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Synthesis and evaluation of some novel thiomers as mucoadhesive polymer

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ABSTRACT

The purpose of the present study was to synthesize and characterize novel thiolated polymers mediated by carbodiimide. Cysteine was covalently linked to sodium carboxymethyl starch (CMS) and carboxymethyl guar gum (CMG). Depending on the weight-ratio polymer to cysteine during the coupling reaction, the resulting CMG-Cysteine conjugates displayed 80% ± 2.6 thiol groups per gm of polymer were as CMS-Cysteine conjugates displayed 78% ± 2.1. (mean ± S.D.; n=3).In aqueous solution above pH 6.0 polymers were capable of forming inter- and/or intra-molecular disulfide bonds. Mucoadhesion studies carried out on freshly excised sheep intestinal mucosa. The CMS-Cysteine displayed less mucoadhesion where as CMG-Cysteine conjugate were displayed increased mucoadhesion. The swelling behaviour of the CMS-Cysteine conjugate was influenced by the covalent attachment of the sulfhydryl compound. In contrast the swelling behaviour of CMG-Cysteine was not influenced by the immobilisation of cysteine. Furthermore, in aqueous solutions the cumulative % drug release time of tablet based on the CMG-Cysteine was prolonged 1.5 fold as compared to tablet containing CMS-Cysteine conjugate. Due to a high crosslinking tendency by the formation of disulfide bonds stabilizing drug carrier systems based on thiolated polymers and permeation enhancing effect, CMG-Cysteine conjugates represent promising excipients for the development of novel drug delivery system.

Keywords: CMS-Cysteine conjugates; Mucoadhesion; Permeation enhancement; CMG-Cysteine conjugates; Thiomers.

INTRODUCTION

The concept of mucoadhesion has been pioneered in the 1980s; numerous attempts have been taken in order to improve the adhesive properties of polymers. These attempts include approaches such as the use of linear poly (ethylene glycol) as adhesion promoter for hydrogels, the neutralization of ionic polymers, and mucoadhesion by a sustained hydration process and the development of polymer–adhesin conjugates, providing a specific binding to epithelium. However, all these systems are based on the formation of non-covalent bonds such as hydrogen bonds, Van der Waal's forces, and ionic interactions. Accordingly, they provide only relative weak mucoadhesion, in many cases insufficient to guarantee the localization of a drug delivery system at a given target site. Mucoadhesive polymers have therefore in many cases not proven to be effective as pharmaceutical glue. A presumptive new generation of mucoadhesive polymers is thiolated polymers or designated thiomers. In contrast to well established mucoadhesive polymers these novel polymers are capable of forming covalent bonds [1].

In recent years the interest in bioadhesion has been inspired by the development of novel bioadhesive polymers for mucosal delivery. Bioadhesive, or more precise mucoadhesive drug delivery systems are aimed to adhere to various mucosal tissues. All traditionally used mucoadhesive polymers, e.g. poly(acrylates) or chitosan, are based on the formation of noncovalent bonds such as hydrogen bonds and ionic interactions with the mucus layer. These polymers provide only a weak adhesion being in many cases insufficient to guarantee the localisation of a drug at a given target site. According to this, various attempts have been undertaken to improve the mucoadhesive properties of polymers. A promising new approach was the generation of thiolated polymers—or the so-called thiomers. Due to immobilized thiol groups these polymers are capable of forming covalent bonds with the mucus layer covering mucosal tissues, subsequently leading to improved mucoadhesive properties [2-3]. The responsible mechanism for this effect is based on thiol/disulfide exchange reactions between the thiol groups of the polymer and the cysteine-rich subdomains of mucin glycoproteins [4]. First success was achieved with polycarbophil–cysteine and chitosan–thioglycolic acid conjugates displaying a 2.75- and 10.3-fold, respectively, higher mucoadhesion on freshly excised porcine intestinal mucosa than the corresponding unmodified polymers. Due to these strongly improved mucoadhesive properties, thiomers seem to be advantageous over so far used polymers. The better adhesion to mucosal tissues should provide the localisation of the delivery system in specified regions, like the buccal, nasal or vaginal epithelium. Furthermore, a comparatively longer residence time at the site of drug absorption can be achieved. A limiting factor thereby seems to be the rapid turnover of the mucus. But the intensified contact with the mucosal absorption membranes provided by thiomers should additionally guarantee an increased drug concentration gradient representing the driving force for a passive drug uptake. In order to make further progress in investigating the potential of thiolated polymers, it was the aim of this study to evaluate the properties of two recently generated thiomers: carboxymethyl starch –cysteine and carboxymethyl guar gum –cysteine in a common dosage form [5].

Among delivery systems tablets provide an accurate dosage and are easy to manufacture. Accordingly, the features of tablets based on CMS–cysteine and CMG–cysteine conjugates were evaluated focusing on swelling and disintegration behaviour as well as on mucoadhesion. Obtained results should contribute to a better understanding of the function of thiomers in delivery systems.

MATERIALS AND METHODS

Materials

The gift sample of Guar gum was obtained from Premchem Gums Private Limited, Mumbai, India. Metformin Hydrochloride was received from Emcure Pharmaceuticals, Pune, India. All the other chemicals were of analytical grade and purchased locally.

Synthesis of Carboxymethyl Guar Gum (CMG) and Carboxymethyl Starch (CMS)

For a typical synthesis of CMG / CMS, 5 g (8.69 mmole; 19.92 mmole) of guar gum / starch was suspended in 150 ml isopropanol. The reaction mixture was vigorously stirred at room temperature for 1 h after addition of 10 ml of 15% aqueous NaOH solution (1.5 g, 37.8 mmole). Then 4.39 g (37.8 mmole) SMCA was added and the temperature of the reaction bath was raised to 55^0C. The etherification was performed for 3 hrs. The product was filtered off, suspended in 80% (v/v) aqueous methanol, neutralized with dilute acetic acid, and washed five times with 100 ml ethanol. The product was dried in oven [6-10].

Synthesis of thiolated polymers

5 g of CMS/CMG was hydrated in 100 ml of distilled water to form a homogeneous solution. The carboxylic acid groups of the polymer were activated by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) to a final concentration of 50 mM. The reaction was allowed to proceed for about 45 min. The sulphur containing amino acid cysteine was added to a weight-ratio of 1:2 (polymer: amino acid) and the pH was adjusted to around 4.0 using 1M HCl solution. The reaction mixture was incubated for 2 hrs. under stirring at room temperature. The pH was raised to 6.0 using 1 M NaOH solution and the reaction proceeded for an additional 1 hr. The resulting thiomer conjugated was isolated by dialyzing against 1 mM HCl aqueous solution at room temperature, followed by 2 cycles of dialysis against 1% NaCl in 1 mM HCl aqueous solution and then thoroughly against 1 mM HCl aqueous solution. Samples were lyophilized by drying frozen aqueous polymer solutions at-40°C [11-14].

Characterization of polymers

FT-IR

FT-IR spectra of synthesised polymers (4 mg) blended with solid KBr (100 mg) were scanned from 400 to 4000 cm^{-1} in a Shimadzu FTIR-8400S. IR solution software was used to analyze the sample.

Proton NMR

^1H NMR spectrum of the sample was recorded in Varian Mercury YH-300 NMR spectrometer at a constant temperature of 22±2^oC using deuterated carbon tetrachloride ($CDCl_3$) as the solvent.

Determination of degree of substitution (DS)

CMG / CMS (5 g) was dispersed in acetone (150ml) and 5M HCl (15 ml) was added to the dispersion which was stirred for 30 min. During this process, the CMG / CMS which was in sodium form was converted to the H-CMG / H-CMS (carboxymethyl guar gum/starch in hydrogen form). H-CMG / H-CMS was washed four times with 80% (v/v) methanol until the solution became neutral with pH test. The neutral dispersion was filtered again, suspended in acetone and it was stirred for another 15 min, following which it was filtered, and dried for 24 h in a desiccator over silica gel. The dried acid form samples made in the previous step (1g) were transferred to 200ml Erlenmeyer flasks and suspended in distilled water (100 ml) until they dissolved completely. An excess of 1.0 N NaOH solution was added with stirring and stirring was continued for 15 more minutes before solution was heated to boil for 15- 30 min. While the solution was hot excess NaOH was back titrated with 0.5 N HCl to a phenolphthalein end point. The amount of acid consumed was recorded and the D.S. was calculated according to equation,

$$DS = \frac{0.162\,A}{(1 - 0.058A)}$$

Where $A = (BC-DE)/F$

Where *A* was acid consumed per gram of sample, *B* was NaOH solution Added, *C* was Normality of NaOH, *D* was HCl required for titration of the excess NaOH, *E* was Normality of HCl, *F* was CMG / CMS used, 162 was grams molecular mass of anhydroglucose unit of Guar Gum / Starch and 58 was net increase in molecular mass of anhydroglucose unit for each Carboxymethyl group substituted [15-17].

Solubility

Solubility of the derivatives in different buffers and organic solvents was gravimetrically determined. Approximately 2 g of material with 50 ml of solvent was placed in an airtight screw-capped tube and agitated for 24 h at 25 °C. Two milliliters of supernatant was withdrawn in a tared dish. Solvent was evaporated by a mild heat and the tared dish was weighed again. The difference in weight gives the amount of material dissolved in the solvent. Various buffers of pH 1.6, 4.0, 6.8, and 8.0 and the organic solvents acetone, chloroform, ethanol, and isopropyl alcohol, were used for this study. The experiment was repeated three times for each solvent/buffer solution. Buffers of different pH were prepared by the method described in Indian Pharmacopoeia (Pharmacopoeia of India) [18].

Differential scanning calorimetry (DSC)

The glass transition temperature (Tg) of polymers was determined by differential scanning calorimetry (DSC-Shimadzu, METTLER, TA4000, London, England). Approximately 15 mg samples were placed in an aluminium pan and scanned over a temperature range of 25°C to 250°C at the rate of 10°C/min. Scanning was performed in triplicate.

Preparation of tablets

Metformin Hydrochloride tablets of 200 mg weight based on thiolated polymer were compressed into 8.0 mm diameter flat-faced discs using 8 station tablet punching machine. (Rimek minipress machinery Co.Pvt.ltd., India).The tablet contains 50% of drug, 25% of synthesised polymer, 15% of SCMC, 5% of HPMC, and 2.5% of magnesium state, talc. The pressure was kept constant during the preparation of all tablets [19-20].

Determination of surface pH

The surface pH of the mucoadhesive tablets was determined in order to investigate the possibility of any side effects in vivo. As an acidic or alkaline $_pH$ may irritate the intestinal mucosa, we sought to keep the surface $_pH$ as close to

neutral as possible. A combined glass electrode was used for this purpose. The tablet was allowed to swell by keeping it in contact with of distilled water in petri plate for 2 hours at room temperature. The $_p$H was identified by bringing the electrode into contact with the tablet surface and allowing the surface to equilibrate for 1 minute [21].

Evaluation of the swelling behavior

The swelling properties and the erosion characteristics of tablets were evaluated by determination of % of Hydration. Each tablet was weighed (W1) and immersed in distilled water for predetermined times (4 hr) [22]. After immersion, tablets were wiped off by the excess of surface water by the use of filter paper and weighed (W2).The swelling index was calculated by using formula,

$$\text{Swelling index} = \frac{W2 - W1}{W1} \times 100$$

Determination of ex-vivo Mucoadhesive strength

A modified balance method was used for determining the ex vivo mucoadhesive strength. Fresh sheep intestinal mucosa was obtained from a local slaughterhouse and used within 2 hours of slaughter. The mucosal membrane was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with phosphate buffer $_p$H 6.8. The fresh sheep intestinal mucosa was cut into pieces and washed with phosphate buffer $_p$H 6.8. A piece of intestinal mucosa was tied to the open mouth of a glass vial, which was filled completely with phosphate buffer $_p$H 6.8, and held on the left side of the balance. The glass vial with rubber stopper was placed and tightly fitted in the center of glass beaker containing phosphate buffer ($_p$H 6.8, 37°C ± 1°C) just touching the mucosal surface. The tablet was stuck to the lower side of the rubber stopper of the glass vial by applying some force through fingertip for five minutes. The left and right pans were balanced by adding a 5g weight on the right hand pan. When the 5-g weight was removed from the right-hand pan, the left-hand pan along with the tablet was lowered over the mucosa. The balance was kept in this position for 5 minutes. Water was added slowly at 100 drops/min to the right-hand pan until the patch detached from the mucosal surface. The weight (gram force) required to detach the tablet from the mucosal surface gave the measure of mucoadhesive strength [21, 23].

Determination of ex-vivo Mucoadhesion Time

The ex vivo mucoadhesion time was performed after application of the Mucoadhesive tablet on freshly cut sheep intestinal mucosa. The fresh sheep intestinal mucosa was tied on the glass slide and a mucoadhesive core side of each tablet was wetted with 1 drop of phosphate buffer $_p$H 6.8 and pasted to the sheep intestinal mucosa by applying a light force with a fingertip for 30 sec. The glass slide was then put in the beaker, which was filled with 200 ml of the phosphate buffer $_p$H 6.8 and was kept at 37 ± 1°C. After 2 min, a 50 rpm stirring rate was applied to simulate the intestinal cavity environment and tablet adhesion was monitored for 6 hrs. The time for the tablet to detach from the sheep intestinal mucosa was recorded as the mucoadhesion time [24-26].

In Vitro Drug Release

The USP XXIII dissolution apparatus 2 (Veego scientific, Mumbai, India) was used to study the drug release from the mucoadhesive tablet. The dissolution medium consisted of 900 mL of 0.1N Hydrochloric Acid. The release study was performed at 37°C ± 0.5°C, for 2 hrs. with a rotation speed of 50 rpm. Then the dissolution medium was replaced with phosphate buffer $_p$H 6.8 and release study performed for additional 4 hrs. Samples of 5 mL were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through 0.2-µm Whatman filter paper and analyzed after appropriate dilution by UV spectrophotometer (Double beam spectrophotometer 2203, Systronics) at 233 nm. % cumulative drug release (%CDR) in 6 hours was calculated from the UV absorbance values of each aliquot. A plot of time versus % CDR was studied to determine the drug release pattern for each batch of tablet [21,26].

RESULTS AND DISCUSSION

Synthesis of thiolated polymers

The thiolated polymers of starch and gaur gum namely, CMS-C, CMG-C, respectively were synthesised as per the scheme of synthesis. The intermediate carboxymethyl starch (CMS) and carboxymethyl gaur gum (CMG) were synthesised by reacting starch/gaur gum with monochloroacetic acid. The first step is an alkalization where the hydroxyl groups of the starch molecules are activated and changed into the more reactive alkoxide form (St–O-) / (GG-O-). This is followed by etherification in the second step by using monochloroacetic acid.

The resulted CMS/CMG was hydrated in distilled water to form a homogeneous solution. The carboxylic acid groups of the polymer were activated by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) to a final concentration of 50 mM. Then sulphur containing amino acid L-cysteine was added to a weight-ratio of 1:2 (polymer: amino acid) and the pH was adjusted to around 4.0 using 1M HCl solution. After 2 hrs the pH was raised to 6.0 using 1 M NaOH solution and the reaction proceeded for an additional 1 hr. The resulting thiomer conjugated was isolated by dialyzing and allow to freeze dry.

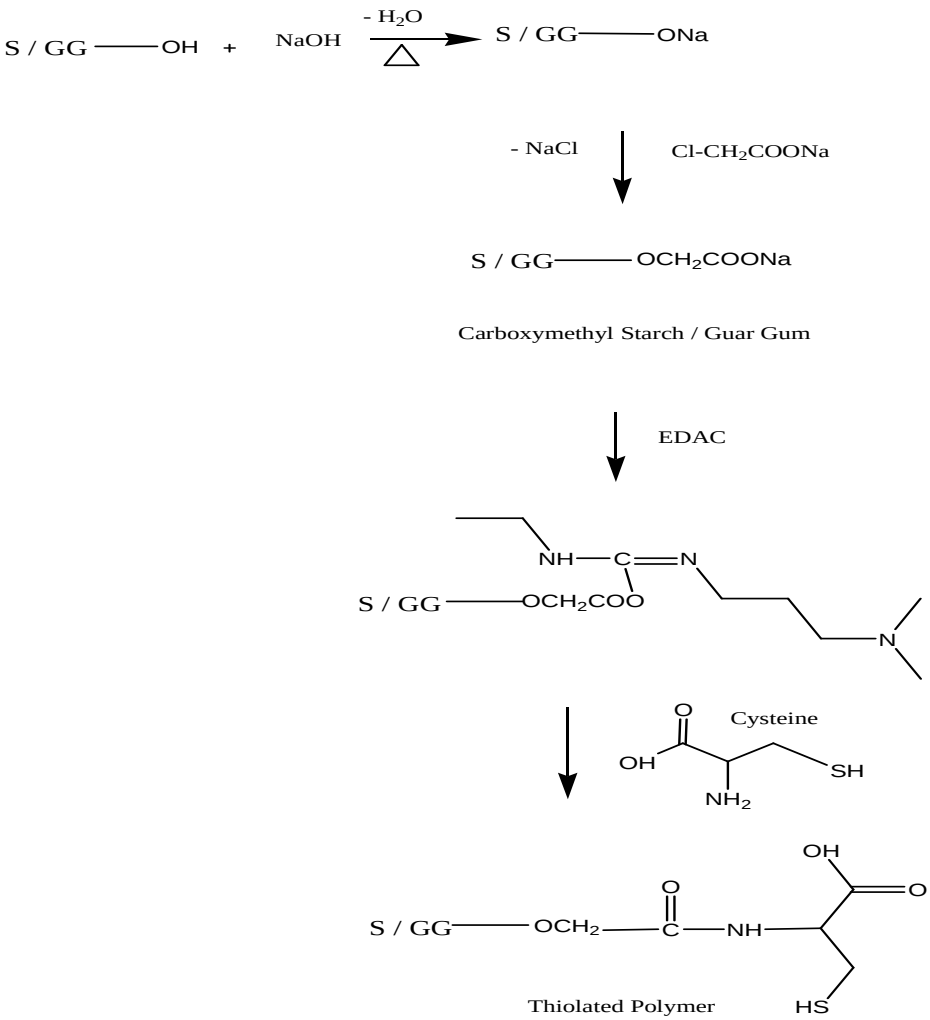


Fig 1: Reaction for the synthesis of thiolated polymers

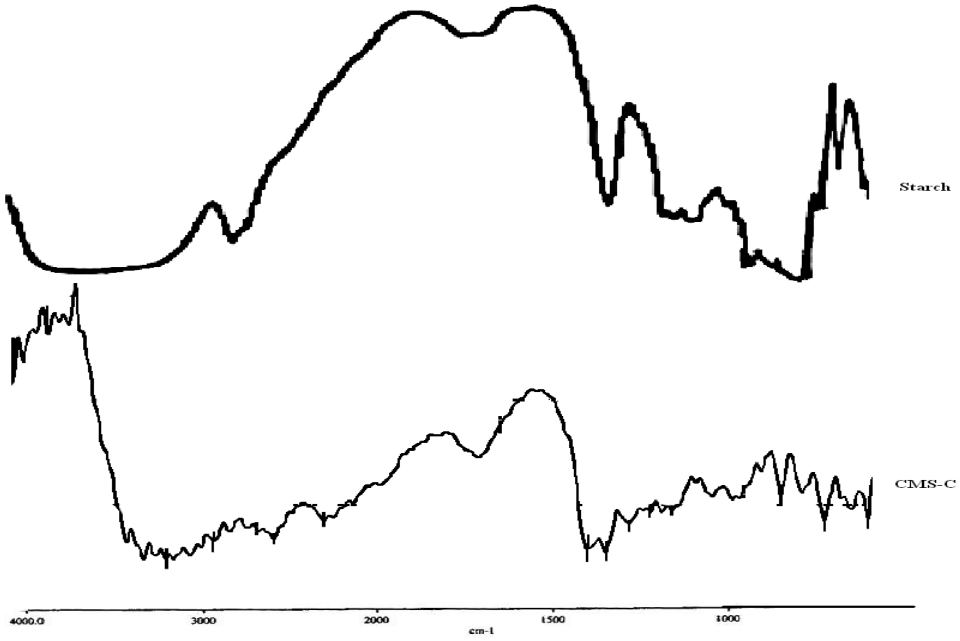


Fig.2 Comparative IR spectra of Starch and CMS-C

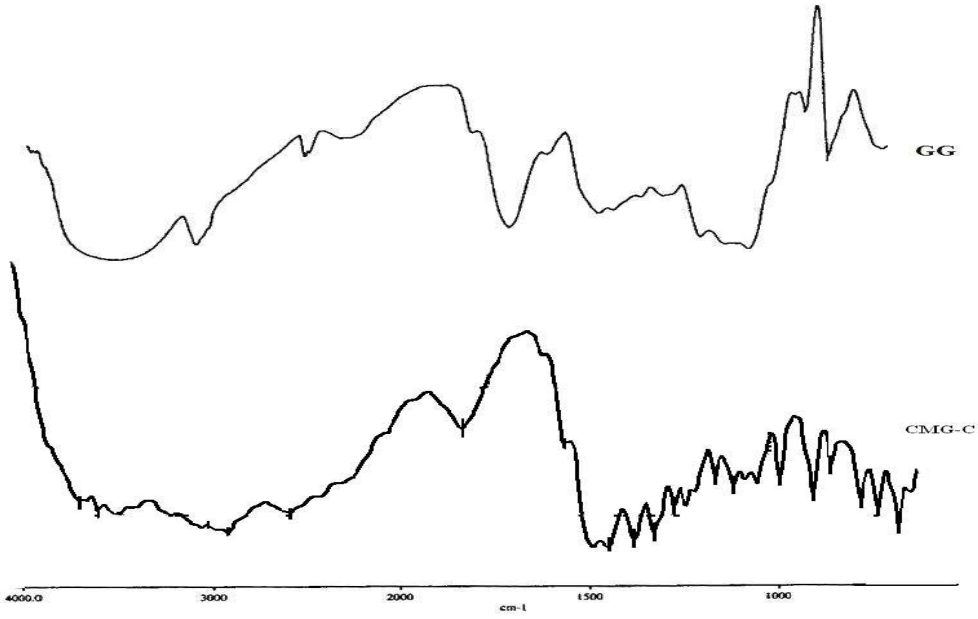


Fig.3 Comparative IR spectra of Guar gum and CMG-C

The formation of CMS-C and CMG-C was confirmed by the FT-IR spectrum (Fig. 2 and 3) of the derivative. Comparison of the FT-IR spectra of native starch with CMS-C indicated the introduction of substituent groups -C=O absorption around 1750-1754 cm^{-1}. As the thiolation reaction continued there was an increment in the absorption due to carbon–sulphur stretching (C-S) at 1365 cm^{-1}.Also stretching due to nitrogen-hydrogen (N-H) at 3276 $cm^{-1.}$

Similarly Comparison of the FT-IR spectra of Guar gum with CMG-C indicated the introduction of substituent groups -C=O absorption around 1700–1711 cm^{-1}. As the thiolation reaction continued there was an increment in the

absorption due to carbon–sulphur streching (C-S) at 1287 cm^{-1}.Also streching due to nitrogen-hydrogen (N-H) at 3390 $cm^{-1.}$

Since, three hydroxyl groups are present in each monomeric moiety of GG the theoretical maximum for GG is 3.0.The obtained D. S. using the equation was 2.3(theoretical maximum, 3.0).These values proved almost complete thiolation of GG. Similarly one hydroxyl groups are present in each monomeric moiety of starch the theoretical maximum for starch is 1.0.The obtained D. S. using the equation was 0.8(theoretical maximum, 1.0)High DS was essential for controlled release properties of the polymer.

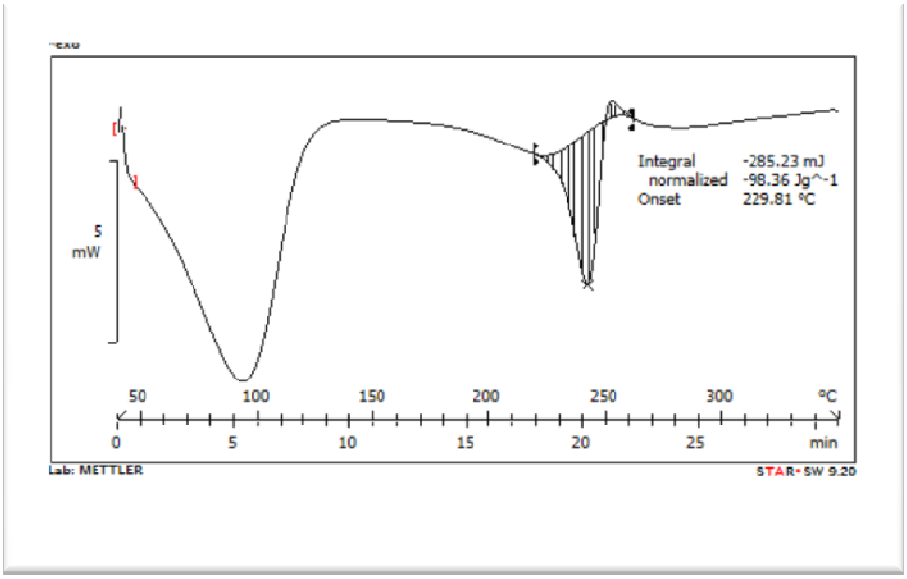


Fig.4 DSC curve of CMS-C

The onset of temperature (T_O) of polymer was 229.81°C, where (T_P) peak temperature was 245°C.The enthalpy was (ΔH), -98.36 J/g.

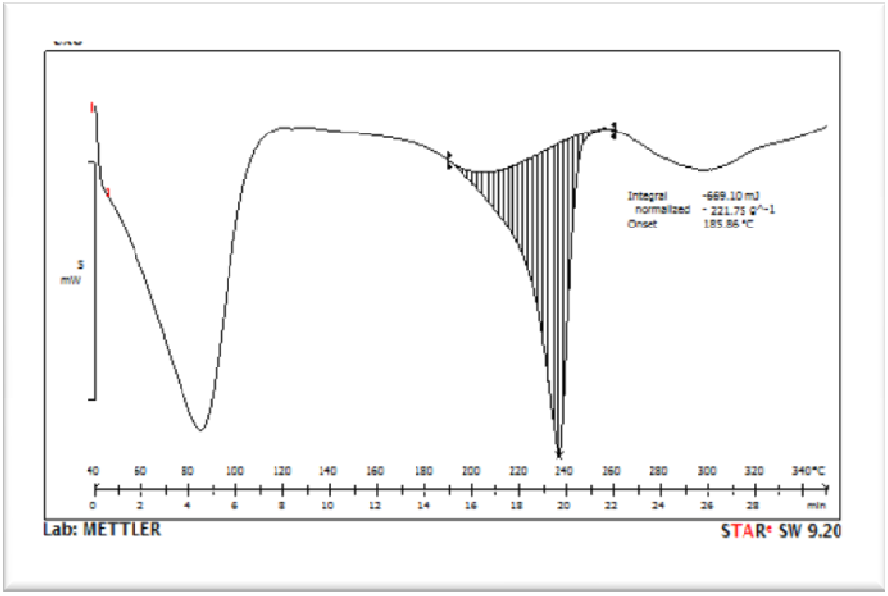


Fig.5 DSC curve of CMG-C

The onset of temperature (T_O) of polymer was 185.86°C, where (T_P) peak temperature was 238°C.The enthalpy was (ΔH), -185.86 J/g.

Solubility of polymer in different solvents and buffer solutions

A study of relative solubility was carried out in different solvents and under different pH conditions. Synthesised polymer was found to be insoluble in water and soluble in all organic solvents tested as shown in Table 1. The solubility increases with increase in the pH of the solution. A low solubility of 3.8×10^{-3} and 4.14×10^{-3} (g/ml) was found at a lower pH of 1.6 for CMS-C and CMG-C respectively. This indicates a minimum drug releasing property in the gastric acid environment. Similarly a higher solubility of 23×10^{-3} and 37.4×10^{-3} (g/ml) was found in the basic pH of 8.0 for CMS-C and CMG-C respectively this indicates a maximum drug release in the intestine.

Table.1: Solubility profile of polymer in different solvent

Solvent	Solubility in gm/ml	
	CMS-C	CMG-C
Acetone	2.3×10^{-2}	2×10^{-2}
Chloroform	2.9×10^{-2}	4×10^{-2}
Ethanol	2.3×10^{-2}	4.04×10^{-2}
IPA	1×10^{-2}	3.2×10^{-2}
Water	Insoluble	Insoluble

Table.2: Solubility profile of polymer in different $_P$H solvent.

$_P$H Solvent	Solubility in gm/ml	
	CMS-C	CMG-C
1.6	3.8×10^{-3}	4.14×10^{-3}
4.0	4.8×10^{-3}	9.04×10^{-3}
6.8	9.2×10^{-3}	25.8×10^{-3}
8.0	23×10^{-3}	37.4×10^{-3}

Determination of thiol group content

Weigh about 0.2 gm of polymer and placed in iodine flask. Add 50 ml of standard 0.1 N iodine solution into flask shake it vigorously. Introduce 20 ml of ethanol and shake for 10-15 min. Titrate excess of iodine with 0.1 N sodium thiosulphate solution using starch indicator near end point [17].

$$\% = \frac{(V1-V2) \times N1 \times M \times 100}{W \times 1000}$$

Where,
V1 = volume of sodium thiosulphate for blank
V2 = volume of sodium thiosulphate for sample
N1 = normality of sodium thiosulphate
M = molecular weight of sample
W = weight of sample.

Table.3: % of thiol group content

Polymer	% thiol group
CMS-C	76.15 ± 1.30
CMG-C	80.0 ± 2.60

(mean ±S.D,n=3)

Swelling behavior

The swelling behavior of mucoadhesive polymer has a considerable influence on their adhesive properties and cohesiveness. The hydration theory postulates that mucoadhesive polymers take water from the underlining mucosal tissue by absorbing, swelling and capillary effect, leading to a considerably strong adhesion [26].

Mucoadhesive drug delivery system can be attached in dry form to buccal, ocular, nasal, intestinal or vaginal mucosa. A prolonged residence time of the system on the mucosa leads to an extended period of absorption and consequently of improved bioavailability. When it is directed to the small intestine, it reaches its target site already

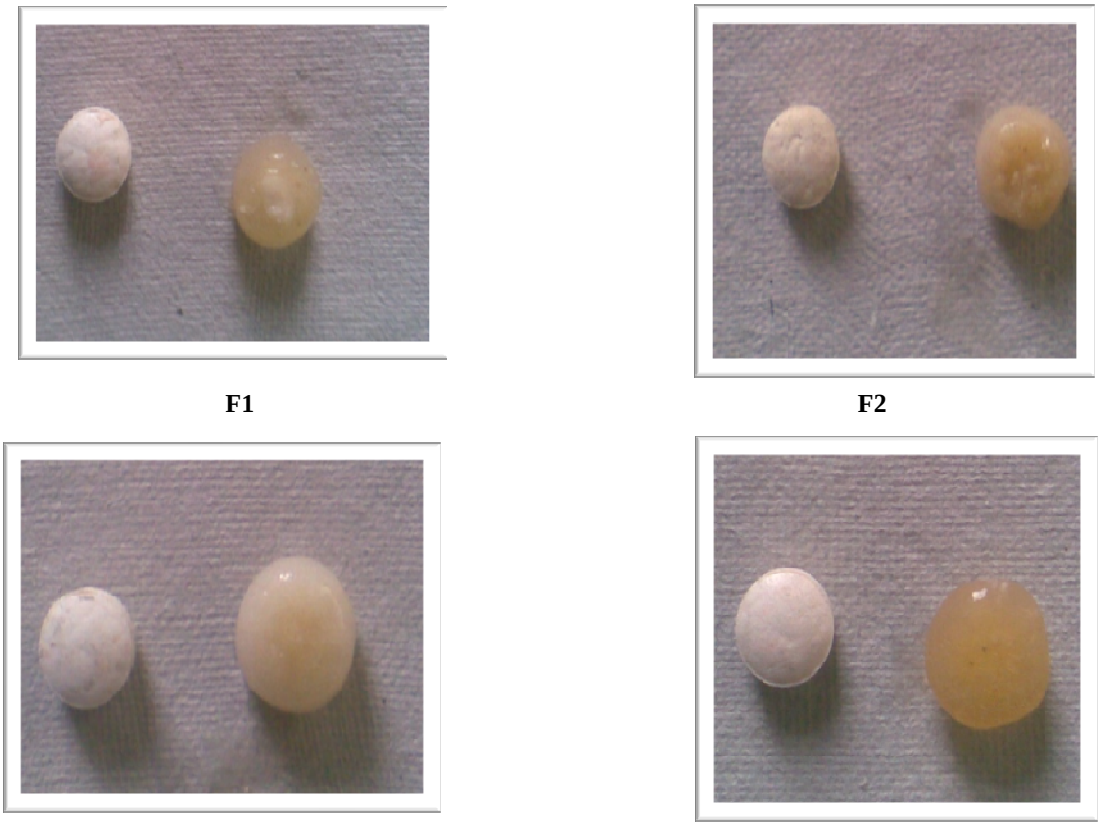
in at least partially hydrated form. Therefore, slow swelling is a requisite to avoid the formation of an over hydrated form that losses its mucoadhesive properties before reaching the target.

In order to evaluate this effect for the new polymers, water uptake studies were carried out with tablets based on the modified polymer. Thereby the obtained results are shown in Fig.7. As shown in Fig.6 the covalent attachment of cysteine to CMS had no much influence on the swelling behavior of the polymer. The swelling behavior of the tablets based on CMS-cysteine conjugate had been evaluated with the result that the immobilization of cysteine displayed less effect on the water uptake of the polymer. Whereas, the swelling behavior of CMG was improved due to the attached cysteine. The more thiol groups introduce to the polymer, the higher was the water uptake rate.

Table.4: Evaluation of thiolated polymer

Batches	pH	Swelling index (%)	Mucoadhesive Strength(gm)	Mucoadhesive Time (hrs.)
F1 (CMS-C)	6.28±0.02	92.84±2.84	17.82±0.03	4.30 hrs.
F2 (CMS-C)	6.40±0.01	100.66±2	17.43±0.02	4.40 hrs.
F3 (CMG-C)	6.89±0.06	131.21±0.91	19.79±0.08	5.40 hrs.
F4 (CMG-C)	7.02±0.03	136±4.39	19.63±0.02	5.20 hrs.

(mean ±S.D,n=3)



F1 **F2**

F3 **F4**

Fig. 6: Swelling behavior of different batches

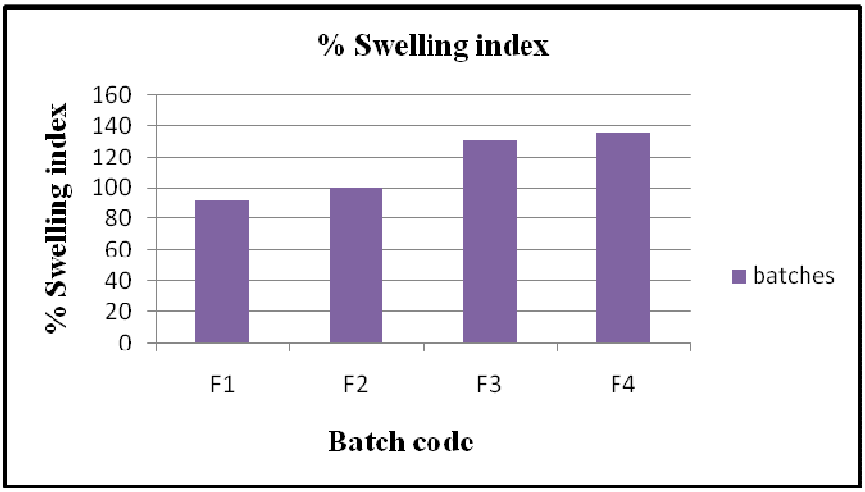


Fig 7: Swelling behavior of different batches

Mucoadhesion studies

Tensile studies represent the most widely employed in vitro test method for the measurement of adhesion strength of mucoadhesives. In present study, the maximum detachment force (MDF) for the CMS-cysteine was low as compared to CMG-cysteine. Tensile studies with tablets based on thiolated CMS-C showed no significant improvement in the mucoadhesive properties. This observation can be explained on the one hand by the amount of attach a thiol group which was relatively low compared with CMG-C and on the other hand, showed that disulfide bond formation between two molecules take placed rapidly if the thiol groups of both reactants are surrounded by the opposite charges. By immobilisation of cysteine to CMS many negative charges from the polymer will be lost. As a result disulfide bond formation of the cysteine structure and the thiol groups of mucus layer seems to be very slow process.

Where as the tensile test with the tablets of CMG-cysteine conjugate demonstrated a clear correlation between the amounts of polymer linked cysteine and the adhesive properties of the polymer. Results of adhesion studies are shown in (Fig.8)

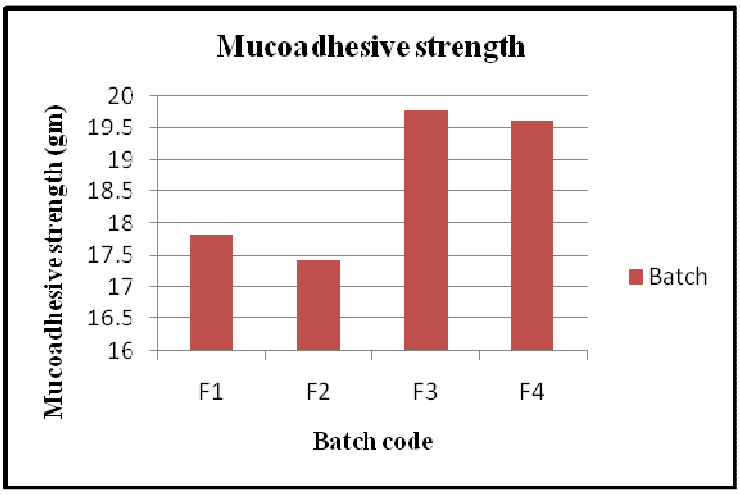


Fig 8: Mucoadhesive strength of different batches

Drug release study

The another advantage of the thiolated polymer CMG-C, CMS-C in drug delivery system is a controlled drug release which can be reach by using polymer as a carrier matrix.

One reason for the relatively slow release may be the possibility of the formation of disulfide bonds within the matrix tablet. This cross-linking process leads to lowering the velocity of diffusion of drug molecules. The polymer matrix probably combines two major types of mechanism for the drug release: control diffusion and swelling. Release of drug from the tablet is varied according to the ratio and type of the polymer used.

The *in vitro* drug release profile of tablets containing CMS-cysteine show cumulative percent drug release for formulation F1 to F2 were ranging from 0.96 % to 0.60 % during first 2 h. Also at the end of 6 h, the cumulative percent drug releases were found to vary from 93.18 % to 88.94 %. On physical examination of tablets during dissolution study, it was found that tablets were initially swell and slowly eroded over the period of time (Fig.9).

The *in vitro* drug release profile of tablets containing CMG-cysteine show cumulative percent drug release for formulation F3 to F4 were ranging from 1.39 % to 1.17 % during first 2 h. Also at the end of 6 h, the cumulative percent drug releases were found to vary from 97.82 % to 94.96 %. Tablets from CMG-cysteine show the % drug release highest, this is due to the higher hydration. On physical examination of tablets during dissolution study, it was found that tablets were initially swell and slowly eroded over the period of time (Fig.10).

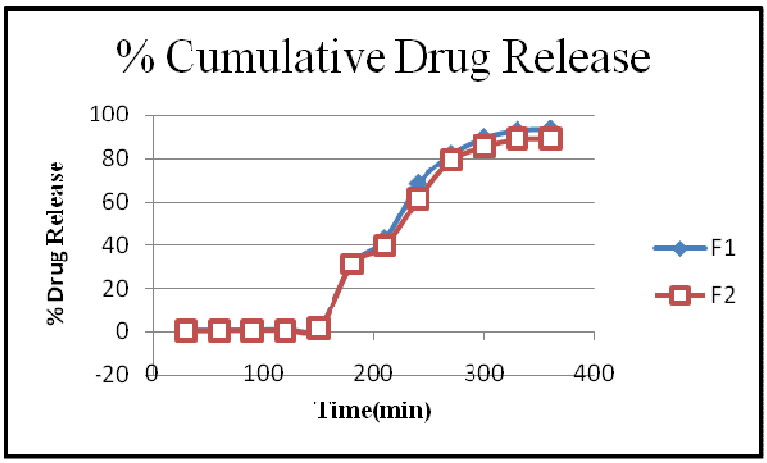


Fig 9: % Cumulative drug release of F1 and F2

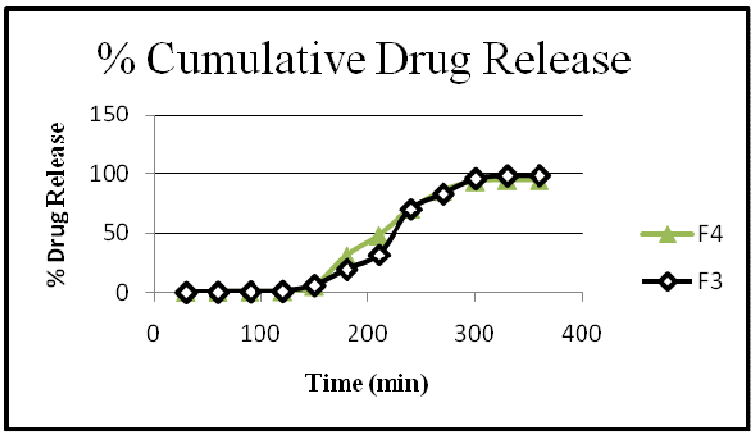


Fig 10: % Cumulative drug release of F3 and F4

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