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۴۰ درصد تخفیف نوروزی ویژه کارگاه‌ها و فیلم‌های آموزشی



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## Preparation and Characterization of Tamarind Gum/Sodium Alginate Composite Gel Beads

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### ABSTRACT

A two-step preparation and the characterization of composite gel beads of tamarind gum (2.0 wt%) and sodium alginate (0.6 wt%) as spherically well-shaped forms are reported. In the first step, the prepared solution containing tamarind gum and sodium alginate was extruded as small drops by means of syringe into a stirred calcium chloride ( $\text{CaCl}_2$ , 3.0 wt%) at 4°C and then in the second step the beads were soaked in solidified agent solution ( $\text{Na}_2\text{B}_4\text{O}_7$ , 2.0 wt%). Thus, we obtained composite gel beads with diameter range between 2 and 3 mm. We have demonstrated the properties of the composite beads, such as morphological, thermal stability and functional groups characterized by different techniques (i.e., SEM, DSC, and FTIR). The swelling behaviour in response to pH variation as well as the mechanical strength of the composite gel beads are examined and reported. The results have demonstrated that the composite gel beads not only have the advantages of rather rough surface, three-dimensionally network structure, and high anti-acid and anti-alkali properties, they are not prone to breakage under load. The composite gel beads prepared are potentially useful as polymeric carriers or supports in biotechnology and biochemistry applications.

### Key Words:

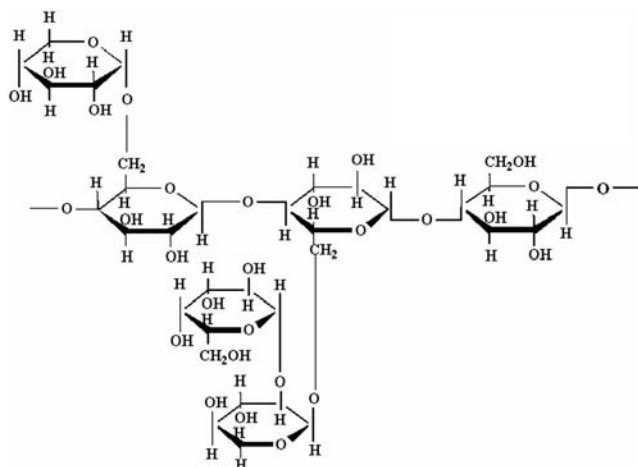
composite gel beads;  
tamarind gum;  
sodium alginate;  
anti-acid and anti-alkali properties.

### INTRODUCTION

Tamarind gum, derived from the seeds of *Tamarindus indica linn.* a common and most important tree of India and Southeast Asia, is composed of (1→4)-b-D-glucan backbone substituted with side chains of a-D-xylopyranose and b-D-galactopyranosyl (1→2)-a-D-xylopyranose linked (1→6) to

glucose residues (Scheme I). The glucose, xylose, and galactose units are present in the ratio of 2.8/2.25/1.0 [1]. The molecular weight of tamarind gum is within the range of  $2.5 \times 10^5$  and  $6.5 \times 10^5$ . It can spread in water easily and change to mucilaginous liquid when heated up, as an acid and

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**Scheme 1.** Chemical structure of tamarind gum.

thermal resistant solution. Tamarind gum can change into gel in neutral and acidic pH. The gel can be used as a thickening and stabilizing agent in food industry [2].

Alginate which is a linear polysaccharide obtained from marine algae and various bacteria, consists of 1,4-linked  $\beta$ -D-mannuronate (M) and  $\alpha$ -L-guluronate (G) residues. Alginates are an interesting family of natural polymers that have been studied for a variety of biomedical applications [3-5]. This interest in the alginates is primarily due to their high biocompatibility and low toxicity, and the relative ease with which they undergo gelation with divalent cations under very mild conditions suitable for biomacromolecules and living cells [6,7]. The gelation behaviour has been investigated in various ways [8]. However, cross-linked alginate gel beads are relatively rigid, but usually fragile [9,10] which may be a disadvantage particularly for reduplicated fermentation industry.

Thus, to our knowledge, few studies have been carried out by using tamarind gum and sodium alginate as a support for the immobilization of cells carrier. In this work, a new way is described in two steps for preparing composite gel beads by using tamarind gum (2.0 wt%) and sodium alginate (0.6 wt%) complex. In the first step, the prepared solution containing tamarind gum and sodium alginate was extruded as small drops by means of syringe into a stirred  $\text{CaCl}_2$  solution, where  $\text{CaCl}_2$  was used as both cross-linking and coagulating agent to cross-linked sodium alginate. Gelation of alginates occurs when  $\text{Ca}^{2+}$  takes part in the interchain bindings between G blocks

leading to a three-dimensional network [11]. Then, in the second step the beads were soaked in  $\text{Na}_2\text{B}_4\text{O}_7$  solution, as an accelerating agent, to lower the solubility of tamarind gum rapidly and by expelling water it becomes a semi-transparent solid [12] similar to a sponge. The technique [13] not only can avoid the agglomeration of tamarind gum gel beads simultaneously but also can improve and control tamarind gum network structure efficiently. This process presents the advantages of a simple reaction which can be carried out at room temperature, and retain the elasticity and mechanical strength of the gel. The properties, such as morphology, functional groups, thermal stability, and anti-acid and anti-alkali properties of the beads were characterized by SEM, FTIR, and DSC techniques. The results have indicated that the beads are potentially useful as polymeric carrier or support in biotechnology and biochemistry applications.

## EXPERIMENTAL

### Materials

Tamarind gum was obtained from AB Co. (Tianjin, China). Sodium alginate was purchased from Mingyue Co. (Shandong, China). The solidified agent was composed of  $\text{CaCl}_2$  and  $\text{Na}_2\text{B}_4\text{O}_7$  solutions. All chemicals were analytical grades with no need of purification stages.

### Preparation of Composite Gel Beads

The process was consisted of the following two steps. Step I: A solution, containing tamarind gum (2.0 wt%) and sodium alginate (0.6 wt%) prepared with distilled water, was stirred for 30 min at  $85^\circ\text{C}$ . Afterwards, the solution was then extruded as small drops by means of syringe into a stirred solution of calcium chloride (3.0 wt%) at  $4^\circ\text{C}$ , where spherical gel beads were formed with a size of 2-3 mm [14,15]. Each composite gel bead contained about  $5.7 \times 10^{-4}$  g and  $1.7 \times 10^{-4}$  g of tamarind gum and sodium alginate, respectively. The gel beads were subsequently soaked for 1 h at  $4^\circ\text{C}$  in calcium chloride solution while stirring. No significant difference in shape was detected between the diameters of gel beads prepared from unmodified concentration of alginate and tamarind gum or each operation of injection [16] by means of syringe. Then,

the resulting gel was removed from the solidified agent and washed several times with distilled water to remove the calcium ions [17].

Step II: The beads were soaked in the solidified agent ( $\text{Na}_2\text{B}_4\text{O}_7$ , 2.0 wt%) at 4°C. After standing for 4 h, the cross-linked beads were rinsed thoroughly with sodium chloride (0.9 wt%) three times and stored at 4°C in the same solution.

### Characterization of Composite Gel Beads

#### Morphology

The morphological characterization of the composite gel beads was carried out with a scanning electron microscope (JSM-5600LV, Kever Ltd., American). A certain amount of composite gel beads was dropped onto a sample holder by liquid nitrogen flash freezer and placed in a vacuum oven at refrigeration to dry. For examining the surface morphology, sample beads were mounted on a metal grid with a double-sided adhesive tape, followed by coating the bead surfaces with gold under vacuum. To examine the internal morphology, sample beads were cut in halves with a steel blade before treatments and the SEM micrographs were obtained.

#### Mechanical Strength

Mechanical strength of the gel beads was determined using an easy method as follows:

After being removed from sodium chloride solution, a certain amount of the composite gel beads was trapped with a filter paper to remove excess water from its surface and then weighed by an electronic microbalance. We gradually put weights on the composite gel beads to observe the crack initiation time.

$$\text{Mechanical strength (g/g)} = W_L/W_b$$

where,  $W_L$  and  $W_b$  are the weights of the loads and the beads, respectively. We have reported an average value for each sample which was analyzed at least six times.

#### Evaluation of pH-sensitive Properties of the Composite Gel Beads

The evaluation of pH sensitive behaviour was determined by the equilibrated swelling ratio (ESR) at given pH data. The desired basic and acidic pHs were

adjusted by sodium hydroxide and hydrochloric acid solutions, respectively. The pH values were precisely checked by a pH-meter (Leici/E-201-C, accuracy  $\pm 0.01$ ). The excess surface-adhered liquid was removed by blotting paper and the samples weft into separate tubes of buffer solution (pH = 1, 3, 5, 7, 9, 11, 13) for at least 24 h at room temperature. During this process, the beads were removed from the buffer solution and frequently weighed by an electronic microbalance after trapped with a filter paper to remove its excess surface water. Then, these composite gel beads were dried in a vacuum oven at 60°C to constant weight. ESR is calculated from the following formula:

$$\text{Equilibrated swelling ratio (ESR) (g/g)} = W_e/W_d$$

where  $W_e$  and  $W_d$  are the weights of the swollen beads at each predetermined buffer solution under the equilibrium state and the beads at dry state, respectively [18].

#### FTIR

We recorded the FTIR spectra of the composite gel beads to identify the functional groups. FTIR spectra of the beads were obtained by using a FTIR spectrophotometer from Alpha-Centauri (Nicolet Avatar 360 FTIR). The dry sample was mixed with potassium bromide and pressed into plate for measurement. The FTIR spectrum was then recorded.

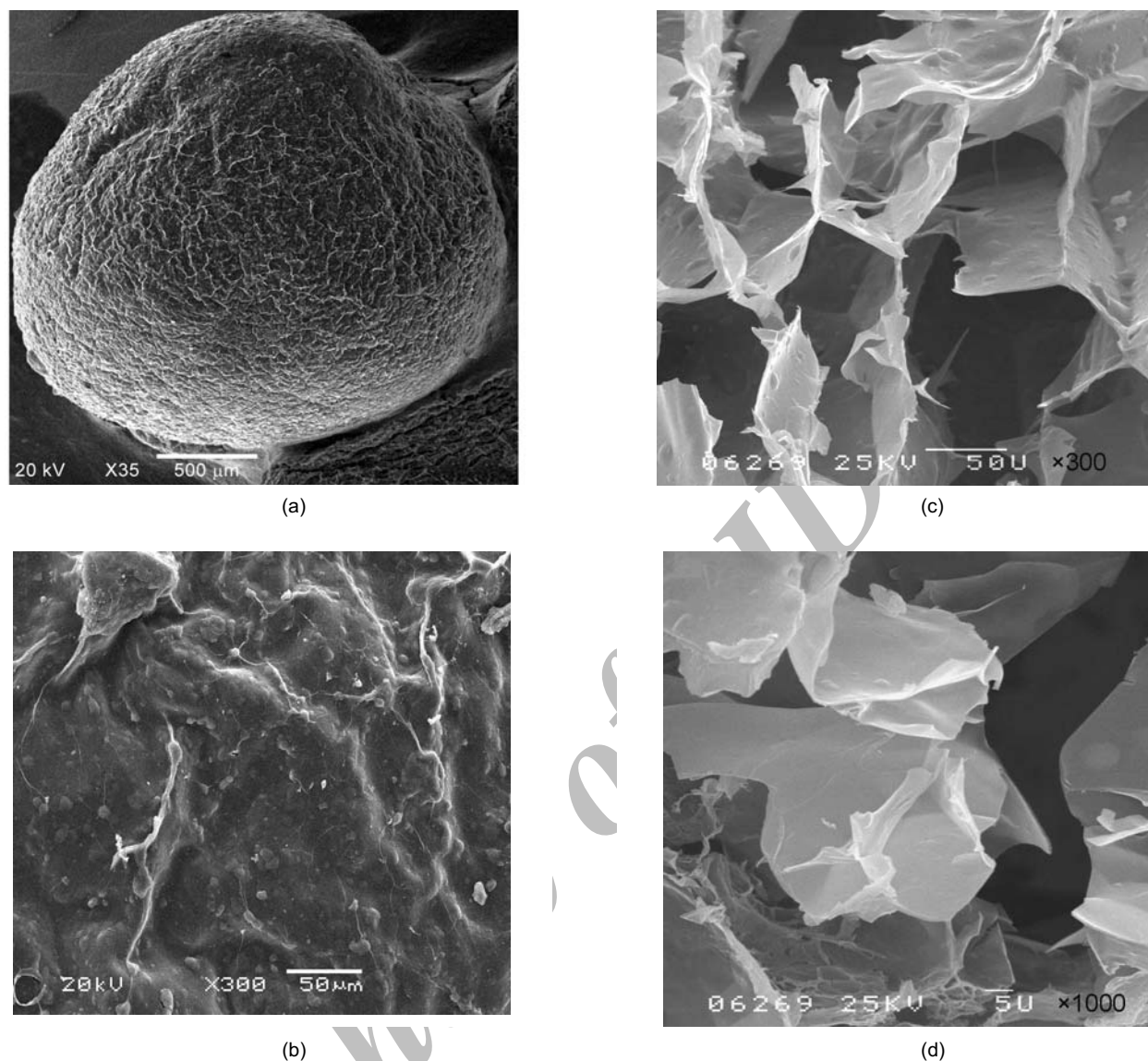
#### DSC Methods

Thermograms were obtained using a Perkin-Elmer DSC instrument equipped with an in-house cooling system. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The powdered sample of beads was hermetically sealed in an aluminium pan and heated at a constant rate of 10°C/min, over a temperature range of 25-800°C. Inert atmosphere was maintained by purging nitrogen at the flow rate of 10 mL/min.

## RESULTS AND DISCUSSION

### Morphology of the Composite Gel Beads

The morphological characteristics of the composite



**Figure 1.** SEM micrographs of the surface of a composite gel bead with magnification: (a)  $\times 35$  and (b)  $\times 300$  and its interior with magnification: (c)  $\times 300$  and (d)  $\times 1000$ .

gel beads are shown in Figures 1a-1d. The outer surface of the composite gel beads are textured and contoured whilst the internal morphology of the beads clearly shows numerous cavities that are formed as a result of the freeze-drying process. Because of the interphase solubility of sodium alginate and tamarind gum in solution, the Ca-alginate gels were formed prior to that of tamarind gum in the experiment, and  $\text{Ca}^{2+}$  rapidly replaced  $\text{Na}^+$  [13] to form the chelate complexation of alginate chains leading to a three-dimensional network (Figure 1c). This has had no relationship, however, with the improvement of tamarind gum network [19]. In the process, Ca-algi-

nate gels might have restricted the fluidity of tamarind gum molecules to enhance the conglomeration of tamarind gum molecules. Later,  $\text{Na}_2\text{B}_4\text{O}_7$ , as an accelerating agent, decreased the solubility of tamarind gum and gave rise to the contraction of the network with expulsion of water to produce a sponge like semi-transparent solid [12]. All these events have paved the way for formation of Ca-alginate-tamarind gum interpenetrating network with large pore size within the range of 60-100  $\mu\text{m}$ . The interconnectivity of pores determine the convective flow of solvent while large pore size demonstrates the potential of the composite gel beads for particles processing, e.g.,

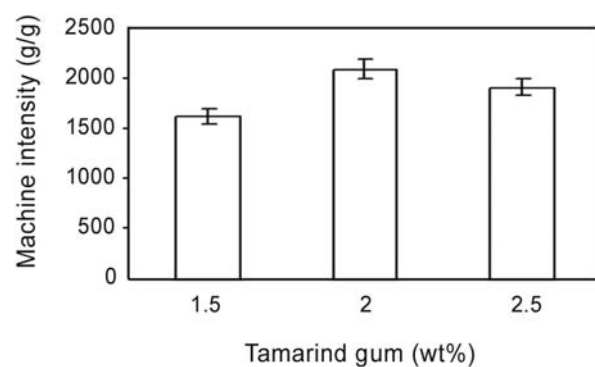
cells, cell organelles and inclusion bodies [20]. Highly porous structure and sufficiently large pore size of the composite gel spherical beads provide non-hindered diffusion of all solutes including macromolecules [21]. The three-dimensional structure obtained was composed of a polymer backbone, water, and cross-linking agents [22] which substantially enhanced the growth of microorganisms, the transfer of substrate, and greatly promoted biological oxidation rate.

### Machine Intensity

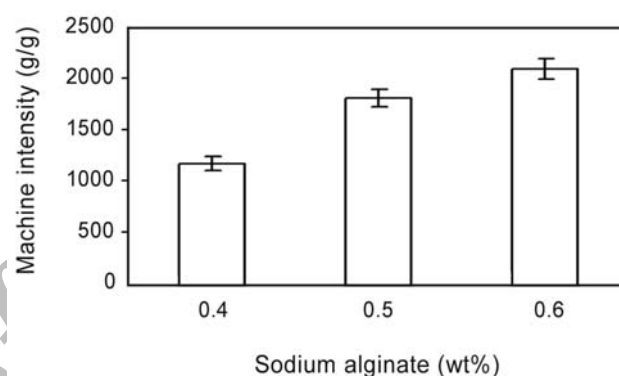
The most effective parameters determining the machine intensity was the tamarind gum/sodium alginate ratio. The results obtained are summarized as follows:

Firstly, sodium alginate was fixed at 0.6 wt%, while tamarind gum was varied in the range of 1.5-2.5 wt% for the investigation of the effects of the tamarind gum on the machine intensity. The results are shown in Figure 2a. As can be seen, when the concentration of the tamarind gum is below 2.0 wt%, the machine intensity increases significantly. It is easy to explain this behaviour such that the higher the amount of tamarind gum, the more semi-transparent solid constricted at the presence of sodium tetraborate leading to the better compactness of the above interpenetrating network. However, with the higher concentration of the tamarind gum beyond 2.0 wt%, the machine intensity decreased. The reason is that the suspension containing tamarind gum (>2.0 wt%) and sodium alginate (0.6 wt%) were rather thick and difficult to extrude small drops into the solidified agent to form well shaped spherical gel beads during preparation. Therefore, the capability of standing against tension became slightly weaker.

Secondly, tamarind gum was fixed at 2.0 wt%, while sodium alginate was varied in the range of 0.4-0.6 wt% for investigating the effects of sodium alginate on the machine intensity. The results are shown in Figure 2b. As can be seen, the machine intensity increases significantly with sodium alginate content. It is induced by a cooperative binding of  $\text{Ca}^{2+}$  with polyG chain segments in sodium alginate, as it is widely accepted that the gel network forms more stable junctions consisting mainly of dimers (egg-box model) [23]. In addition, a larger abundance of



(a)



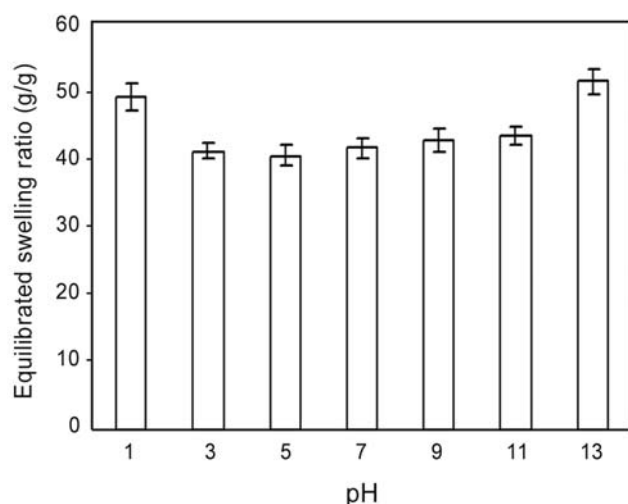
(b)

**Figure 2.** Machine intensity: (a) effects of the concentration of tamarind gum on the machine intensity; (b) effects of the concentration of sodium alginate on the machine intensity.

interpenetrating networks via Ca-alginate and tamarind gum in the gel beads may increase cross-linking density and therefore the machine intensity of the composite gel beads increases significantly. Furthermore, similar behaviour involving this parameter has already been reported [16].

### Swelling Behaviour in Response to pH Variations

The swelling behaviour of a support under specified experimental conditions is a very important parameter for immobilized cell carriers [24]. Figure 3 shows the swelling behaviour responding to pH of the composite gel beads. As is shown in Figure 3, the composite gel beads were very stable over the experimental pH range of 3.0-11.0. With regard to alginate, in acidic medium, such as pH 1.0, as the chelate complexing of chains was destroyed as a result of the high concentration of  $\text{H}^+$ , hydrogels swelled rapidly and even partially dissolved. But, as the pH varied from 3.0 to 7.0, the destruction due to  $\text{H}^+$  was not evident. With the

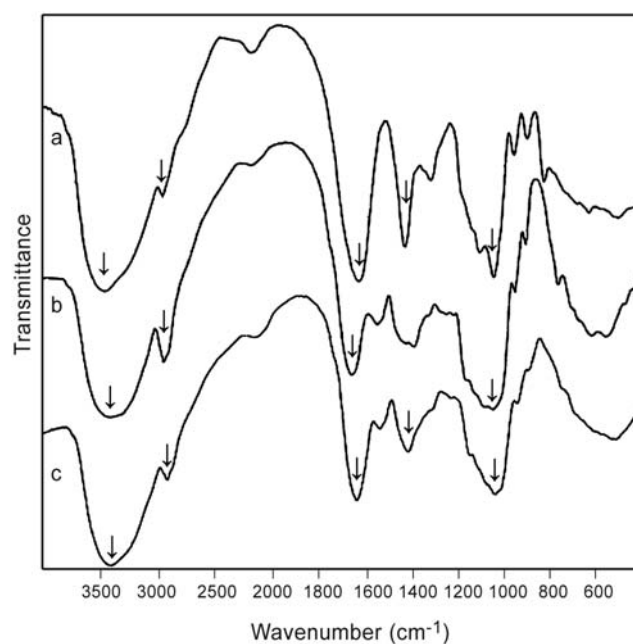


**Figure 3.** Swelling behaviour vs. pH.

continuous increase in the degree of alkalinity, calcium hydroxide was formed gradually as a result of the chelate complexing due to the disintegration of the alginate chains, which resulted in simultaneous increase in the degree of swelling. As for tamarind gum, which is highly acid resistant [25] is not sensitive to external acidic environment. In alkaline medium, as the pH increased, the main reaction is the interactions of hydroxyl groups of the gum with the sodium hydroxide to give alkoxides groups [26]. By variation of sodium hydroxide concentration, the network structure becomes more and more hydrophilic. On the basis of the above events, the swelling degree of the composite gel beads reaches a maximum at  $\text{pH} < 3.0$  or  $\text{pH} > 13.0$  which are beyond the experimental pH range of 3.0-11.0. The results demonstrated that the composite gel beads are adaptable to fermentation at acidic or alkaline conditions.

### Functional Groups

The spectra are shown in Figure 4 for all forms: (a) sodium alginate, (b) tamarind gum, and (c) the composite gel beads. The -OH groups present in tamarind gum and sodium alginate are clearly seen at  $3400\text{ cm}^{-1}$  and they are also present in the case of the beads forms. The peaks attributed to the  $-\text{CH}_2$  groups present at  $2931\text{ cm}^{-1}$  and  $2926\text{ cm}^{-1}$  in sodium alginate and tamarind gum, respectively. In sodium alginate some distinct peaks such as carboxyl group showed strong absorption bands at  $1614\text{ cm}^{-1}$ ,  $1416\text{ cm}^{-1}$  and  $1306\text{ cm}^{-1}$ , due to carboxyl anions

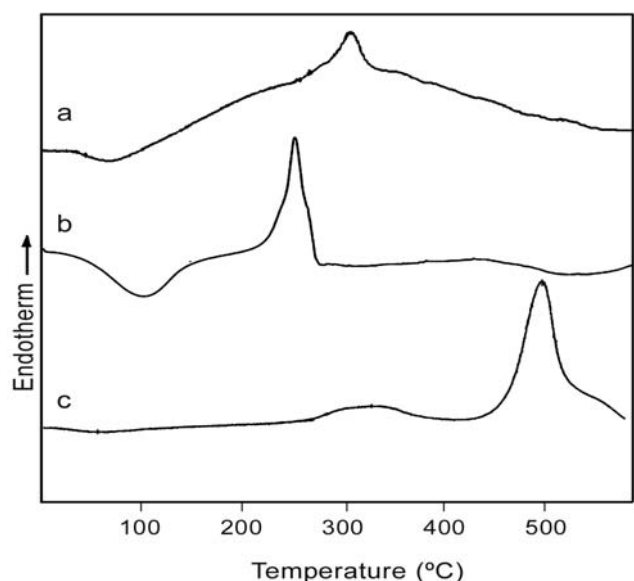


**Figure 4.** FTIR spectra of: (a) sodium alginate, (b) tamarind gum, and (c) the composite gel beads.

asymmetric and symmetric stretching vibrations [27,28] disappearing or becoming weak at  $1421\text{ cm}^{-1}$  in the composite gel beads. The reason for it is that within the beads, more tamarind gum is combined with very low alginate concentration and another may be that there were multi-interactions (hydrogen bonding and electrostatic interaction) among tamarind gum and alginate molecules [29]. This can also be seen from the spectrum of tamarind gum that a band at  $1648\text{ cm}^{-1}$  is attributed to the absorption band of the carbonyl ( $-\text{HC}=\text{O}$ ) stretching [29]. It may be explained by the fact that each monosaccharide of tamarind gum has two isomers, comprising of a ring system and a chain system, which results in the appearance of the carbonyl absorption band. The other band at  $1041\text{ cm}^{-1}$  that was assigned to the stretching vibration of (CH-OH) appeared at  $1643\text{ cm}^{-1}$  and  $1045\text{ cm}^{-1}$  for the composite gel beads. Other peaks were similar for the raw materials and for the beads. As a result, it can be concluded that all the components which were used to form the composite gel beads are present.

### DSC Analysis

The DSC thermogram of tamarind gum (a), native alginate (b) and the composite gel beads (c) are shown



**Figure 5.** DSCs of: (a) tamarind gum, (b) sodium alginate, and (c) the composite gel beads.

in Figure 5. The endothermic peaks from 50 to 100°C are due to removal of the moisture absorbed by three samples. The exothermic peaks at 300.1°C (a) and 498.2°C (c) can be attributed to thermal decomposition of tamarind gum and the composite gel beads, respectively. Melting peaks at 254°C (b) gained from native alginate were observed which were the same as those obtained by Smitha [30] and Miura [31]. According to the results, the thermal stability of the composite gel beads becomes stronger than that of each tamarind gum and sodium alginate individually which can be attributed to the gel formation via Calcium alginate and tamarind gum. In addition, the interactivity between molecules plays a vital role in the stability of the gel beads. These observations have demonstrated that the composite gel beads have a better thermal stability.

## CONCLUSION

In this study, spherically well shaped composite gel beads were produced using very cheap biopolymers (i.e., tamarind gum and sodium alginate). The composite gel beads were evaluated based on morphology, mechanical strength, FTIR data, thermal stability, and acid/alkali resistance. All these special characteristics make the tamarind gum-sodium alginate beads partic-

ularly interesting for biomedical applications. In addition, we would like to underline that our results are of good reproduction and all the experiments were conducted in triplicates. Therefore, composite gel beads are promising as a potentially good support to be employed in immobilized cell carrier technology and fermentation industry with good economical feasibility and good quality.

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