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RESEARCH PAPER

Preparation and In Vitro Evaluation of Chitosan Matrices Cross-Linked by Formaldehyde Vapors

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ABSTRACT

Rifampicin-chitosan matrices were prepared by a chemical cross-linking method to develop a sustained-release form. The effects of cross-linking agent (formaldehyde) on the drug release rate and release kinetics were investigated in this study. Moreover, the kinetics of rifampicin released from chitosan matrices exposed to formaldehyde vapors for predetermined time intervals were analyzed using Ritger and Peppas exponential equation. The in vitro release kinetics exhibited a non-Fickian transport model. Increasing the exposure time to formaldehyde vapors decreased the release rate of rifampicin from chitosan matrices as a result of formation of greater structural strength and tighter texture.

Key Words: *Chitosan; Formaldehyde; Matrices; Release models; Rifampicin.*

INTRODUCTION

Tuberculosis is still one of the most important infectious diseases worldwide. Most of the antitubercular drugs have been known to produce toxic side effects (1). The long period of treatment with conventional drug delivery systems often leads to discontinuation of treatment by a substantial proportion of patients as soon as they begin to feel better (2). In the last several years, many different types of rifampicin controlled-release formulations have been developed to improve clinical efficacy of the drug and patient compliance (3–11).

Rifampicin is a first-line drug recommended by the World Health Organization (WHO) in the treatment of tuberculosis. Relatively high doses of the drug are required to maintain therapeutic concentrations for longer periods, which leads to several side effects (12). Because of its high cost and adverse side effects, it is used mainly in intermittent therapy (13,14). In the present investigation, the drug was directly compressed with chitosan, and

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the tablets were exposed to formaldehyde (cross-linking agent) vapors. The effects of cross-linking agent on the drug release and release kinetics were studied.

The use of natural hydrophilic polymers as drug carriers has received considerable attention in the last few years, especially from cost, biocompatibility, and environmental concerns. The biopolymer chitosan, β-(1 → 4)-2-amino-2-deoxy-D-glucose, is prepared by *N*-deacetylation of chitin, one of the polysaccharides widely distributed in nature as a principle component of the shells of crustaceans and insects (15). It has structural characteristics similar to glycosaminoglycans. It is tough, biodegradable, and nontoxic. Chitosan has reactive hydroxyl and amino groups that can be modified chemically for various biomedical and pharmaceutical applications (16,17).

MATERIALS AND METHODS

Materials

Rifampicin I.P. was obtained from Indoco Remedies Limited, India. Other materials were chitosan powder (60 mesh), formaldehyde solution I.P., potassium dihydrogen orthophosphate, sodium hydroxide, and ascorbic acid; all were analytical grade.

Preparation of Matrices

Different ratios of rifampicin and chitosan sufficient for a batch of 30 tablets were mixed thoroughly to ensure complete mixing. The mixture was compressed into tablets using 11-mm flat and plain punches (surface lubricated with talc) on a single-stroke tableting machine (Cadmach Machinery Co., Pvt. Ltd., India). The amount of drug in each tablet was 300 mg.

Tablets were exposed to formaldehyde vapor in a desiccator containing a solution of formaldehyde at the bottom. After exposure for predetermined time intervals, the tablets were removed from the desiccator, exposed to air to remove adhering free formaldehyde and moisture, and finally dried in a vacuum desiccator over fused calcium chloride.

Estimation of Formaldehyde

A vapor-hardened tablet was powdered and transferred to a 100-ml volumetric flask; 30 ml of 0.1 N hydrochloric acid was added, and the solution was held overnight. Then, to dissolve the remaining portion, the volumetric flask was heated in a water bath, then cooled; the volume was adjusted to the mark with distilled water. Next, 5 ml of the dissolved sample was taken, and the drug was extracted with 2 ml of chloroform. From that, 1 ml of supernatant was removed, and 9 ml of chromotropic acid reagent was added. An intense purple color was fully developed in 30 min on heating and was still stable for at least 36 hr. The formaldehyde content was estimated spectrophotometrically at 490 nm (18).

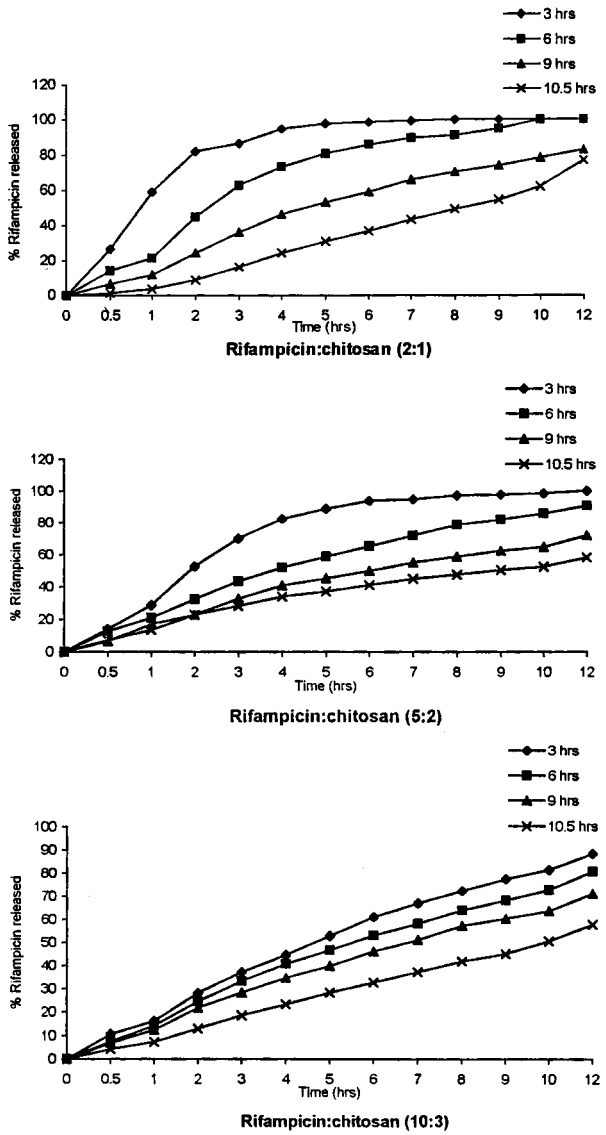

Figure 1. Dissolution profiles of rifampicin from chitosan matrices ($n = 3$) hardening with formaldehyde vapors for 3 hr, 6 hr, 9 hr, and 10.5 hr.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) scans were performed using a Shimadzu DSC-50 thermal analyzer (Shimadzu, Kyoto, Japan) to obtain the melting endotherms of pure rifampicin, pure chitosan, and chitosan matrix treated with formaldehyde vapors under static air atmosphere. The instrument was calibrated using indium standards. Approximately 5 mg of each sample were weighed into small aluminum pans. Samples were heated from 30°C to 225°C at a rate of 10°C per min.

Infrared Spectroscopy

Infrared spectra of the pure rifampicin, chitosan, and chitosan matrix exposed to formaldehyde vapors were determined from mineral acid mull using an infrared (IR) spectrophotometer. The scanning range used was 4000 to 600 cm^{-1}.

In Vitro Dissolution Studies

The dissolution test was carried out using the USP rotating basket method at 100 rpm with 900 ml of pH 7.4 phosphate buffer containing 0.02% w/v of ascorbic acid (dissolution medium) at 37°C ± 1°C. Samples were withdrawn at predetermined time intervals with a pipette fitted with a filter and were analyzed spectrophotometrically at 475 nm.

Data Treatment

Experimental results were fitted according the following exponential equations:

$$M_t/M_\alpha = Kt^n$$

where M_t/M_α is the fractional solvent absorbed or drug released at time t, K denotes a constant incorporating the properties of the macromolecular polymeric system and the drug, and n is a kinetic constant that depends on and is used to characterize the transport mechanism. For example, $n = 0.45$ for case I or Fickian diffusion, which is characterized by a dependence on square root of time in both the amount diffused and the penetrating diffusion front position; $n = 0.89$ for case II transport, which is completely governed by the rate of polymer relaxation and exhibits a linear time dependence in both the amount diffused and the penetrating swelling front position; $n =$

Table 1

Coefficients and Exponents of Drug Release Functions

	First-Order Model		$M_t/M_\alpha = Kt^n$	
Matrices	r^2	$K(hr^{-1})$	r^2	n
Rifampicin:chitosan (2:1) treated for				
3 hr	0.994	7.003×10^{-1}	0.939	0.420
6 hr	0.998	3.224×10^{-1}	0.980	0.663
9 hr	0.947	1.213×10^{-1}	0.992	0.801
10.5 hr	0.973	1.152×10^{-1}	0.998	0.893
Rifampicin:chitosan (5:2) treated for				
3 hr	0.998	4.400×10^{-1}	0.956	0.601
6 hr	0.997	1.925×10^{-1}	0.997	0.629
6 hr	0.993	0.993×10^{-1}	0.985	0.698
10.5 hr	0.991	0.657×10^{-1}	0.993	0.627
Rifampicin:chitosan (10:3) treated for				
3 hr	0.995	1.729×10^{-1}	0.999	0.686
6 hr	0.998	1.305×10^{-1}	0.997	0.736
9 hr	0.999	0.997×10^{-1}	0.994	0.729
10.5 hr	0.998	0.697×10^{-1}	0.999	0.820

$0.45 < n < 0.89$ for anomalous behavior or non-Fickian transport, which is exhibited whenever the rates of Fickian diffusion and polymer relaxation are comparable (19).

RESULTS AND DISCUSSION

The dissolution profiles of rifampicin from chitosan matrices cross-linked with formaldehyde vapors for various time intervals are shown in Fig. 1. The time of exposure to formaldehyde vapor markedly affected the drug release properties of the chitosan matrices. It is notable that the release rates of rifampicin from chitosan matrices were related inversely to the time of exposure with formaldehyde vapors. It is suggested to be due to the decrease in solubility and permeability of the chitosan matrices by the cross-linking of the chitosans (20,21), and it can be assumed that a certain volume of the dissolution medium is required for dissolving the drug incorporated in the chitosan matrices. Both the penetration of the dissolution medium and the diffusion of the dissolved drug through the chitosan matrices are limited. Cross-linked chitosans have been established as sustained drug release matrices (21).

To understand the mode of release of drug from chitosan matrices, the data were fitted to a power law equation (19). The linear correlation coefficients of the slopes and slope values shown in Table 1 indicate that the drug release from chitosan matrices is non-Fickian. This kind of diffusion corresponds to a more predictable type of swelling-controlled system.

It can be observed from the data that the linear correlation coefficients of the first-order model provided an adequate fit to the release profile of chitosan matrices cross-linked with formaldehyde vapors. The release rate constants of the formaldehyde-exposed chitosan matrices are also shown in Table 1. The release rate decreased as the time of exposure to formaldehyde vapor increased. Compared with the plotting method in Fig. 2, the linearity was obtained for the instance when log percentage drug remaining was plotted as a function of t. The formaldehyde residues in tablets treated with formaldehyde vapors are shown in Table 2.

The chitosan matrices were cross-linked by formaldehyde vapors in a closed chamber for various time intervals at ambient temperature. This process was done to retard the chitosan degradation rate. The amino group in the chitosan molecular chain could react with aldehyde groups of formaldehyde by a Schiff base condensation. It has been reported that the drug release rate could be slowed by increasing the time of the cross-linking reaction.

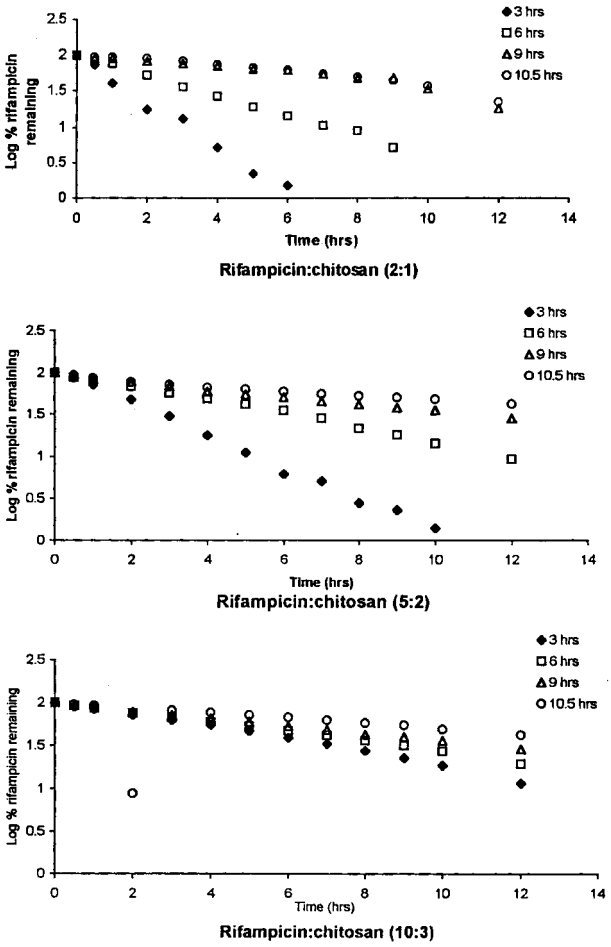

Figure 2. First-order plots of rifampicin release from chitosan matrices ($n = 3$) hardening with formaldehyde vapors for 3 hr, 6 hr, 9 hr, and 10.5 hr.

DSC scans of the pure rifampicin and formaldehyde-treated chitosan matrix are reported in Fig. 3. The pure drug showed a sharp melting peak at 184.1°C. The pure chitosan showed a peak at 112.1°C, which indicates a dehydration peak. The melting peak of rifampicin in the matrix was at 181.6°C. Formaldehyde-treated rifampicin and the rifampicin in matrices showed no shift in the characteristic peak in comparison with the pure rifampicin. The DSC thermograms reveal that there is no interaction between rifampicin and the formaldehyde, no degradation in the rifampicin molecule, or no interaction between rifampicin and chitosan.

IR spectra of the pure drug and formaldehyde-treated chitosan matrix were obtained (not shown). A character-

Table 2

Formaldehyde Content in Hardened Chitosan Matrices

Matrices	Formaldehyde Content (mg/tablet)
Rifampicin : chitosan (2 : 1) treated for	
3 hr	0.576
6 hr	0.987
9 hr	1.222
10.5 hr	1.579
Rifampicin : chitosan (5 : 2) treated for	
3 hr	0.146
6 hr	0.201
9 hr	0.280
10.5 hr	0.335
Rifampicin : chitosan (10 : 3) treated for	
3 hr	0.035
6 hr	0.105
9 hr	0.210
10.5 hr	0.223

istic absorption stretch for the C═O group at 1554 cm^{-1} and broad bands between 3200 and 2300 for N–H stretch were obtained. The fingerprint region of IR spectra showed a characteristic sharp peak at 1243 and 1040 cm^{-1} for the C–O–C acetyl group. In comparison with pure drug, the absorption peaks of the spectra for rifampicin in the treated matrix showed no shift and disappearance of characteristic peaks, suggesting that there is no interaction between drug and formaldehyde, no degradation in rifampicin, or no reaction between chitosan and rifampicin.

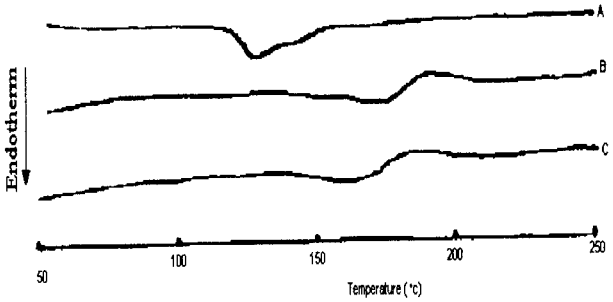

Figure 3. DSC thermograms of (a) pure chitosan, (b) pure rifampicin, and (c) chitosan matrix treated with formaldehyde vapors.

CONCLUSION

In conclusion, rifampicin-chitosan matrices prepared using cross-linking methods are suitable for a controlled-release system. The in vitro release kinetics of rifampicin from chitosan matrices exhibited a non-Fickian transport model. Thus, by varying the extent of the cross-linking reaction, it is feasible to obtain chitosan matrices with different in vitro release kinetics depending on the different requirements.

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