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Poly (Methacrylic Acid)-Grafted-Okra Gum: Synthesis, Characterization and Evaluation as Mucoadhesive

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ABSTRACT

Recently, worldwide extensive attention is being paid in exploration and exploitation of pharmaceutical excipients from natural resource. Various natural polysaccharides have been significantly reported as prospective drug delivery carriers. These natural gums are preferred over synthetic polymers because of their biocompatibility, low cost, free availability and biodegradability. But due to variable chemical composition, microbial load, microbial growth and change in viscosity upon aging, acceptability is low compared to commercial synthetic products. Tailoring or modification may be an approach to make them smart as drug carriers specially in order to modulate the site of drug release and it's kinetic. The chemical modification of okra gum (OG) was the main objective of the present study in order to make it potential mucoadhesive for the application in mucoadhesive drug delivery. In this study, methacrylic acid was grafted onto okra gum. At first, okra gum has been isolated from the fruits of *Hibiscus esculentus*. Poly (methacrylic acid)-grafted-okra gum (PMAc-g-OG) was synthesized employing a microwave-promoted and redox-initiated method. Potassium persulphate was used as free- radical-initiator. Methacrylic acid was mixed to 1% solution of OG and then 30 ml of potassium per sulphate aqueous solution was added to the previous mixture along with continuous stirring. The mixture was exposed to microwave in a domestic micro-oven. The mixture was kept overnight and the copolymer was collected and purified using acetone and aqueous methanol (30% v/v) subsequently. The copolymer was characterized by elemental analysis, FTIR, DSC-TGA, and ^{13}C NMR study. Ex-vivo mucoadhesion test was performed using goat stomach. A highest % grafting of 448.32% was found in the synthetic procedure employed in the study. The characterization studies also substantiate the successful grafting. Ex-vivo mucoadhesion study also showed excellent mucoadhesive capacity over a period of 16 hours. The study exhibited that the method employed was very simple, less time consuming, one-pot and without N_2 atmosphere. The copolymer also exhibited excellent mucoadhesivity which might be applied in different mucoadhesive drug delivery systems such as prolonged release gastroretentives, buccal gels, etc.

Keywords: Okra gum; poly (methacrylic acid)-grafted-okra gum; graft-copolymerization; mucoadhesive.

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INTRODUCTION

The natural polysaccharides have recently gained attention of excipients scientist as pharmaceutical additives for their easy availability, low cost, biodegradability and biocompatibility. [1] But microbial load, contamination proneness and unrestrained swelling are the main disadvantages in their application in drug formulation. These drawbacks can be prevailed over by chemical modification such as polymeric hybridization via grafting of synthetic monomer etc. [2] The graft copolymerization also imparts different functional properties to the polymers that may make them applicable to different purposes such as mucoadhesive, sustained release, etc. [3-4] A graft-copolymer is defined as hybrid molecule in which one or more side chains remain joined to the main polymeric backbone. [5] The Ceric (IV) ions start grafting of vinyl monomers onto polymeric backbone abstracting hydrogen atom from hydroxyl groups and thereby generating oxygen free radicals in free radical induced graft copolymerization technique. [6] Application of microwave irradiation in grafting reaction has also been useful nowadays because it provides more energy that shortens the reaction time. [7] The objective of the present investigation was to explore the potentiality of poly (methacrylic acid) grafted okra gum (PMAc-g-OG) as mucoadhesive. Okra gum (OG) is a water-soluble polysaccharide obtained from *Hibiscus esculentus* belonging family, Malvaceae, composed of D-galactose, L- rhamnose and L-galactouronic acid. [8] It has been used as vegetable food. [9] It has been reported as film coating polymer [10], as tablet matrix [11], etc. In the present study, poly (methacrylic acid) was grafted onto OG using a microwave-promoted and redox-initiated grafting technique. PMAc-g-OG was characterized by FTIR, elemental analysis, DSC and ^{13}C NMR. Its mucoadhesive potential was also evaluated.

MATERIALS AND METHODS

Materials

OG was isolated from fresh okra fruits. Ceric ammonium nitrate (CAN) was purchased from Qualigens Fine chemicals, Mumbai, India. Methacrylic acid was bought from Merck India Pvt. Ltd., Mumbai, India. All other reagents and chemicals used were of laboratory reagent grade and used without further purification. Triple-distilled water was used throughout the experiment.

Isolation of okra gum

Fresh fruits were collected, washed with water, sliced to small pieces and soaked in cold water for overnight. Then the slurry was strained and acetone is added to the strained liquid containing okra gum. The precipitate was collected, dried at 50°C in a hot air oven and finely powdered.

Synthesis of PMAc-g-OG

PMAc-g-OG was synthesized using a microwave-promoted and redox-initiated method reported earlier by Sarkar *et al*., 2017. [12] Methacrylic acid (MAc), CAN and microwave irradiation time (MW) were the synthetic variable parameters that may affect the yield. The synthetic conditions of total 8 batches of PMAc-g-OG were presented in Table 1. Methacrylic acid was mixed to an aqueous solution of OG (0.5% w/v). CAN was added to the previous mixture and stirred for 1 min and then microwaved for specific time following 1min heating and 1 min cooling cycle. Then it was kept for tonight and the grafted OG was then precipitated by addition of acetone and washed with 80% v/v aqueous methanol. The precipitate was dried at 40°C to a constant weight in a hot air oven. % grafting (%G), % grafting efficiency (%GE) and % conversion (%C) were calculated using following relations. [12]

$$\%G = \frac{(W_1 - W_0) \times 100}{W_0} \quad (1)$$

$$\%GE = \frac{(W_1 - W_0) \times 100}{W_2} \quad (2)$$

$$\%C = \frac{W_1 \times 100}{W_2} \quad (3)$$

Where, W_0, W_1 and W_2 are the weight of okra gum, grafted okra and methacrylic acid, respectively.

Characterization of PMAc-g-OG

Elemental analysis

The percentage of carbon, hydrogen and oxygen contents of okra gum and 8 batches of PMAc-g-OG were determined using a Perkin Elmer CHN 2400 microanalyzer.

Fourier Transform Infrared Spectroscopy

For the purpose of detection of the probable changes of functional groups of OG due to grafting reaction, FTIR study was done with OG and PMAc-g-OG (S6) using FTIR (Bruker, Alpha T, Germany) in the scanning range of 550 - 4000 cm^{-1} preparing KBr discs.

NMR study

A ECX 400 – Jeol high resolution multinuclear FT-NMR spectrometer operating at 100 MHz was used for ^{13}C solid state NMR analysis of okra gum and PMAc-g-OG (S6).

DSC and TGA study

DSC-TGA curves of OG and PMAc-g-OG (S6) were obtained by Perkin Elmer Pyris Diamond TG/DTA, Singapore. The rate of nitrogen flow and rate of heating were 150 mL/min and 10°C/min, respectively. 3 - 5 mg of each sample was taken in a platinum crucible with alpha alumina powder as reference. The heating range was from 10°C to 300°C.

Evaluation of mucoadhesion

Mucoadhesion capacity of PMAc-g-OG was evaluated by determining the ex-vivo mucoadhesive strengths of its different synthetic batches using Texture Analyzer (TA.XT plus, Stable Micro Systems Ltd., UK). A freshly collected stomach mucosa was washed with distilled water followed by normal saline, soaked in simulated gastric fluid (SGF, pH 1.2) for 2 minutes and then fixed on the base surface of Texture Analyzer.

Table 1: Synthetic details and elemental analysis of poly (methacrylic acid) grafted okra gum.

Batch code	Amt. of methac-rylic acid (g)	Amt. of CAN (mg)	MW irradiation time (min)	%G	%GE	$\%C_n$	Elemental analysis			Mucoadhesive Strength (Newton, N)
							%C	%H	%O	
OG	-	-	-	-	-	-	34.42	5.14	53.28	14.6±0.7
S1	5	200	1	120.5	23.78	43.5	38.85	7.31	56.83	54.8±2.3
S2	5	400	1	244.2	48.16	67.89	43.31	8.35	60.15	66.2±3.4
S3	5	200	5	4.41	0.87	20.59	34.57	5.23	53.37	14.4±1.4
S4	5	400	5	156.7	30.91	50.63	41.62	8.11	57.33	58.7±2.8
S5	10	200	1	7.86	0.775	10.63	34.71	5.51	53.61	16.9±4.1
S6	10	400	1	448.5	44.24	54.09	44.97	9.25	63.12	90.2±5.3
S7	10	200	5	12.0	1.18	11.04	35.26	5.75	54.19	16.2±3.8
S8	10	400	5	34.94	3.34	13.31	36.05	6.19	55.67	23.5±2.4

%G, % grafting; %GE, % grafting efficiency; $\%C_n$, % conversion; %C, carbon percentage; %H, hydrogen percentage; %O, oxygen percentage.

The approach surface of a sheet of PMAc-g-OG was wetted with a drop of SGF and then fixed to the movable probe of the texture analyzer using a both sided tape. The probe was lowered at a rate of 0.5 mm/s to make a contact with the mucosa and a force of 1 Newton (N) was applied for 60 s after contact. The probe was then moved upward to detach the polymer sheet from the mucosa at a rate of 0.5 mm/s to a height of 15 mm. The peak detachment force was obtained from the analyzer, which was taken as mucoadhesive strength. It is repeated for three times in each batch (n=3). [13]

RESULTS AND DISCUSSION

Synthesis of PMAc-g-OG

Table 1 represents the values of different grafting parameters. It is exhibited that MW has most positive effect on the higher grafting efficiency with respect to other factors, which is indicated by very low and insignificant grafting and significantly higher grafting found in batch S3 to S1 and in S4 to S2, respectively. In the batches, (S1; S2), (S3; S4) and (S5; S6), %G was found to be proportional to the concentration of CAN. Degree of grafting was also found to be proportional to the concentration of monomer. Grafting starts from the anomeric –CHOH of the monosaccharide repeating unit of okra gum. A OG-ceric complex is formed as a result of attack of okra backbone by ceric (IV) ions. The cerric (IV) ions create free radicals onto okra-backbone by reducing itself to cerric (III) ions and oxidizing the hydrogen atom of the anomeric –CHOH group. Grafting of methacrylic acid onto okra gum was initiated by the free radicals reacting with the methacrylic acid molecules and results in formation of a covalent bond between methacrylic acid and okra gum, which subsequently is responsible for chain propagation. Poly (methacrylic acid) may also be formed in similar way. Finally, two propagating chain free radicals join together and termination occurs. The proposed reaction scheme is as follows:

Initiation

$Ce(NH_4)_2(NO_3)_6 \rightarrow Ce^{4+} + 2NH_4^+ + 6NO_3^-$

$Ce^{4+} + OG\text{-}OH \rightarrow \text{complex} \rightarrow OG\text{-}O^\bullet + Ce^{3+} + H^+$

$OG\text{-}O^\bullet + M \rightarrow OG\text{-}OM_1^\bullet$

$Ce^{4+} + M \rightarrow M^\bullet + Ce^{3+} + H^+$

$M^\bullet + M \rightarrow M_2^\bullet$

Propagation

$OG\text{-}OM_1^\bullet + M \rightarrow OG\text{-}OM_2^\bullet$

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$OG\text{-}OM_{n-1}^\bullet + M \rightarrow OG\text{-}OM_n^\bullet$

$M_2^\bullet + M \rightarrow M_3^\bullet$

- - - - - - -   - - -   - - - - - -

- - - - - - -   - - -   - - - - - -

$M_{p-1}^\bullet + M \rightarrow M_p^\bullet$

Termination

$OG\text{-}OM_n^\bullet + OG\text{-}OM_m^\bullet \rightarrow OG\text{-}OM_{(m+n)}$ (graft copolymer)

$OG\text{-}OM_n^\bullet + M^\bullet \rightarrow OG\text{-}OM_{(n+1)}$ (graft copolymer)

$OG\text{-}OM_n^\bullet + M_p^\bullet \rightarrow OG\text{-}OM_{(n+p)}$ (graft copolymer)

$M_p^\bullet + M_q^\bullet \rightarrow M_{(p+q)}$ (homopolymer)

(OG-OH= okra gum; M= methacrylic acid)

Elemental analysis

The results of elemental analysis are shown in Table 1. %C, %H and %O were found to be higher in case of microwave synthesis batches, which corroborates the higher % grafting, and shows that monomer and CAN have proportional effect on the % grafting whereas an inversely proportional effect is shown by MW resulting breakage of propagated side-chains from OG-backbone and premature advance termination after a particular time point of microwave-irradiation.

FTIR

Infrared spectra of OG and PMAc-g-OG are shown in Fig. 1 (a) and (b), respectively. Characteristic peaks at 1609 cm^{-1} for carbonyl group indicating C=O stretching, at 1415 cm^{-1} for methyl C-H bonding, at 3395 cm^{-1} for –OH group, at 2924 cm^{-1} for –COOH group, at 1302 cm^{-1} for alcoholic –C-O group, at 1230 cm^{-1} for carboxylic –C=O group and at 895 cm^{-1} for C-O stretching for alkyl ether, were shown by FTIR spectra of okra gum in Fig. 1 (a).

The infrared spectra of PMAc-g-OG (Fig.1 (b)) shows characteristic bands at 1696 cm^{-1} for stretching vibration of carbonyl group of methacrylic acid, 1161 cm^{-1} for –C - O streching, and 1378 cm^{-1} for C – O – H bending vibration substantiating the grafting of methacrylic acid onto okra gum. A significant band observed at 1030 cm^{-1} for $CH\text{-}O\text{-}CH_2$ group ratifies grafting reaction between OH group of okra gum and π bond of methacrylic acid.

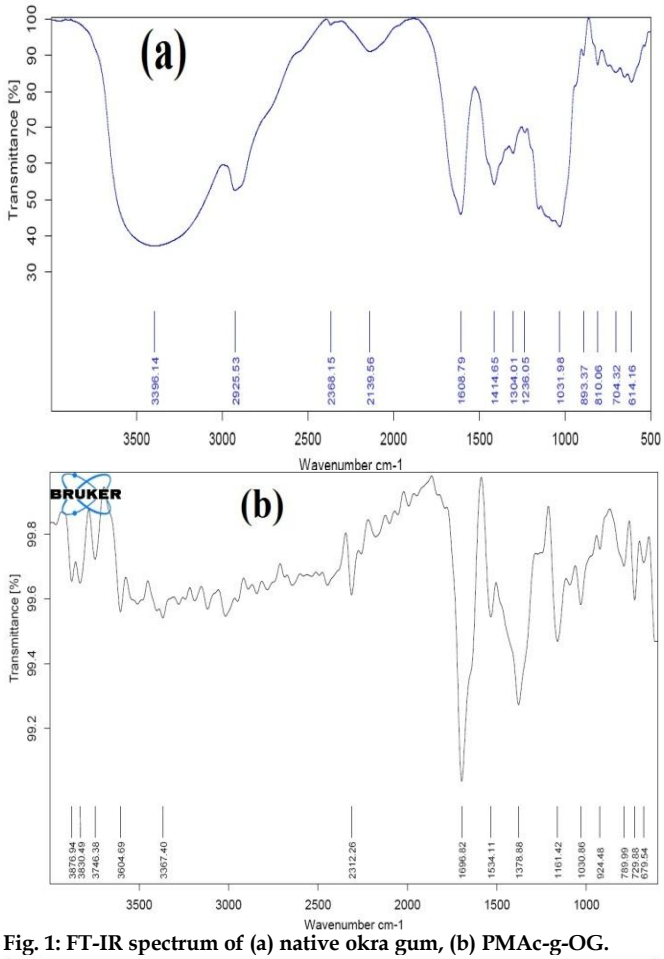

Fig. 1: FT-IR spectrum of (a) native okra gum, (b) PMAc-g-OG.

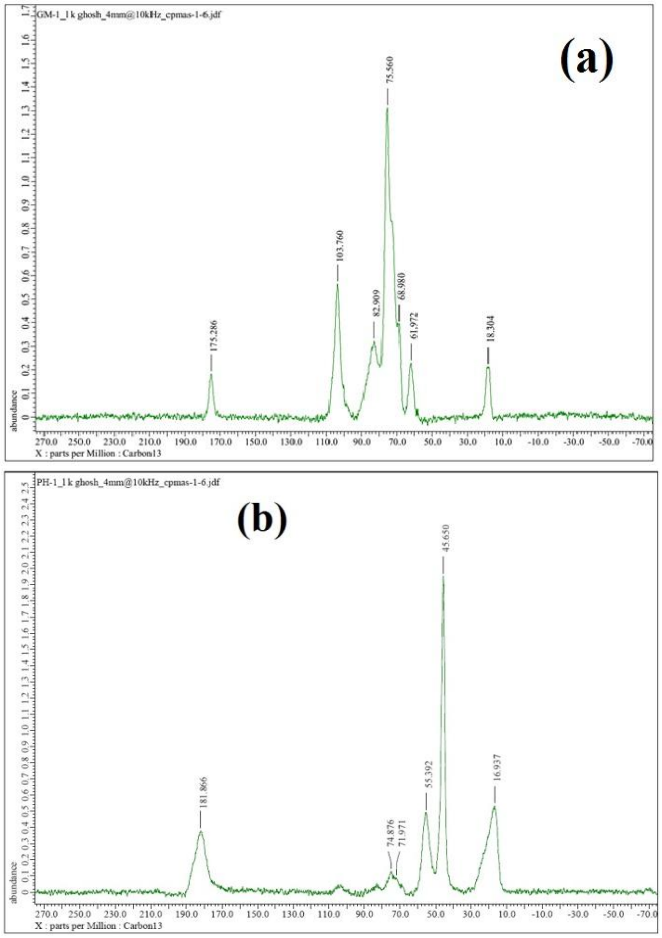

Fig. 2: Solid state ^{13}C NMR of (a) okra gum and (b) PMAc-g-OG.

NMR study

The solid state ^{13}C NMR spectra of OG and PMAc-g-OG are shown in Fig. 2(a) and (b), respectively. The spectrum of OG exhibited seven distinct peaks: the absorption peak at δ =18 ppm for the CH_3 group of rhamnose, at δ = 61 ppm for -CH_2OH group of glucose, at δ = 75 ppm (high intensity) for aromatic carbons of sugar moieties, at δ = 103 ppm for ring anomeric carbons, at δ = 175 ppm for -COOH group of glucuronic acid residue of OG. [14] The ^{13}C NMR spectrum of PMAc-g-OG shows additional bands present, compared with OG. The peak at δ = 45 ppm for $(-CH-CH_2-CH-)_n$ groups those have been formed during the polymerization reaction of methacrylic acid. The presence of very intense peak at δ = 181 ppm indicates carbon atoms of -COOH group. The shape of these bands suggests that it is composed of multiple signals as well as the signals for -C = O in glucuronic acid moiety. Absorption peak at δ = 74 ppm indicates the aromatic carbon –C - OH groups and at δ = 16 ppm for presence of CH_3 groups of rhamnose, at δ = 71 ppm for the carbon which is attached with the oxygen of ring -C - OH group. Peak at δ = 133 ppm and δ = 130 ppm which represented sp^2 carbons of methacrylic acid gets converted to the sp^3 hybridized carbon atom attached with oxygen (δ = 74 ppm), thus proving grafting reaction.

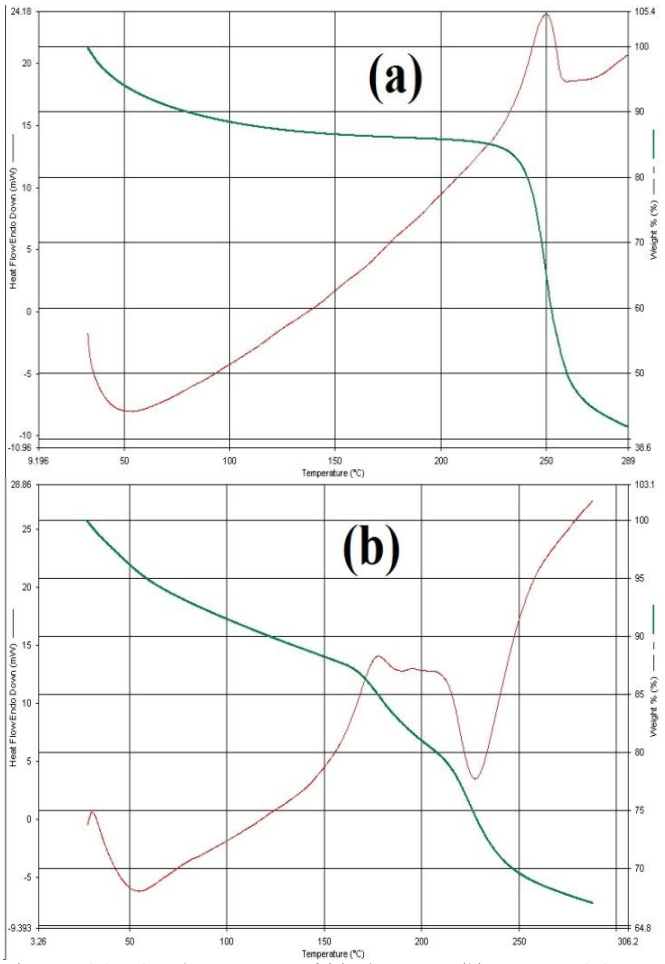

Fig. 3: DSC-TGA thermogram of (a) okra gum, (b) PMAc-g-OG.

DSC-TGA

DSC-TGA curves of OG and PMAc-g-OG are shown in Fig. 3(a) and 3(b), respectively. The DSC thermogram of OG reveals an endothermic peak at 50°C and an exothermic peak at around 250°C where TGA thermogram shows a nearly 13% weight drop at around 80°C and about 47% reduction in weight within the range from 228°C to 298°C. The relationship between endothermic peak and corresponding weight loss at 50°C indicates loss of moisture present in the OG. The exothermic peak at 250°C and corresponding decrease in weight in the temperature range from 228°C to 298°C could be ascribed due to depolymerization of OG with its degradation into water, carbon monoxide and methane.

DSC thermogram of PMAc-g-OG reveals an endothermic peak at 55°C signifying moisture-loss, which is further supported by the 14% fall in weight at 55°C observed in corresponding TGA curve. An additional endothermic peak is observed at 235°C, which may be attributed to degradation of polymeric chains of the PMAc-g-OG.

Mucoadhesive strength

The mucoadhesive strengths showed by different batches are given in Table 1. OG sheet showed very low strength (14.6 ± 0.7 N), which may be due to its high attraction to water and presence of very less no of –COOH groups in its moiety. The sheets of PMAc-g-OG exhibited maximum strength of 90.2 ± 5.3 N indicating their strong adhesion to the goat stomach mucosa. This may be due to the presence of numerous –COOH groups in PMAc-g-OG, which have the ability to form numerous hydrogen bondings with mucus molecules. Again, acidic pH prevents ionization of –COOH groups leading to increase the affinity of –COOH towards mucin molecules rather than water. S1, S2, S4 and S6 batch of PMAc-g-OG with higher % grafting exhibited significant mucoadhesion strength, which may be due to the fact that higher number of –COOH groups are present in side chain of PMAc-g-OG having higher % grafting, form more hydrogen bonding with mucin molecules and thereby result in stronger attachment with mucus. [15]

The study demonstrated the microwave-promoted free-radical initiation grafting of methacrylic acid onto okra gum which was found to be very simple, less time consuming and one-pot synthesis without any nitrogen environment. The graft copolymer has been characterized by elemental analysis, NMR, FTIR and DSC. The study also revealed that the copolymer possesses excellent mucoadhesiveness. This copolymer may be useful in the formulation of different mucoadhesive drug delivery systems along with gastroretentive and buccal preparations.

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