the capillary pores of the adsorbent. So, when the drug is taken along with chitosan and amine oxide, it is stirred well to ensure complete absorption of drug molecules into the pores of chitosan. The mecha-

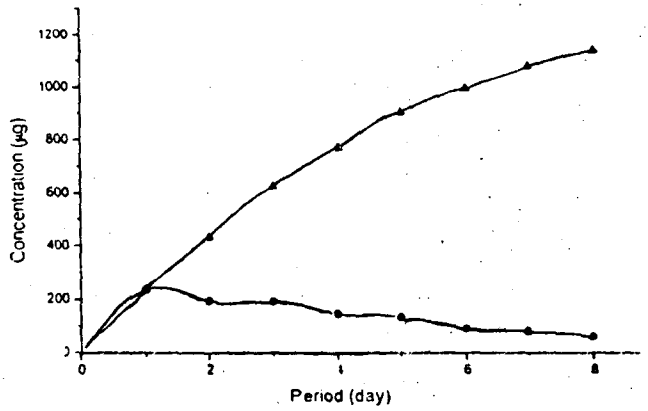

Fig. 3 : Release of ampicillin trihydrate from chitosan gel in pH 7.4 phosphate buffer. Per day release was shown on —●— and cumulative release as --▲—

nism of drug release may be due to diffusion through swollen gel in the pH-controlled solution[13,14].

The release of drugs from the gels depends on their structure or their chemical properties[11,15] in response to environmental pH. In pH 7.4, after the maximum swelling is attained, the degree of swelling of the gel begins to decrease, which may indicate dissolution of chitosan from the gel[14,16,17]. The swelling of crosslinked chitosan-amine oxide gel is dependent on the protonization of amino groups and dissociation of hydrogen bonding within the network, which is related to the pH of solution.

The size of the polymer matrix is found to decrease gradually with time, and towards the end of the drug release, the matrix disintegrates into pieces[18]. This indicates that the erosion takes place from the surface as well as from the bulk of the matrix. The homogeneous erosion of the matrix and nearly zero-order release kinetics of the drug suggests drug release by a combination of both diffusion and erosion[18].

The decreasing rate with time (Fig. 3) expected in a purely diffusional release due to increasing diffusion path counterbalanced by an increasing diffusion coefficient of the drug due to increasing polymer permeability resulting from gradual erosion of the matrix by crosslink cleavage. Thus a zero order release becomes a reality[19]. A slightly higher rate of release in one day arises from the fact that the drug particles near the surface go into solution as soon as the matrix is placed in the buffer and that the initial rate of penetration of the swelling interface is very high. Such high initial rate of drug release has also been observed for other systems[13,14,15].

In conclusion, chitin derivatives are polysaccharides that hold promise for medical use because they are biocompatible. Chitin derivatives including partially acetylated chitosan can be easily molded into various forms and are digested *in vivo* by lysozomal enzymes. The *in vitro* release of ampicillin trihydrate from chitosan-amine oxide gel was very slow. Thus this material can be a very interesting candidate for use as a carrier of a variety of drugs for controlled-release applications. Further work of *in vitro* studies of ampicillin trihydrate from chitosan-amine oxide gel is now in progress.

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Validation of Antifertility Activity of Various Rubus Species in Female Albino Rats

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Alcoholic extracts of leaves of various species of Rubus, *Rubus ellipticus, R. niveus, R. racemosus* and *R. rugosus var.* thawaitesii (Rosaceae) were tested for antifertility activity in female Wistar albino rats. The results indicate decreased implantation sites and increased resorption sites, which denotes antiimplantation and early abortifacient activities of Rubus species. The results are in agreement with the traditional use of this plant as abortifacient by the tribals of Nilgiris.

Use of plant preparations by ancient physicians of India for pregnancy interception is evident from the available reports and reviews[1,2]. One such plant is *Rubus ellipticus* (Rosaceae) a shrub found in Nilgiri hills[3]. The roots and shoots of *Rubus ellipticus*, commonly known as "Zardanchu" on "Hinsalu" were reported to have therapeutic uses in the conditions like colic pain, diabetes and hyperthermia[4]. A recent report indicates antiprotozoal activity of this plant against *Entamoeba histolytica*[5]. The antifertility activity of *R. ellipticus* has been reported in Ayurvedic and Unani literature[6]. Whereas, Sharma *et al*[7] reported antiimplantation activity in roots and aerial parts, ethnomedically, root decoction of *R. ellipticus* were used to treat diarrhoea and dysentry[8,9] and also as antifertility agent[10]. Other Rubus species *R. niveus, R. racemosus* and *R. rugosus var. thawaitesil* (Rosaceae) has been used widely by tribals of Nilgiris for abortifacient action. Validation of these plants for its activity are not available in the plethora of literature. Since antifertility activity of this genus has been reported in *R. ellipticus*, the present study was undertaken to find out the antifertility activity of other unexplored Rubus species available in Nilgiri hills including Rubus ellipticus.

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Fresh leaves of various species of *Rubus viz., Rubus ellipticus, R. niveus, R. racemosus* and *R. rugosus var. thawaitesil,* family Rosaceae were collected from different parts of Nilgiri hills. The leaves were dried under shade, mechanically reduced to a coarse powder and then extracted with Petroleum ether (60-80°) to remove fatty matter. Then subjected to hot continuous extraction in Soxhlet apparatus using ethyl alcohol (90%). Extracts were concentrated below 60° and further drying was carried out under reduced pressure. The dried extracts were used for pharmacological screening. Overnight fasted albino mice of either sex (24-30 g) were used to evaluate behaviour and toxicity studies. Ethanolic extracts were administered orally as a fine suspension in PEG 400 at a graded dose level (1000 mg to 5000 mg/kg). Behavioural changes were observed for 4 h, and mortality rate was recorded after 72 h[11].

Proven fertile male and female Wistar strain albino rats (150-200 g) were used for antiimplantation and early abortifacient studies. The rats were maintained at room