Acta Pharm. Sci. Vol 59:(1), 2021
DOI: 10.23893/1307-2080.APS.05903

Thiol Functionalization of *Sesbania* Gum and Its Evaluation for Mucoadhesive Sustained Drug Delivery

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ABSTRACT

The objective of present study was to improve muco-adhesiveness of *sesbania* gum by thiol functionalization. Thiolated *sesbania* gum was synthesized by reacting *sesbania* gum with mercaptoacetic acid in the presence of catalytic amount of acids. The modified gum was characterized physic-chemically and for biocompatibility. Thiolated *sesbania* gum was tested as mucoadhesive polymer for pharmaceutical applications by formulating its composite beads in the sodium alginate using metformin as a model drug. Thiolation onto *sesbania* gum was confirmed by Fourier transform infrared spectroscopy and energy dispersive X-ray –scanning electron micrographs. The degree of thiol substitution was found to be 1.72mmol/gm. The results of thrombogenic and haemolytic potential studies confirmed the biocompatibility of Thiolated *sesbania* gum. The comparative evaluation of composite beads of thiolated *sesbania* gum with *sesbania* gum and alginate alone beads revealed that thiolation of *sesbania* beads improves the bioadhesion property of *sesbania* gum.

Keywords: *Sesbania* gum, thiolation, bead, biocompatible, mucoadhesive

INTRODUCTION

A group of naturally occurring neutral polysaccharides i.e. galactomannans are most abundant raw material for industrial and pharmaceutical application due to easy availability, biodegradability, sustainability and non-toxic characteristics[1].

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(Received 11 February 2020, accepted 13 April 2020)

Galactomannans are composed of linear β– (1 ⟶ 4) glycosidic linked mannan backbone with α-galactose side chain residues at C-6 of mannose[2]. The various properties of galactomannans including molecular weight, mannose: galactose ratio (M:G) and attachment of galactose side chain residue on mannan backbone are believed to be responsible for their different rheological and physicochemical properties. Despite of various potential advantages of natural galactomannans, they owned certain limitations including less microbial stability and biodegradability. In order to overcome these problems and to enhance their utility, galactomannans are chemically modified via thiolation, carboxymethylation, microwave assisted grafting and many more. Thiolated polymers designated as thiomers havethiol group bearing side chain along the polymeric backbone[3-5]. These thiol groups are able to form disulfide linkage with mucosal glycoproteins resulting in higher mucoadhesiveness which further improve the therapeutic efficacy of the drug delivery system[6]. Numerous studies conducted earlier reported that thiolation modification of natural polysaccharide such as xanthan gum[7], gellan gum[8], pectin[9], tamarind seed polysaccharide[10], Psyllium husk[11], chitosan[12], alginate[13] and hyaluronic acid[14] improved their mucoadhesive properties.

Sesbania gum,a seed galactomannan, belongs to genus *Sesbania* and family *Faboideae*. The M:G ratio and average molecular weight of *Sesbania* gum is 2:1 and 2.3-3.4×105Da, respectively[15]. The gum form highly viscous suspension in aqueous system. *Sesbania* gum have been explored as diclofenac sodium loaded topical gelling agent[16] and for colon targeting drug delivery of metronidazole[17-18]. Carboxymethyl functionalized *sesbania* gum has been evaluated as thickening agent in printing cotton fabrics with reactive dyes[19]. Also, the cross linking on *sesbania* gum using dialdehyde group was performed with changed thermal and swelling properties[20]; *Sesbania* gum was evaluated as filter-aid agent with enhancing leachability of rare-earth ore with ammonium sulphate solution as lixiviant[21]; Oxidized *sesbania* gum was formed using sodium hypochlorite and evaluated as wrap sizing agent for fine cotton yarns[22]; High adsorption capacity towards metal ions having epoxy functional groups into *sesbania* gum was evaluated[23]. However, there are no reports on thiol modification of *sesbania* gum.

In the present investigation thiol functionalization of *sesbania* gum was carried out. The modified gum was characterized usingfourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), energy dispersive X-ray micro-analysis (EDX) and thermo gravimetric analysis (TGA) studies. Thiolated *sesbania* gum was tested as mucoadhesive polymer by formulating composite beads with sodium alginate by ionic gelation method. The beads were evaluated for entrapment efficiency, *in vitro* release and mucoadhesive study. It was aimed

to prepare metformin hydrochloride (model drug) loaded *sesbania* gum-sodium alginate (SG-Alg) and thiolated *sesbania* gum-sodium alginate (TSG-Alg) composite beads to achieve controlled drug delivery system.

METHODOLOGY

Materials

Sesbania gum and metformin hydrochloride were obtained as gift samples from Badar Enterprises (Jodhpur, Rajasthan, India). Mercaptoacetic acid, sodium alginate and methanol were purchased from Thomas Baker Chemicals Pvt. Limited (Mumbai, India). Sodium dihydrogen phosphate, hydrochloric acid and calcium chloridewere purchased from SD Fine-Chem Limited (Mumbai, India). Ellman's reagent [5,5'–dithiobis-(2-nitrobenzoic acid); M_v = 396.34 g/mol; 0.03% w/v], di-sodium hydrogen phosphate, and *L-Cysteine* were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).Freshly excised chick intestine was procured from local butcher house (Hisar, India).

Synthesis of thiolated *sesbania* gum

The synthesis of thiolated *sesbania* gum was done by esterification of native *sesbania* gum employing mercaptoacetic acid (80%, w/v) in the presence of hydrochloric acid as catalyst. A dispersion of *sesbania* gum was prepared by adding 2 g of *sesbania* gum powder in 200 ml cold distilled water with the aid of magnetic stirrer. The dispersion was reacted with mercaptoacetic acid (7.56 g) in the presence of 5 ml of 7N HCl by refluxing at temperature of 70 ± 2°C for 2h. The above reaction mixture was poured in 500ml methanol. White precipitate of thiolated *sesbania* gum so obtained was filtered using Whatman filter paper, washed with methanol and dried in oven at a temperature of 50±2°C[24].

Calculation of thiol group content

The content of thiol group substitution in TSG was determined by well-establishedEllman'smethod[25]. An aqueous dispersion (0.2% w/v) of native *Sesbania* gum (control) and thiolated *Sesbania* gum was prepared. A volume of 2.5 ml of each prepared suspension was diluted with 2.5 ml of phosphate buffer (0.5 M, pH 8.0) followed by addition of 5 ml of Ellman's reagent in it and allowed to react for 2 h at an ambient temperature in dark. Absorbance of the above reaction mixture was measured at 450 nm using UV spectrophotometer (UV-1800, Shimadzu, Japan). The number of thiol group substitution was calculated using calibration curve of *L-cysteine* (standard) with Ellman's reagent as detailed above.

Physiochemical characterization of *sesbania* gum and thiolated *sesbania* gum

Both the gums i.e. native *sesbania* gum and thiolated *sesbania* gum were characterized for organoleptic and physical properties. Color, odour and taste like organoleptic characterization were done manually, while the physical properties such as density (bulk and tapped), angle of repose, Hausner's ratio, Carr's index and swelling index were calculated using standard procedures as follows-

pH determination

For pH determination of native and thiolated *sesbania* gum, a 2% w/v dispersion of each of the gum in distilled water was mixed with vigorous shaking for 10 min[26]. The pH was measured using calibrated pH meter (Waterproof *pHTestr 10,* EUTECH instruments, OAKTON®, Singapore).

Bulk density and tapped density

Accurately weighed amount of 5 gm of *sesbania*/thiolated *sesbania* gum powder sample was introduced into 100 ml measuring cylinder and the volume occupied by the powder was recorded as bulk volume. The measuring cylinder was tapped on a wooden frame till obtaining constant volume, which was taken as tapped volume[27]. The bulk density and tapped density was calculated as follows:

$$Bulk\ p = \frac{Amonut\ of\ sample\ taken}{Bulk\ volume} \quad (1)$$

$$Tapped\ p = \frac{Amonut\ of\ sample\ taken}{Tapped\ volume} \quad (2)$$

Angle of repose

The angle between horizontal surface and apexof cone shaped pile of powder is characterized as the angle of repose. A glass funnel having the orifice diameter of 5 cm was fixed using a stand, 4 cm above the horizontal surface. The weighed amount of powder was then allowed to pass through the glass funnel followed by the measurement of diameter and height of the pile of powder[27]. The angle of repose was found using the equation:

$$\tan \Theta = \frac{Height\ of\ pile}{Diameter\ of\ pile} \quad (3)$$

Where, Θ is the angle of repose

Hausner ratio and Carr's Index

Hausnerratio and Carr's index provide flow properties and compressibility of powders. The values of bulk density and tapped density were used to determine these parameters using the formula:

$$Hausner\ Ratio = \frac{\rho_{Tapped}}{\rho_{Bulk}} \quad (4)$$

$$Carr's\ Index = \frac{\rho_{Tapped} - \rho_{Bulk}}{\rho_{Tapped}} \quad (5)$$

Swelling Index

Swelling behaviour of *sesbania*/thiolated *sesbania* gum was determined by using modified method reported in previous literature[28]. A dispersion (1% w/v) of *sesbania* gum/thiolated *sesbania* gum powder was prepared and the initial (at 0 h) and final volume (at 24 h) occupied by the powder sediment was noted. The swelling index was determined using the given below equation:

$$Swelling\ Index = \frac{V_{Final} - V_{Initial}}{V_{Initial}} \times 100 \quad (6)$$

Where, $V_{initial}$ and V_{final} are initial and final volume of powder, respectively

Moisture content

Moisture content affects the quality and stability of the product. However, it can be determined using thermogravimetric approach so, the moisture content of s*esbania* gum and chemically modified *sesbania* gum was calculated by evaporating them in a petridish (4g each) at 80°C in an oven until constant weight obtained[29]. The percentage moisture content was calculated via given formula:

$$Moisture\ content\ (\%) = \frac{W_{Initial} - W_{Final}}{W_{Initial}} \times 100 \quad (7)$$

Where, $W_{initial}$= Initial weight of sample and W_{final}= Final weight of sample after evaporation

Fourier transformInfrared spectroscopy (FT-IR)

Native *sesbania* gum and thiolated s*esbania* gum were subjected to spectrophotometer (IR-Affinity-1, Shimadzu, India)for functional properties confirmation at an ambient temperature using KBr pellets (Pellets were prepared by compress-

ing the material for 30 sec at the pressure of 75 kg/cm^{2}in IR hydraulic press, CAP-15T, PCI Analytics, Mumbai, India) and scanned in the wave number range of 4000-400 cm^{-1}.

Thermal Analysis

Thermogravimetric analysis study was conducted to investigate thermal stability and decomposition of *sesbania* gum and thiolated *sesbania* gum with the elevation of temperature. TGA analysis was performed employing Mettler Toledo TGA analyser (DSC3 PLUS, California, USA) in temperature ranging from 30-410°C under nitrogen atmosphere at a heating rate of 2°C per minute.

Scanning electron microscopy- Energy dispersive X-ray micro-analysis (SEM-EDX)

The size and surface morphological analysis of *sesbania* gum and thiolated *sesbania* gum were determined using scanning electron microscope (Apreo SEM, Thermo Scientific™). The specimens were coated with gold and mounted on specimen stubs using double adhesive carbon tape. Electron micrographs were captured at an accelerating voltage of 20kV at different magnifications. Presence of different element in native *sesbania* gum and thiolated s*esbania* gum were recorded using EDX image (AZtech,Oxford X –Max^N).

Biocompatibility studies

A comparative biocompatibility study of thiolated s*esbania* gum was performed against native *sesbania* gum for evaluation of its clot formation capability using thrombogenic and haemolytic potential. Gravimetric method was used to determine thrombogenic potential as discussed in previous literature[30]. The equal amounts of *sesbania* gum and thiolated *sesbania* gum (500 mg) was dispersed in phosphate buffer (20 ml, pH 7.2) for 24 h at room temperature. After complete hydration of both gums, samples were kept in whole citrated human blood (0.2 ml) followed by mixing of 0.1M $CaCl_2$ (0.2 ml) and then distilled water (5 ml) after 45 min. A volume of 5 ml of formaldehyde (38 %) was added for fixing the clot formed which was dried further and weighed. The following equation was used to determine percentage thrombose:

$$Thrombose = \frac{Wt.\ of\ sample - Wt.\ of\ negative\ control}{Wt.\ of\ postive\ control - Wt.\ of\ negative\ control} \times 100 \quad (8)$$

Weight of positive control indicates weight of the clots without sample while weight of negative control represents weight of residue without blood and samples

ASTM (American Society for Testing and Materials) standard was used to determine haemolytic potential as described in literature[30]. The same procedure was followed as mentioned above for hydration and clot formation of pure gum with its thiolated form. After incubating the samples in B.O.D. incubator (NSW- 152, Super Deluxe Automatic, India), centrifugation (Research centrifuge, TC 4100 D, Khera Instruments Pvt. Ltd., Delhi) was done at 10,000 rpm for 15 min for complete leaching of the unclotted blood. The absorbance of obtained supernatant fluid was analysed at λ_{max} of 540 nm in UV Visible spectrophotometer (UV-1800, Shimadzu, Japan). The following equation was used to calculate the haemolytic index:

$$\textit{Haemolytic index (\%)} = \frac{\textit{Absorbance of sample - Absorbance of negative control}}{\textit{Absorbance of postive control - Absorbance of negative control}} \text{X100} \quad (9)$$

Fabrication of drug loaded composite beads of thiolated *sesbania* gum with sodium alginate

Composite beads of thiolated s*esbania* gum (TSG-Alg) and s*esbania* gum with sodium alginate (SG-Alg) were prepared by extrusion through a hypodermic needle in a crosslinking solution of calcium chloride. Previously, *sesbania* gum/ thiolated *sesbania* gum (500mg) and sodium alginate (500mg) was dispersed in 0.1 N NaOH (20 ml) and deionized water, respectively. Both the suspensions i.e. *sesbania* gum/thiolated *sesbania* gum and sodium alginate were mixed followed by addition of model drug i.e. metformin hydrochloride (150mg) with continuous stirring. To obtain the adequate composite beads, the prepared suspensions were dropped into $CaCl_2$ solution (30 ml) using hypodermic needle (24 #) from a height of 26 cm over the period of 2 min, and the beads formed were kept for 10 min so that cross-linking could take place[31]. On completion of cross-linking reaction, obtained composite beads were filtered, taken out and dried in petridish at ambient temperature. For comparison purpose, metformin hydrochloride loaded sodium alginate beads (Alg) were also prepared using above mentioned procedure.

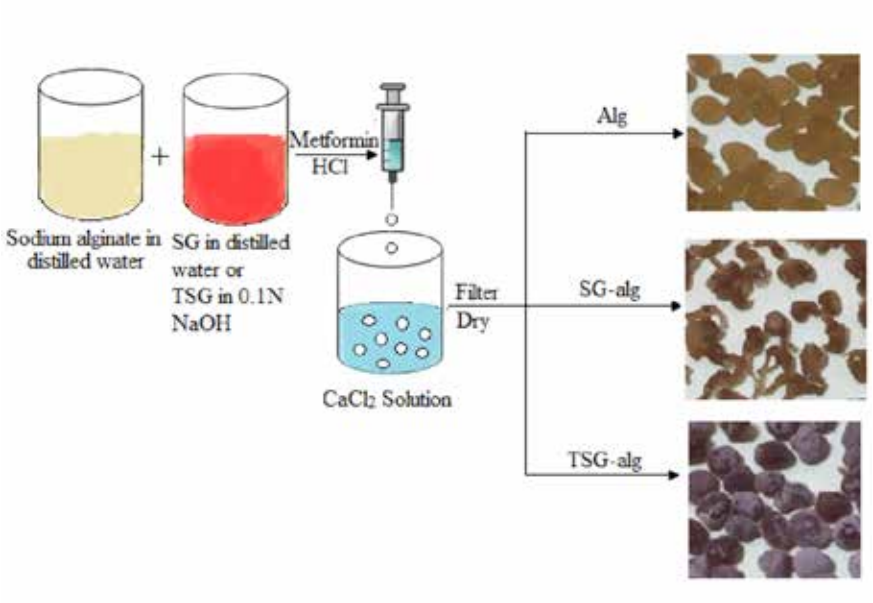


Figure 1: Schematic representation of bead formulation.

Characterization of drug loaded composite beads

The beads of various batches so obtained were evaluated for percentage yield, percentage entrapment efficiency, swelling behaviour, Fourier-transform infrared spectroscopy, scanning electron microscopy and *in-vitro* release behaviour.

Percentage yield

The yield (%) of SG-Alg,TSG-AlgandAlgwere calculated by using the given formula:

$$Yield\ (\%) = \frac{Wt._{Beads}}{Wt._{Polymer+Metfor.HCl}} X100 \quad (10)$$

Where,

$Wt._{Beads}$ = Total weight of beads produced

$Wt._{Polymer+Metfor.HCl}$ = Total weight of *sesbania* gum/thiolated*sesbania* gum/sodium alginate and drug used in the formulation of beads

Entrapment efficiency

The amounts of drug in gum included or not included in the beads were evaluated spectrophotometrically.Briefly, about 100 mg of crushed beads from each batch i.e. composite beads and sodium alginate beads were digested in 50 ml phosphate

buffer (pH 6.8) using probe sonication (Q55, QSonica, USA) for 5 min (amplitude 40%). Aliquots from the filtrate, remaining after filtration of polymer debris were assayed using a spectrophotometer at 234 nm (UV 1800, Shimadzu, Japan).The amount of entrapped metformin hydrochloride was calculated as follows:

$$\textit{Entrapment efficiency (\%)} = \frac{\text{Metfor.}HCl_{Practical}}{\text{Metfor.}HCl_{Theoritical}} \text{ X100} \quad (11)$$

Where,

$\text{Metfor.HCl}_{\text{Practical}}$= amount of metformin hydrochloride found in beads

$\text{Metfor.HCl}_{\text{Theoritical}}$= amount of metformin hydrochloride calculated to be present in thebeads

In-vitro release study

The release of metformin hydrochloride from SG-Alg, TSG-Alg and Alg beads were performed using USP dissolution test apparatus (Paddle Type, TDL-08L, Electrolab, Mumbai, India) in 900 ml dissolution medium (0.2 M phosphate buffer, pH 6.8). The beads having metformin hydrochloride equivalent to 100 mg were tied in muslin cloth and suspended under the paddle[32]. Then the paddle was immersed in phosphate buffer solution for 24 h at 50 rpm and the temperature was maintained at (37±0.5°C. At predetermined interval of time, aliquots of 5ml sample were removed and replaced by the same volume of fresh dissolution medium to maintain the sink condition during the whole test. The withdrawn samples were filtered using syringe filter (0.45µm) and analysed using UV-Visible spectrophotometer (UV 1800, Shimadzu, Japan) at 234 nm.

Swelling study

The swelling behaviour of SG-Alg, TSG-Alg and Alg beads were determined in solutions of different pH (1.2, 6.8 and 7.2) at (37±0.5)°C up to 24 h. Briefly, about 100 mg of beads from each batch was kept in 200 ml buffer solution[31]. Weight of swollen beads after blotting the excess liquid adhered on the surface was recorded at different interval of time until constant weight. The % swelling was calculated using the given formula:

$$\textit{Swelling (\%)} = \frac{\text{Wt.}_S\text{-Wt.}_D}{\text{Wt.}_D} \text{ X100} \quad (12)$$

Where, Wt._S and $\text{Wt.}_D$are the weights of swollen and dry beads, respectively.

Mucoadhesive study

Mucoadhesivestudy of SG-Alg, TSG-Alg and Alg beads were carried out by wash off method using freshly excised chick intestine purchased from local butcher house (Hisar, India)[9]. Adipose and connective tissue of the isolated intestine was removed properly. Then, tissue was adhered with mucosal surface (facing outside) on glass slide using cyanoacrylate glue. About 150 beads of each batch were adhered by pressing lightly on mucosal surface. The prepared glass slide was hung into the beaker having phosphate buffer solution (pH 6.8) on the USP tablet disintegration test machine[33] for 24 h. Total number of beads detached was noted at specific time interval. The whole study was carried out in triplicate manner.

RESULTS AND DISCUSSION

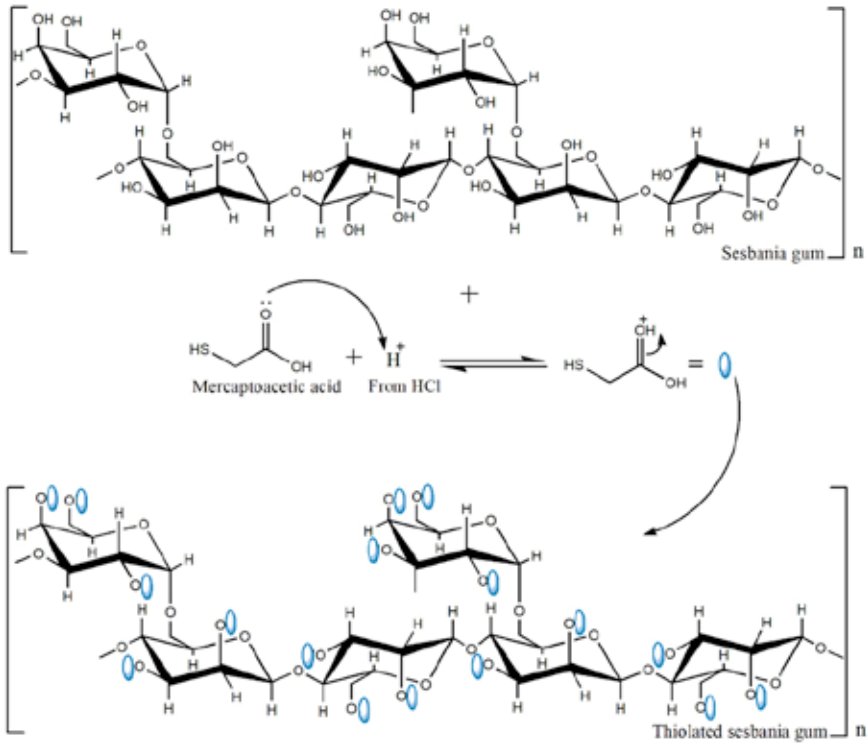


Figure 2: Schematic diagram for thiolation of *sesbania* gum.

Sesbania gum, a galactomannan has been chemically modified by employing mercaptoacetic acid under acidic conditions for thiol derivatization as shown in figure 2. In the first step of thiolation process, the hydroxyl groups (-OH) exhibited on *sesbania* gum are substituted by chlorine which further react with the carboxyl

groups (-COOH) of mercaptoacetic acid having sulfhydryl (-SH) present at terminal to form thiolated s*esbania* gum[3]. The air dried reaction product so obtained was copper red in colour with the characteristic odour. It was soluble in alkaline medium. The % yield of modified s*esbania* gum was found to be 89.65%. The number of thiol groups substitution was found to be 1.72mmol/gm, which was determined by Ellman's method.

The physicochemical properties of s*esbania* gum were considerably altered after thiolation. An aqueous dispersion (2.5% w/v) of thiolated*sesbania* gum was found to be more acidic than the *sesbania* gum. The pH of *sesbania* gum gets decreased after thiolation as shown in table 1. Similar results were earlier reported for thiolated starch, which were attributed to the higher affinity of thiol group for accepting electron pair and donation of H^+ ions[29]. The bulk and tapped densities after chemical modification of *sesbania* gum were increased. The Hausner ratio and Carr's index were calculated from the bulk and tapped density. The Hausner ratio is indicator of interparticulate friction. The lower values of Hausner ratio on thiolation of *sesbania* gum points towards lesser friction among the thiolated*sesbania* gum particles as compared to the *sesbania* gum[27]. Further the Carr's Index which refers to the bridge strength and stability of powder was also found to diminish on thiol functionalization. The angle of repose which is traditionally used to characterize the flow properties of powders also shows a decrease in value on thiolation. On the basis of the result of Hausner ratio, Carr's Index and angle of repose measurements, it can be concluded that the *sesbania* gum can be characterized as passable to very poor flow properties powder, whereas thiolated *sesbania* gum powder exhibit fair to good flow behaviour.

Table 1. Different parameters of *sesbania* and thiolated *sesbania* gum

Parameters	Sesbania gum	Thiolatedsesbania gum
pH	6.2±0.1	2.9±0.1
Bulk density (g/cm^3)	0.51±0.02	0.81±0.02
Tapped Density (g/ cm^3)	0.75±0.01	1±0.01
Angle of repose (°)	42.26±0.5	21.8±0.7
Hausner's ratio	1.49±0.01	1.22±0.01
Carr's index	32.65±0.03	19±0.04
Swelling (%)	30±1.24	10±1.11
Moisture content (%)	7.7±0.6	2.8±0.4

Data are presented as mean± SD (n=3)

In terms of swelling, thiolated *sesbania* gum has found less swelling power than pure *sesbania* gum as shown in table 1. It can beexplained by the fact that the native *sesbania* gumhas large numbers of hydroxyl groups which form intermolecular hydrogen bond with water molecules while in case of thiolated *sesbania* gum,-SH groups present in thiolated *sesbania* gumform weak H-bonds as compared to –OH groups because the thiol groups have less polarity and dipole moment as compared to the corresponding alcohols[34].

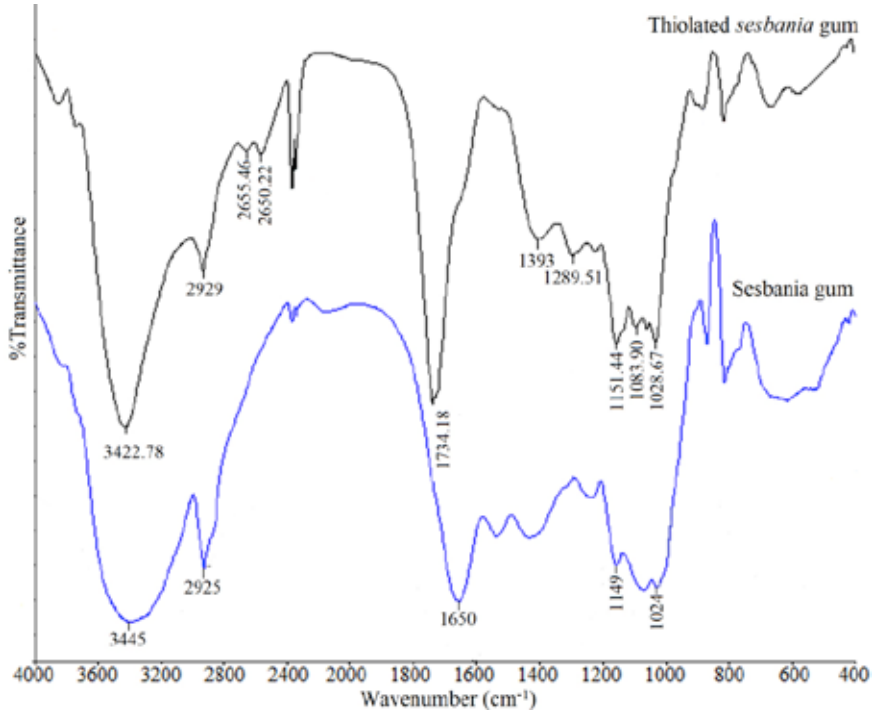


Figure 3: FT-IR spectrum of thiolated *sesbania* gum and *sesbania* gum.

Figure 3 represent FT-IR spectra of *sesbania* and thiolated *sesbania* gum. The broad and strong absorption band in native s*esbania* gum which appears at 3445 $cm^{-1}$is due to O-H group stretching. Thepresence of C-H linkage of alkane is shown at 2925 cm^{-1}, while the peak at 1650 cm^{-1} is attributed to C=O stretching of primary alcohols and two small peaks at 1149 cm^{-1} and 1024 cm^{-1} are due to C=O stretching of tertiary alcohol[22]. FTIR spectrum of thiolated *sesbania* gum showed a narrow band at 3442.78 cm^{-1}which is due to free O-H group stretching of mercaptoacetic acid, while the presence of C-H linkage of alkane is shown at 2929 cm^{-1}; C-H bending at 1734.18 cm^{-1}; O-H bending at 1393cm^{-1}; C=O stretching at 1289.51cm^{-1} and 1028.67cm^{-1};C=O stretching of primary and tertiary alcohol at

1083.90cm^{-1} and 1151.44cm^{-1}, respectively; small peak at 2655.46cm^{-1} is related to O-H stretching;one extra stretch at 2650.22cm^{-1} is due to –SH stretching of thiol group which confirms thiolation of *sesbania* gum. Thiol bands are not easy to detect using FT-IR spectroscopy; therefore, various other studies were performed.

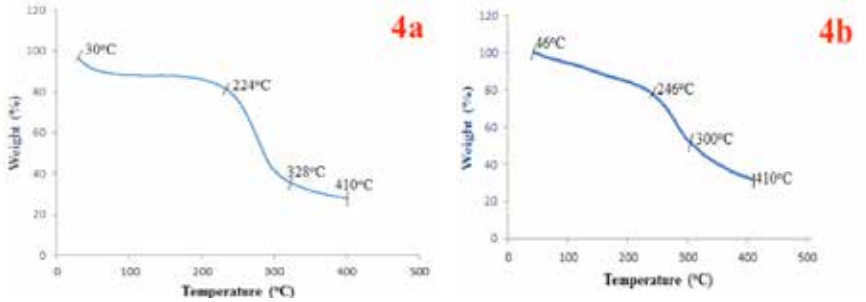
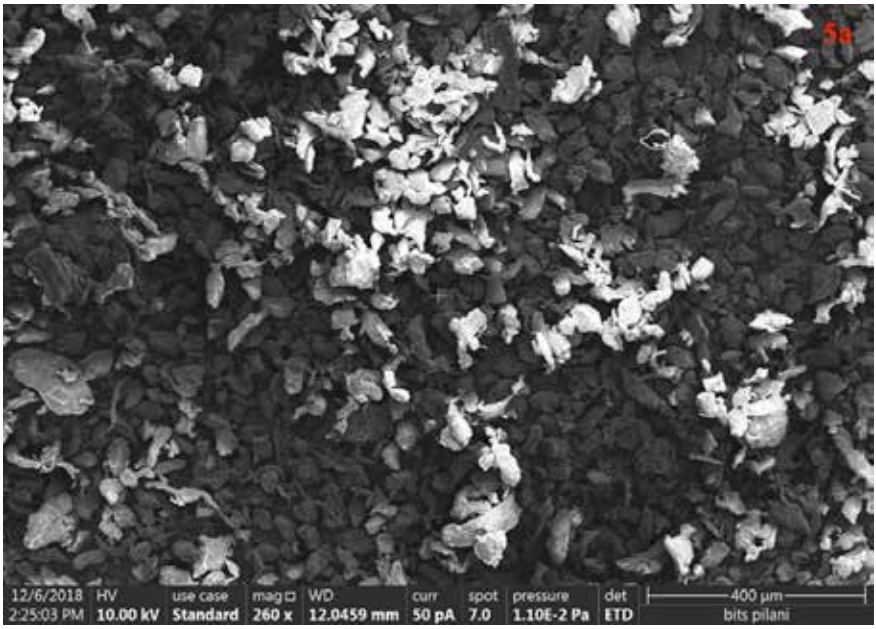


Figure 4: TGA plot of *sesbania* and modified thiolated *sesbania* gum.

Figure 4 exhibits thermal plots of *sesbania* and thiolated *sesbania* gum showing weight loss (%) with temperature while reviewing the thermogram, it was observed that decomposition occurs in three successive phases showing sigmoid curve. In native *sesbania* gum, first stage of decomposition occurs from 30°C to 224°C with 14% weight loss while 48% weight loss occurred during second stage in temperature range of 225°C-328°C. In third stage of thermal degradation from 329°C-410°C, 8% loss of weight was observed. In thiolated *sesbania* gum, first stage of decomposition was from 46°C to 246°C in which 24% weight loss occurred. In second stage of degradation which occurred between 247°C and 300°C, 24% weight loss occurred while in third stage (301°C-410°C), 21% weight loss occured. At the end of the thermal study at 410°C, a residue of 30% of *Sesbania* gum and 31% of thiolated *sesbania* gum was left which indicate that there is no difference between thermal stability of *sesbania* gum and thiolated *sesbania* gum[24]. The weight loss during first stage of degradation is due to desorption of bound water and dehydration because of loss of hydroxyl groups from the polysaccharide backbone[35], while the weight loss during the second and third stages can be ascribed to depolymerisation and the pyrolysis resulting in the evolution of gaseous products such as carbon monoxide, carbon dioxide, methane etc[36].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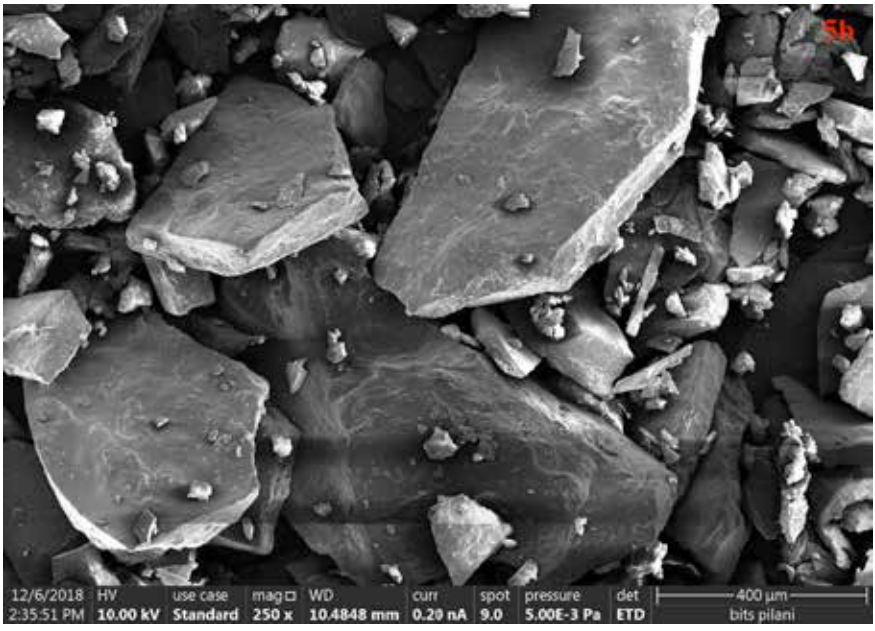
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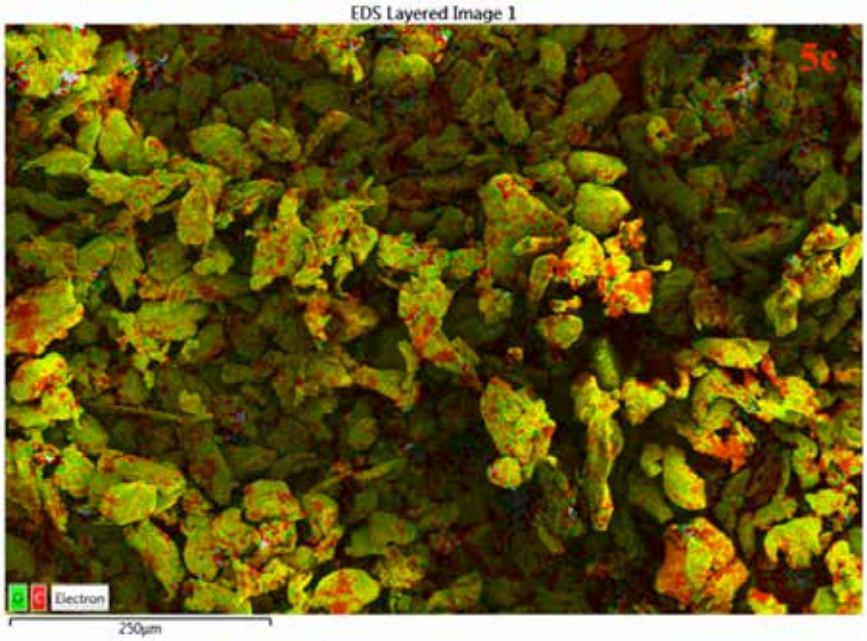
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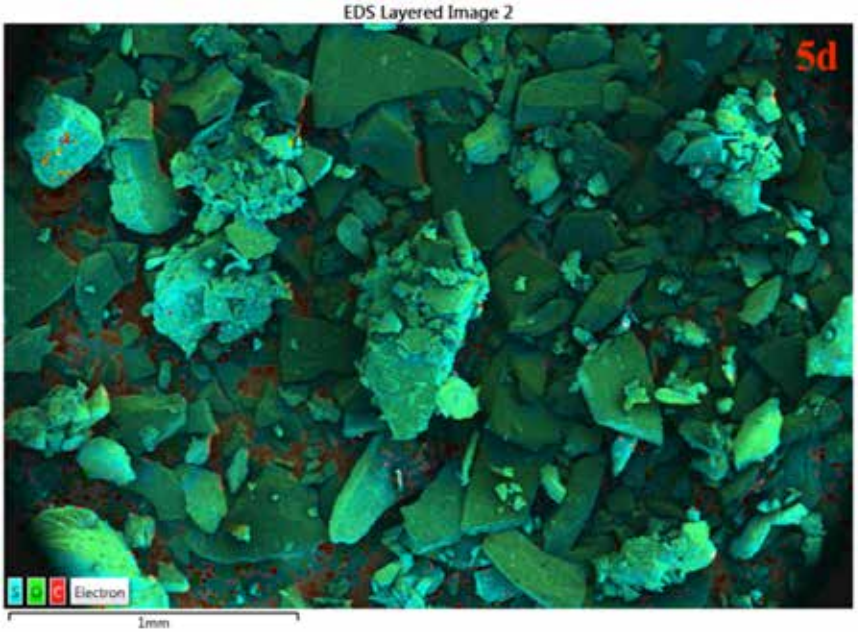
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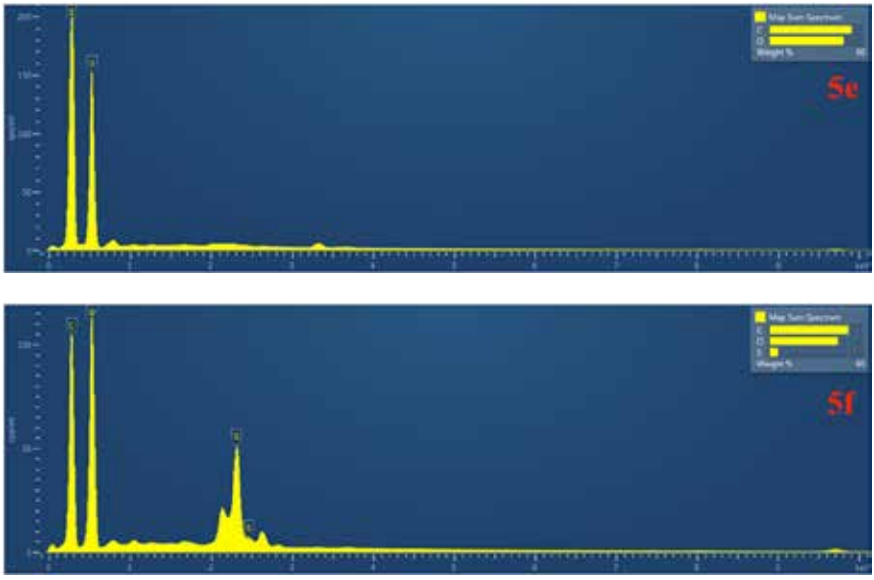

Figure 5: Field emission-scanning electron micrographs – Energy dispersive X-ray analysis (FE-SEM-EDX) of *sesbania* gum (a, c, e) and modified *sesbania* gum (b, d, f).

Scanning electron micrographs of *sesbania* and thiolated s*esbania* gum have been shown in figure 5. As clearly shown in photographs of s*esbania* gum (fig. 5a), polyhedral flakes are present while SEM micrographs of thiolated *sesbania* gum (fig 5b), show presence of plate shape particles. Fig 5shows the EDX layered images of *sesbania* (c) and thiolated s*esbania* gum (d). The presence of carbon is indicated by red color while green color represents oxygen in figure 5(c). In the EDX layered image of thiolated s*esbania* gum (fig. 5d), additional blue color shows the presence of sulphur. Further, the EDX spectrum of thiolated s*esbania* gum (fig. 5f) also shows the additional peak of sulphur at 2.3keV, which confirms the presence of sulphur in thiolated s*esbania* gum. This peak is not present in the EDX spectrum of *sesbania* gum (fig 5e).

Biocompatibility study was performed on native *sesbania* and thiolated *sesbania* gum for analysis of its clot formation capability. The test revealed that clot formation in native *sesbania* gum (0.132 g in 2 ml citrated whole blood) and thiolated-*sesbania* gum (0.157 g in 2 ml citrated whole blood) was less than as compared to positive control clot weight (0.181 g in 2 ml of citrated whole blood). The % thrombose of *sesbania* and thiolated *sesbania* gum was calculated i.e. 71% for *sesbania* gum and 85% for thiolated *sesbania* gum which concludes that both *sesbania* and thiolated *sesbania* gum can be non-thrombogenic. Further, hemolytic index of *sesbania* and thiolated *sesbania* gum was found to be 2.85% and 1.30% respectively which is also considered as safe and suitable for drug delivery applications[30].

Thiolated *sesbania* gum was further tested for drug delivery applications by using it as a mucoadhesive polymer. Since thiolated *sesbania* gum as such does not form ionically gelled beads, it was used in combination with sodium alginate to prepare composite beads using metformin hydrochloride as a model drug and $CaCl_2$ as a cross-linking agent. For comparative study, composite beads of *sesbania* gum with sodium alginate and the beads of sodium alginate alone were also prepared. The composite beads of thiolated *sesbania* and alginate beads (TSG-Alg), *sesbania* gum and sodium alginate (SG-Alg) and alginate alone beads (Alg) were obtained in a percentage yield of 122.3%, 141.2% and 97.4%, respectively. The entrapment efficiency of TSG-Alg, SG-Alg and Alg beads was found to be 99.96%, 99.26% and 89.03%, respectively.

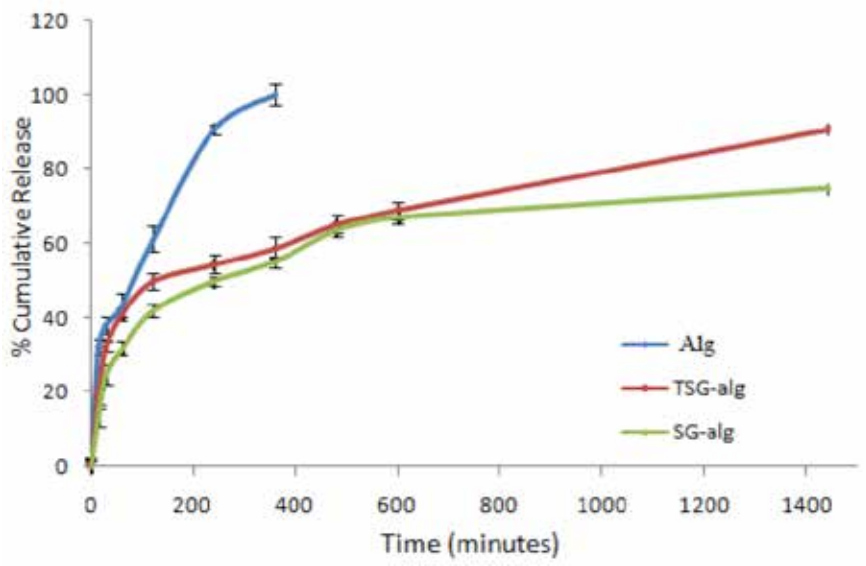

Figure 6: *In-vitro* drug release of Alg, SG-Alg and TSG-Alg composite beads.

Figure 6 represents the comparative *in-vitro* release profile of metformin from the Alg, TSG-Alg and SG-Alg composite beads. The drug release behaviour was studied in phosphate buffer (pH 6.8) to evaluate its release kinetics. It can be seen in the figure thatTSG-Alg composite beads released 90.68% of drug in 24 h whereas SG-Alg composite beads released 74.85% of metformin in the same time. As compared to composite beads, Alg beads released almost 100% of the drug in 6 h. This indicates that composite beads of *sesbania* gum/thiolated*sesbania* gumwith alginate are more effective in sustaining the release of metformin as compared to the beads of alginate alone. It can be observed from the profile

that almost similar pattern of release is observed for the release of metformin from composite beads of SG-Alg and TSG-Alg. Only at 24th h there was somewhat greater difference in the release of metformin from the TSG-Alg beads. Further to check whether there is any significant difference in the release of metformin from the two composite beads, the release data was evaluated for determining f_1 and f_2 value. On comparing the release data of metformin from TSG-Alg and SG-Alg beads, the f_1 and f_2 value were found to be 13.07 and 55.58, respectively which indicates that the release profile of metformin from the two beads can be considered to be similar. Further the release rate data was fitted into various kinetic models for determining the release kinetic and mechanism of release (Table 2). The results revealed that the release of metformin from the alginate (Alg) and composite beads of *sesbania* gum and alginate beads (SG-Alg) follows Higuchi square-root kinetics while in case of composite beads of thiolated sesbania gum ad alginate (TSG-Alg), the release data fits best into 1st order kinetics. The value of '*n*' the release exponent of Korsemeyer and Peppas equation (n<0.45) which indicates that the release of metformin from all batches of the beads occurs primarily by diffusion through matrix.

Table 2 Modelling and release kinetics of TSG-Alg,SG-Algand Alg.

Formulation	R^2				'n'
	Zero order	Istorder	Higuchi square-root	Korsemeyer-Peppas	
TSG-Alg	0.693	0.950	0.908	0.900	0.330
SG-Alg	0.626	0.782	0.887	0.937	0.421
Alg	0.871	0.966	0.981	0.969	0.300

Table 3 lists the results of swelling behaviour and *ex-vivo* bioadhesion study of TSG-Alg, SG-Alg composite beads and alginate beads in phosphate buffer (pH 6.8).The results of swelling study support the release rate profile. The Alg beads dissolved within 6h releasing almost all the drug. On the other hand, SG-Alg beads continued to swell till 24h sustaining the release of metformin, while TSG-Alg beads continued to swell till 12h and then started to erode thereby releasing drug at slightly faster rate than the SG-Alg beads.

Table 3. Swelling behaviour and *Ex-vivo*bioadhesion study of Alg, SG-Alg and TSG-Alg

Time (h)	Swelling (%)			Ex-vivobioadhesion time (%)		
	Alg	SG-Alg	TSG-Alg	Alg	SG-Alg	TSG-Alg
0.5	45.21±1.23	77.41±2.55	20±1.32	100	100	100
1	223.48±1.44	183.81±2.12	153.33±1.22	100	100	100
2	486.67±2.13	294.23±2.34	320.34±1.12	95.4	100	100
4	256.45±1.47	483.87±2.17	520±1.25	50.9	100	100
6	21.11±1.69	516.13±1.99	706.67±1.09	30	86.6	100
12	-	541.93±1.33	893.33±1.11	28	80	100
24	-	554.83±1.76	320±2.05	5	73	93.3

The mucoadhesive ability of the metformin loaded beads of the different batches of the beads was evaluated comparatively by determining bioadhesion (table 3). It can be observed from the results that Alg beads could adhere to the intestinal mucosal tissue only upto 6h. On the other hand, composite beads of SG-Alg shows 100% adhesion till 4 h and at the end of 24 h of the study 73% of the beads still adhered to the intestinal mucosal tissue. In case of composite beads of TSG-Alg, 100% of the beads were found adhering till 12 h and at the end of 24 h study period 93.3% of beads were found adhering to the intestinal mucosal tissue. The results thus conform the higher mucoadhesivity of TSG-Alg as compared to the SG-Alg beads. The literature already reported that sulfhydryl group (-SH) present in thiolated polymers form strong covalent disulphide bond with glycoproteins present in mucus which was clearly indicated in its results also[33]. However, hydroxyl group (-OH) present in s*esbania* gum form weak hydrogen bond or show weak Van der Waal's interaction with mucus glycol-proteins and show less mucoadhesionproperty[37]. Similar results were earlier observed in thiolated pectin[9] and thiolated alginate beads[38].

This study introduces the thiolation modification on *sesbania* gum with their characterization. The *sesbania* gum was esterified using mercaptoacetic acid to formulate thiolated *sesbania* gum which was characterized physiochemically, structurally, morphologically, and thermally. Thiolated *sesbania* gum was also found biocompatible as compared to native *sesbania* gum in the biocompatibility study. Fabrication of metformin drug loaded composite beads with sodium alginate i.e. Alg, SG-Alg and TSG-Alg beads were done with characterization. The thiolated *sesbania* gum shows 90.68 % *in-vitro* drug release following 1st order kinetics. Thiol group on *sesbania* gum enhance the mucoadhesive strength which can also be explored for pharmaceutical applications.

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