PREPARATION AND CHARACTERIZATION OF GLUTARALDEHYDE CROSS-LINKED CHITOSAN- XANTHAN GUM HYDROGEL MATRIX OF CELECOXIB

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ABSTRACT

In present study celecoxib, a selective COX-2 inhibitor was incorporated into glutaraldehyde cross-linked chitosan- xanthan gum hydrogel matrix system. Inter penetrating network of chitosan and xanthan gum was prepared for sustained release of celecoxib by using various concentrations of polymers and cross-linking agent. The drug and formulation were separately studied for FT-IR along with other preformulation studies. Entrapment efficiency for all the formulations were found to be 81.67 ± 1.68 % to $95.45 \pm 0.54\%$ (n=3). Swelling study was done and water uptake by hydrogel matrix at pH 1.2 and at pH 7.4 were studied and significant swelling of hydrogel matrix were observed. *In vitro* drug release study for celecoxib matrix tablets was carried out in three different buffer solutions, (pH 1.2 HCl buffer, pH 6.8 and pH 7.4 phosphate buffers) which showed a typical sustained release pattern throughout the GIT. The effect of xanthan gum on drug release from hydrogel matrix was investigated. The drug release was modified by changing the concentrations of glutaraldehyde and xanthan gum and chitosan. Among all the formulation, F7 was considered as best formulation based on the entrapment efficiency and drug release pattern from polymeric matrix. Kinetics study was done for all the formulations and best fit model for drug release was determined depending on R^2 values. Release kinetics of F7 was found to follow zero order kinetics which fits the criteria of drug release from hydrogel matrix system.

KEY WORDS: Glutaraldehyde, chitosan, xanthan gum, cross-linking, hydrogel.

1. INTRODUCTION

The term sustained release indicates an initial release of drug sufficient to provide a minimum effective concentration immediately after administration and then a gradual release over an extended period of time.[1] Sustained release dosage forms are designed to enhance the therapeutic activity of a drug in order to achieve a prolonged duration of action with the benefit of safe and effective concentration in plasma. Being more flexible in modulating oral dosage form, sustained release delivery has gained more significance.[2]

Hydrogels are loosely cross-linked polymeric network having the ability to absorb large amount of fluid without disturbing their three dimensional structure. They can absorb fluids more than 15 times of their dried weight. The fluid may be water, electrolyte solution, and synthetic urine, biological fluid such as sweat, urine or blood.[3] In swollen state hydrogels shows excellent biocompatibility after becoming soft and rubbery.[4] It can release the drug at a controlled rate over an extended period of time.

Inter penetrating networks (IPNs) are composites of at least two polymers in which at least one network is synthesized and/or cross-linked in the presence of the other.[5] IPNs are also known as entanglements of polymer networks that are ideally held together only by permanent topological interactions.[6] It can assure a slow release of drug from a polymeric matrix for a prolonged period of time. This fits the criteria of sustained release dosage forms. Generally IPNs are created for the purpose of combining individual properties of two or more polymers. In some cases, entirely new properties are exhibited by the IPN that are not observed in either of the two single networks alone.[7] The development of interpenetrating network polymers is attractive because IPNs provide free volume space for the easy encapsulation of drugs in the three-dimensional network structure which are obtained by cross-linking of two or more polymer networks.[8] Various properties of IPNs such as porosity, bio-adhesiveness, elasticity, swelling and stimuli-responsive behavior can be controlled by the appropriate choice of the network-forming polymers and suitable cross-linking agent and its proportion.[9-10]

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-H- pyrazol-1-yl) is a new generation non-steroidal anti-inflammatory drug which is effectively used in treatment of osteoarthritis and rheumatoid arthritis.[11] The mechanism of action of Celecoxib is inhibition of prostaglandin synthesis by inhibiting cyclooxygenase-2 isoenzyme. Being a selective COX-2 inhibitor, it causes lesser ulceration and gastric bleeding in stomach. Celecoxib binds with its

polar sulphonamide side chain to a hydrophilic side pocket region close to the active COX-2 binding site. This selectivity allows the drug to reduce inflammation.[12]

Xanthan gum is a polysaccharide secreted by the bacterium Xanthomonascampestris. It is widely used as food additive and rheology modifier. It is also used to form o/w type of emulsion to stabilise the oil droplet against coalescence. In general 0.2% by weight of xanthan gum result in an slight thickening. Too much of xanthan gum can result in an unpleasant and undesirable slimy texture. It is very stable under a wide range of temperature and p^H.[13-14]

Chitosan has natural favourable biological properties such as biocompatibility, biodegradability, anticancer, haemostatic, low toxicity, and it displays mucoadhesive properties, rendering this molecule very biodegradable polymer obtained by partial deacetylation of chitin.[15] It is a cationic polysaccharide composed of β [1→ 4]-2-amino-2-deoxy-D-glucopyranose and some *N*-acetylglucosamine units depending upon its degree of deacetylation. Chitosan is soluble in acidic solution. This polymer has Attractive properties for drug delivery applications.[16-17]

2. MATERIALS AND METHOD

2.1 Materials: Celecoxib was obtained as a gift sample from MSN Labs, Hyderabad; Chitosan, Gluteraldehyde and Xanthan Gum was purchased from Loba Chemie Ltd, ;Mannithol, glacial acetic acid, ethanol, lactose, Magnesium Sterate, and Talc was purchased from S.D.Fine Ltd, (Banglore, India).

2.2 Preparation of celecoxib loaded microparticles: Required amount of chitosan was dissolved in 1% aqueous solution of glacial acetic acid. Specific amount of celecoxib (500 mg) was properly mixed with chitosan solution by continuous stirring. Xanthan gum solution was separately prepared in another beaker. Then the xanthan gum solution was poured into the chitosan-celecoxib solution with addition of glutaraldehyde. Stirring was continued up to 5 hrs. Celecoxib loaded microparticles were formed which was kept in hot air oven at 40˚C. Same procedure was followed for all the 8 formulations by changing the concentrations of chitosan, xanthan gum and glutaraldehyde.

2.3 Characterization of celecoxib loaded microparticles

2.3.1 Fourier transformed-infrared (FT-IR) spectroscopy: Samples were powdered and KBr pellets were prepared and analysed by using a Fourier transform infrared (FT-IR) spectroscope (spectrum BX, Perkin-Elmer® Instrument, USA). The prepared pellets were placed in the sample holder compartment and spectral scanning was done in between wavelength region $4000 - 550$ cm^{-1} at a resolution of 4 cm^{-1} at a scanning speed of 2 mm/s.

2.3.2 Estimation of drug entrapment efficiency: 20 mg of dried microparticles were taken in 100 ml phosphate buffer (pH 7.4) and kept for 48 hours. Then it is sonicated for 15 minutes in a sonicator (Frontline Sonicator, FS-600, Frontline Electronics and Machinery Pvt. Ltd., India). The polymer debris was removed by filtering the solution through Whatman® filter paper (No. 40). The drug content was determined by using a UV-VIS Spectrophotometer (Thermo Spectronic UV-1, USA) by measuring the absorbance at λ_{max} 254 nm. The entrapment efficiency was calculated by using the following formula.[18]

$$\text{Drug entrapment efficiency} = \frac{\text{Actual drug content in microparticles}}{\text{theoritical drug content in microparticles}}$$

2.3.3 Particle size determination: Particle size of the prepared celecoxib microparticles were determined by digital slide caliperse (CD-6" CS, Mitutoyo Corporation, Japan). 100 particles were randomly collected and kept between two plates. Diameters of the particles were displayed on the digital screen.

2.3.4 Surface morphology analysis: Surface morphology of the prepared celecoxib microparticles were analysed by scanning electron microscope (SEM). Microparticles are deposited in double sided adhesive carbon tape (NEM Tape, Nisshin Em. Co. Ltd. Tokyo, Japan) and then kept under saturated vapour of palladium to make them conductive. It was mounted on SEM instrument (JEOL-JSM-6360, Jeol Datum Ltd, G5/IL/42/08. Tokyo, Japan) and their morphology was examined by secondary electro image (SEI) detector.

2.3.5 Swelling study: The swelling characteristics of cross linked chitosan microparticles were studied by placing dried samples separately in dialysis membrane containing simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4). The solutions were stirred at 50 rpm at $37° \pm 0.5°$ C for 2 hours. The samples were withdrawn periodically (30 mins interval)

and net weight was determined after blotting the surface water. The degree of swelling was calculated by using the following formula.

$$Degree\ of\ swelling = \left[\frac{Wt - Wo}{Wo}\right]X100$$

Where Wt is the weight of microparticles after time t, and Wo is the initial weight of the microparticles.

2.4 Preparation of sustained release tablets of celecoxib: Sustained release tablets of Celecoxib were prepared by direct compression method. A total of 8 formulations were made by changing the concentrations of chitosan, xanthan gum and glutaraldehyde (Table no 1). The required ingredients were weighed accurately and blended uniformly to distribute the active ingredient evenly in the powder blend. Weigh accurately about 500 mg of the mixture blend and fed into the die of single punch tablet press and compressed at 1.5 N compression force using 8 mm concave punches.

Table 1. Formulation of Celecoxib sustained release matrix tablets

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Celecoxib (mg)	500	500	500	500	500	500	500	500
Chitosan (gm)	1	1.25	1.5	1	1	1	1.25	1.5
Glutaraldehyde (ml)	-	-	0.1	0.2	0.3	0.4	0.5	0.5
Xanthan gum (mg)	100	100	100	150	200	250	200	250
Lactose (gm)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Microcrystalline cellulose (mg)	350	350	350	350	350	350	350	350
Talc (mg)	25	25	25	25	25	25	25	25
Magnesium stearate (mg)	25	25	25	25	25	25	25	25

2.5 Characterization of prepared sustained release tablets of celecoxib

2.5.1 *In vitro* drug release study: In vitro drug release study of celecoxib sustained release tablets were performed by using a USP X1X paddle apparatus 2 under sink condition. The dissolution studies were carried out with a paddle speed of 50 rpm in three different buffer solutions, (pH 1.2 HCl buffer, pH 6.8 and pH 7.4 phosphate buffers) at 37.4 ± 0.5 °C. The tablets were tested for drug release up to 2 hours in pH 1.2 (500 ml) and pH 6.8 (500 ml) separately and in phosphate buffer pH 7.4 up to 8 hours. All experiments were done in triplicate and mean values were calculated. At regular time interval 5 ml aliquots was collected and the same amount of fresh dissolution medium was replaced. The collected

aliquots were filtered through Whatman filter paper and finally absorbances were measured by using a UV-VIS spectrophotometer (Thermo Spectronic UV-1, USA) at λ_{max} 254 nm.

2.5.2 Determination of *In Vitro* release kinetics and mechanism: The drug release from prepared chitosan- xanthan gum IPN system were studied for various different kinetic model like zero order, first order, Higuchi, Hixson-Crowell and Korsemeyer-Peppas model.[19]
Will come as a sub heading

Zero order kinetics: $Q_t - Q_0 = K_0 t$; where Q_t is the amount of drug released after time t, Q_0 is the initial concentration of the drug in solution; K_0 is the zero order rate constant.[20]

First order kinetics: $\text{Log } Q_t = \text{Log } Q_o - K_1 t / 2.303$; where Q_t is the amount of drug released after time t, Q_0 is the initial concentration of the drug in solution; K_1 is the first order rate constant.

Higuchi model: $Q = K t^{1/2}$; where Q is the amount of drug released after time t; and K is the rate constant.[21]

Hixson-Crowell model: $Q^{1/3} = kt + Q_0^{1/3}$; where Q is the amount of drug released after time t, Q_0 is the initial concentration of the drug in solution; and K is the rate constant.

Korsemeyer-Peppas model: $Q = kt^n$; where Q is the amount of drug released after time t, K is the rate constant and n is diffusional exponent for drug release.

3. RESULT AND DISCUSSION

3.1 Fourier transformed-infrared (FT-IR) spectroscopy: FT-IR spectrum Celecoxib and formulation were shown fig. 1. FTIR spectrum of pure drug Celecoxib shows peaks at 1345.37(N-O symmetric stretching), 1161.11(C-H wagging of alkyl halides), 1133.43 (C-N stretching of aliphatic amine), 791.05 (N-H wagging for 1°,2° amine), 631.66 (C-F stretching for alkyl halides). FTIR spectrum of Celecoxib loaded formulation showed peaks at 1346.81, 1162.33, 1135.26, 791.81, and 632.23 for N-O, C-H wagging, C-N stretching, N-H wagging, C-F stretching respectively. So it can be said that Celecoxib was successfully entrapped in formulated particles and no significant interaction occurred in drug properties.

3.2 Estimation of drug entrapment efficiency: Entrapped amount of celecoxib was determined in chitosan- xanthan gum hydrogel system and it was expressed in percentage.

The entrapment efficiency varies from 81.67±1.68 % to 95.45 ± 0.54 % (Table No. 2) depending on the amount of polymers and cross-linker. Highest drug entrapment efficiency was observed for formulation 7 (F-7). As the amount of cross-linker increases, an increase in entrapment efficiency was also observed.
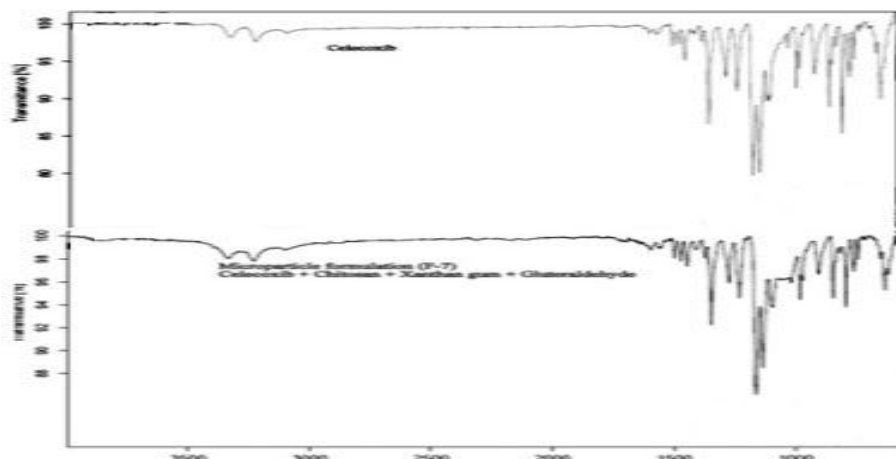

Fig. 1. FTIR spectra of celecoxib and celecoxib loaded glutaraldehyde cross-linked chitosan- xanthan gum microparticles.
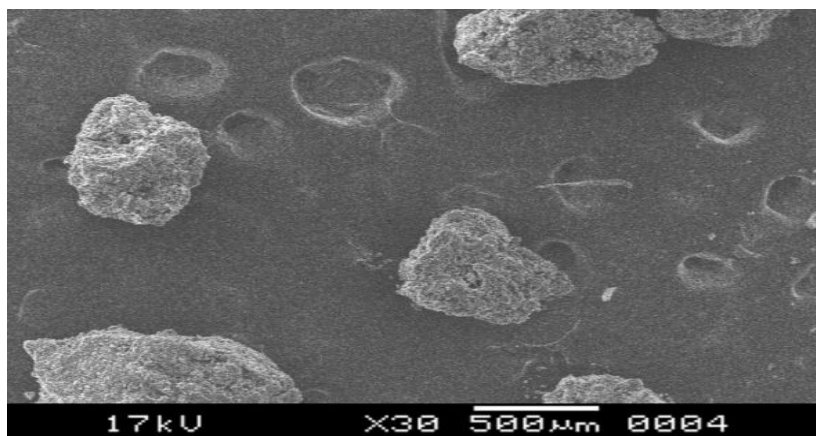

Fig. 2. SEM photograph of celecoxib loaded chitosan-xanthan gum microparticles

Table 2. Result of swelling index study and particle size determination

Formulation code	Swelling index % ± S.D. at SGF pH=1.2 (n = 3)	Swelling index % ± S.D. at SIF pH=7.4 (n = 3)	Mean size of particles (μm) (n = 100)
F1	87 ± 7	93 ± 5	683.19 ± 37.25
F2	92 ± 4	91 ± 4	686.43 ± 46.81
F3	113 ± 6	108 ± 8	636.82 ± 63.15
F4	116 ± 4	113 ± 7	602.16 ± 51.34
F5	115 ± 6	129 ± 5	569.53 ± 52.33
F6	124 ± 7	122 ± 6	581.39 ± 27.67
F7	139 ± 5	146 ± 7	527.37 ± 31.82
F8	134 ± 7	144 ± 4	573.74 ± 68.17

3.3 Particle size determination and surface morphology: The average particle size of celecoxib loaded chitosan- xanthan microparticles were found to be in the range between 527.37 ± 31.82 μm to 686.43 ± 46.81. An increase in cross-linker concentration resulted decreased particle size for the prepared celecoxib microparticles due to more rigid network formation. The celecoxib loaded chitosan- xanthan gum microparticles are found hemispherical in shape. The surfaces of the particles are rough and porous in nature and no aggregation found in group of particles.

3.4 Swelling study: Swelling behaviour of cross-linked chitosan microparticles were studied in simulated gastric fluid (pH 1.2) and in simulated intestinal fluid (pH 7.4). There was no disintegration of swollen microparticles during the study period. In both the cases swelling index was found to be almost similar.

3.5 Characterization of powder blends: Angle of repose, bulk density, tapped density, compressibility index were found to be satisfactory (data not shown in table).

3.6 *In-vitro* drug release: *In vitro* drug release from the prepared celecoxib tablets were carried out in three different buffer solutions. In HCl buffer (pH 1.2), cumulative release of the drug were found in between 11-15 % for all the formulation after 2 hours of dissolution. In phosphate buffer (pH 6.8), cumulative percentage release were found in between 10-14 % after 2 hours of dissolution. At pH 7.4 phosphate buffer, dissolution was carried out up to 8 hours. The release of the drug from hydrogel matrix was varied in between 60.365 – 74.271 %. If the amount of chitosan were kept constant, a decrease in drug release is observed with the increase in glutaraldehyde concentration. The degree of cross-linking increases with the increase amount of cross-linker resulting in more sustained release of celecoxib.
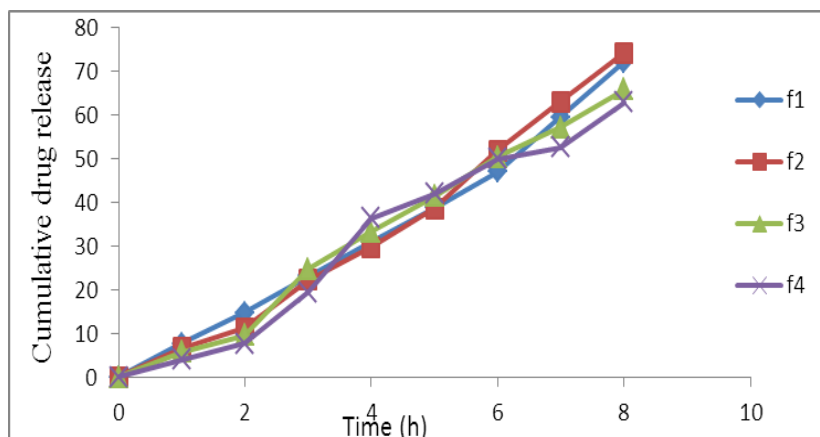

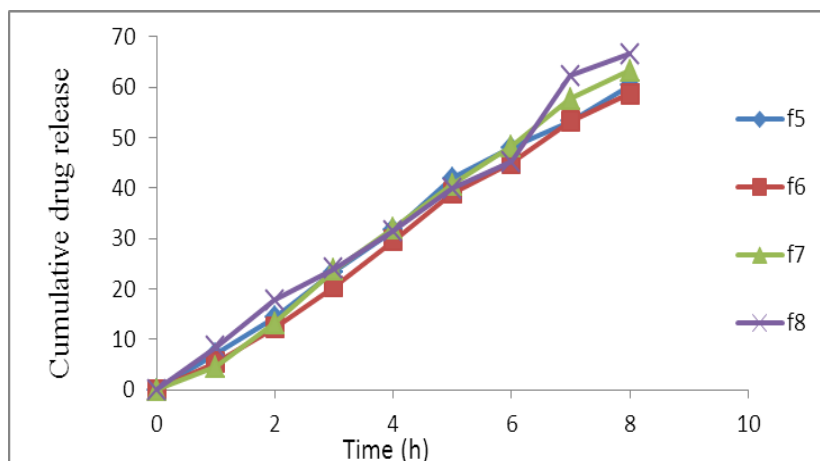**Fig. 3. Comparative *in vitro* drug release of F1, F2, F3 and F4**

Fig. 4. Comparative *in vitro* drug release of F5, F6, F7 and F8

Table 3. Result of curve fitting of various formulation of chitosan- xanthan gum of celecoxib tablet.

Formulation Code	Zero order R^2	First order R^2	Higuchi R^2	Hixson Crowell R^2	Koresmeyer - Peppas		Drug entrapment efficiency in %
					R^2	n	
F1	0.982	0.923	0.946	0.882	0.996	0.803	81.67 ± 1.68
F2	0.968	0.932	0.945	0.873	0.988	0.768	83.87 ± 0.36
F3	0.983	0.986	0.980	0.925	0.976	0.733	88.23 ± 0.32
F4	0.957	0.979	0.970	0.891	0.968	0.593	91.33 ± 1.78
F5	0.992	0.994	0.988	0.905	0.996	0.857	90.55 ± 1.21
F6	0.992	0.992	0.984	0.913	0.997	0.746	92.43 ± 0.72
F7	0.992	0.991	0.989	0.926	0.988	0.710	95.45 ± 0.54
F8	0.984	0.943	0.952	0.826	0.993	0.928	93.27 ± 0.43

3.7 *In-vitro* release kinetics: The *in-vitro* drug release study for prepared celecoxib tablets were evaluated kinetically by using different mathematical models like zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer- Peppas models. The correlation coefficients (R^2) were determined based on the accuracy of the models and values as nearer to 1 are considered as the best fit model for the respective formulation. F1, F2, F5, F6, F8 are found to follow Korsmeyer- Peppas release kinetics. The value of release exponent (n) determined from the data obtained from various celecoxib formulation indicating anomalous (non-Fickian) diffusion for drug release. F3 and F4 followed first order release kinetics. F7 showed zero order release kinetics which suits the controlled release profile.

4. CONCLUSION

Glutaraldehyde cross-linked chitosan microparticles were prepared successfully through a simple method for sustained release of celecoxib. The prepared microparticles were almost

spherical in nature with average particle size of 527.37 ± 31.82 μm to 686.43 ± 46.81. The drug entrapment efficiency of celecoxib was found to be within the range of 81.67 ± 1.68 to 95.45 ± 0.54 %. The IPN structure of celecoxib microparticles was studied for compatibility by FT-IR and no such significant interaction was found between celecoxib and chitosan. Swelling study of celecoxib microparticles shown almost similar swelling index in simulated gastric fluid (pH 1.2) and in simulated intestinal fluid (pH 7.4) without any sign of disintegration of swollen microparticles. The in vitro drug release of celecoxib tablets showed a sustained release of celecoxib over a period of 8 hrs. Most of the formulation was following Korsmeyer- Peppas model for non-Fickian release of drug. Overall these results confirmed that the ability of glutaraldehyde cross-linked chitosan microparticles is effective for the successful sustained release of celecoxib. As a conclusion, formulating chitosan-xanthan gum mixture as a hydrophilic polymer matrix resulted in a superior pharmacokinetic parameters translated by better rate and extent of absorption of celecoxib.

ACKNOWLEDGEMENTS

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