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Comparative Studies on Two Methods of Preparation of Chitosan Matrix Tablets of Rifampicin

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Rifampicin-chitosan matrices were prepared by direct compression method and wet granulation method to develop a sustained release form. The effects of methods of preparation 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 analysed using Ritger and Peppas exponential equation. The *in vitro* release kinetics of directly compressed matrices exhibited a non-Fickian transport model and matrices prepared by wet granulation method showed Fickian diffusion 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 more tightly texture.

The use of controlled release technology in the formulation of pharmaceutical products has become increasingly important in the last few years. In particular, the interest awakened by hydrophilic matrices is completely justified in view of their biopharmaceutical and pharmacokinetic advantages over conventional dosage forms. Since its introduction, the hydrophilic matrix system is becoming an interesting industrial method to prepare controlled release dosage forms for oral administration. Its convenience and easiness to manufacture cuts down the cost of the final product. In principle, when this hydrophilic matrix is exposed to an aqueous medium it does not disintegrate, immediately after hydration it develops a highly viscous gelatinous surface barrier, which controls the drug release from and the liquid penetration into the center of the hydrophilic matrix system.

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Rifampicin, the semi synthetic hydrazine derivative of rifampicin B, one of the most potent and powerful mycobactericidal drug that is used mainly in intermittent therapy, both in tuberculosis and leprosy. The biological half-life varies from 1.5 to 5 h. High cost, prolonged treatment and adverse side effects with rifampicin in conventional chemotherapy of tuberculosis, prompted the development of controlled release formulations. In the last several years many different types of rifampicin controlled release formulations have been developed to improve clinical efficacy of drug and patient compliance[1-9].

Chitosan, [β-(1→4)-2-amino-2-deoxy-D-glucose], a biopolymer, is prepared by N-deacetylation of chitin, one of the polysaccharides widely distributed in nature as a principle component of crustaceans shells insects[10]. It has similar structural characteristics as that of glycosaminoglycans. It is tough, biodegradable and non-toxic. Chitosan has reactive hydroxyl and amino groups, which can be modified chemically for various biomedical and pharmaceutical applications[11,12].

MATERIALS AND METHODS

Rifampicin I.P. was a gift from Aristo Pharmaceuticals, India. Chitosan powder (passed through sieve No. 60) obtained from C.I.F.T., Kochi, India. Formaldehyde AR, hydrochloric acid, chloroform, potassium dihydrogen orthophosphate, sodium hydroxide, sulphuric acid, chromotropic acid, ascorbic acid and acetic acid were purchased from the market and used as such.

Preparation of matrix tablets-Direct compression method:

The drug and different proportions of chitosan powder shown in Table 1, sufficient for a batch of 100 tablets were mixed thoroughly to ensure complete mixing. Tablets containing rifampicin equivalent to 300 mg were compressed using 11 mm flat, round and plain punches (surface lubricated with talc) on single stroke tabletting machine (Cadmach Machinery Co. Pvt. Ltd., India). The compressed tablets were exposed to formaldehyde vapor in a desiccator containing formaldehyde (40% w/v) solution 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 for one day and were finally dried in a vacuum desiccator over fused calcium chloride for 24 h.

Wet granulation method:

Different ratios of drug and chitosan powder shown in Table 1, sufficient for a batch of 100 tablets were mixed thoroughly for complete mixing. 1.5% v/v glacial acetic acid solution in water was added drop wise for granulating the powders. After each addition, the contents of the mortar were triturated well to ensure uniform distribution. When enough cohesion was obtained the mass was passed through sieve no. 14 and the granules were dried at 45° for 30 min. The dried mass was again passed through a sieve no. 14. The 14 mesh passed granules, equivalent to 300 mg of rifampicin were taken for compression using 11 mm round, flat and plain punches (surface lubricated with talc) on Cadmach single stroke tabletting machine set at an appropriate compression pressure (hardness 4-5 kg/cm^2).

Estimation of drug content:

From each batch of prepared tablets, five tablets were randomly collected and powdered. A quantity of powder equivalent to 100 mg of drug was transferred into a 100 ml volumetric flask, added sufficient amount of methanol to produce 100 ml, shaken for 20 min and filtered. Two millilitres of the filtrate was diluted to 100 ml with phosphate buffer (pH 7.4) containing ascorbic acid (200 µg/ml) and the drug content was analysed spectrophotometrically at 475 nm using a double beam UV spectrophotometer[13].

Estimation of formaldehyde:

Vapor hardened matrix tablet was powdered and transferred into a 100 ml volumetric flask and to that 30 ml of 50% aqueous sulphuric acid was added and kept overnight. The volume was adjusted to the mark with distilled water and filtered. The filtered sample (5 ml) was extracted with 2 ml of chloroform. To 1 ml of supernatant aqueous layer 9 ml of chromotropic acid reagent was added. An intense purple color was fully developed in 30 min on heating in boiling water. The content of formaldehyde was estimated spectrophotometrically at 490 nm[14].

Differential Scanning Calorimetry (DSC):

DSC scans were performed using a Shimadzu DSC-50 Thermal Analyzer to obtain the endotherms of pure rifampicin, pure chitosan and formaldehyde vapors treated chitosan matrix under static air atmosphere.

TABLE 1: COMPOSITION OF CHITOSAN MATRIX TABLETS PER TABLET

Ingredients (mg)	Direct compression method			Wet granulation method		
	CH	CH1	CH2	CHW	CHW1	CHW2
Rifampicin	300	300	300	300	300	300
Chitosan (60 mesh passed)	150	120	90	150	120	90

Compositions of various formulations prepared by direct compression (CH, CH1 and CH2) and wetgranulation (CHW, CHW1 and CHW2)

Approximately 5 mg of each sample was weighed into small aluminium pans. Samples were heated from 30° to 225° at a rate of 10° per min with an empty pan as reference.

Infrared spectroscopy (IR):

Infrared spectra of pure rifampicin and the chitosan matrix exposed to formaldehyde vapors were determined from mineral acid mull using a Perkin-Elmer 841 IR spectrophotometer. The scanning range used was 4000 to 600 cm^{-1}

***In vitro* dissolution studies:**

Dissolution test was carried out using the USPXXI rotating basket method. The stirring rate was 100 rpm. The pH 7.4 phosphate buffer having ascorbic acid (200 µg/ml) was used as the dissolution medium (900 ml) and maintained at 37±1°. Samples of 5 ml were withdrawn at regular time intervals, filtered, diluted suitably and assayed spectrophotometrically. An equal volume of fresh medium was immediately added to maintain the dissolution volume. The samples were analyzed spectrophotometrically at 475 nm using double beam UV spectrophotometer to assay the amount of rifampicin dissolved at each time interval. Dissolution studies were performed in triplicate and mean values were calculated.

At the end of 12 h of testing, the drug content remaining in the tablet was estimated by collecting the remains of tablet from basket. This is to conform that the amount of drug remained, when added to the cumulative amount of drug released upto 12 h equals the average drug content of the tablets estimated prior to the drug release studies.

Data treatment:

Experimental results were fitted according the following exponential equations.

$$\frac{M_t}{M_\alpha} = Kt^n$$

Where $\frac{M_t}{M_\alpha}$ 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 which depends on and is used to characterize the transport mechanism. For example n=0.45 for case I or Fickian diffusion which is characterised by a square root time dependence 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, exhibits a linear time dependence in both the amount diffused and the penetrating swelling front position; n=0.45<n<0.89 for anomalous behavior or non-Fickian transport, which exhibits whenever the rates of Fickian diffusion and polymer relaxation are comparable[15].

RESULTS AND DISCUSSION

In hydrophilic matrix tablets, drug release occurs in two steps that take place simultaneously and consecutively, just after the ingestion the first drug portions are released before the macromolecules gellification is complete. During this step, the drug release depends on the penetration capacity of the dissolution medium and on the gellifying characteristics of the matrix. When the macromolecule hydration is completed, the drug release depends on the drug diffusion through the tablet core, i.e., on the physico-chemical properties of the viscous layer.

The drug content was estimated in the prepared matrices was found to be less than ± 5% variation of the stated amount of rifampicin. The drug release from chitosan matrices depended on proportion of chitosan present in the tablets. The release rate constants shown in Table 2 indicated a proportional increase in the release rates with increase in chitosan content. Total amount of drug was found to be released within 3 h. Chitosan is having low cohesive property and increase in the quantity of chitosan causes quick disintegration of tablets resulting in higher release rates. This may also be attributed to its low hydration property also.

Chitosan has free primary amino groups and a strong cross-linking agent like formaldehyde readily reacts with chitosan by Schiff's condensation reaction. Literature reports[16,17] also confirmed the view of cross-linking. Hence, the prepared tablets of rifampicin with chitosan were exposed to formaldehyde for controlling the release rates. The chitosan matrices were cross-linked by formaldehyde vapors in a closed chamber for various time intervals at ambient temperature. The primary amino group in the chitosan molecular chain could react with aldehyde groups of formaldehyde by a Schiff's base condensation. It has been reported that the drug release rate could be slowed by increasing the time of cross-

TABLE 2: COEFFICIENTS AND EXPONENTS OF DRUG RELEASE FUNCTIONS

Matrices	First order model		$M_t/M_\alpha=Kt^n$	
	r^2	$K(h^{-1})$	r^2	n
Rifampicin:chitosan (CH)				
3 h treated	0.994	7.003×10^{-1}	0.939	0.420
6 h treated	0.998	3.224×10^{-1}	0.980	0.663
9 h treated	0.947	1.213×10^{-1}	0.992	0.801
10.5 h treated	0.973	1.152×10^{-1}	0.998	0.893
Rifampicin:chitosan (CH1)				
3 h treated	0.998	4.400×10^{-1}	0.956	0.601
6 h treated	0.997	1.925×10^{-1}	0.997	0.629
9 h treated	0.993	0.993×10^{-1}	0.985	0.698
10.5 h treated	0.991	0.657×10^{-1}	0.993	0.627
Rifampicin:chitosan (CH2)				
3 h treated	0.995	1.729×10^{-1}	0.999	0.686
6 h treated	0.998	1.305×10^{-1}	0.997	0.736
9 h treated	0.999	0.997×10^{-1}	0.994	0.729
10.5 h treated	0.998	0.697×10^{-1}	0.999	0.820
CHW	0.996	2.700×10^{-1}	0.983	0.427
CHW1	0.998	1.440×10^{-1}	0.997	0.469
CHW2	0.997	0.284×10^{-1}	0.989	0.483

Where M_t/M_α- drug fraction released at time t, K is the kinetic constant characterizing the polymeric system, t-time (h), n-diffusion co-efficient and r^2-regression co-efficient

TABLE 3: FORMALDEHYDE CONTENT IN HARDENED CHITOSAN MATRICES

Matrices	Formaldehyde (mg/tablet)*
Rifampicin:chitosan (CH)	
3 h treated	0.576
6 h treated	0.987
9 h treated	1.222
10.5 h treated	1.579
Rifampicin:chitosan (CH1)	
3 h treated	0.146
6 h treated	0.201
9 h treated	0.280
10.5 h treated	0.335
Rifampicin:chitosan (CH2)	
3 h treated	0.035
6 h treated	0.105
9 h treated	0.210
10.5 h treated	0.223

* Average of three determinations

linking reaction. The formaldehyde residues in formaldehyde vapors treated tablets were shown in Table 3.

The dissolution profiles of rifampicin from formaldehyde vapors treated chitosan matrix tablets 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 rate of rifampicin from chitosan matrices was inversely related to the time of exposure with formaldehyde vapors. It is due to the decrease in solubility and permeability of the chitosan matrices by cross-linking of the chitosan. Penetration of the dissolution medium and diffusion of the dissolved drug are the rate limiting factors.

In order to establish the mechanism of release of drug from chitosan matrices prepared by direct compression technique, the dissolution data was fitted to power law equation ($M_t/M_\alpha = Kt^n$). The linear correlation coefficients of the slopes and slope values are shown in Table 2 indicated that the drug release from chitosan

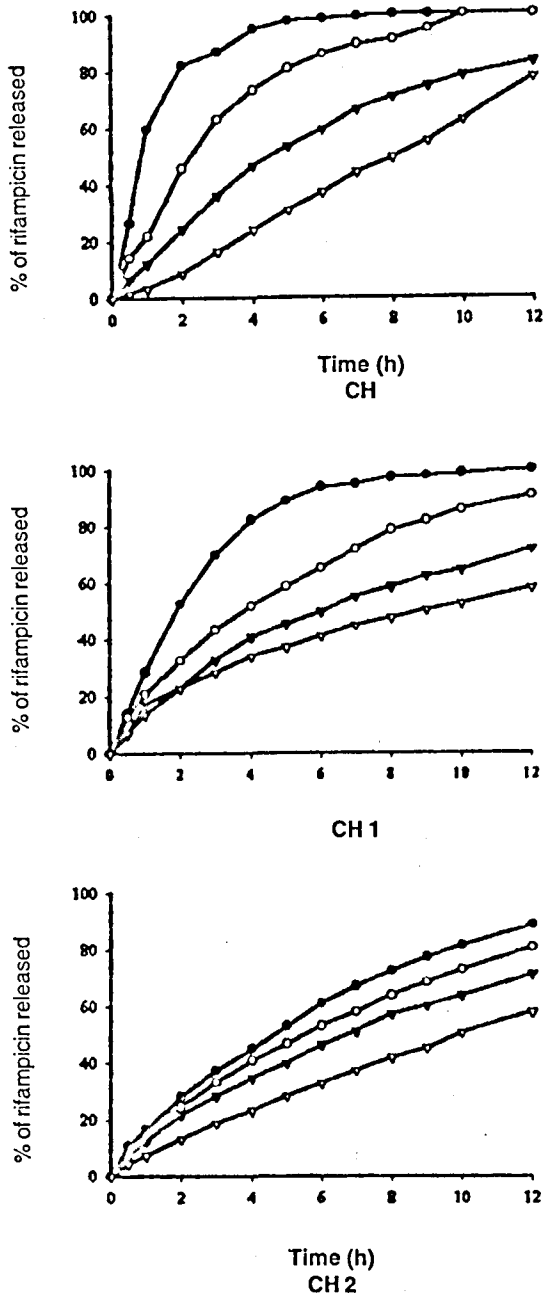

Fig 1: Dissolution profiles of rifampicin from chitosan matrices hardened with formaldehyde

Dissolution profiles of rifampicin from chitosan matrices (n=3) hardening with formaldehyde vapors for 3 h (-O-), 6h (-O-), 9h (-▼-) and 10.5 h (-∇-)

matrices is non-Fickian or anamalous diffusion. This kind of diffusion corresponds to a more predictable type of swelling controlled system.

It can be observed from the data, the linear correlation coefficients of the first order model provided an adequate fit to the release profile of rifampicin from chitosan matrices cross-linked with formaldehyde vapors. The release rate constants of the formaldehyde exposed chitosan matrices are also shown in Table 2.

The DSC thermograms of pure drug showed a sharp melting peak at 184.1°. Rifampicin in the prepared formulations also showed no shift in the characteristic peak in comparison with the pure rifampicin. From these studies it was concluded that there is no interaction of rifampicin with either formaldehyde or chitosan or no degradation of rifampicin in the prepared formulations.

The IR spectra of pure Rifampicin gave the characteristic absorption stretch for C=O group at 1572 cm^{-1} and broad bands between 2800-2300 cm^{-1} for N-H stretch. In comparison with pure drug, rifampicin in treated matrix showed no shift or disappearance of characteristic absorption peaks suggesting that there is no interaction or degradation of rifampicin in the presence of formaldehyde or chitosan.

The *in vitro* dissolution data of matrix prepared by wet granulation method were shown in Fig. 2, exhibited that the release rate is increased as the proportion of chitosan increased. In order to understand the mechanism of drug release, the data were fitted to power law equation ($M_t/M_\alpha=Kt^n$). The linear correlation coefficients of the

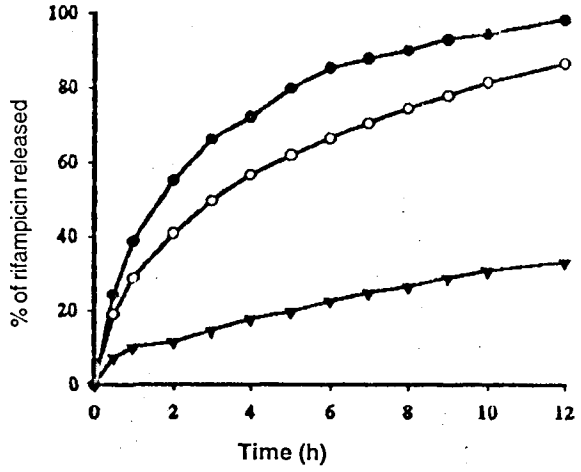

Fig 2: Dissolution profiles of rifampicin from chitosan matrices prepared by wet granulation

Dissolution profiles of rifampicin from chitosan matrices prepared by wet granulation method (n=3) CHW (O) CHW1 (O) CHW2 (▼)

slopes and slope values are shown in Table 2, indicated that the release kinetics confirm predominantly to case I or Fickian diffusion (i.e. square root of time profile). This classical Higuchi type of release mechanism can be explained as a result of the rapid hydration of the polymer molecules on the surface of the tablets, which results in a gel or a highly viscous solution surrounding the matrix that restricts water penetration into the center. The net result is a reduction of the rate of drug release as a function of time.

It can be observed from the data, the linear correlation coefficients of the first order model provided an adequate fit to the release profiles of rifampicin from chitosan matrices prepared by wet granulation method. The release rate constants of these matrices are given in Table 2.

In conclusion, rifampicin-chitosan matrices prepared using cross-linking methods are suitable for a controlled release system. Formaldehyde is the cross linking agent most frequently used to prepare chitosan matrices. It is obvious, however, that formaldehyde provides only a model system, which will not be amenable to human use due to formaldehyde related toxicity. Matrices prepared with wet granulation technique are most suitable for controlled release formulations of rifampicin. The *in vitro* release kinetics of rifampicin from chitosan matrices crossed linked with formaldehyde exhibited a non-Fickian transport model and matrices prepared with wet granulation showed Fickian diffusion model.

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