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Preparation and Characterization of Crosslinked Gum Acacia Microspheres by Single Step Emulsion In-Situ Polymer Crosslinking Method: A Potential Vehicle for Controlled Drug Delivery.

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Research Article

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ABSTRACT

Because of its biodegradability several natural polymers, such as plant polysaccharides, have been proposed as appropriate excipients for the development of controlled drug delivery systems for oral administration. In this work microspheres of crossinked gum acacia were prepared by single step emulsion crosslinking method using glutaraldehyde (GL) as the crosslinking agent and Hydrochloric acid (HCl) as the catalyst.. Aqueous gum acacia solution (10% w/v) was emulsified with castor oil using span 80 as emulsifier (3%) and reacted with GL (25% solution) at different temperature. After a certain time of reaction the mixture was cooled and the oily phase decanted. The microspheres were washed with isopropyl alcohol (IPA) and dried at 40^{o}C to constant weight. The FTIR spectra and the swelling properties of obtained microspheres showed that gum acacia could be crosslinked using glutaraldehyde. Degree of swelling and % weight loss decreased with increase in amount of GL and severity of reaction conditions (temperature and time). Crosslinking with higher amount of glutaraldehyde produced microspheres with lower swelling degree. These results lead to the conclusion that crosslinked GA presents good perspectives for its use in modified release pharmaceutical formulations.

INTRODUCTION

Controlled release of active ingredients in specified regions of the digestive tract is a most challenging area in the field of development of the controlled drug delivery systems. Site-specific drug therapy based on polymer matrix and coating systems is a growing field of research and application technology. Several natural polymers, such as those found in the diet, have recently been proposed as appropriate excipients for the development of controlled drug delivery devices for oral administration based on their microbial biodegradability [1, 2]. A large number of these polysaccharides and oligosaccharides may form the basis for a suitable biodegradable carrier. The administration of drugs directly to the colon, particularly to the proximal portion of the large intestine, has been evaluated as a site for local colonic pathologies but also for systemic drug delivery. Colon targeting still remains one of the most challenging systems to drug delivery. Many diseases of the colon, such as bowel disease, constipation, carcinomas and infections could be benefit from colon specific delivery.[1-3] In these cases, local administration of drugs is advantageous as it promotes reduced exposure of the organism to the drug, as a result of smaller doses employed and reduced systemic absorption, which minimizes the occurrence of side effects related to another use for colon-specific release is shown in the case of drugs degraded on upper portions of the GIT (stomach and small intestine), such as proteins and peptide hormones, [3] successful oral administration of these agents is conditioned to the protection of the pharmaceutical form against gastric and duodenal enzyme attack. Various options for colon-specific delivery have been proposed, including the use of pro-drugs, which release the active drug on the colon, and the production of hydrogels, matrices and coated solid forms, employing biodegradable polymers such as polysaccharides [2,3,4].

The drug release from the system activated by colonic microflora appears to be more suitable with regard to selectivity. Also due to the toxicity associated with the synthetic polymeric systems, a wide variety of natural polymers are being used for the design and development of colon-targeted delivery systems. Most of these systems are based on the knowledge that anaerobic bacteria in the colon are able to recognize the various substrates and degrade them with the enzymes. The application of biodegradable natural polymers, which are resistant to degradation in the upper GIT (above the colon), has gained tremendous importance in pre-biotic

food systems. Most of the recent research includes natural polysaccharides, especially from plant origin, being applied to create degradable colon-specific substrates. A number of colon-targeted delivery systems based both on polysaccharides and synthetic polymers, such as acrylics, have been designed and developed by various research groups [5, 6, 7]. Polysaccharides with a large number of derivatizable groups, a wide range of molecular weight, varying chemical composition and above all being stable, safe and biodegradable, offer properties preferable over all the other approaches. Exploiting the use of these naturally occurring dietary polysaccharides for colonic drug carrier means that issues of safety, toxicity and availability are simplified. An important pre-requisite for a colon-specific drug carrier made of natural and modified polysaccharide hydrogel, is its ability to hydrate and resultant swelling which creates a diffusion barrier at the surface of the solid dosage form during its passage through the GI tract. These hydrated layers of polymers allow the penetration of colonic enzymes/bacteria which leads to the degradation of the polysaccharide barrier, hence releasing the drug at the target site [1, 3].

Most of the natural gums are safe enough for oral consumption in the form of food additives or drug carriers. Among the advantages of natural gums over their synthetic counterparts are their biocompatibility, low cost, low toxicity (ecofriendliness) and relative widespread availability. Gums are metabolized by the intestinal microflora and ultimately degraded to their individual component sugars. In addition, enzymes available in the intestine can cleave the gums at specific sites. For example, α-galactosidase can hydrolyze terminal non reducing galactose residues to produce free α-d-galactose. However, there are certain problems associated with the use of gums. These include uncontrolled rates of hydration, pH dependent solubility, thickening, drop in viscosity on storage, and the possibility of microbial contamination. Chemical modification of gums not only minimizes these drawbacks but also enables their use for specific drug delivery purposes. Crosslinked or derivatives of gums are widely being investigated for the design of new delivery systems with tailor-made drug release profiles. An additional advantage of biodegradability confers the property of complete drug release to the dosage form due to the degradation of gums by colonic bacteria and enzymes present in the distal portion of the gastro-intestinal tract.

Gum Acacia; Indian Gum is the air-hardened, gummy exudation from the stem and branches of *Acacia nilotica* (Linn.) Del. subsp. *Indica* (Benth.) Brenan (syn. *A. arabica* Willd. var. *indica* Benth.) (Fam. Leguminosae), or other species of *Acacia.* It is available as pieces (tears) or in the form of a powder acacia. The powder form is white or yellowish-white ; odourless; on treatment with *water* it dissolves to give a mucilaginous liquid which is colourless or yellowish, dense, viscous, adhesive and translucent [8]. Chemically GA consists of a mixture of high molecular weight polysaccharide (major component) and hydroxyproline rich glycoprotein (minor component). The basic structural units of the gum are L-arabinose (45-65%), D-galactose (23-36%), L-rhamnose (2-3%) and uronic acid (8-14%) (Fig 1&2) [9]. GA enjoys a wide range of applications in industries such as paper, textile, food and pharmaceutical, due to its water binding capacity and high thickening efficiency. GA is hydrophilic and susceptible to easy biodegradation [10]. In the pharmaceutical industry, GA is used in pharmaceutical preparations and as a carrier of drugs since it is considered a physiologically harmless substance. Additionally, recent studies have highlighted GA antioxidant properties [11, 12], anticancer properties [13], its role in the metabolism of lipids [14] and its positive results when being used in treatments for several degenerative diseases such as kidney failure [15, 16], cardiovascular [17] and gastrointestinal [18]. GA has high water solubility and a relatively low viscosity compared with other gums. Most gums cannot dissolve in water in concentrations above 5% due to their high viscosity. Instead, GA can get dissolved in water in a concentration of 50% w/v, forming a fluid solution with acidic properties (pH ~ 4.5). GA has been extensively tested for its properties as non-digestible polysaccharide which can reach the large intestine without digestion; in the small intestine. GA is slowly fermented by the bacterial flora of the large intestine producing short chain fatty acids [19]. However, since natural GA is highly water-soluble it becomes impossible to use it as carrier for controlled release oral dosage form, because the polysaccharide would promptly dissolve in the aqueous content of the digestive tube, completely releasing the drug. In order to overcome this disability of the natural gum, modification of the polymer solubility through crosslinking reaction can be employed. This kind of reaction reduces the amount of hydroxyl groups on the polysaccharide chain bonding the chains together, hence decreasing its affinity by water. Chemical modifications applied to polysaccharides can produce new compounds that can be used for novel drug delivery system. However, these structural modifications should maintain the potential biodegradability of the polymer by colonic micro flora at a specific portion of the GIT. Aldehyde derivatives such as formaldehyde, glutaraldehyde or other bifunctional reactants have been used to produce insoluble biodegradable microspheres [20]. Glutaraldehyde is used as a crosslinking agent to obtain rigid microspheres. This method has been widely studied in various formulations by different researchers [21, 22]. In this method, it is important to remove excess oil by washing the particles with solvents such as isopropyl alcohol (IPA). Otherwise, the oil retained in the microspheres may cause aggregation and alter the morphological properties of the microspheres. This washing procedure is also said to remove excess of the cross-linking agent [27]. IPA used to remove excess glutaraldehyde, extracts the water content of microspheres to obtain hardened microspheres that are easily filtered and dried.

Crosslinked Gum acacia microspheres were prepared by single step emulsion in-situ crosslinking technique in which gluataraldehyde 25% solution was used as crosslinking agent. The obtained crosslinked GA microspheres were analyzed through FTIR which allows us to identify certain characteristic bonds on the compounds that indicate the formation of new products. In addition, equilibrium swelling study of microspheres was carried out to study the effect of variables (temperature, amount of GL and reaction time).

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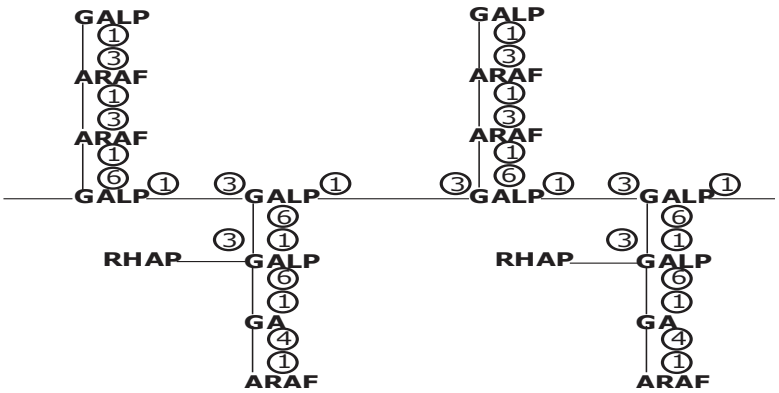

Figure 1: Molecular structure of Gum Acacia:GALP=D-Galactopyranose, ARAF=L-Arabofuranose GA= D-Glucuronic acid, RHAP= L-Rhamnopyranose [23].

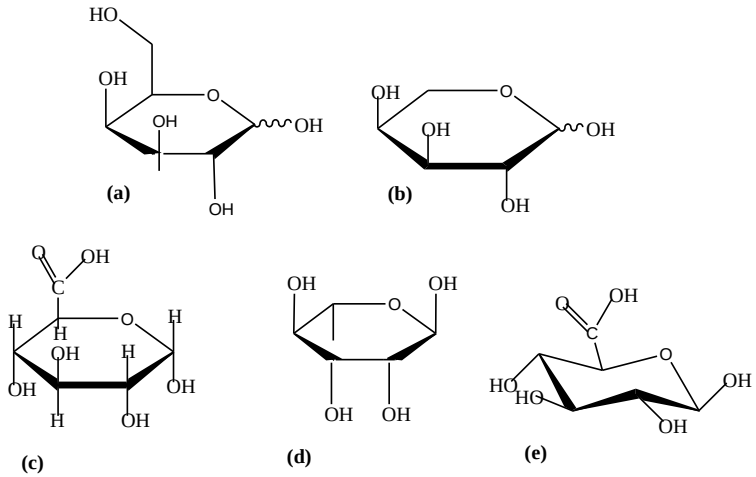

Figure 2: Chemical structures of (a) D-galactose, (b) L-arabinose, (c) D-glucuronic acid, (d) L-rhamnose and (e) uronic acid forming the mixture of gum acacia [24].

MATERIAL AND METHODS

Material

Gum Acacia IP was purchased from Loba Chemie Pvt Ltd (Lot No.: SL 12981107), Mumbai, Glutaraldehyde was purchased from S.D. Fine chemicals, Mumbai. All other chemicals used in the study were of analytical grade

Method of Preparation of Crosslinked Gum Acacia Microspheres

The chemical crosslinking method for preparation of gum acacia microspheres involves emulsification followed by crosslinking with a suitable crosslinking agent (glutaraldehyde). An aqueous solution of gum acacia was prepared in distilled water The aqueous phase was dispersed in castor oil containing 3% span 80 (emulsifying agent), using overhead stirrer with three blade paddle. The biphasic system was stirred with heating (45^{o}C, 50^{o}C, 55^{o}C, 60^{o}C, 70^{o}C), at 2000 rpm. The pH of the emulsion was adjusted to 3 with hydrochloric acid. Glutaraldehdye solution (25%w/v) was added as crosslinking agent to the above emulsion. Fifteen batches were prepared by varying the amount of crosslinking agent, temperature and reaction time, one at a time. The oily phase was decanted and the product was filtered, washed with distilled water followed by isopropyl alcohol. The microspheres were dried at 40^{o}C. Upon drying, an off white colored free flowing, fine powder was obtained. The product was kept in desiccators till further evaluation.

Fourier Transform Infrared Spectroscopy

The spectra were recorded for product using FTIR spectrophotometer (Jasco FTIR-410). Samples were prepared by KBr disk method and scanned over the range of 400-4000 cm^{-1}; the resolution was 2/cm. The spectra were recorded for Gum acacia and crosslinked gum acacia microspheres to identify certain characteristic bonds on the compounds that indicate the formation of new products

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Differential Scanning Calorimetry

The thermal behavior of gum acacia before and after crosslinking was examined with Perkin Elmer, Model: Pyris 1, DSC. Indium std was used for calibration and done every once in a week. Approximately 2.5 mg of sample was weighed in an aluminium pan and crimped with aluminium lid by using crimper. Sample was placed in a cell against a reference cell which having empty aluminium pan. DSC curves were recorded at a temperature range of 60-300^0 C. with heating rate of 20^{0}C per min.

Table 1: Batches prepared by varying the amount of crosslinking agent, temperature, and reaction time

Batch code	Gum acacia solution (%w/v)	Stirring speed (RPM)	Glutaraldehyde solution (25%w/v) in ml	Temperature(^{0}C)	Reaction time (hrs)
P1	10	2000	0	60	5
P2	10	2000	1	60	5
P3	10	2000	2	60	5
P4	10	2000	3	60	5
P5	10	2000	4	60	5
P6	10	2000	5	60	5
P7	10	2000	3	45	5
P8	10	2000	3	50	5
P9	10	2000	3	55	5
P10	10	2000	3	60	5
P11	10	2000	3	70	5
P12	10	2000	3	60	4
P13	10	2000	3	60	5
P14	10	2000	3	60	6
P15	10	2000	3	60	7

Shape and Surface Morphology

Surface and shape characteristics of microspheres were evaluated by means of scanning electron microscopy. The scanning electron microscopy samples were prepared by lightly sprinkling the microsphere powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å using a sputter coater, and the photographs of samples were taken. Also Photomicrographs of crosslinked gum acacia microspheres obtained from various batches were taken using a digital trinocular microscope (Axioplan microscope, MPM-400 with image analyzer, Zeiss, Oberkochen, Germany). A small volume of ethereal suspension of microspheres was taken on a clean slide and was allowed to air-dry. The slide containing the dry film of microspheres was mounted on the stage of the microscope for observation.

Percentage Yield

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer used to prepare that batch multiplied by 100.

Equilibrium Swelling Study and % Weight Loss

To evaluate the effectiveness of in-situ chemical crosslinking equilibrium swelling and % weight loss of treated GA was studied.

Equilibrium Swelling

The equilibrium swelling ratio which signifies the expanding and retracting forces between crosslink's at equilibrium, was determined by water uptake measurement. The terms 'swelling ratio', [25] 'equilibrium degree of swelling' (EDS) [26] or 'degree of swelling' [27] has been used for more or less similar measurements. A pre-weighed amount (100 mg) of microspheres was placed in PBS (pH 7.4) and allowed to swell for 24 hrs, which is sufficient to reach the equilibrium state. The weight of swollen samples was measured after blotting excessive water gently with filter paper. The degree of swelling (α) was then calculated from the following formula [28]

$$\alpha = W - W0 / W0$$

Where W0 is the initial weight of the microspheres and W is the weight of the microspheres at equilibrium swelling in the medium.

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% Weight Loss

To evaluate the effectiveness of in-situ chemical crosslinking % weight loss of the product in Phosphate buffer saline (Ph 7.4) was studied. The weight (W1) of a 25mm glass fibre paper (pore size 2 micron) was determined following drying in an oven at 105°C for 1 hour and subsequently cooled in a desiccators containing silica gel. Dispersion of pre-weighed (S) sample from each batch was prepared in Phosphate buffer saline (Ph 7.4) followed by overnight hydration at room temperature. The hydrated dispersion was then centrifuged for 2-5 minutes at 2500 rpm prior to filtration. Drying of the filter paper with retained amount was carried out in an oven at 105°C followed by cooling to a constant weight (W3).

Amount retained (R) = W3-W1

Weight loss = S-R

% Weight loss = (S-R/S) X100

RESULT AND DISCUSSION

FTIR and DSC

The evaluation of the FTIR spectra and DSC curves of products compared to natural Gum Acacia as reference, strongly suggests the occurrence of the proposed crosslinking reaction.

The FTIR spectrum of both Gum acacia and crosslinked gum acacia (Fig 4) depict a characteristic absorption band at 3420 cm-1 representing the presence of a hydrogen bonded OH group. The amino group which shows a characteristic absorption band in the region of 3400 - 3500 cm-1 must have been masked by an O-H group absorption band. The polymers also showed the characteristic bands of amine stretch (NH bend) around 1650 cm^{-1}. The ether linkage is manifested as a characteristic band at 1074 cm-1. The intense absorption band at 1746.33 cm^{-1} in IR spectra of microspheres expresses the carbonyl group which could be due formation of new acetal groups. This new peak indicates that glutaraldehyde had been reacted with hydroxyl of gum acacia. According to previous authors [29, 30, 31, 32] in crosslinking reaction, the aldehyde groups from glutaraldehyde reacts with hydroxyl group from polymer under acidic condition, and then forms acetal bridges. In the present work, spectra IR indicated that microspheres based on glutaraldehyde-crosslinked gum acacia were prepared successfully. Broader peak at 1263 cm-1 and 1145 in spectra of crosslinked gum acacia microspheres as compared to that of gum acacia indicates ether linkages (cyclic ether large ring -C-O- stretch), reflective of the glutaraldehyde-crosslinked gum acacia. The mechanism of cross linking reaction was predicted as in Fig. 3.

DSC thermal profiles for gum acacia (Fig 5) with low water content (<15%) showed an endothermic event at about 87.44°C. This can be related to water evaporation. No degradation DSC was observed for gum acacia, meanwhile, microspheres of gum acacia cross-linked with glutaraldehyde showed a different pattern of the DSC thermogram (fig 6) with a broad endothermic peak between 80 and 120°C and a new endothermic peak at 160.81° C reflective of formation of new compound.

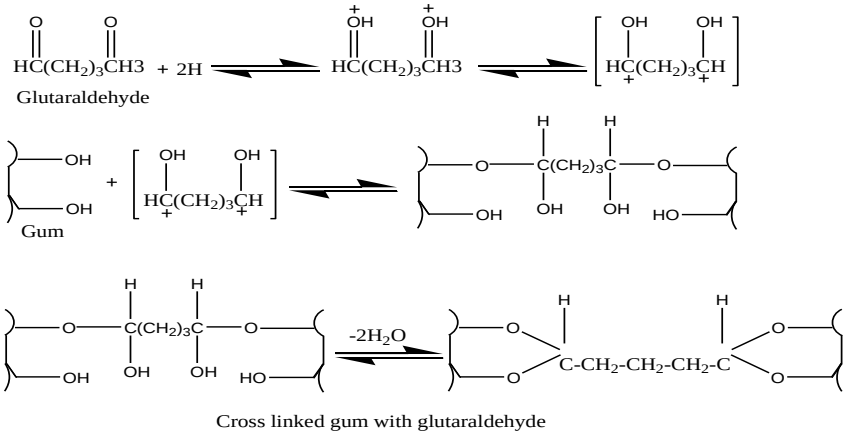

Figure 3: Reaction between gum and glutaraldehdye

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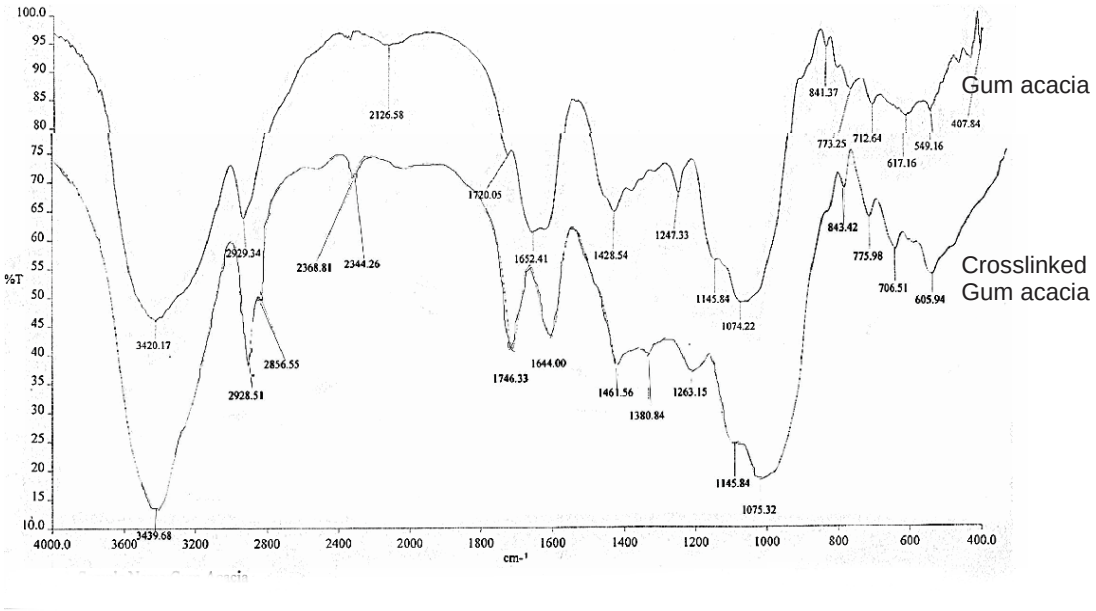

Figure 4: IR spectra of Gum acacia and crosslinked gum acacia

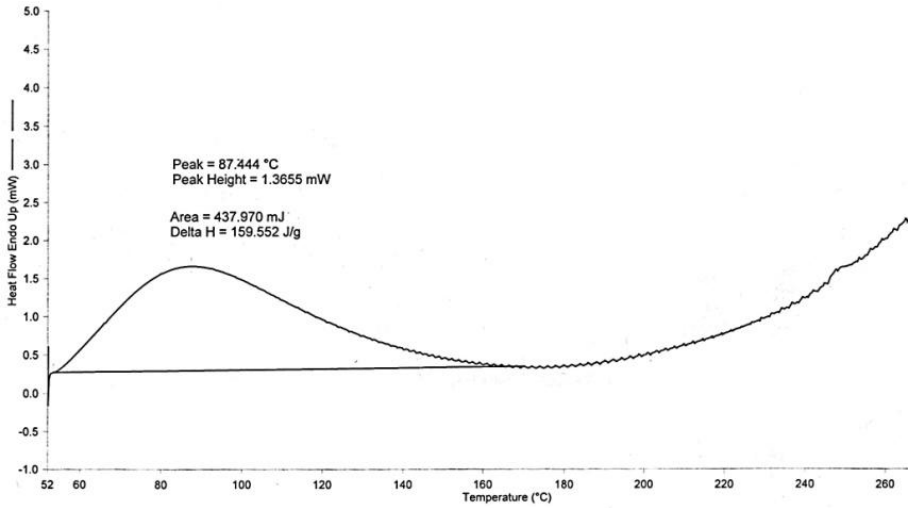

Figure 5: DSC of Gum Acacia

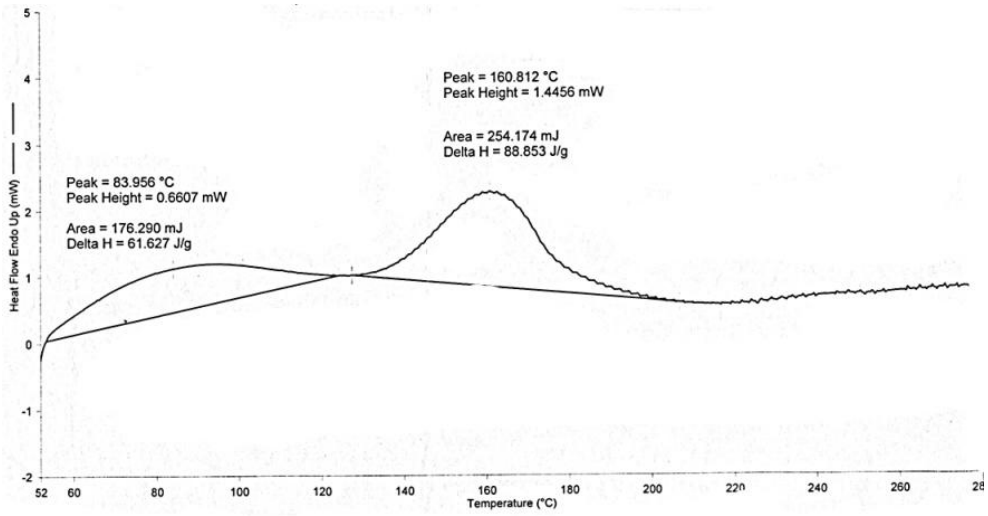

Figure 6: DSC of crosslinked gum acacia microspheres

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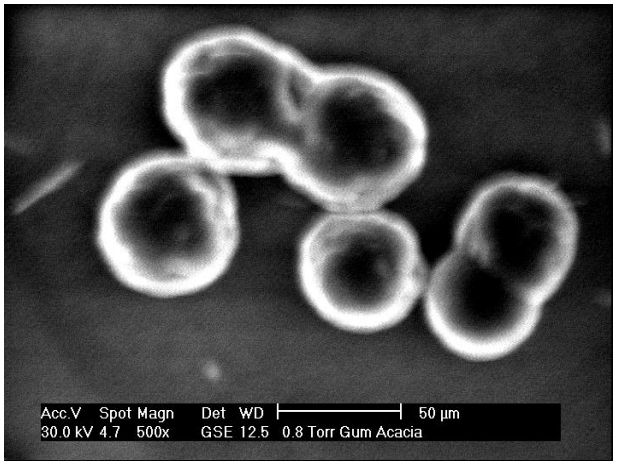

Figure 7: Scanning electron micrographs of crosslinked gum acacia microspheres (P-5)

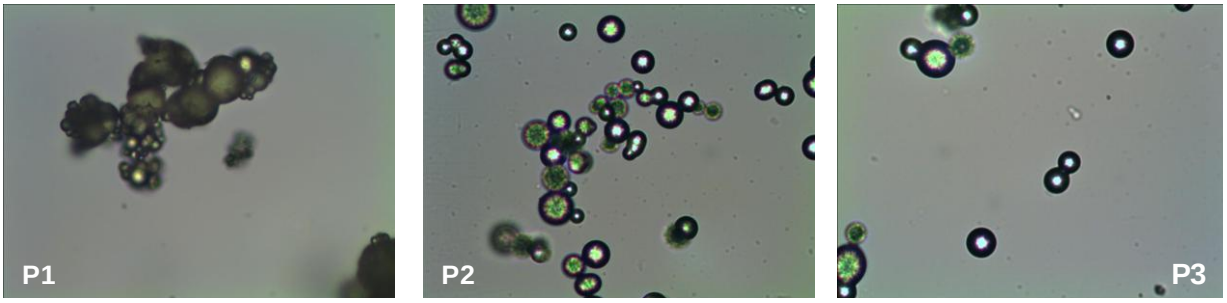

Figure 8: Trinocular photomicrographs of crosslinked gum acacia microspheres

Evaluation of Gum Acacia Microspheres

Percentage yield

The percentage yield obtained for in various batches is between 55.0- 92.16%. The product obtained at temperature lower than 60^{o}C was mucilaginous in nature could not be separated from the oily phase. At 60^{o}C and above granular product was obtained with phase separation which could be filtered and dried after adequate washing with IPA. Minimum time taken for complete evaporation of aqueous phase with appearance of granular product was found to be 5 hours at 60^{o}C. Further increasing the time, while maintaining constant temperature slightly decreased the product yield. The product yield was found to increase with increase in the concentration of gum acacia in aqueous phase (P15-P17) as well as with increase in amount of glutaraldehyde (P1-P5) suggesting increase crosslink density and therefore increase in water insoluble fraction in the product.

Equilibrium Swelling Study

Gum Acacia is known to swell in aqueous environments due to hydration. As a new polymeric structure is formed by introducing bridges between polymeric chains during the cross-linking procedure, the extent of the swelling process depends on the degree of cross-linking. Therefore, the denser the cross-linking bridges between the molecules, the more packed is the structure. Such a structure can be characterized by lower and slower penetration of the solvent through the chain structure of the polymer, suggesting that the swelling ratio and hence the release characteristics of the microsphere can be controlled by varying the content of the cross-linking agent used during the manufacturing process. Since glutaraldehyde is responsible for the formation of crosslinks, increasing the amount of glutaraldehyde and the crosslinking time increased the polymer density, resulting in reduction of the macromolecular chains mobility, and the formation of more stable and rigid spheres that showed a lower tendency to swell. However increase in the severity of the treatment conditions (i.e. increasing treatment temperature or time) also resulted in product exhibiting a darker color.

% Weight loss

Upon crosslinking, GA should not be dissolved completely in aqueous solvent. Table 2 shows the effects of crosslinkng agent (GL), temperature and time of treatment on weight loss and swelling behavior of the treated GA after submersion in Phosphate Buffer Saline at room temperature for 24 hrs. Weight loss and degree of swelling was highest in batch treated without GL (P-1) which subsequently decreased with increase in amount of GL and finally reached a plateau. Weight loss, for a fixed treatment temperature

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(P10-P15), decreased appreciably with initial increase in the treatment time and finally reached a plateau value at longer treatment times. With increasing treatment temperature, the weight loss was found to decrease.

Table 2: Result of evaluation parameters of crosslinked gum acacia microspheres

Batch code	Product yield (percent)	Degree of swelling	%Weight loss	Particle shape
P1	75.43	8.813	65.77	Spherical
P2	66.41	4.443	42.77	Spherical
P3	77.72	3.64	33.14	Spherical
P4	82.64	3.49	25.47	Spherical
P5	86.41	3.50	23.55	Spherical
P6	88.16	3.51	20.17	Spherical
P7	0*	NA	NA	NA
P8	0*	NA	NA	NA
P9	0*	NA	NA	NA
P10	86	4.44	26.32	Spherical
P11	77.63	3.41	20.34	Spherical
P12	74.82	4.78	36.14	Spherical
P13	76.65	3.49	26.33	Spherical
P14	77.84	3.40	23.21	Spherical
P15	80.53	3.34	22.56	Spherical

*Product was not obtained for evaluation

Shape and Surface Morphology

Scanning electron micrograph and images of Trinocular microscope (Figure 7&8) indicate that cross-linked gum acacia microspheres possessed a fairly smooth surface and spherical shape.

CONCLUSION

Gums are abundantly found in nature. Among the advantages of natural gums over their synthetic counterparts are their biocompatibility, low cost, low toxicity (ecofriendliness) and relative widespread availability. GA has been extensively tested for its properties as non-digestible polysaccharide which can reach the large intestine without digestion; in the small intestine. GA is slowly fermented by the bacterial flora of the large intestine producing short chain fatty acids. However, the highly swellable nature of their putative form often restricts their use for delivering drugs to distal parts of the gastrointestinal tract. Swelling properties are strongly influenced by the crosslinking reaction parameters, like amount of crosslinking agent and reaction time. The FTIR spectra of obtained crosslinked gum acacia microspheres showed the new peak that indicated the formation of crosslinked structure. Crosslinking reduced the solubility of the natural gum which is indicated by decrease in weight loss on increasing temperature and reaction time. The increase in the severity of the treatment conditions (i.e. increasing treatment temperature and time) also resulted in product exhibiting a darker color. The increasing amount of glutaraldehyde will increase the crosslinked density, indicated based on the reduction of swelling ability and % weight loss of obtained gum acacia microspheres. Crosslinked gum acacia microspheres can be a potential carrier for colon specific drug delivery since reduced hydrophilicity due to crosslinking can prevent premature drug release. The drug release can be controlled by controlling the density of crosslinking by varying the amount of GL, temperature and time of reaction, and hence the degree of swelling.

REFERENCES

1. Philip AK, Philip B. Colon Targeted Drug Delivery Systems: A Review on Primary and Novel Approaches. Oman Med J. 2010; 25(2): 70-78.
2. Vandamme Th F, Lenourry A, Charrueau C. Chaumeil JC. The use of polysaccharides to target drugs to colon. Carbohyd Polym. 2002; 48: 219-31.
3. Sinha VR, Rachna K. Polysaccharides in colon-specific drug delivery-Review. Int J Pharm. 2001; 224: 19-38.
4. Rubinstein A, Nakar D, Sintov A. Chondroitin sulfate: A potential biodegradable carrier for colon-specific drug delivery. Int J Pharm. 1992; 84: 141-50.
5. Semde R, Amighi K, Devleeschouwe MJ, Moes AJ. Effect of pectinolytic enzymes on the theophylline release from pellets coated with water insoluble polymers containing pectin HM or calcium pectinate. Int J Pharm. 2000; 197: 181-192.
6. Macleod GS, Fell JT, Collett JH, Sharma JL Smith AM. Selective drug delivery to the colon using pectin, chitosan, hydroxypropyl methylcellulose film coated tablets. Int J Pharm. 1999; 187: 251-257.

7. Chourasia MK, Jain SK. Design and development of multiparticulate system for targeted drug delivery to colon. Drug Del. 2004a; 11: 201-207.
8. Indian Pharmacopoeia: Acacia. 6th ed., Indian Pharmacopoeia Commission; Ghaziabad, India 2010; 2469.
9. Kapoor VP, Farooqui Mohammad IH, Taravel FR, et al. Studies on *Acacia nilotica* gum exudates. Structural variations due to different habits. Carbohyd Res. 1991; 221: 289-293.
10. Samui S, Ghosh AK, Ali Md A, Chowdhury P. Synthesis, characterization and kinetic studies of PEMA grafted acacia gum. Indian J Chem Tech. 2007; 14: 126-133.
11. Trommer H, Neubert RH. The examination of polysaccharides as potential antioxidative compounds for topical administration using a lipid model system. Int J Pharm. 2005; 298: 153-163.
12. Ali BH, Al Moundhri MS. Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: a review of some recent research. Food Chem Toxicol. 2006; 44: 1173-1183.
13. Meena PD, Kaushik P, Shukla S, et al. Anticancer and Antimutagenic Properties of *Acacia nilotica* (Linn.) on 7,12-Dimethylbenz(a)anthracene-induced Skin Papillomagenesis in Swiss Albino Mice. Asian Pacific J Cancer Prev. 2006; 7: 627-632.
14. Tiss A, Carrière F, Verger R. Effects of gum arabic on lipase interfacial binding and activity. Anal Biochem. 2001; 294: 36-43.
15. Matsumoto N, Riley S, Fraser D, Al-Assaf S, et al. Butyrate modulates TGF-beta1 generation and function: potential renal benefit for Acacia (sen) SUPERGUM (G.A.)?. Kidney International 2006; 69: 257-265.
16. Ali AA, Ali KE, Fadlalla A, Khalid KE. The effects of GA oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in Central Sudan. Nat Prod Res. 2008; 22: 12-21.
17. Glover DA, Ushida K, Phillips AO, Riley SG. Acacia(sen) SUPERGUMTM (Gum arabic): An evaluation of potential health benefits in human subjects. Food Hydrocolloids. 2009; 23: 2410-2415.
18. Wapnir RA, Sherry B, Codipilly CN, et al. Modulation of rat intestinal nuclear factor NF-kappaB by gum Arabic. Dig Dis Sci. 2008; 53: 80-87.
19. Annison G, Trimble RP, Topping DL. Feeding Australian acacia gums and gum arabic leads to non-Starch polysaccharide accumulation in the cecum of rats. J Nutr. 1995; 125: 283-292.
20. Vandelli MA, Rivasi F, Guerra P, Forni F, Arletti R. Gelatin microspheres cross-linked with D,L-glyceraldehyde as a potential drug delivery system: Preparation, characterization, in vitro and in vivo studies Int J Pharm. 2001; 215: 175-184.
21. Fan H, Dash AK. Effect of cross-linking on the in vitro release kinetics of doxorubicin from gelatin implants. Int J Pharm. 2001; 213: 103-116.
22. Sivakumar M, Panduranga K, Rao K. Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite-gelatin composite microspheres. Biomaterials. 2002; 23: 3175-3181.
23. Alpatova AL, Shan W, Babica P, Upham BL. Single-walled carbon nanotubes dispersed in aqueous media via non-covalent functionalization: Effect of dispersant on the stability, cytotoxicity, and epigenetic toxicity of nanotube suspensions. Water Res. 2010; 44: 505-520.
24. Onder E, Sarier N, Cimen E. Encapsulation of phase change materials by complex coacervation to improve thermal performances of woven fabrics. Thermochimica Acta. 2008; 467: 63-72.
25. Liu P, Peng J, Li J, Wu J. Radiation crosslinking of CMC-Na at low dose and its application as substitute for hydrogel. Rad Phy Chem. 2005; 72: 635-638.
26. Valles E, Durando D, Katime I, Mendizabal E, Puig JE. Equilibrium swelling and mechanical properties of hydrogels of acrylamide and itaconic acid or its esters. Poly Bull. 2000; 44:109-114.
27. Liu P, Zhai M, Li J, Peng J, Wu J. Radiation preparation and swelling behavior of sodium carboxymethyl cellulose hydrogels. Rad Phy Chem. 2002a; 63: 525- 528.
28. Nagasawa N, Yagi T, Kume T, Yoshii F. Radiation crosslinking of carboxymethyl starch. Carbohyd Poly. 2004; 58: 109-113.
29. Lee C, Kung PH, Lee YD. Preparation of Poly(vinyl alcohol)-Chondroitin Sulfate Hydrogel as Matrices in Tissue Engineering. Carbohyd Polym. 2005; 61: 348-354.
30. Mansur HS, Sadahira CM, Souza AN, Mansur AP, FTIR spectroscopy characterization of poly(vinil alcohol) hydrogel with different hydrolysis degree and chemically crosslinked with glutaraldehyde. Mater Sci and Eng C. 2008; 28: 539-548.
31. Kim K, Lee S, Won HN. Kinetics of Crosslinking Reaction of PVA Membrane with Glutaraldehyde. Korean J Chem Eng. 1994; 11(1): 41-47.
32. Lee C, Kung PH, Lee YD. Preparation of Poly (vinyl alcohol)-Chondroitin Sulfate Hydrogel as Matrices in Tissue Engineering. Carbohydr Polym. 2005; 61: 348-354.