REPORT

FABRICATION OF POTENTIAL MACROMOLECULAR PRODRUGS OF ASPIRIN AND DICLOFENAC WITH DEXTRAN

MUHAMMAD AJAZ HUSSAIN[1,*], ZAHID HASSAN[2], MUHAMMAD TAHIR HASEEB[2], MOHAMMAD SAEED IQBAL[3], MUHAMMAD SHER[1], MUHAMMAD NAWAZ TAHIR[4], WOLFGUNG TREMEL[4], SAJID BASHIR[2] AND RIAZ AHMAD[5]

[1]*Department of Chemistry,* [2]*Department of Pharmacy, University of Sargodha, Sargodha, Pakistan*
[3]*Department of Chemistry, GC University, Lahore, Pakistan*
[4]*Institute of Inorganic & Analytical Chemistry, Johannes Guttenberg University, Duesbergweb 10-14, Mainz, Germany*
[5]*Department of Physics, GC University, Lahore, Pakistan*

ABSTRACT

Aspirin and diclofenac conjugates with dextran were synthesized as potential macromolecular prodrugs under homogeneous reaction conditions by using 4-methyl-benzenesulfonyl chloride as an acylating agent in the presence of triethylamine as a base. Highly pure conjugates with good yields were synthesized by this acylation method. All of the products were found soluble in aqueous medium as well as in dimethylsulfoxide and *N,N*-dimethylacetamide. The UV/Vis spectrophotometry has indicated the incorporation of drugs in conjugates and extent of substitution of drug onto dextran polymer. Covalent attachment of the drug onto the drug carrier polymer (dextran) was verified by ^{1}H NMR and Fourier transform infrared (FTIR) spectroscopic analysis. The prodrugs were analysed by powder X-ray diffraction (XRD) measurements. Phase changes were noticed by powder XRD for all macromolecular prodrugs indicating the change of state of matter towards more crystallinity. Therefore, fabricated macromolecular prodrugs are potential candidates to show better pharmacokinetic profile. All of the products were thoroughly characterized by using different spectroscopic techniques.

Keywords: Acylation, dextran, NSAIDs, macromolecular prodrugs, polysaccharides, X-ray diffraction.

INTRODUCTION

The development of macromolecular prodrugs of non steroidal anti-inflammatory drugs (NSAIDs) is advantageous because of the fact that these formulations show sustained release of drug, colon-targeted drug delivery, reduction in the administration frequency and better patient compliance (Gac *et al*., 2000; Jain *et al*., 2007). These days, fabrication of macromolecular prodrugs of NSAIDs by the covalent attachment onto polysaccharides as an ester moiety is getting greater attention (Dhaneshwar *et al*., 2006). In general, such ester conjugates of NSAIDs (especially onto dextran polymer) can easily be hydrolysed in the basic medium of colon in particular and such linkages affected less by acidic hydrolysis in stomach. Hence, by this conversion of NSAIDs, stomach can be kept secure from the harmful effects of NSAIDs (Julian *et al*., 1996; Larsen *et al*., 1989; Dorsch *et al*., 2007; Gerald and Pronto, 2001). A significant number of publications have been reported about macromolecular prodrug design of different drugs with polysaccharides and other synthetic polymers in which useful results were obtained (Hussein *et al*., 1996; Parejo *et al*., 1998; Babazadeh, 2006).

Corresponding author: majaz172@yahoo.com

The polysaccharides, especially, glycopolymers made possible to deliver a wide variety of drugs, e.g. peptides, proteins, nucleic acid, NSAIDs, anti-cancer drugs, resin and sensitive drugs to colon (Hussain *et al*., 2009; Hussain, 2008; Sandrine *et al*., 2005; Mehvar, 2003; Ohya *et al*., 2001). The literature shows that dextran, pullulan and mannan glycopolymers are of greater interest (Mehvar, 2003; Ohya *et al*., 2001; Peng *et al*., 2006). Dextran is a glycopolymer (based on 1,6-linked β-D-glucose) produced by *Leuconostoc mesenteroides* strain is commonly used as an excipient in several pharmaceutical formulations due to its non toxic nature and biocompatibility (Khalikova *et al*., 2005).

Nevertheless, in present study, dextran was selected for the fabrication of macromolecular prodrugs of aspirin and diclofenac. An easily applicable and powerful esterification reagent 4-methyl-benzenesulfonyl chloride (Shimizu and Hayashi, 1988; Hussain and Heinze, 2008; Heinze *et al*., 2003; Liebert *et al*., 2006) is being used for the one pot and homogeneous conversion of available free hydroxyl groups onto dextran with aspirin and diclofenac. Aim was also focused to fabricate these novel conjugates to get double advantage, i.e. fabrication of novel colon-targeted macromolecular prodrugs and such macro-molecular prodrug itself is a very good system for

controlled/sustained release of drugs as it can release pure drug after basic hydrolysis in intestine slowly. Sustained release studies and formulation development of these newly fabricated conjugates are the future plans.

MATERIALS AND METHODS

Materials

Dextran (40000, Fluka) was dried under vacuum at 110°C for 8 h prior to use. Analytical grade *N,N*-dimethylacetamide (DMAc), 4-methyl-benzenesulfonyl chloride (tosyl chloride), triethylamine, lithium chloride, solvents and other chemicals were used as obtained from Fluka. The drugs used were pure according to USP standards.

Measurements

The ^{1}H NMR spectra (δ, ppm; *J*, Hz) of the dextran conjugate esters were acquired in D_2O on Bruker 400 MHz machine. The Fourier transform infrared (FTIR) spectra (ν, cm^{-1}) were measured on IR Prestige-21 (Shimadzu, Japan) by using KBr pellet technique. Thermogravimetric analysis (TGA) was used to evaluate the thermal stability and to determine the decomposition temperature (T_d) of samples and dextran. The T_d of the macromolecular prodrugs and standards were determined on a SDT Q 600 (TA Instruments, USA) thermal analyzer. The T_d was reported as the onset of significant weight loss from the heated sample. The thermograms of heated samples (10 mg) were recorded under nitrogen atmosphere at a constant heating rate of 10 °C/min from 35 upto 600 °C. Powder X-ray diffraction measurements were carried out over a range of 5-100° (2θ) in steps of 0.020° on an Xpert Pro MPD, (PANalytical, The Netherlands) diffractometer equipped with mono-chromatic X-rays.

Dissolution of dextran in N,N-dimethylacetamide (DMAc)

Pre-dried dextran (2 g) was added in 40 mL DMAc and mixed, followed by the addition of 4 g lithium chloride to dissolve the polymer. All the mixtures were kept under stirring for 2 h to obtain optically clear solution of dextran at room temperature.

Esterification of dextran with diclofenac, a typical example (Sample 1)

The triethylamine base (5.19 mL, 37 mmol) and tosyl chloride (3.52 g, 18.5 mmol) were added to the solution of dextran (2 g, 12.33 mmol) dissolved in DMAc/lithium chloride. The diclofenac (5.48 g, 18.5 mmol) was added in parts to the reaction mixtures. The reaction mixture was kept under stirring for 24 h at 80°C under nitrogen atmosphere. The reaction mixture was precipitated in 150 mL methanol. The precipitates of sample **1** were filtered and washed with methanol three times. The white precipitates after filtration were dried under vacuum at 50°C overnight. Yield: 1.51 g (49%). Degree of Substitution (DS): 18 mg drug/100 mg sample. UV (0.1 N NaOH in H_2O): λ_{max} 276.5 nm. FTIR (KBr, cm^{-1}): 3483 (O-H stretching), 2985 (C-H stretching of aromatic ring), 1730 (C=O stretching of ester), 1452 (CH_2 stretching of sugar units). ^{1}H NMR: (400 MHz, D_2O, ppm): 3.18-5.21 (anhydroglucose unit-H); 6.90-7.80 (aromatic-H).

Esterification of dextran with aspirin, a typical example (Sample 5)

To the solution of dextran (2 g, 12.33 mmol) in DMAc/lithium chloride, triethylamine (10.38 mL, 74 mmol) base and tosyl chloride (7.05 g, 37 mmol) were added under stirring. After the dissolution of tosyl chloride, aspirin (6.66 g, 37 mmol) was added and reaction mixture was kept under stirring under nitrogen for 24 h at 80°C. The reaction mixture was precipitated in 150 mL *n*-propanol and precipitates of sample **5** were filtered. The precipitates (white and powdery) were washed three times with *n*-propanol. The precipitates were dried under vacuum at 50 °C over night. Yield: 1.84 g (53%). DS: 23 mg drug/100 mg sample. UV (0.1 N NaOH in H_2O): λ_{max} 297 nm. FTIR (KBr, cm^{-1}): 3400 (O-H stretching), 2990 (C-H stretching of aromatic), 1752 (C=O stretching of ester), 1454 (CH_2 stretching of sugar unit). ^{1}H NMR (400 MHz, D_2O, ppm): 2.21 (methyl-H); 3.17-5.21 (anhydroglucose unit-H); 7.2-7.8 (aromatic-H).

UV/Vis spectrophotometric analysis

The UV Pharmspec 1700 (Schimadzu, Japan) was used to quantify the degree of substitution (DS) of pendant group on dextran conjugates. The solutions of standard drugs were prepared in 0.1 N NaOH and UV spectra were recorded from 190-400 nm. For the generation of calibration curve of standards, six different concentrations were prepared and absorbance was recorded at wavelength (λ_{max}) 276.5 and 297 nm for diclofenac and aspirin, respectively. For UV analysis of samples, 10 mg of each sample was taken in a round bottomed flask followed by the addition of 10 mL 0.1N NaOH. Reaction mixture was stirred for 8 h at 80°C for the complete hydrolysis of the sample. After filtration, the volume of reaction mixture was made upto 10 mL and absorbance was recorded at wavelength (λ_{max}) 276.5 and 297 nm for dextran diclofenac and dextran aspirin conjugates, respectively.

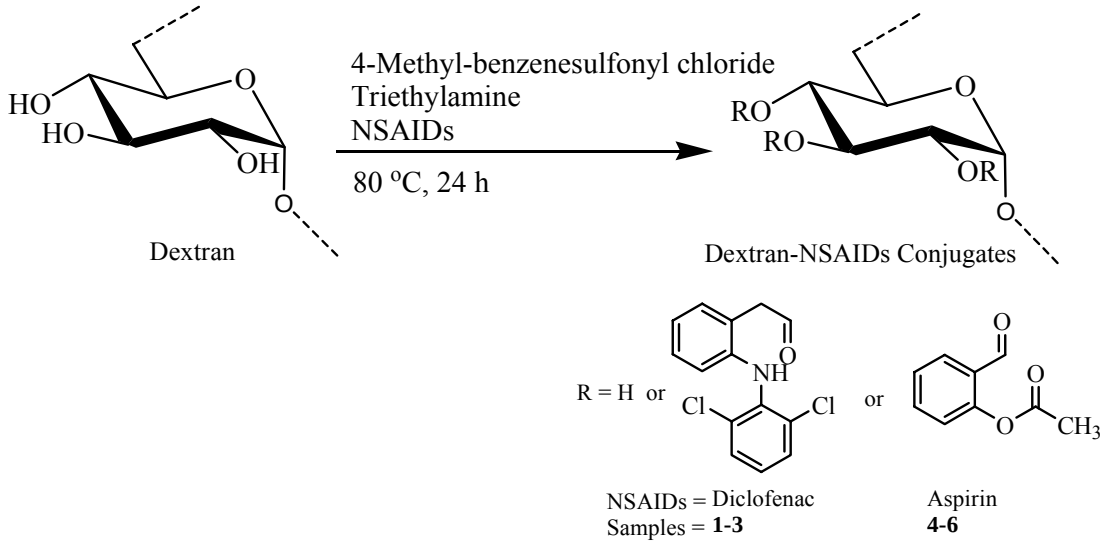
Calculations of degree of substitution (DS)

For the purpose of DS, 10 mg sample (dextran conjugates) was hydrolysed and the resulting drug was analysed by UV spectrophotometry as described above. The DS in terms of 'mg of drug bound per 100 mg' of dextran-drug conjugate was determined from the calibration curves of standards. The DS in term of substituted drug per AGU of polymer was also calculated by standard acid base titration followed by the saponification as adopted in references (Klemm *et al.,* 1998; Hussain *et al.,* 2010a).

Table 1: Reaction conditions and results of the esterification of dextran dissolved in DMAc/LiCl using *in situ* activated aspirin and diclofenac with tosyl chloride at 80°C for 24 h.

Sample	Drugs	Molar Ratio[a]	Conc.[b] (ppm)	DS[c]	Absorbance (λmax)	DS[d]	Yield, g (%)
1	Diclofenac	1:1.5:1.5:3	17.5	0.31	0.6263(276.5)	18	1.51 (49)
2	Diclofenac	1:3:3:6	25.5	0.52	0.9272(276.5)	26	1.83 (48)
3	Diclofenac	1:6:6:12	35.5	0.91	0.6105[e](276.5)	35	2.35 (46)
4	Aspirin	1:1.5:1.5:3	12.5	0.50	0.2193(297)	12	1.75 (58)
5	Aspirin	1:3:3:6	22.5	0.74	0.4351(297)	23	1.84 (53)
6	Aspirin	1:6:6:12	40.8	0.94	0.8107(297)	41	2.02 (52)

[a]Dextran (anhydroglucose unit): Drug: 4-methyl-benzenesulfonyl chloride: Triethylamine. [b]Concentration of drug was calculated by UV spectrophotomery. [c]DS (degree of substitution) was calculated by acid base titration after saponification. [d]DS was calculated by UV/Vis spectroscopy as mg drug attached per 100 mg sample. [e]Absorbance was recorded by 50 % dilution.



Scheme 1: Synthesis of macromolecular prodrugs of aspirin and diclofenac with dextran applying *in situ* activation of carboxylic acid groups with tosyl chloride.

Calculations of yield

Following formulae were used to calculate the percentage yield of the product as adopted in reference (Hussain *et al.,* 2010b):

$W_P + [(W_P/MW_{AGU}) \times DS \times MW_{Sub}]$ = theoretical yield

Practical yield/theoretical yield × 100 = %age yield

Where, W_P is weight of polymer, MW_{AGU} is molecular weight of anhydroglucose unit, DS is degree of substitution, MW_{Sub} is molecular weight of substituent.

RESULTS

Macromolecular prodrugs of aspirin and diclofenac with neutral, non-ionic and biocompatible dextran polysaccharide were synthesized homogeneously as a one pot synthesis. The results of reactions are summarized in table 1.

All of the products were thoroughly characterized by means of different identification and characterization techniques, i.e. UV, Fourier transform infrared (FTIR), ^{1}H NMR, powder X-ray diffraction (PXRD) measurements, thermogravimetric (TG) analysis and differential thermogravimetry (DTG).

Dextran-drug conjugates **1**-**6** have shown a characteristic peak in FTIR (KBr) spectra typical for the ester moieties at about 1730-1752 cm^{-1} ($C=O_{Ester}$) for different samples. A typical FTIR spectrum of dextran aspirin conjugate **5** is shown in fig. 1. The HPMC-aspirin conjugate **5** have shown a characteristic peak in FTIR spectrum typical for the ester moiety at about 1752 cm^{-1} ($C=O_{Ester}$). The spectrum has displayed -OH group absorption at 3400 cm^{-1}, aromatic C-H absorption at 2990 cm^{-1} and polymer CH_2 absorption at 1454 cm^{-1}. The FTIR spectrum of dextran diclofenac conjugate **2** have also shown a characteristic peak typical for the ester moiety at 1730 cm^{-1} ($C=O_{Ester}$)). The spectrum has displayed unreacted -OH group absorptions centred at 3483 cm^{-1}. The stretching of aromatic C-H and dextran -CH_2 absorption were seen at 2985 cm^{-1} and 1452 cm^{-1}, respectively. Nevertheless, all of the FTIR spectra have displayed hydroxyl group absorption, aromatic C-H absorption and CH_2 (polymer)

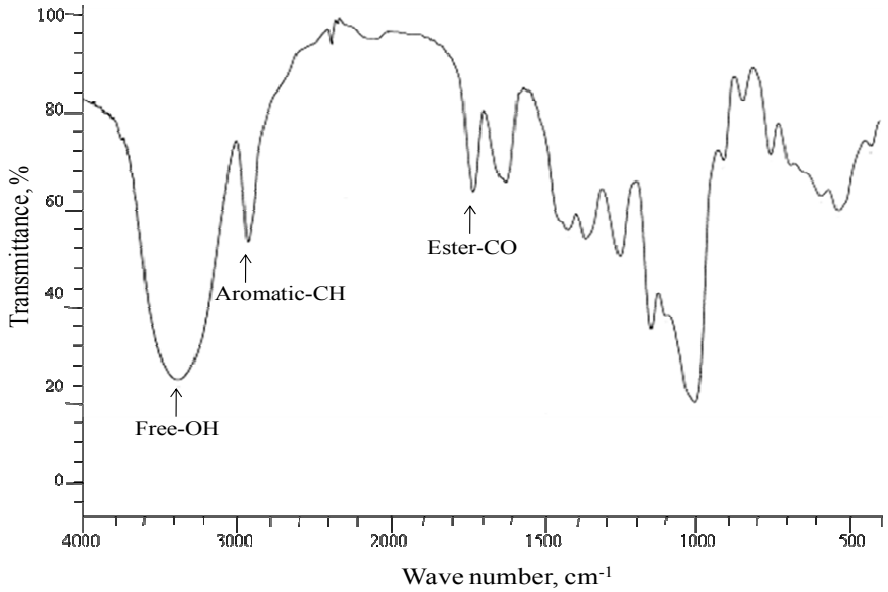


Fig. 1: FTIR (KBr) spectrum of dextran aspirin conjugate **5**.

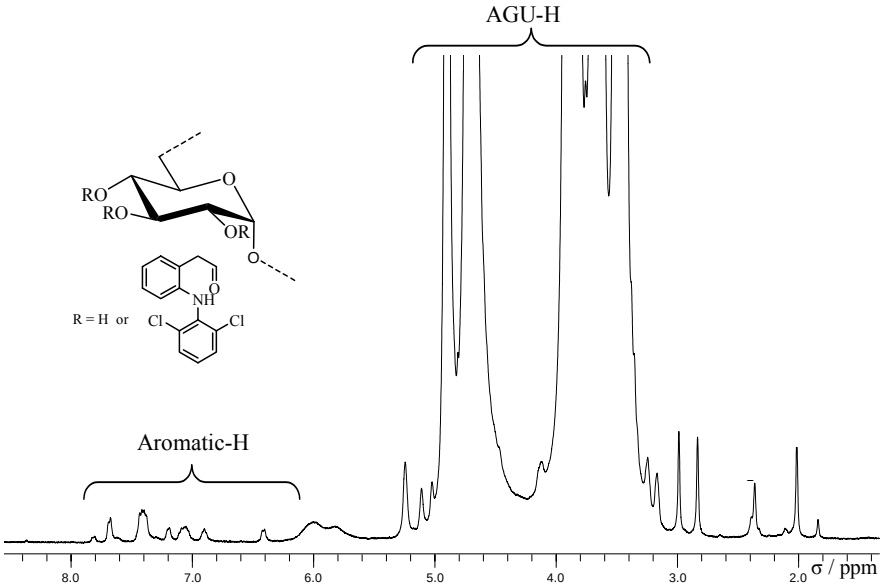


Fig. 2: ^{1}H NMR spectrum (400 MHz, D_2O) of dextran diclofenac conjugate **1**.

absorption signals as well which is indicative of success of reaction and purity of samples.

The ^{1}H NMR spectra of all macromolecular prodrugs were recorded in D_2O. ^{1}H NMR spectrum of dextran-aspirin conjugate **5** has revealed the presence of aromatic rings covalently attached to the dextran polymer backbone detectable at δ 7.2-7.8 ppm. Protons of dextran backbone were detectable at δ 2.02 (unreacted-OH overlapped with methyl protons of aspirin) and 3.17-5.21 (anhydroglucose unit-H) ppm. The ^{1}H NMR spectrum of dextran-diclofenac conjugate **1** is shown in Fig. 2. The presence of aromatic protons was detectable at δ 6.90-7.80 ppm. Unreacted free hydroxyl groups of dextran polymer backbone were detectable at δ 2.0 and protons of the anhydroglucose unit were detected at 3.18-5.21 ppm. The FTIR has already confirmed the aromatic ring absorptions and a typical ester peak. ^{1}H NMR has shown the absence of any impurity in the samples.

Thermal decomposition temperatures T_d 311.5, 312, 312.3, 307.5, 308.83, 310.1 and 304.3°C were obtained respectively for samples **1**-**6** and dextran from thermogravimetric analysis. These results have indicated that all macromolecular prodrugs synthesized are thermally more stable than unmodified dextran polymer. A simultaneous thermogravimetric (TG) analysis and differential thermogravimetric (DTG) analysis are shown

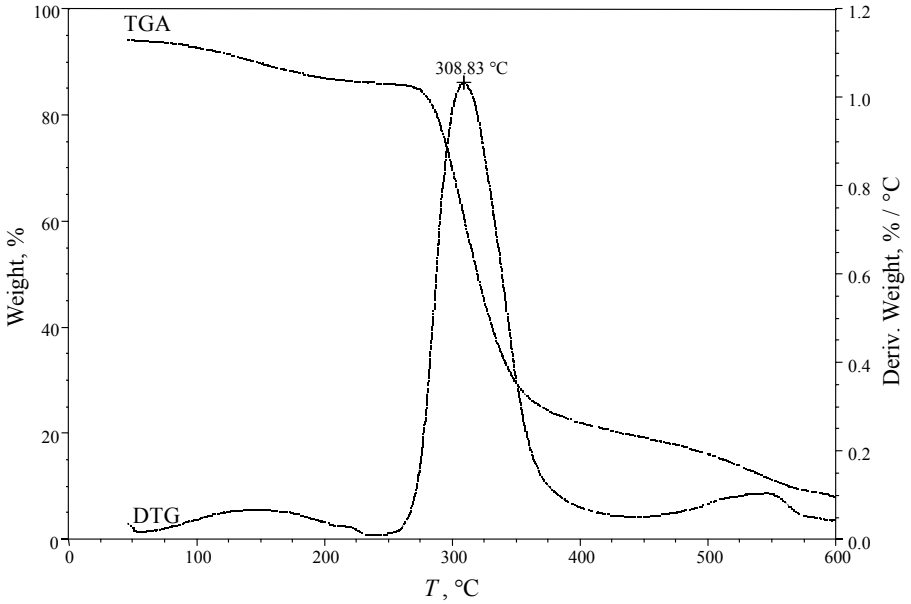


Fig. 3: Simultaneous DTG and TG spectra of dextran aspirin conjugate **5**.

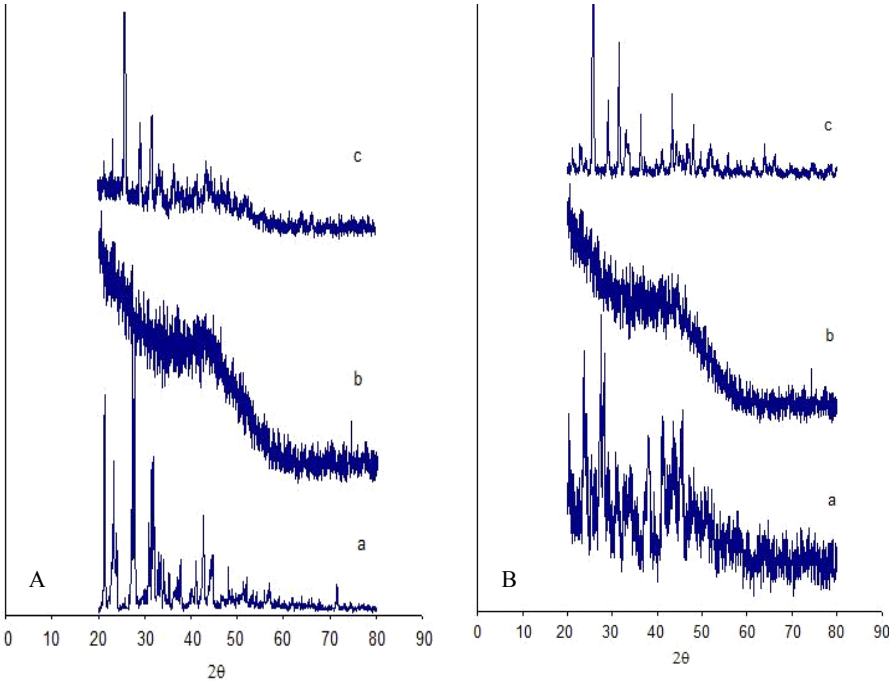


Fig. 4: Powder X-ray diffraction analysis. Spectra A: aspirin (a), dextran (b) and dextran aspirin conjugate **6** (c). Spectra B: diclofenac (a), dextran (b) and dextran diclofenac conjugate **2** (c).

in fig. 3 for sample **5** (dextran aspirin conjugate) as a typical example.

The degree of substitution (DS) of all dextran conjugates in terms of mass ratio was calculated by UV/Vis spectroscopic analysis. All of the samples were analyzed after complete hydrolysis of dextran drug conjugates. The DS is expressed as mg of drug/100 mg of the sample (see table 1) calculated from the calibration curves of the standard drugs. The UV studies have indicated that 18-35 mg/100 mg of diclofenac and 12-41 mg of aspirin per 100 mg of sample. The DS of all dextran conjugates in term of substitution of ester groups per anhydroglucose unit of dextran polymer was calculated by standard acid base titration method followed by saponification. The DS 0.31-0.91 was obtained for diclofenac and 0.50-0.94 for aspirin prodrugs with dextran. These results have indicated that by changing the molar ratio of reactants DS can be

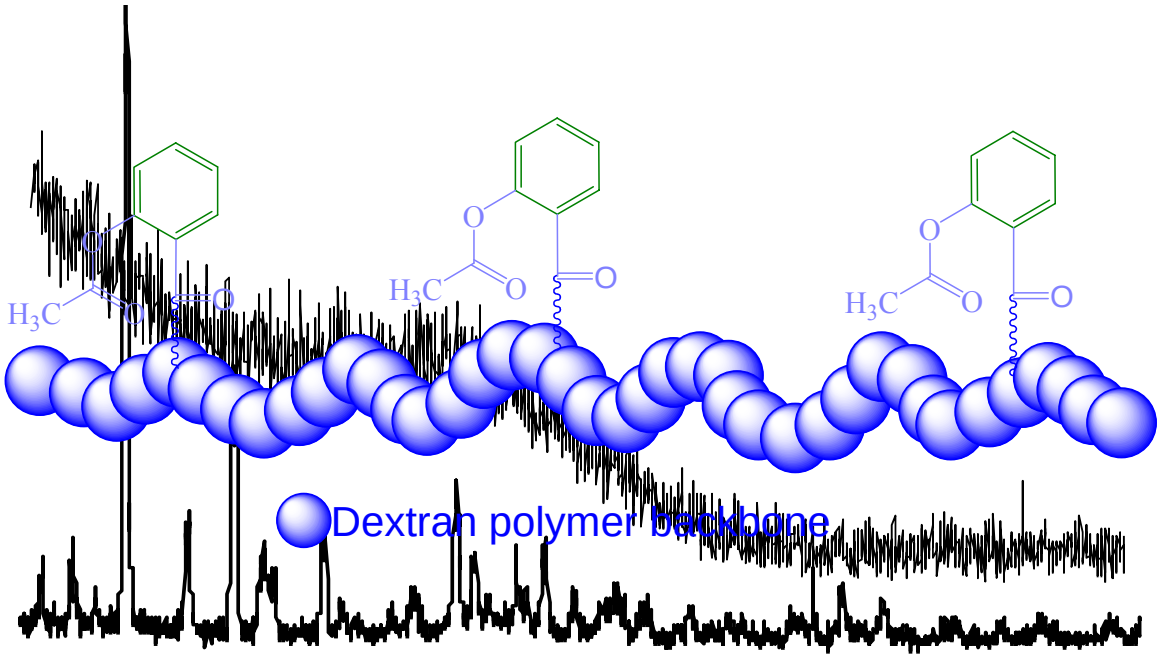


Fig. 5: Illustration of dextran aspirin conjugate: a macromolecular prodrug design with potential for better pharmacokinetic profile due to increased crystallinity in conjugates.

controlled as indicated from table 1. All of the products were obtained in good yield 46-58%.

The powder X-ray diffraction (PXRD) spectra of aspirin dextran conjugate were found to be significantly different (fig. 4), as witnessed by change in peak positions, no. of peaks and intensities, which clearly indicate the change of phase occurring due to bonding of aspirin with dextran.

DISCUSSION

Dextran dissolved in *N,N*-dimethylacetamide (DMAc)/LiCl was allowed to react with aspirin and diclofenac in the presence of highly efficient acylating agent 4-methyl-benzenesulfonyl chloride (tosyl chloride). Triethylamine was used as a base to neutralize the acidic by products and unreacted drugs. Dextran conjugates with aspirin and diclofenac were synthesized using different molar ratios of the reactants according to the reaction scheme given in Scheme 1. It has been established that tosyl chloride first reacts with carboxylic acid groups in NSAIDs (i.e. aspirin and diclofenac) to yield different reactive intermediates, i.e. acid chloride, acid anhydride and mixed anhydride. These intermediates *in situ* react with the -OH groups available on dextran backbone to yield their ester conjugates as macromolecular prodrugs of NSAIDs.

All of the samples were found soluble in aqueous and usual organic solvents, i.e. dimethylsulfoxide and DMAc. Highly pure products were obtained as no sign of any impurity was seen in spectroscopic analysis.

The UV/Vis spectroscopic analyses have shown that covalently attached active drug onto polymer can be released from the samples (prodrugs) *in vitro* by simple hydrolysis.

By comparison of PXRD spectra of the products, drugs and unmodified dextran polymer, it appears that the PXRD pattern changes in products toward more crystalline phase then dextran. The macromolecular prodrugs, i.e. dextran aspirin conjugates with increased crystallinity as compared with pure dextran is illustrated in fig. 5. This is indicative of better formulation of macromolecular prodrug as crystallinity in prodrugs may lead to the better pharmaceutical properties due to the stability of structure while studying its pharmacokinetics. The PXRD spectra of dextran diclofenac conjugates were found comparable to dextran aspirin conjugates regarding change in phase (fig. 4).

CONCLUSIONS

Fabrication of novel macromolecular prodrugs of different NSAIDs with dextran biopolymer was achieved by using homogeneous esterification methodologies. These aqueous and organo-soluble macromolecular prodrugs were purified and characterized. Dextran esters were engineered for acid resistance and favorable basic hydrolysis that leads to the point, by this way, stomach can be kept safe from the harmful effects of such acidic drugs and colon-targeted drug delivery can also be achieved. Nevertheless, esterified drugs will be released at basic pH of colon. It is obvious from literature that dextran macromolecular prodrugs (esterified) can shown

sustained release of drugs with colon-targeted drug delivery.

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