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# USE OF CHITOSAN–DMAc/LiCl GEL AS DRUG CARRIERS

**P.K.DUTTA*[1], M.K.KHATUA[2], JOYDEEP DUTTA[2] and R.PRASAD[2]**

[1]Department of Chemistry, Motilal Nehru National Institute of Technology, Allahabad–211 004 (U.P.)
[2]School of Chemical Sciences, Devi Ahilya University, Indore 452017 (M.P.)

## ABSTRACT

The suitability of chitosan for use as a vehicle for the sustained release of drug was examined. Cefaclor monohydrate was used as a model drug in this investigation. For the study of sustained release of drug, a gel of chitosan–DMAc/LiCl was prepared. Drug dispersed in the chitosan gel was released at a constant rate (zero order). Chitosan–DMAc/LiCl gel could be a useful vehicle for the sustained release of drugs.

**Key words :** Sustained release, Chitosan, Cefaclor monohydrate, DMAc/LiCl, Drug carrier

## INTRODUCTION

Sustained release technology used for pharmaceuticals is now the subject of intensive research[1,2]. Sustained release polymer matrix systems offer a number of potential advantages over the conventional means of application[3,4]. The principal advantage of the sustained release systems is that they allow much less active agent to be used for the desired activity[5,6]. The applicability of natural polysaccharides such as agar, konjac, pectin in the design of dosages for sustained release has been examined elsewhere[7]. Chitin is the most abundant natural polysaccharide just after cellulose[8]. Chitin is basically a polyacetyl amino glucose and when it undergoes deacetylation – the very well known polysaccharide, chitosan is formed. Chitosan is poly (1→4)–β–D–amino glucose and it is much more versatile due to the presence of amino group in comparison to cellulose[9]. The molecular structure of chitosan consists of glucasamine repeating units which allow chain–packing by intermolecular and intramolecular hydrogen bonding[10]. Such strong interactions are responsible for excellent inherent mechanical properties like cellulose, yet at the same time, interface with efforts to process or modify the material is not easy. Controllable, uniform derivatization has been hampered by the lack of suitable, nondegrading solvents or by a limited range of synthetic reactions within these solvents.

In 1977, the dissolution of chitin in N,N–dimethylacetamide (DMAc) and lithium chloride (LiCl) was first reported by Austin[11]. Also a number of synthetic reactions which could be carried out in a facile manner under homogeneous reaction conditions were described. Since then, a number of studies have reported on the utility of the DMAc/LiCl solvent for chitin as

* Author for correspondence

well as cellulose reactions, characterization, and processing. Successful dissolution of cellulose as well as chitin by organic complexes have been limited. DMAc forms complexes with LiCl which in turn is a solvent for chitosan. The nature of complex, the mechanism of dissolution, and utilization of the solvent system is well documented by various researchers[12–17]. The DMAc/LiCl solvent clearly provides a number of potential applications in diversified fields. The current applications in selective membranes, controlled release of bioactive agents are noteworthy. Since chitosan does not present any biological hazard and is inexpensive, this polymer might be suitable for use in the preparation of sustained release dosage form. In this communication, the suitability of chitosan–DMAc/LiCl homogeneous gel as vehicle for sustained release of a model drug was examined.

## EXPERIMENTAL

Cefaclor, was obtained from Lupin Laboratories, Aurangabad, India. Cefaclor comes under cephalosporin group and it is a second generation antibiotic. Chitosan was a gift sample from the Central Institute of Fisheries Technology, Cochin, India. The viscosity –average molecular weight was $9.2 \times 10^5$ and the N–deacetylation degree was 87% and it was used after passing through a 42 mesh screen. Dimethyl acetamide (DMAc) and lithium chloride (LiCl) AR grade) were obtained from BDH, India. All other chemicals were of analytical grade.

### Preparation of chitosan from prawn shell[9] :

The purified prawn shell was reduced in a hammer mill to a particle size of about 600 μm. The preparation involves the following steps.

**(i) Demineralization** The prawn flakes were treated with 1.25 M HCl for 1 h at room temperature, at a ratio of 1g/6 mL acid. The spent liquor was decanted and the prawn flakes were washed with water until neutral.

**(ii) Deproteinization** The prawn flakes, 0.96 g were soaked in 3 mL of NaOH (3%, w/v) in a resin kettle. The mixture was heated to between 90 and 100°C with constant stirring for 0.5 h, the brown layer of NaOH was decanted and flakes were washed with water. The above procedure was repeated twice with the same amount of caustic soda. The solid material left in the kettle was chitin.

**(iii) Conversion of Chitin into Chitosan** Chitin flakes were deacetylated using 10 mL NaOH (50%, w/v) (at a ratio of 1 $kg/dm^3$) in a resin kettle at a 110°C for 1 h. The NaOH layer was decanted and the flakes were washed with water until neutral. This procedure was repeated twice with the same amount of caustic soda. The resulting chitosan flakes were washed with water and dried in an oven at 60°C for 1 week.

## Preparation of chitosan–DMAc/LiCl gel

200 mg chitosan and 4 g DMAc/LiCl (5%, w/v) were taken in a beaker, kept for 48 h at room temperature and then heated to 120°C for about 1 h. A gel of chitosan was obtained. The chitosan gel was then allowed to cool at the ambient temperature. The gel was almost transparent, brownish and rubbery. The gel so prepared was insoluble in water and in common organic solvents and is expected to be highly crosslinked because of polar–polar interaction.

## Drug dispersion technique

375 mg of cefaclor was added to the mixture of swelled chitosan and DMAc/LiCl gel with uniform stirring (100 rpm). Thus the drug was homogeneously distributed in the matrix gel, at temperatures of 35 – 40°C.

## Swelling property

Swelling test was conducted in buffer solution of pH 7.4 at room temperature. The degree of swelling at time t was calculated by the following expression[18].

$$(W_t - W_0)/ W_0 \times 100\%$$

Where $W_t$ and $W_0$ are the samples weight at time t and in the dry state respectively.

## Photomicrographic study

The micrographic study of the drug dispersed in the matrix was studied Scanning Electron Micrography (SEM).

## Determination of *in vitro* drug release

*In vitro* drug release was tested by following method:

## Instrumental set up

A USP apparatus 1 with rotational speed 100 rpm and the solution medium: (1) 0.1N HCl (2) Buffer pH 7.4; sampling time:stage 1 (acid stage) 1 h, stage 2 (buffer stage) 6,8 and 12 h were used through out the experiment. The measurements were made by a UV spectrophotometer, Shimadzu, Japan at $\lambda_{max}$ =264 nm.

## Assay study

The assay was determined by HPLC. The mobile phase was prepared as follows:

1 g of sodium1–pentanesulfonate was dissolved in a mixture of 780 mL of water and 10 ml of triethylamine and pH was adjusted with phosphoric acid to 2.5. 220 mL methanol was added. About 15 mg of USP cefaclor RS was transferred and diluted with mobile phase upto 50 mL. About 15 mg of cefaclor was transferred and diluted with mobile phase upto 50 mL in order to

check the resolution, a solution in mobile phase containing about 0.3 mg of cefaclor and 0.3 mg of USP Delta–3–cefaclor RS per mL was used. The chromatographic system was followed by the liquid chromatograph equipped with a 265 nm detector and a 4.6mm x 25 cm column containing 5 %m packing L1. The flow rate was about 1.5 mL per min. The relative retention times for cefaclor and delta–3–cefaclor were about 0.8 and 1.0, the resolution, R, between the cefaclor peak and the delta–3–cefaclor peak was not less than 2.5, the tailing factor not more than 1.5, and the relative standard deviation for replicate injections was not more than 2%.

## RESULTS AND DISCUSSION

The solubility of chitosan was tested in many solvents. It was found to swell in DMAc/LiCl, and dissolved in hexafluoroisopropanol and hexafluoroacetone. It is also slightly soluble in dilute acids but not capable of forming a gel[7].

The chitosan–DMAc/LiCl gel formation took place at a temperature of 120°C. Chitosan itself is microporous material. Even after dissolution and heat treatment upto 120°C in DMAc/LiCl the microporous structure was not disturbed and a gel formation took place.

The strong Lewis acid character of LiCl, which results in the tight binding of the $Li^+$ to the DMAc carbonyl, leaves the chloride ion free to form hydrogen bonds in the solution. Spectroscopic evidence, from studies of cellulose, chitin, and model compounds, supports the contention that the $Cl^-$ is stabilized by association with any available hydroxyl groups in the polar aprotic medium[14–15,19].

So, chitosan was stirred well with DMAc/LiCl to ensure complete absorption of drug molecules into the pores of the chitosan, resulting in sustained release. In the present case DMAc/LiCl was used since it is a universally accepted non–toxic solvent[20].

The swelling degree for gel swollen in pH 7.4 at room temperature is shown in Fig.1. The swelling degree of the gel begins to decline after the gel was swollen for some time, which may indicate the dissolution tendency of the gel exceeds the swelling degree. This can be confirmed by UV spectroscopy[18].

Fig.2 shows the structure of the surfaces of the DMAc/LiCl chitosan gel (Fig.2a) and drug dispersed in the gel (Fig.2b). The surfaces show the structural integrity in both of the gels. The drug dispersed in the gel homogeneously , which in fact, is reflected in the Fig.2b. The morphology of Fig.2b appears to be different from that of the chitosan due to the above mentioned aspects.

*In vitro* drug release from matrix gives an idea of its ability to function as sustained and controlled release delivery system and its knowledge is a prerequisite for studying its *in vivo* performance. The release of drug from the gel depends on their structure and its chemical properties. The release of drug in 1st hour at pH 1.2 is maximum whereas the total release of drug is caused by the gel deforming ability of chitosan in pH 7.4. The size of the polymer matrix

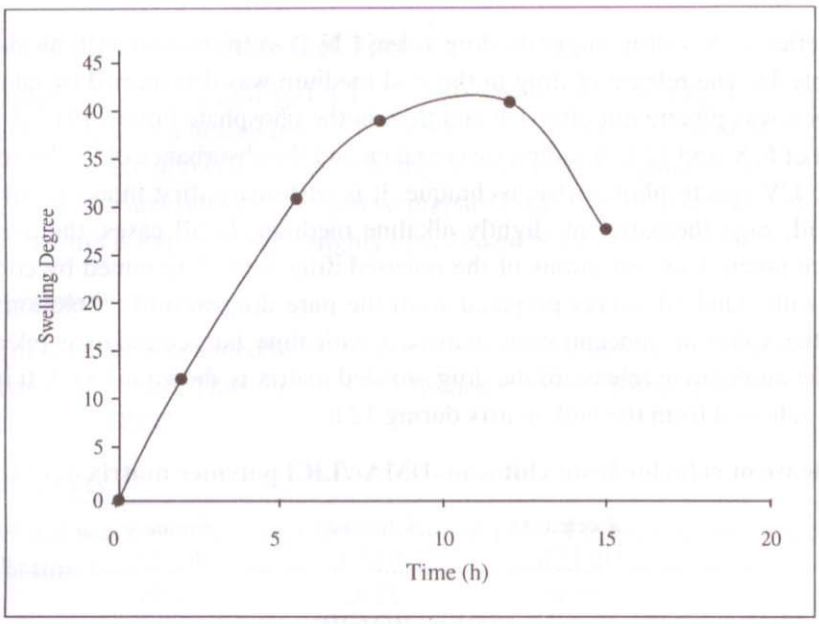


**Fig. 1. Swelling of initial dry gel measured as function of time in pH 7.4 at room temperature**

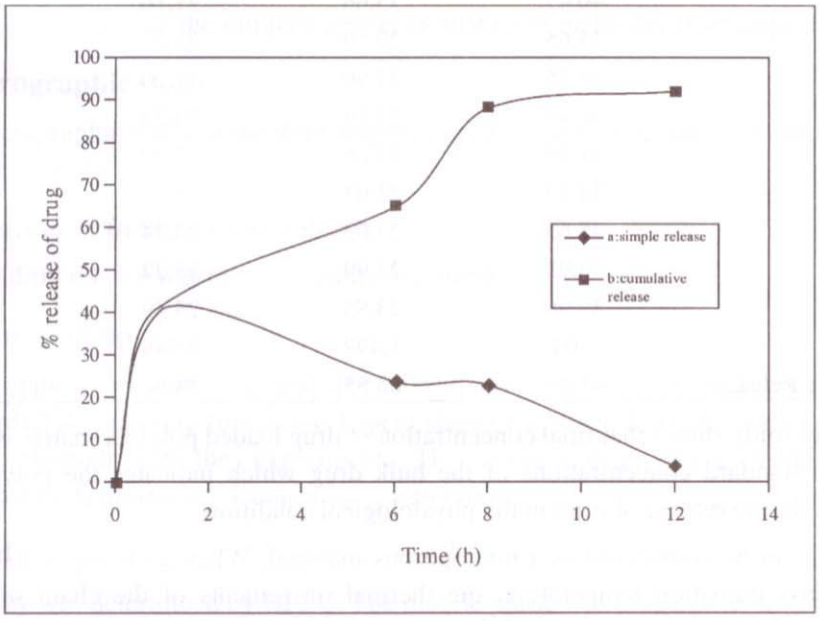


**Fig. 2. Plot of % release of drug over time**

is found to decrease gradually with time and towards the end of the drug release the matrix disintegrates into pieces. This indicates that the erosion takes place from the surface as well as from the bulk of the matrix[21]. The homogeneous erosion of the matrix is nearly zero–order

release. Kinetics of the drug suggests drug release by a combination of both diffusion and erosion (Table 1). The release of drug in the acid medium was determined by taking 3 mL of solution which was pipette out after 1 h and then in the phosphate buffer, pH 7.4 keeping the time interval of 6, 8, and 12 h, 4 samples were taken and the absorbance of all the solution were measured by UV spectrophotometric technique. It is customary, first intake of any food stuff comes in acid stage thereafter in slightly alkaline medium. In all cases, the average of six readings were taken. Concentrations of the released drug were determined by comparing the absorbance with standard curves prepared from the pure drug in buffer solution. The result shows that the value of concentration increased with time i.e., cumulative release of drug increases. The cumulative release of the drug –loaded matrix is shown in Fig.3. It is found that 92% of drug released from the bulk matrix during 12 h.

**Table 1. Release of cefaclor from chitosan–DMAc/LiCl polymer matrix over time**

| | % release in $1^{st}$ h | % release in $6^{th}$ h | %release in $8^{th}$ h | % release in $12^{th}$ h |
|---|---|---|---|---|
| | 39.46 | 22.32 | 24.55 | 3.50 |
| | 43.11 | 21.10 | 25.66 | 3.04 |
| | 40.47 | 25.66 | 21.10 | 3.75 |
| | 45.65 | 20.19 | 22.21 | 4.01 |
| | 38.75 | 27.90 | 21.20 | 3.60 |
| | 41.49 | 27.19 | 24.24 | 4.34 |
| AVG. | 41.49 | 24.06 | 23.09 | 3.71 |
| MIN. | 38.75 | 23.03 | 24.55 | 3.50 |
| MAX. | 45.65 | 23.03 | 24.24 | 4.34 |
| MEDIAN | 40.98 | 23.99 | 23.22 | 3.67 |
| MODE | 39.96 | 23.85 | 23.49 | 3.60 |
| STND.DEV. | 3.04 | 1.492 | 0.549 | 0.556 |
| % Cumulative Release | 41.49 | 65.55 | 88.64 | 92.35 |

The assay study shows that final concentration of drug loaded polymer matrix is near about 1.6 times of standard concentrations of the bulk drug which indicates the polymer matrix disintegrates during release of drug in the physiological condition.

Chitosan can be considered as 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 packaging[22]. 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[22]; transport of the adsorbate to the extent surface area 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 along with chitosan and DMAc/LiCl, it is stirred well to ensure complete absorption of drug molecules into the pores of chitosan. The mechanism of drug release may be due to diffusion through swollen gel[23,24].

The possible mechanism of drug release from chitosan matrix is therefore be summarised as (1) initial release from the micro–sphere surface i.e., drugs which are either loosely bound to the surface or embedded in the surface; (2) release through the matrix pores which depend upon the matrix structure; (3) diffusion through the intact polymer barrier i.e., this would be dependant on the intrinsic polymer properties and core solubility in the polymer membrane; (4) diffusion through a water swollen barrier i.e., this would be dependent on the polymer hydrophilicity, which in turn depends upon polymer molecular weight; (5) polymer erosion and bulk degradation i.e., release would be affected by the rate of erosion and hydrolysis of the polymer chains, leading to the pore–formation in the polymer matrix.

All these possible mechanisms together may play a part in the release process, depending on the nature of the core, the polymer and the final matrix structure. Thus, it is practically impossible to generalise the release kinetics from these polymer matrix. However, several workers attempted to correlate the *in vitro* release data to fit into mathematical model.

Release kinetics for homogeneous spherical matrix followed by Higuichi model[25] is represented by the equation :

$$100 - m = K^* \sqrt{t}$$

where, m = % of drug released; K= dissolution rate constant, t = the time of dissolution.

Release of drug from matrix containing dispersed solid has also been described by Deasy[26].

$$Q = m/a + [\, D\, C_m^2\, (\, C_{tot} - C_s\,)t]^{1/2}$$

Where,

| | | |
|---|---|---|
| Q | = | mass (m) of the drug released /unit area (a) of surface at time, t |
| $C_m$ | = | concentration in matrix |
| $C_{tot}$ | = | total solid concentration as drug loading |
| $C_s$ | = | total solubility of the solute |
| D | = | Diffusivity |

Assuming that diffusivity and other parameters are kept constant, the equation may be simplified as

$$Q = Kt^{1/2}, \text{ where K is a constant}$$

The power law equation for diffusion control release mechanism has been derived by Korsmeyer *et al.*[27] The equation representing their model is

$$M_t/M = Kt^n$$

Where, $M_t/M$ is fraction of drug released, t = time, K = constant. K includes structural and geometrical characteristics of the microsphere and n is the release exponent, which is indicative of drug release mechanism.

Release kinetics model for homogeneous spherical matrix has been determined by Baker[28].

$$2/3\ [1\ -(f)^{2/3}\ ] = K_3{*}t$$

Where, f = fraction of drug released, t = time, $K_3$ = Constant

Zero order release was found to be inapplicable to the data for all the batches in the above model since drug release was non–linear and the release characteristics was not by single mechanism. The principle of diffusion, surface erosion and matrix dissolution were mixed mechanism which were responsible for the drug release in the dissolution medium.

The release of drugs from the gels depends on their structure or their chemical properties in response to environmental pH[18,29]. In pH 7.4, the swelling degree of the gel begins to decline, which may indicate the dissolution tendency[24,30–31] of chitosan from the gel. The swelling of crosslinked chitosan–DMAc/LiCl bulk matrix is dependent on the protonization of amino groups and dissociation of hydrogen bonding within the network, which is related to solution pH. The size of the polymer matrix is found to decrease gradually with time, and towards the end of drug release, the matrix disintegrates into pieces[32]. This indicates that the erosion takes place

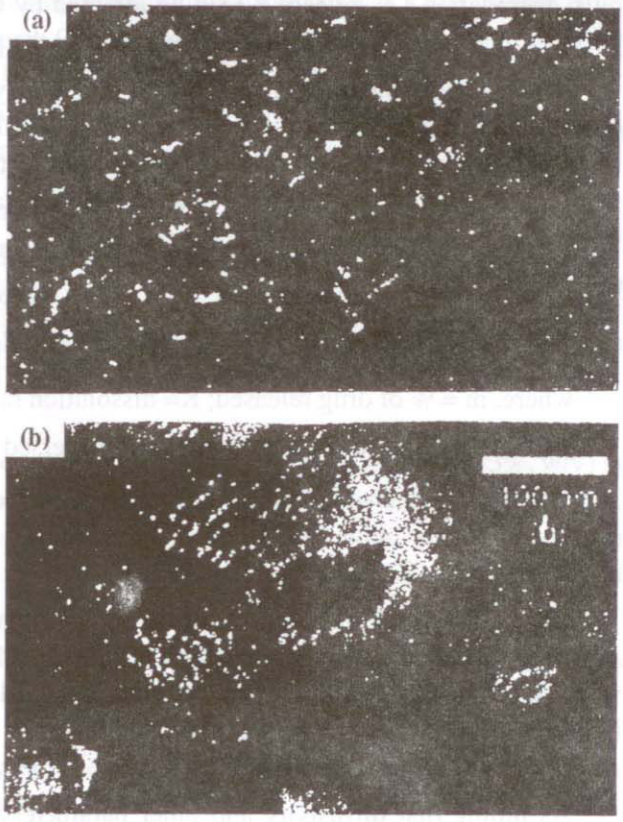


**Fig. 3. Scanning electron micrographs of the surfaces of the gels: (a) chitosan-DMAc/LiCl; (b) drug dispersed in chitosan-DMAc/LiCl**

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 suggest release by a combination of both; diffusion and erosion[32]. The decreasing rate (Fig.3a) with time expected in a purely diffusional release due to increasing diffusion path is counterbalanced by an increasing diffusion coefficient of the drug due to increasing polymer permeability resulting from gradual erosion of the matrix by crosslink cleavage. The release rate decreases with time and polymer matrices exhibit first order release (i.e., rate $\sqrt{t}$) which should be zero order/constant ideally[33]. The reason for fall in release rate includes increasing diffusional distances with time and high initial release[34] also known as "burst effect". A slightly higher rate of release in the 1$^{st}$ h arises from the fact that the drug particles near the surface go into solution as soon as the matrix is placed from the acid medium to 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[24, 35–36].

Thus it can be concluded that chitosan a polysaccharide derived from chitin can hold promise for medical use because it is biocompatible. The *in vitro* release of cefaclor from chitosan–DMAc/LiCl gel was found to be effective for 12 h or might be more. Thus this material can be a very interesting candidate for the release of several drugs in sustained release applications. Further work on comparative study of market available modified release cefaclor with chitosan – DMAc/LiCl matrix based cefaclor is now in progress.

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