SYNTHESIS AND CHARACTERIZATION OF NOBLE ANTICANCER DRUG OF CHITOSAN-5FU CONJUGATED FORMULATION

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ABSTRACT: Cancer is one of the emergings, and best-understood neoplasm's from a genetic perspective, yet it remains the second most common cause of cancer-related death, indicating that some of its cancer cells are not removing completely by current therapies. Chitosan is a polysaccharide β-linked D-glucosamine and N-acetyl-D-glucosamine were used as conjugate to 5Fluorouracil. 5FU is one of the most commonly used drugs to treat cancer. The present invention relates the 5 Fluorouracil drug react with chitosan and formed chitosan – 5FU conjugate and used as Nobel anticancer models in zebrafish as an alternative model. The conjugated drug may highly selective and sustain release of drug to treat effectively colon cancer models in zebrafish. 5-fluorouracil react with chloroacetic acid in presence of potassium hydroxide gives 5-fluorouracil acetic acid. 5-fluorouracil acetic acid reacts with chitosan and formed chitosan -5FU conjugate. Characterization of chitosan folic acid and chitosan 5FU studied details by FTIR and NMR and showed well structural conformation.

Keywords:

5-Fluorouracil, Chloroacetic acid, Chitosan, Chitosan-5FU, Colon cancer

INTRODUCTION: Colon cancer is one of the emerging and best-understood neoplasm's from a genetic perspective [1-3], yet it remains the second most common cause of cancer-related death, indicating that some of its cancer cells are not removing completely by current [4-6]. Chitosan is a polysaccharide consist of β-linked D-glucosamine and N-acetyl-D-glucosamine. It has a number of commercial and possible biomedical uses [7]. 5-Fluorouracil is one of the most commonly used drugs to treat cancer. It is a treatment for many types of cancer including breast cancer, head and neck cancers, anal cancer, stomach cancer, colon cancer, and some skin cancers [8].

The use of the chitosan-5FU conjugate compound is highly significant in case of grade 4 and grade 3 colon cancer. Shrinkage of the high-grade tumor may be possible by this chitosan-5 FU conjugated drug delivery. After colon tumor operation, regular post-operative treatment may be also effective to remove the migrating colon tumor cells present in the body. The conjugated drug may be highly effective for better treatment in different types of cancer including colon cancer. The conjugated drug may highly selective and sustain release of drug to treat effectively Colon cancer models in zebrafish. Further studies will be pharmacological evaluation in Zebrafish Xenotransplant Colon Cancer Model by using Chitosan-5FU conjugated formulation.

MATERIALS AND METHODS:

Materials: The following materials are required for conducting the research. 5-Fluorouracil, chitosan solution, EDC (Ethylene carbodimide), Acetic acid, CM-DiI fluorescence dye, Chloroacetic acid will be procured from Sigma Aldrich. SW620 (colorectal cancer cells), EMEM (collagens in eagle minimum essential medium) will be purchased from the national center for science (NCCS), Pune.

Methods:

Synthesis of 5 Fu-Acetic Acid: In a 250 mL of round-bottomed flask 5-FU (20 mmol) was dissolved in a solution of potassium hydroxide (80 mmol) in 15 mL of water. While this solution was warmed in a 42 °C in water bath, a solution of chloroacetic acid (35 mmol) in 10 mL of water was added over 30 min. After this, the reaction was stirred for 2 h at this temperature. It was allowed to cool to room temperature (15 °C) and the pH was adjusted to 5.5 with conc. HCl. The solution was then cooled in a refrigerator for 2 h. The precipitate formed was removed by filtration. The solution was then adjusted to pH 2 with conc. HCl and put in a freezer for 5 h. The resulted white precipitate product was isolated by filtration, washed with water 2-3 times and dried in vacuum oven at 35 °C for 7 h. The yield 82%, White solid, and M.P. 253–255 °C was obtained according to procedure as described elsewhere [9].

Synthesis of 5 FU-CS conjugates: To synthesize chitosan–5 FU conjugate 100 mg of chitosan powder was dissolved in 1% (w/v) hydrochloric acid. The mixture was vigorously stirred by a magnet stirrer at room temperature until the polymer was completely dissolved. 0.300 g 5 flurouracil-1-yl acetic acid solution was added into the chitosan solution and stirred for 2 h. Then, 0.355 g EDC dissolved in 20 mL of 95% ethanol was added to induce the acylation reaction. The mixture was then stirred for 8 h at room temperature (15 °C). When the reaction finished, the product was then filtered by vacuum and washed with 95% ethanol to remove excessive DCC. The final product was dried in a vacuum oven at 35 °C for 4 h and structural conformation will be evaluated by FTIR and NMR studies [10-12].

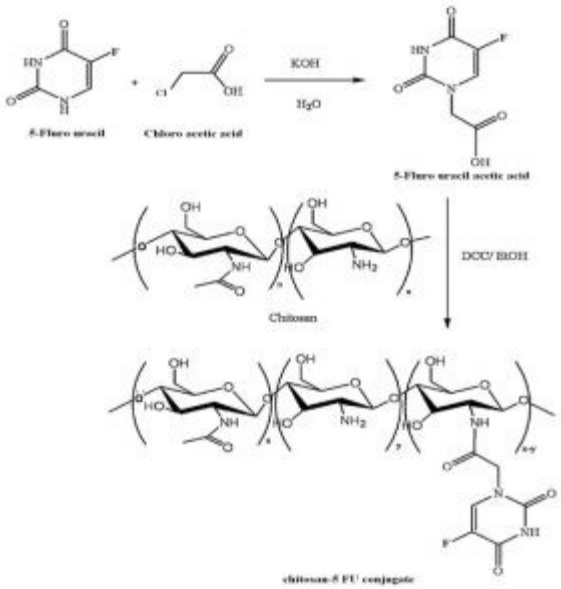

FIG. 1: PREPARATION OF CHITOSAN-5 FU ACETIC ACID CONJUGATE

FTIR Studies: Fourier transform infrared spectroscopy (FTIR) 2.5% w/w of sample, with respect to the potassium bromide (KBr) disc, were mixed with dry KBr (FTIR grade, Aldrich, Germany). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm^{-1} over a wavenumber region of 400 to 4000 cm^{-1} visible spectrophotometer using an FTIR spectrometer (Spectrum RX1 FTIR system, Perkin Elmer, USA). The characteristic peaks of IR transmission spectra were recorded. At least triplicates were carried out for each batch of the sample, and the results averaged [13].

NMR Studies: 1H nuclear magnetic resonance (1H NMR) spectra were determined by Bruker Avance II 400 NMR spectrometer in appropriate solvents and are expressed in parts per million (d, ppm) downfield

from tetramethylsilane (internal standard) NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons [13].

RESULTS AND DISCUSSION:

FTIR of Chitosan-FA: The significant change of FTIR peaks of FA–CS from those of CS confirms the effective formulation of FA–CS conjugates. Strong absorption peaks in 3423, 1694, 1603, and 1520 cm^{-1} can be observed in the FTIR spectrum of FA which is corresponding to the vibration of N–H, C O, amino group in the pteridine ring. In the case of IR spectrum of CS the absorption peaks in 1638 and 1592 cm^{-1} belong to the vibration of amide I and amide II groups, and the wide absorption band in 3451 cm^{-1} is the vibration of OH; the strong absorption peaks in 1087 cm^{-1} belongs to the vibration of C–O–C. The significant difference is observed between the IR spectra of CS and FA–CS. It can be seen that the absorption peak in 3435 cm^{-1} becomes stronger due to the overlapping of the vibration of OH and N–H functional group. The absorption in 1592 cm^{-1} and two new peaks appear in 1633 and 1017 cm^{-1}, which belong to the vibration of C–N. The absorption peak of amide at 1652 cm^{-1} of CS shifts to 1633 cm^{-1}, which is overlapped with the absorption peak of the newly formed C–N bond disappears.

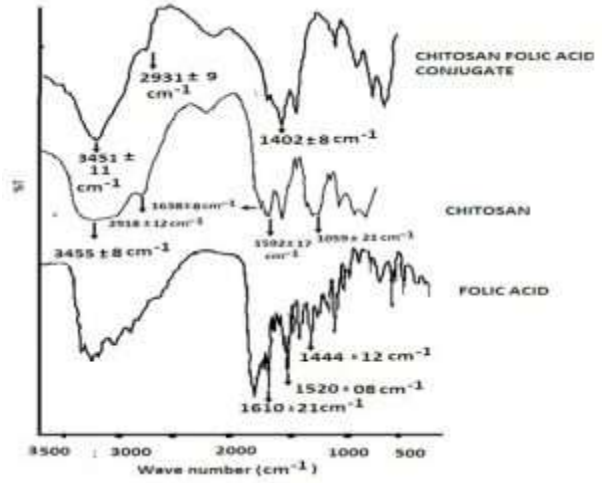

FIG. 2: PEAKS OF CHITOSAN FOLIC ACID CONJUGATE, CHITOSAN, AND FOLIC ACID

NMR of Chitosan-FA: The appearance of characteristic peaks in the 1H NMR spectrum of FA–CS conjugates confirms that FA is successfully incorporated with CS. As shown in the 1H NMR spectrum of FA the signals at 8.67, 8.16, 7.64, 6.94, 6.64, 4.5, 4.34, 2.51, and 2.04 ppm were corresponding to the FA protons of H-18, H-13/15, H-10, H-12/16, H-19, H-19, H-22, and H-21, respectively. The coupling of FA with CS leads to the overlapping of some sympathetic vibration peaks due to the influence of solvent and the interaction between FA and CS. The characteristic peaks at 2.30 ppm (H-21, FA), 2.14 ppm (H-22, FA) and the characteristic peaks at 3.31 ppm (H-2/H-5/H-6'), 1.83 ppm (H-4', CS) can still be observed.

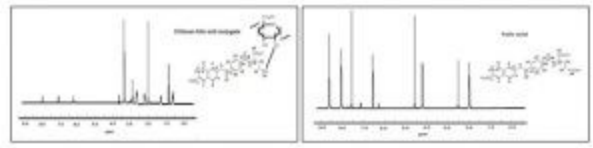

FIG. 3: NMR SPECTRUM OF CHITOSAN FOLIC ACID CONJUGATE AND FOLIC ACID

FTIR of Chitosan-5 FU: Synthesis of 5 Fluorouracil modified chitosan is outlined in **Fig. 4**. Here, the carboxylic group of modified 5FU (5 Fluorouracil-1-yl acetic acids) is reacted with the NH_2 group of chitosan. FTIR spectra of chitosan, 5 Fluorouracil-1-yl-acetic acid, and chitosan–5 Fluorouracil conjugate are shown in **Fig. 4.** Characteristic peaks assignment of chitosan **Fig. 4** are 3360 cm^{-1} (O H stretch overlapped with

N H stretch), 2919 and 2874 cm^{-1} (C H stretch), 1640 cm^{-1} (amide II band, C O stretch of acetyl group), 1592 cm^{-1} (amide II band, N H stretch), 1420–1377 cm^{-1} (asymmetric C H stretch bending of CH_2 group) and 1061 cm^{-1} (skeletal vibration involving the bridge C O stretch) of glucosamine residue. The IR spectral band of 5 Fluorouracil-1yl acetic acids **Fig. 4** 3168 ± 8 cm^{-1} (O H stretch), 1634 ± 12 cm^{-1} (C O stretch). As shown in **Fig. 4** the chitosan–5 Flurouracil conjugate mediated spectral band appear at 3323 cm^{-1} (axial O H group of chitosan), 2928 and 2850 cm^{-1} (C H stretch) which were stronger and sharper in comparison to the chitosan. The peaks at 1624 cm^{-1} (amide linkage), 1436 cm^{-1} (CH stretch bending of CH_2 group), 1570 cm^{-1} (N H bending stretching) and 1087 cm^{-1} (bridge C O C stretch) of chitosan residue were stronger than that of pure chitosan. The above FTIR analysis clearly indicates that the COOH group of 5 Fluorouracil-1-yl acetic acids has been successfully reacted with NH_2 group of chitosan main chain to form amide linkage. The intensity of these bands depends on the amount, type and bulkiness of the acid.

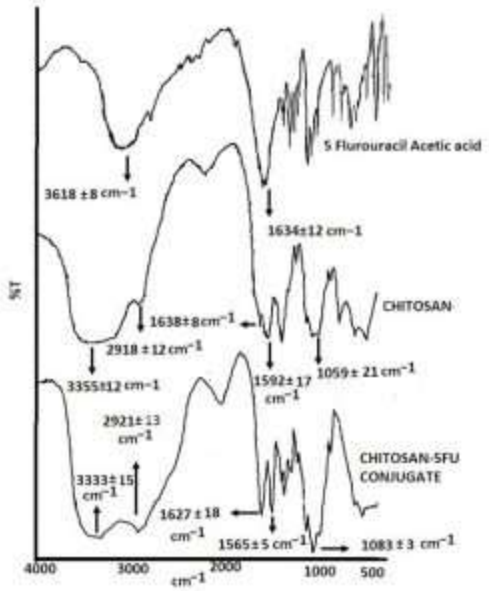

FIG. 4: 5 FLUROURACIL-1-YL-ACETIC ACID AND CHITOSAN–5 FLUROURACIL CONJUGATE AND CHARACTERISTIC PEAKS ASSIGNMENT OF CHITOSAN

NMR of Chitosan-5FU: 1H NMR spectra The 1H NMR spectra of chitosan, 5 Fluorouracil-1-yl acetic acid, and chitosan–5 Fluorouracil conjugate were given in **Fig 4.** Proton assignment of chitosan in **Fig. 4** ı = 4.89 ppm appears for chemical shift of the internal standard, ı = 4.37 ppm is due to chemical shift of the acetal proton (CH) of glucosamine overlaps the chemical shift of the internal standard, ı = 3.27 ppm for CH NH_2 protons (H2), ı = 3.92–3.72 ppm for (H3, H4, H5 and H6) protons of glucosamine ring ı = 3.27 ppm appear for chemical shifts of (H2) proton and upfield ı = 2.04 ppm for (NHCO CH_3) acetamido protons.

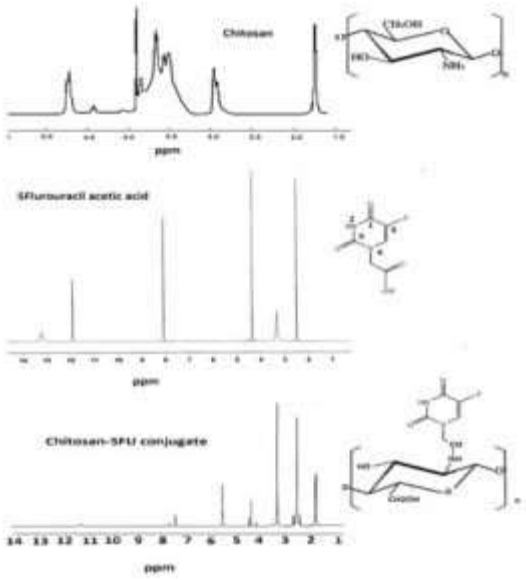

FIG. 5: CHITOSAN, 5 FLUOROURACIL-1-YL ACETIC ACID AND CHITOSAN–5 FLU0ROURACIL CONJUGATE

Proton assignment of 5 Fluorouracil-1-yl acetic acids shown in **Fig. 5**, ı = 1.75 ppm (s, 3H), 4.36 ppm (s, 2H), 7.50 ppm (s, 1H), ı = 11.32 ppm (s, 1H) for N H, ı = 13.10 ppm (s, 1H) for OH proton of carboxylic acid compared with chitosan and 5 Fluorouracil-1yl-acetic acid, the characteristic proton signals of chitosan–5 Fluorouracil conjugate appeared at ı = 5.55 ppm (s) is due to N H proton of amide linkage which is formed between NH_2 group of chitosan and COOH group of 5 Fluorouracil-1yl acetic acid, ı = 7.49 ppm (s) is CH proton of 5 Fluorouracil-1-yl acetic acids, ı = 1.75 ppm (s) CH_3 proton of 5 Fluorouracil, ı = 11.32 ppm (s) N H proton of 5 Fluorouracil, and ı = 2.50 ppm (s, $NHCOCH_3$). ı = 3.30 ppm (H-2 of GlcN residue), ı = 4.36 ppm (m) due to glucosamine unit of chitosan.

The 1H NMR spectra confirm the formation of new amide linkage between the COOH group of 5 Fluorouracil- 1-yl acetic acid and NH_2 group of chitosan. The degree of modified 5 Fluorouracil substitution in chitosan (DS) was found to be 56%. The DS was estimated from the ratio of the integral intensity of modified 5 Fluorouracil proton to the sum of integral intensities of the chitosan protons.

CONCLUSION: 5 Fluorouracil drugs react with chitosan and formed chitosan – 5FU conjugate and will be used as Nobel anticancer models in zebrafish as an alternative model. Characterization of chitosan folic acid and chitosan 5FU studied details by FTIR and NMR and showed well structural conformation.

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CONFLICTS OF INTEREST: Nil

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