

Cross-linked Chitosan-Sodium Sulfate Matrix Systems Using Gel Casting Method for Sustained Drug Release of Doxorubicin Hydrochloride

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Abstract

The purpose of this study was to design a chitosan matrix systems made with cross-linking agents that targets control release of anticancer drug. Chitosan (CS) with cross-linking agent sodium sulfate (SS) was used for entrapping the model drug Doxorubicin Hydrochloride (DOX) through novel gel casting method. Scanning Electron Microscopy (SEM), Fourier Transforms Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), swelling index, drug entrapment efficiency and *in vitro* drug release studies were also done for physicochemical characterization of the formulations. Statistically significant different t50% and MDT values were noted between SS (5% w/v), SS (10% w/v) matrix systems and the control, as well as between SS (5% w/v) matrix systems and all the other formulations at pH 5.8 and pH 7.4. DOX release was slower in matrix systems without Explotab® and also at higher dissolution media pH (7.4). Statistically significant dissimilarity was observed between the control and the SS (5% w/v) $f_2 = 11.32 \pm 2.54$; SS (10% w/v) $f_2 = 12.16 \pm 0.82$ at pH 7.4. The findings of the study suggested that the matrix formulation is a promising carrier for DOX delivery.

Keywords: Chitosan, Sodium Sulfate, Cross-linking Agents, Gel Casting Method, Drug Delivery.

Introduction

Typically, hydrophilic polymer matrix systems are swells on contact with water to form a hydrated matrix layer [1, 2]. This outer hydrated matrix layer erodes, as it becomes more dilute. The rate of erosion depends on the nature of the polymer [3-8]. Drugs that are dispersed in a matrix formed by soluble polymer molecules rely on slow swelling and dissolution of the matrix to give continuous release of the drug. A true gel is a matrix system where interactions between the polymer chains are more than just random hydrogen bonds [9]. The cross-link between polymer chains in such a gel can either be chemical bonds or physical bonds, which limit mobility of the polymer chains and give a rigid structure to the gel. Matrix drug delivery systems can also be referred to as wafers or matrices discs, biscuits or cookies etc [10-13].

For release of modifiers and lubricant/glidants requires a combination of suitable hydrophilic colloid along with drug in hydrophilic polymer matrix systems. Distribution of drug during hydrated matrix is limited step for drug release [14-16]. There are two universal types of true gels hydrophilic matrix systems which

are formed either chemical (covalent) or physical bonds (helix formation based on hydrogen bonds or ionic interactions) by the cross-linked polymeric structures. Hence, chitosan is one of the exceptional polymeric viscous matrices easy entanglements of adjoining polymer chains [10]. The use of chitosan in the development of drug delivery preparations is based on studies with chitosan intra-gastric tablets and chitosan-coated drug delivery systems [17, 18]. Drugs dispersed in chitosan were released at a constant rate, thus highlighting its potential as a sustained-release matrix polymer [19, 20].

To the best of our knowledge, no study has been reported on chitosan matrix systems made with sodium sulfate cross-linking agents using gel casting method. Here, we present the drug release properties of cross link chitosan matrix systems. The aim of this study is to investigate the cross-linking ability and the influence of the type of cross-linking agent on the drug release properties of chitosan matrix systems prepared by a unique gel casting method. Physicochemical characterization of the formulations has been done by *in vitro* drug swelling index study. Schematically presentation of DOX release from cross link chitosan matrix System was shown in Figure. 1.



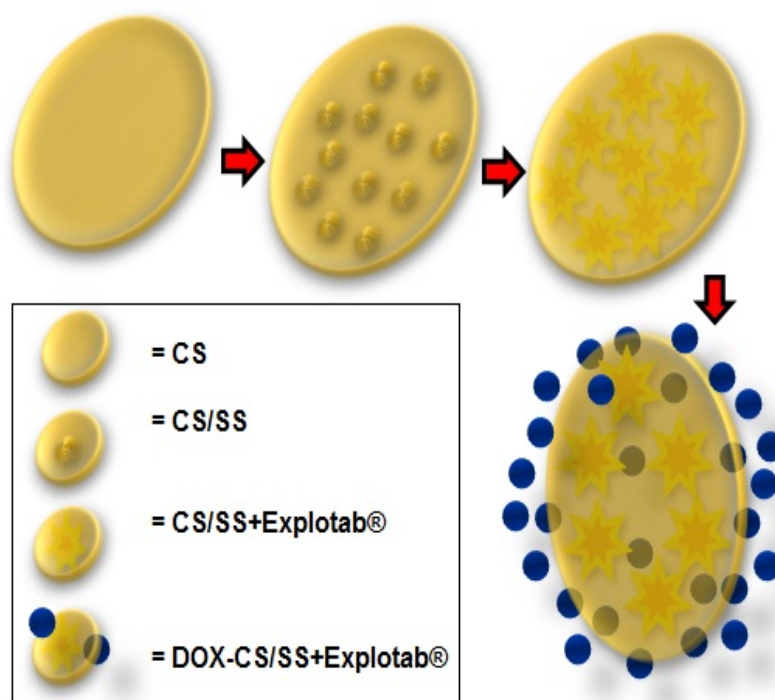


Figure 1: Schematically Presentation of DOX Release from Cross Link Chitosan Matrix System.

Materials and Methods

Materials

Chitosan (75% deacetylated), sodium sulfate, acetic acid, Explotab® and doxorubicin Hydrochloride were purchased from Sigma Aldrich, India. The other entire reagent used was of analytical grade.

Preparation of Chitosan Cross-link Matrix with Loaded Doxorubicin

Chitosan solution was prepared by dissolving 3g of chitosan in 100ml of 2% (v/v) acetic acid solution at 60°C with continuous stirring for 30 minutes. After complete dissolution of the CS, DOX was dispersed into CS solution using a high-speed rotary mixer. This mixer was run at 800rpm for 10 minutes followed by at 1000rpm for 20 minutes at 30°C. Then accurately measured quantities of the cross-linking agent sodium sulfate with different concentrations (5% and 10%) were dissolved in distilled water. The sodium sulfate of these two different concentrations were added separately to the chitosan-DOX suspension to cross-link the chitosan over a period of 1 hour to form true gels under continuous stirring with a magnet stirrer at 30°C. Another batch of these formulations was prepared with the addition of a disintegrant, Explotab®, at a concentration of 3% w/v shown in Table-1. The gels were dried in a drying oven for 60 minutes at 37°C and further forced through a 2mm sieve while still damp to obtain granules.

Equal aliquots of the cross-linked chitosan wet granular gels were loaded using a spatula into the moulding compartments of 24-well polystyrene plates. The moulding frames containing cross-linked matrix systems were dried in a drying oven at 37°C for another 48 hours. The dry matrix systems that were formed inside the moulding frames were removed and stored in light and moisture protected containers.

Table -1: Composition of the Samples:

Formulation code	CS (w/v %)	SS (%w/v)	Explotab®	DOX mg/ml
F1	3	5	-	2
F2	3	10	-	2
F3	3	5	3	2
F4	3	10	3	2

Drug Content in Chitosan Matrix System

Exactly 500mg powder of crushes matrix systems was taken and then stirred for 24 hours in 100ml of 0.1M NaOH. The solution which was ultrasonicated for 15minutes then filtered. From this solution, 5ml of sample was taken to find out the amount of DOX present in the sample by UV-vis Spectroscopy. Both concentration



and quantity of DOX was calculated by using the equation of standard curve.

Methods

Physical Properties of the Chitosan Matrix Systems (Weight Variation, Hardness)

Total 20 matrix systems of each formulation were calculated for weight variation to evaluate conformity with standard specifications for solid dosage forms. Twenty matrix systems were arbitrarily chosen and weighed one at a time and their standard weight was determined. For each formulation, the hardness of 20 randomly selected matrix systems was examined using a hardness tester (Erweka, Germany). Each matrix system was subjected to an increasing compressional force and the value at which the mobile jaw of the hardness tester started to bounce back was recorded as the tensile strength on recoil (hardness) of the matrix system.

Scanning Electron Microscopy (SEM)

The surface morphology of the chitosan matrix was examined using scanning electron microscopy (Jeol Datum Ltd., G5/IL/42/08, Tokyo, Japan). Samples were deposited on stub using one side of a double-sided adhesive dried carbon tape (NEM Tape, Nisshin Em. Co. Ltd., Tokyo, Japan). The working distance 25mm was maintained and acceleration voltage used was 17 kV with secondary electro image (SEI) as a detector.

Fourier Transforms Infrared Spectroscopy (FT-IR)

Fourier Transform Infrared Spectrum of pure DOX, chitosan, sodium sulfate, SS-CS physical mixture and drug loaded matrix were recorded in range of 4000–400 cm⁻¹ using FT-IR (Perkin Elmer) from KBr pellet to find out the drug excipient compatibility study.

X-ray diffraction Analysis (XRD)

This study is useful to investigate crystallinity of the drug in cross-linked matrix. It was performed with X-ray diffractometer (ULTIMA-III, Rigaku, Japan), using nickel filtered Cu Ka radiation (a voltage of 40 kV and a current of 30 mA) for pure drug and formulated matrix.

Swelling Studies

The swelling ratios of three matrix systems were determined of each formulation [21]. Then, it was weighed and kept in a glass beaker containing 500ml of phosphate buffer at pH 5.8 ± 0.1 and pH 7.4 ± 0.1 respectively at 37°C with continuous stirring at 50rpm. After that, every one hour intervals removed matrix systems from buffer, maximum of 8 hours and then taken weight by removing excess surface moisture using blotting paper. The variation of dry weight and swollen state was calculated on the basis of the degree of swelling (S_{wt}).

$$S_{wt} = \frac{W_s - W_d}{W_d} \times 100$$

Where: W_s = Weight of Swollen Matrix Systems.
 W_d = Weight of Dry Matrix Systems.

Friability Studies

The matrix systems were weighed together and then put in a friabilator (Erweka, Germany) [22]. The apparatus was run at 25rpm for 4minutes, i.e. rotated 100 rotations, according to the United States Pharmacopoeia (USP) 23, (1995:1981). After 100 rotations, the matrix systems were removed, dusted and their total weight determined. The percentage friability was calculated.

$$F = \frac{W_1 - W_2}{W_2} \times 100$$

Where: W_1 = Initial Weight of Twenty Matrix Systems.
 W_2 = Weight of Twenty Matrix Systems after the Friability Test.

In vitro Drug Release Studies

Phosphate buffers of pH 5.8 ± 0.1 and 7.4 ± 0.1 were prepared according to the method of the USP 23 (1995:2049-2050). The experimental conditions for the dissolution tests were as follows: Dissolution volume = 900 ml, Rotation speed = 60 rpm, Sample intervals = 0, 15 minutes to 24 hours; Temperature = 37°C, and Sample volume = 5 ml. The amount of DOX released was analyzed using a UV spectrophotometer at the λ_{max} value of 234 nm [23].

Analysis of Dissolution Data

Dissolution experiments on the different matrix system formulations and the control were performed in triplicate on a fully calibrated eight station dissolution auto sampling apparatus using the paddle method (USP Apparatus II, Electro lab TDT 08L, India). Statistical analysis of the data was performed on the calculated t50% values and mean dissolution times (MDT) using a one-way repeated analysis of variance (ANOVA) with Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) to calculate the p -values. The differences between the values (both t50% and MDT) of the experimental groups were considered statistically significant if $p < 0.05$.

Results and Discussion

Physical Properties of the Chitosan Matrix

The average weights, hardness and friability of the different matrix systems are given in Table - 2. The matrix systems prepared from 5% w/v SS had higher hardness values than those made from the 10% w/v SS formulation. Matrix formation and physical bonding between the chitosan chains and the anionic cross-linking agents depend therefore on both the type of cross-linking agent and its concentration. An optimum concentration clearly exists for SS in terms of the hardness of the matrix systems and increasing the



concentration of SS results in a decrease in the hardness. Addition of Explotab® to the cross-linked chitosan formulations resulted in an increase, although relatively small, in the hardness of the matrix systems as compared to those without Explotab®. This may be explained by a contribution of the Explotab® molecules to the cross linking of the cationic chitosan chains in the matrix systems.

This probable higher density of cross-linking resulted in matrix systems with increased hardness. The matrix systems showed a decrease in friability with the addition of Explotab®, which is in accordance with the physical strength of the matrices as observed during the hardness tests and swelling experiments.

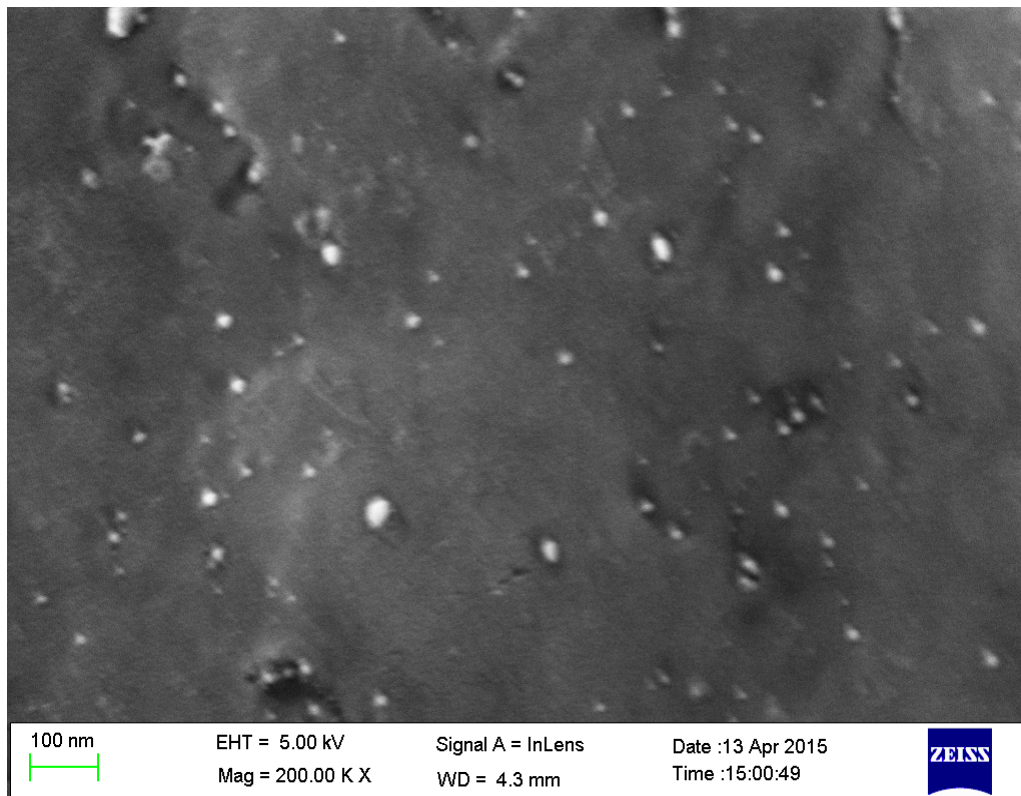
Table -2: Physical Properties and Drug Content of the Chitosan Cross-link Matrix System.

F.C No	Weight (mg)	Hardness (N)	P.I ± SD	Friability (%)	Drug content: mg/0.5 g unit
F1	505.090 ± .765	54.20 ± 12.5	0.160± .027	1.23	124.708±1.981
F2	498.090 ± .453	49.34 ± 5.72	0.140± .023	0.47	93.363 ± 1.706
F3	501.090 ± .843	57.12 ± 8.65	0.160± .026	1.08	104.591 ±0.780
F4	478.090 ± .385	52.63 ± 2.76	0.140± .022	0.43	97.554±2.106

SEM (Scanning Electron Microscopy)

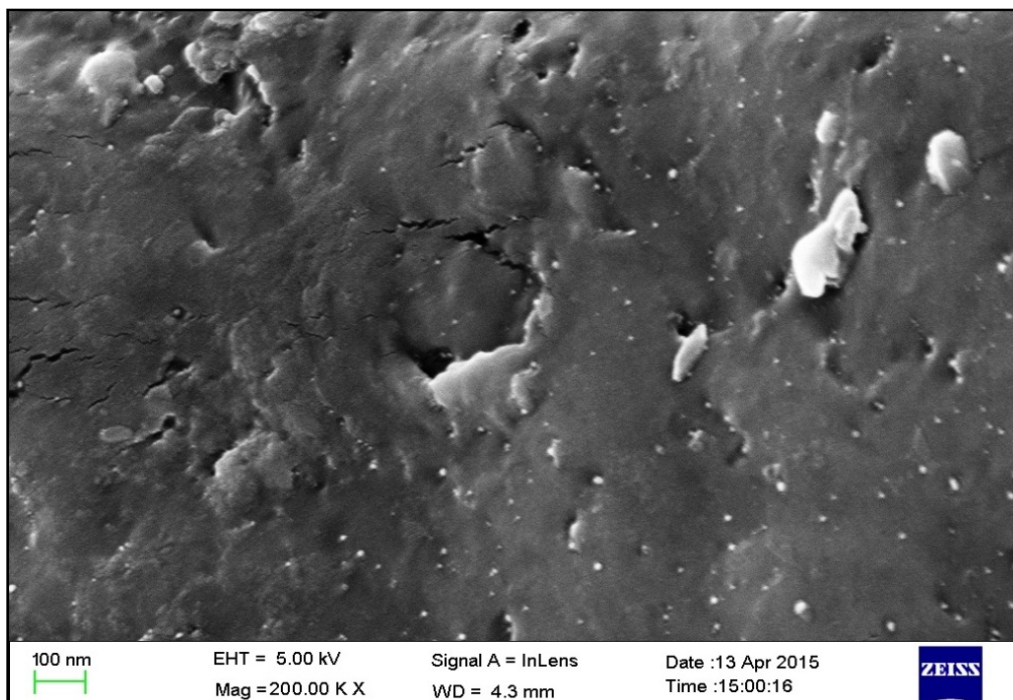
In Figure. 2, the SEM of original F1, F2, F3 and F4 are demonstrated. The cross sections of the matrix systems it is clear that the morphology changed with the use of different types of cross-linking agents [24, 25]. Addition of Explotab® influenced the

structure of the matrices; the presence of the Explotab® seems to cause bigger crevices and pores to form in the internal structure of the matrix systems. These pores may contribute to a faster rate of fluid penetrating the matrices with a subsequent faster dissolution of the incorporated drug.

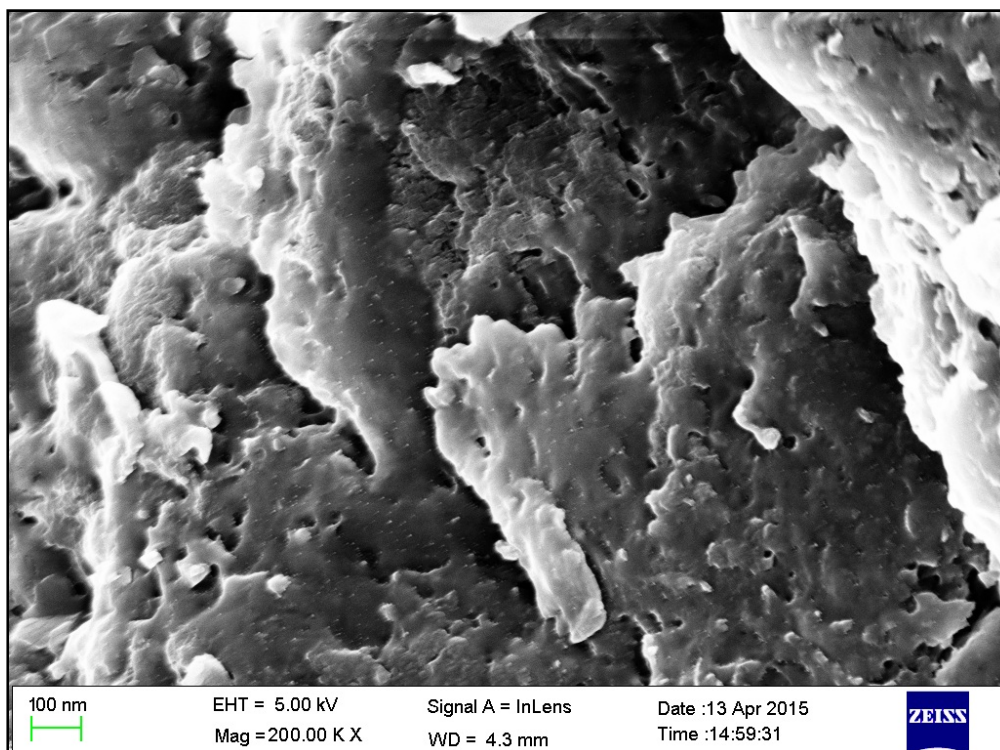


2F1



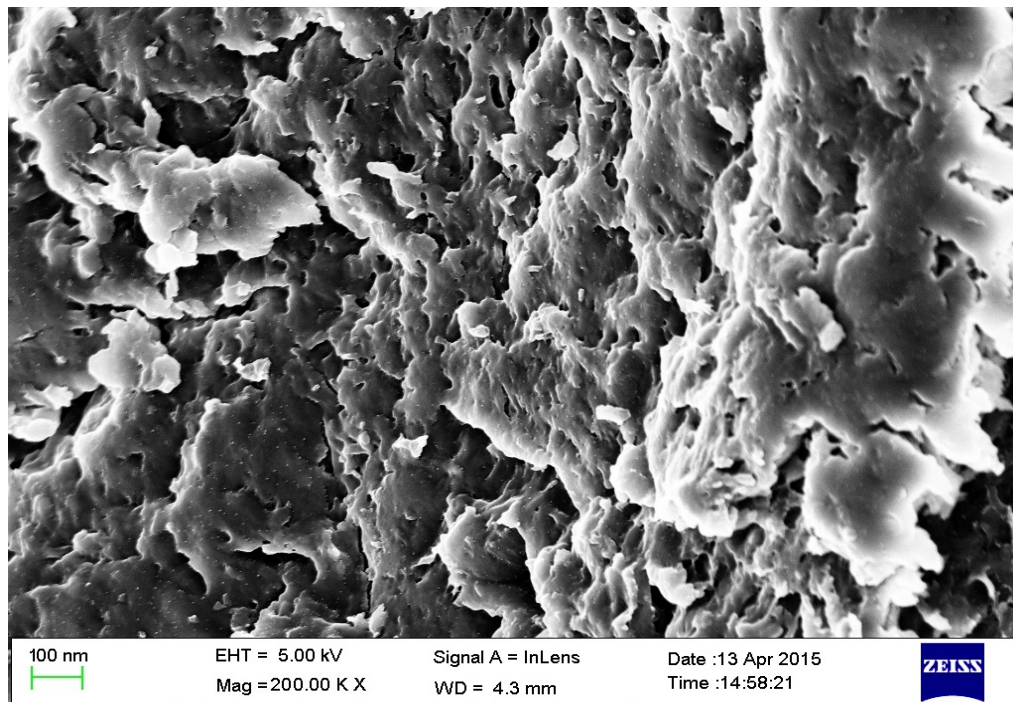


2F2



2F3





2F4

Figure 2: SEM of Cross Link Chitosan Matrix System.

FTIR (Fourier Transforms Infrared Spectroscopy)

FTIR analysis was performed to characterize any chemical changes that occurred during the preparation of cross-link chitosan matrix. Figure. 3 shows the FTIR spectra of CS, CS-SS. It shows N-H stretching at 3500-3200 as well N-H bends at 1550-1450 nm in the primary amine functional group. It was observed that the symmetric stretching for SO_4 at 1123nm as well as asymmetric bending of SO_4 at 615nm in Sodium sulfate. Here, it was observed that in case of chitosan sulfate, the two peaks could match up in

the regions of 615nm and 1100nm due to presence of sulfate. On the other hand, the shifting of 1513nm of the amino group bends into higher wave number which may signify a higher energy level for cross-linked chitosan sulfates. This was confirmed that the bending of the N-H group could limit in sulfate anion by chemical interaction. The shifting of the N-H deformation band to higher frequencies in hydrogen bonding which was already been reported earlier. This shift was implied due to the occurrence of cross-linking reaction among sulfate anions and ammonium groups of chitosan [26].

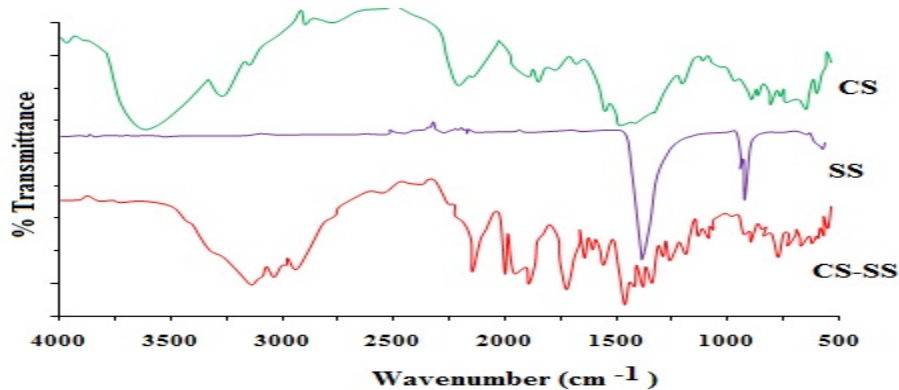


Figure 3: FTIR of Cross Link Chitosan with Sodium Sulfate Matrix System.



XRD (X-ray diffraction) Analysis

The cross link chitosan matrix was used for x-ray diffraction analysis. The diffracted intensities were recorded from 5 to 80 at 2θ angles. The diffraction pattern in Figure. 4 corresponds to CS, F1, F2, F3 and F4 were studied. The X-ray diffraction of the raw CS shows two characteristic peaks at around $2\theta=11.281$ and at $2\theta=21.181$. After being prepared into cross-link with sodium

sulfate, the CS shows two similar diffraction peaks but with decreased intensity. This is due to that part of the crystallized CS may transfer into amorphous state during the phase parathion process. When Explotab® was added to the chitosan cross-link matrix solution, irrespective of amount, the peaks with decreased intensity remained at the same position indicating that the formation of intercalation and exfoliated hybrids.

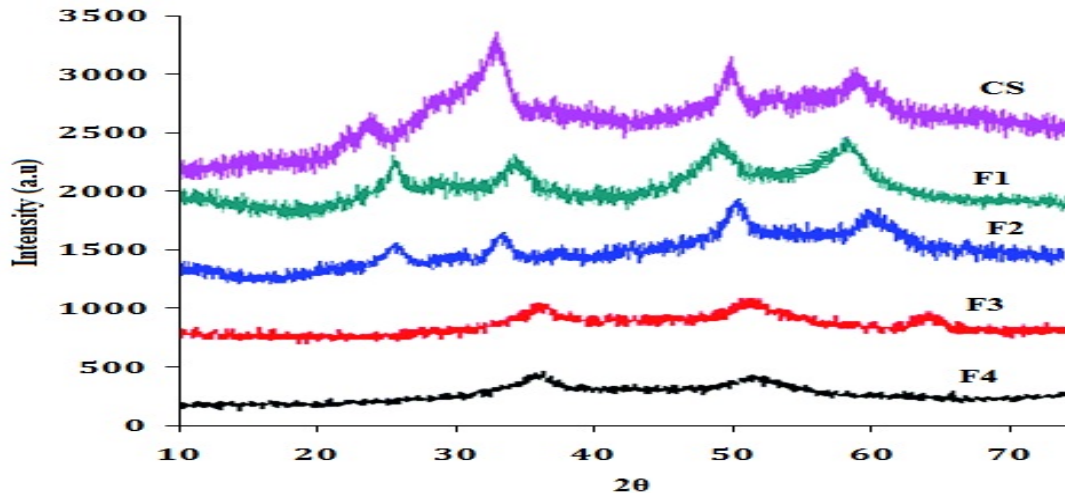


Figure 4: XRD of Cross Link Chitosan Matrix System.

Drug Content of the Matrix Systems

A plot of absorbance versus the concentration of DOX (mg/100 ml) in 0.1 M NaOH at 234 nm is shown in Figure. 5. This standard curve of DOX was obtained by the spectrophotometrical determination of the absorbencies of the serial dilutions performed. The r^2 value of the curve was found to be equal to unit (i.e. perfect straight line). This implies a 100% confidence interval in predicting the value of the concentration corresponding to any extrapolated

absorbance value along the curve. The DOX content values (mg/0.5 g of product mass) of the different matrix systems are shown in Table -2. The DOX content of the different types of matrix systems ranged from 93.3mg/0.5g to 124.7mg/0.5g, which represents 15 to 25% of the product weight. The variation in drug content between the different types of matrix systems may be attributed to the uneven loss of DOX during the cross-linking phase of matrix preparation into the supernatant.

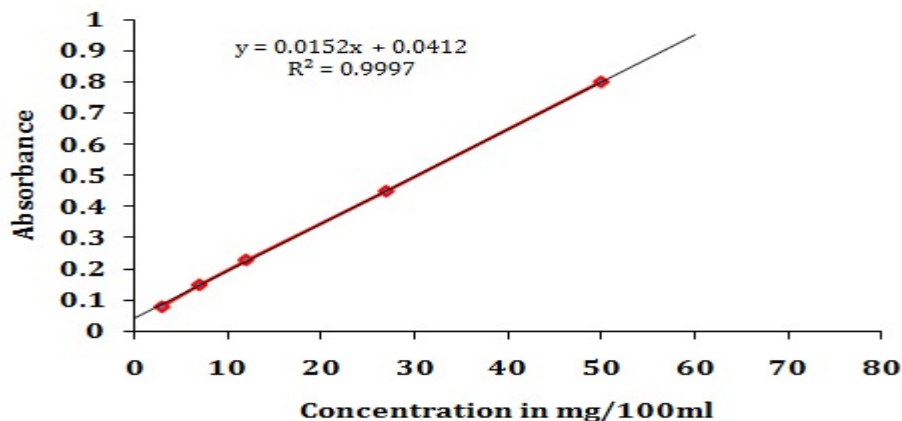


Figure 5: DOX Standard Curve.



Swelling Properties at 5.8 and 7.4

Evaluation of the swelling properties of the chitosan cross link matrix systems F1, F2, F3 and F4 at pH 5.8 and 7.4 in Figure. 6a and 6b. In pH 5.8, the F1 formulation showed a swelling ratio of 75% within 2 hours. SS is a highly reactive surfactant, which means it has a tendency to enhance fluid penetration and wetting of the matrix systems. Addition of Explotab® to F3 formulation increased the swelling ratios to values close to 225%, which represent a 150% increase in the swelling ratio of the matrix systems without Explotab®. The matrix systems F2 reached a swelling ratio of about 100% within three hours and then disintegrated. This sudden disintegration suggests a quick reversing of the bonds after a hydration time of three hours. Addition of Explotab® to F4 formulation, however, changed the swelling properties of the matrix systems in such a way that relatively high swelling ratios of between 125 to 175% were reached. Additionally, the matrix systems containing Explotab® did not disintegrate during the total period of the swelling test. This is in accordance with the hardness results that showed an increase in strength with addition of Explotab®.

In Figure. 6b, the F1 formulation showed a swelling ratio of 83% within 2 hours compared to the 75% at pH 5.8. On addition of Explotab® to F3 formulation, the swelling ratio increased to values

close to 225%, which represents an increase of 125% as compared to the formulation without Explotab®. The swelling curves for the F1 and F3 matrix systems that incorporated Explotab® at pH 5.8 and pH 7.4 resembled each other closely as depicted in Fig. 6a and 6b, respectively.

The F3 formulation swelled to a ratio of about 100% within 1 hour, but slowly started to erode and eventually disintegrated after 5 hours. This indicated an influence of pH on the swelling properties and reversibility of the bonds between the cross-linked chitosan molecules, which lead to disintegration of the matrix. Furthermore, addition of Explotab® to the F4 formulation influenced the swelling properties of the matrix systems, attaining swelling ratios between 125 to 175%. From the results of the swelling experiments, it is clear that the Explotab® increased the physical strength of the matrix systems to such an extent that they remained intact throughout the 8 hour swelling test.

The relatively high increase in the swelling ratio of the matrix systems during the first hour may be indicative of a possible high drug release rate over the initial period after administration. The drug release properties are discussed under the results of the dissolution tests. Swelling ratios were lower than those at pH 5.8, suggesting probably slower drug release rates at pH 7.4.

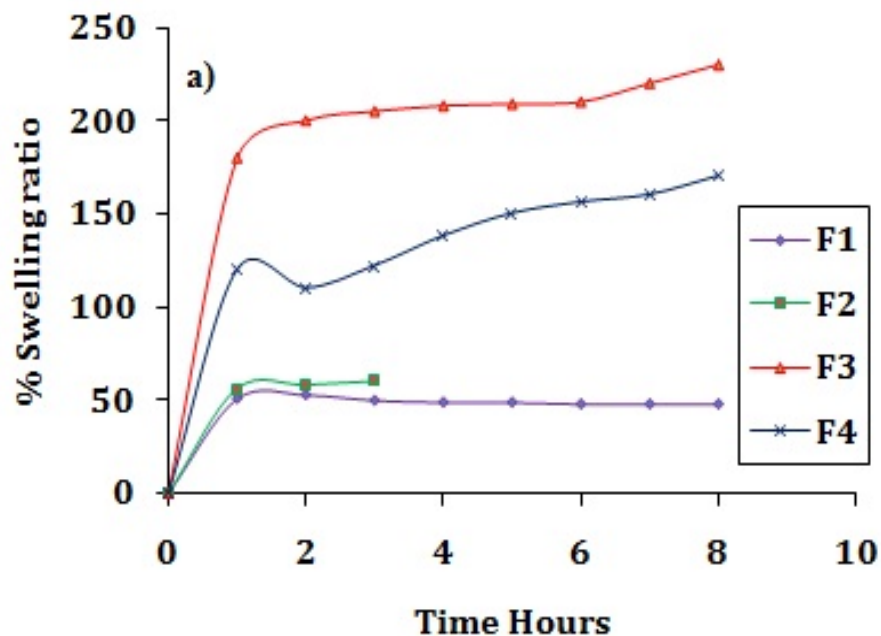


Figure 6a: Variation in the Swelling Ratio with time (hours) for the different Chitosan Matrix Systems at pH 5.8.



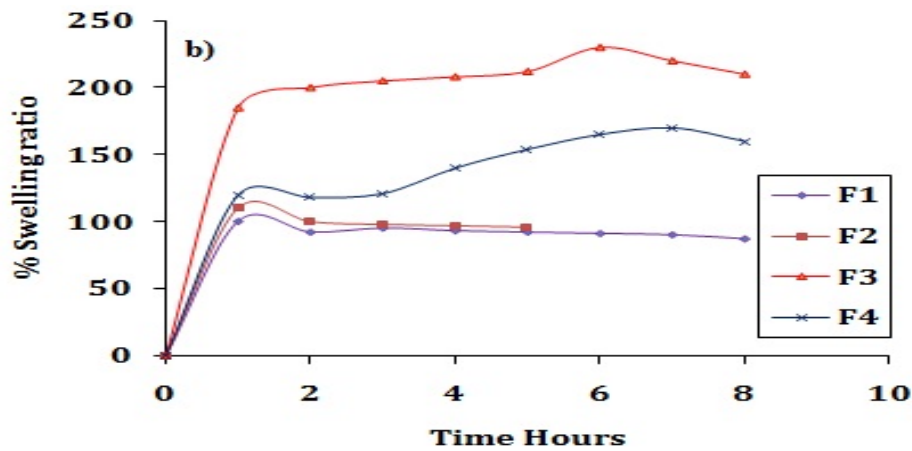


Figure 6b: Variation in the Swelling Ratio with time (hours) for the different Chitosan Matrix Systems at pH 7.4.

Drug Release Studies at pH 5.8 and pH 7.4

The cumulative percentages of DOX obtained from the *in vitro* drug release experiments at pH 5.8 and 7.4 are depicted in Figure. 7a and 7b. In pH 5.8, the release of DOX from the matrix systems F1 increased gradually from values as low as 30% in the first hour to a maximum percentage of approximately 81% in 8 hours. In case of the matrix F2 the matrix systems released about 77% of their DOX load in 8 hours, F3 and F4 systems that contained Explotab® accomplished a maximum release value of approximately 80% and 94% respectively by the end of the test.

Although inclusion of Explotab® in the cross-linked chitosan matrix systems did not cause disintegration of the delivery systems that is a known effect in the case of compressed tablets, it managed to increase the drug release rate. Therefore, this disintegrates exhibited ideal properties by increasing the hardness, swelling characteristics and drug release rate of the matrices without affecting their integrity.

In pH 7.4, on the contrary, drug release from the matrix systems of chitosan cross-linked with F1 and F2 respectively, showed slower rates compared to that of the control, and their respective formulations at pH 5.8. The cross-linked chitosan matrix systems released between 64-69% of their DOX within eight hours. On addition of Explotab®, the amount of DOX released increased to between 80-85%. Therefore, it was postulated that the pH of the dissolution media affects the rate of drug release from a cross-linked chitosan formulation [27]. This can be explained by the possible higher protonation of the free amino groups (i.e. those not involved in the cross-links) on the chitosan molecules at pH 5.8 as well as those involved in the cross-links, which might influence the reversibility of the ionic cross-links. At a neutral pH, the protonation of the amino groups occurs to a lower extent with a consequent lower extent of cross-links reversed that results in a slower drug release rate [28, 29].

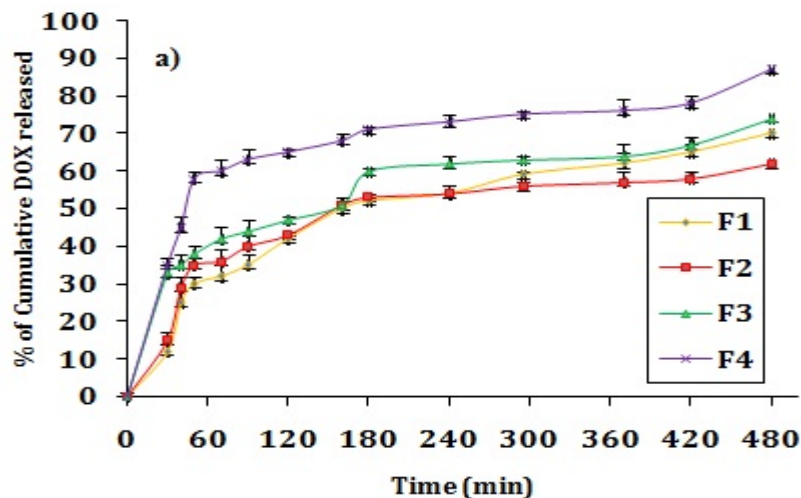


Figure 7a: Cumulative % DOX released versus time (minutes) at pH 5.8.



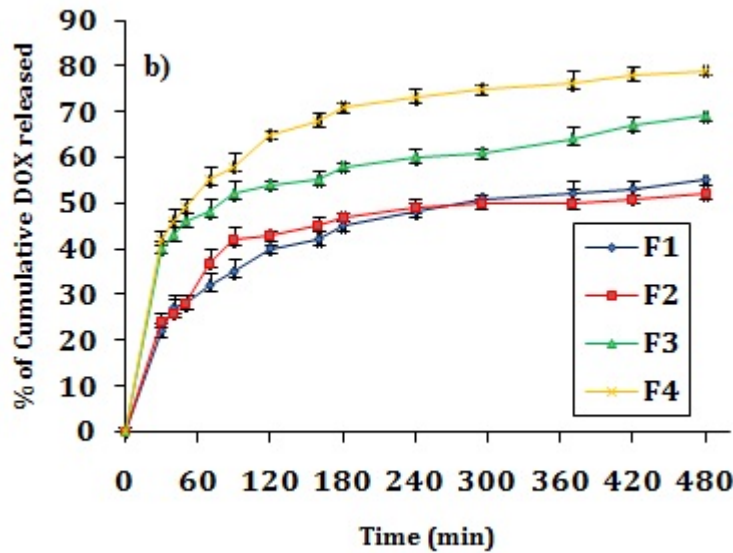


Figure 7b: Cumulative % DOX released versus time (minutes) at pH 7.4.

Analysis of Dissolution Data

In vitro dissolution experiments were statistically analyzed using the techniques described under analysis of results in Table - 3. Complete sets of values of the $t_{50\%}$, MDT, and the similarity factor (f_1) as well as the difference factor (f_2) for all the different matrix systems at pH 5.8 and pH 7.4 in triplicates are presented. A high MDT value indicates a slow rate of drug release and a lower MDT value means the rate of DOX release was higher. The mean values (\pm standard deviations) for the $t_{50\%}$ ($n=3$), the fit factors $\{f_1$,

f_2 ($n=3$) at pH 5.8 and pH 7.4 respectively, and their corresponding standard deviations. The following criteria were adapted in the determination and the interpretation of f_1 and f_2 values: $f_1 < 15$ and $f_2 > 50$ for profiles that were statistically significantly not similar to the control up to an end point equal to six intervals corresponding to 120 minutes. Not more than one point with a cumulative % DOX value higher than 85% should be considered as this could yield an f_2 value above 100.

Table 3: Statistical Analysis for the Dissolution Data at pH 5.8 and pH 7.4

F.C Code	pH5.8			pH7.4		
	* $t_{50\%}$ (min)	f_1	# f_2	* $t_{50\%}$ (min)	f_1	# f_2
F1	136.3 \pm 10.3	83.32 \pm 7.11	16.11 \pm 3.37	199.2 \pm 80.0	94.60 \pm 12.20	11.32 \pm 2.54
F2	130.0 \pm 25.0	80.06 \pm 5.91	17.02 \pm 2.51	162.7 \pm 15.5	91.00 \pm 1.01	12.16 \pm 0.82
F3	102.5 \pm 2.5	84.40 \pm 2.57	16.25 \pm 1.95	55.3 \pm 21.6	70.77 \pm 5.47	17.70 \pm 0.91
F4	31.2 \pm 3.2	50.84 \pm 11.12	27.11 \pm 3.58	35.3 \pm 9.6	57.43 \pm 0.45	21.97 \pm 1.00

Conclusion

From the above research the higher protonation of amino groups at a lower pH value (5.8) with reversing of the cross-link and faster release of the incorporated drug was observed. Addition of Explotab® to the matrix systems significantly reduced their $t_{50\%}$ thus indicating a faster release of the drug. This was expected as Explotab® increases the swelling properties of the matrices and showed a more porous structure. Based on the MDT values and the fit factors f_1 and f_2 , it was concluded that the anionic surfactant

SS are good cross-linking agents for chitosan. Matrix systems formed with these cross-linking agents released the drug significantly slower as compared to an immediate release dosage form. The effect of Explotab® and a lower dissolution medium pH on the release of DOX from the different chitosan matrix systems was increased the rate of drug release from the chitosan matrix systems.

Disclosure

The authors report no conflicts of interest in this work.

Acknowledgments

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