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RESEARCH ARTICLE

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PREPARATION OF UNIFORM-SIZED AGAROSE BEADS FOR AFFINITY CHROMATOGRAPHY MATRIX

ABSTRACT

Uniform-sized agarose beads were prepared by emulsification technique in this study. Agarose (4% w/v) was dissolved in boiling water containing 0.9% sodium chloride and used as water phase. A mixture of liquid paraffin containing 3 wt% of tween 80 emulsifier was heated to 70-80^{o}C and used as oil phase. Then the heated oil phase was poured into the water phase and the mixture was stirred with a homogenizer to form uniform W/O emulsion. Then the emulsion was cooled down to room temperature under gentle agitation to form gel beads. The effect of emulsifier on the uniformity of the beads was investigated. The beads with different size can be prepared by using different concentration of emulsifier and the best result was found to be 3% w/v tween 80. Under optimized condition, the coefficient variation (C.V.) showing the size distribution of the beads was under 17%. Beads with diameter from 40 to 80 μm were obtained in this study

Keywords: Agarose beads, Uniform-sized, Emulsification technique.

INTRODUCTION

Agarose is a natural polysaccharide obtained from red seaweeds, and it is composed of alternating residues 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose. It has a characteristic of forming heat-reversible gel in dilute aqueous solution. The formed gel is hydrophilic and macroporous, and it does not contain charged groups or other adsorption centers. In addition, hydroxyl groups available on the polysaccharide chains can be activated and then covalently bound ligand, it provides extensive application to agarose gel in chromatographic separation(1-3), e.g., ion exchange, affinity, and hydrophobic interaction chromatography. On the other hand, in the field of microbiology, agarose gel is also used in cell encapsulation(4-6), the macroporous gel allows cell to distribute inside uniformly, and favors the free diffusion of nutrition and substance produced by metabolism. Therefore, the gel can enhance permeability of microcapsule membrane and facilitates the growth of cell.

There are a few methods for preparing spherical agarose gel, such as suspension gelation(7), spraying gelation(8,9), etc. The basic process of these methods is that the emulsion is firstly prepared by dispersing agarose aqueous solution into organic solvent containing surfactants by mechanical stirring or spraying and then droplets are solidified by cooling. The disadvantages of these methods are that the size distribution of agarose beads is very broad and the diameters are difficult to control. However, the uniformity and controllability of beads are important for application. If the beads are not uniform, when packed into column for chromatographic separation, small beads tend to locate in the middle of bed, this will result in that the flow rate in the middle is slower than that at the edge, which must be avoided in chromatographic separation. Furthermore, beads with broad size distribution may result in high backpressure because small beads will block the interspaces among large beads. As microcapsules, if the beads are not uniform, the number of cells encapsulated in each capsule is not the same, after incubating for a period of time, the density of cell in each capsule is quite different, it will result in some difficulties in interpretation of cell characterization since the interpretation of monodispersed microcapsule is much simpler than polydispersed microcapsule. So before used as microcapsules or chromatographic medium, agarose beads must be sieved to obtain microbeads within a certain size range. Some researchers have proved that the uniform-sized particles were responsible for improving the purity and recovery of the product. The most recent method of preparation of uniform-sized agarose beads is membrane emulsification technique(10), which is not suitable for all laboratories.

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In this study, shaking emulsification method was applied in preparation of uniform-sized agarose gel beads. Furthermore, it possesses other advantages, such as easy to scale-up, the excellent reproducibility and lower energy consumption. The difficulty of preparing agarose beads is that the water phase (agarose aqueous solution) has to be kept at high temperature during the whole emulsification process, because agarose aqueous solution will form gel rapidly under room temperature. However, temperature cannot be too high because this will lower the interfacial tension between the membrane and the water phase, resulting in broad size distribution of the droplets. In this study, we investigated the effects of oil-soluble emulsifier on the size distribution, and obtained uniform emulsion and beads under optimum condition. The agarose beads prepared by this method are sieved for separating uniformed sized beads. Furthermore, the uniform beads with different size can be obtained by using different concentration of emulsifier.

MATERIALS AND METHODS

Preparation of uniform-sized agarose beads

The agarose beads were prepared by emulsification technique combined with cooling solidification process. Agarose was dissolved in boiling water containing 0.9 wt% sodium chloride and used as water phase. The liquid paraffin containing emulsifier was heated to 70-80^{o}C and was used as oil phase. Then the heated oil phase was poured into the water phase and the mixture was stirred with a homogenizer to form uniform W/O emulsion. The emulsion thus obtained was cooled down to room temperature with cold liquid paraffin under gentle stirring to obtain solid agarose beads. After filtration of emulsion mixture, agarose beads were collected and screening was carried out for separating uniform-sized agarose beads. Finally uniform sized agarose beads were washed successively with ethanol and water.

Standard conditions were 4 wt% agarose containing 0.9% NaCl water, 3 wt% emulsifier in liquid paraffin and temperature 70-80^{o}C.

Measurement of size distribution of agarose beads

Agarose beads were dispersed in distilled water and diameters were measured by an optical microscope. The beads size distribution was characterized as C.V. (coefficient variation) value(10), defined as follows,

$$C.V = \left(\sum_{i=1}^{n} \frac{(d_i - \bar{d})^2}{N} \right)^{1/2} / \bar{d},$$

$$\bar{d} = \sum_{i=1}^{n} d_i / N$$

where d_i is the diameter of the *i*th diameter, $\bar{d}$ is the number mean diameter, and N is the total number of beads.

RESULTS AND DISCUSSION

Preparation of agarose beads

Agarose beads were prepared by emulsification technique where 4% w/v agarose was dissolved in boiling water containing 0.9% sodium chloride and used as water phase. A mixture of liquid paraffin containing 3 wt% of Tween 80 emulsifier was used as oil phase. Then the heated oil phase was poured into the water phase and the mixture was stirred with a homogenizer to form uniform W/O emulsion. Then the emulsion was rapidly cooled bellow the gelation temperature by mixing with a second liquid paraffin at –20°C under gentle agitation to form gel beads. Beads with diameter from 40 to 80 μm were obtained in this method.

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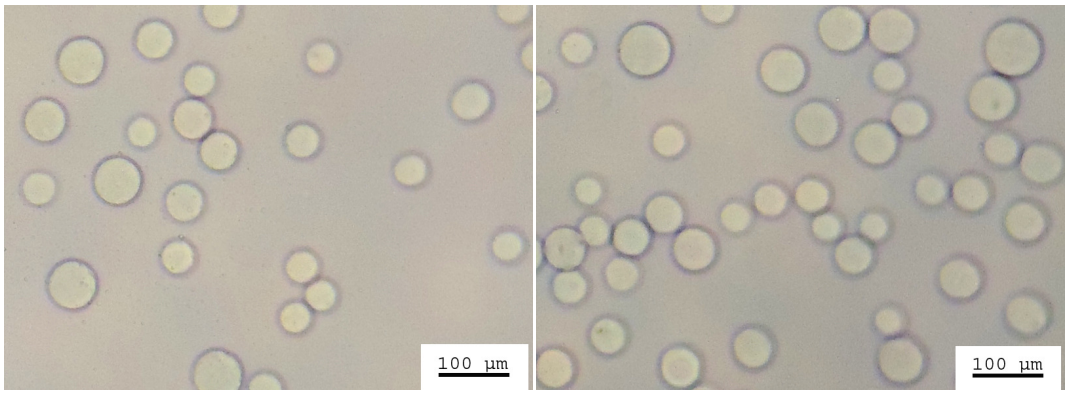
Effect of emulsifier on uniformity of agarose beads

Tween 80 and titan X 100 were chosen as emulsifier in our study, the other preparative conditions were the same as the standard condition. The optical microscopic photographs of emulsion droplets prepared with different emulsifier are shown in **Figure 1**. It was apparent that the droplets by using titan X 100 was not as uniform as that by the tween 80. In order to interpret the difference on uniformity, the size distributions and mean droplets diameter of beads were investigated, the result is shown in **Table 1**. Therefore, the concentration of tween 80 was investigated. The preparative conditions were the same as the standard condition. The optical microscopic photographs of emulsion droplets prepared with different concentration of tween 80 are shown in **Figure 2**. The size distributions and mean diameters of the gel beads prepared with different concentration of tween 80 are shown in **Figure 3**. The size distribution of the gel beads decreased with increase of the concentration of tween 80 from 1 to 3 wt% and remained almost constant when emulsifier concentration increased to above 3 wt%. When concentration of tween 80 was above 3 wt% the emulsion showed the best uniformity, where the C.V. value of the droplet was under 17%. The trend of mean diameters of the gel beads was same as the C.V. value as shown in **Figure 3b**. The higher was the C.V. value, the less was the uniformity, and larger was the mean diameter of droplets.

Emulsifier	**C.V. value (%)**	**Mean diameter (µm)**
Tween 80	16.12	49.4
Titan X 100	17.8	51

Table 1: The emulsification results of W/O emulsion prepared by emulsification technique with different emulsifier.



(a) (b)

Figure 1: Optical microscopic photographs of agarose beads prepared using different emulsifier: (a) Tween 80; (b) Titan X 100.

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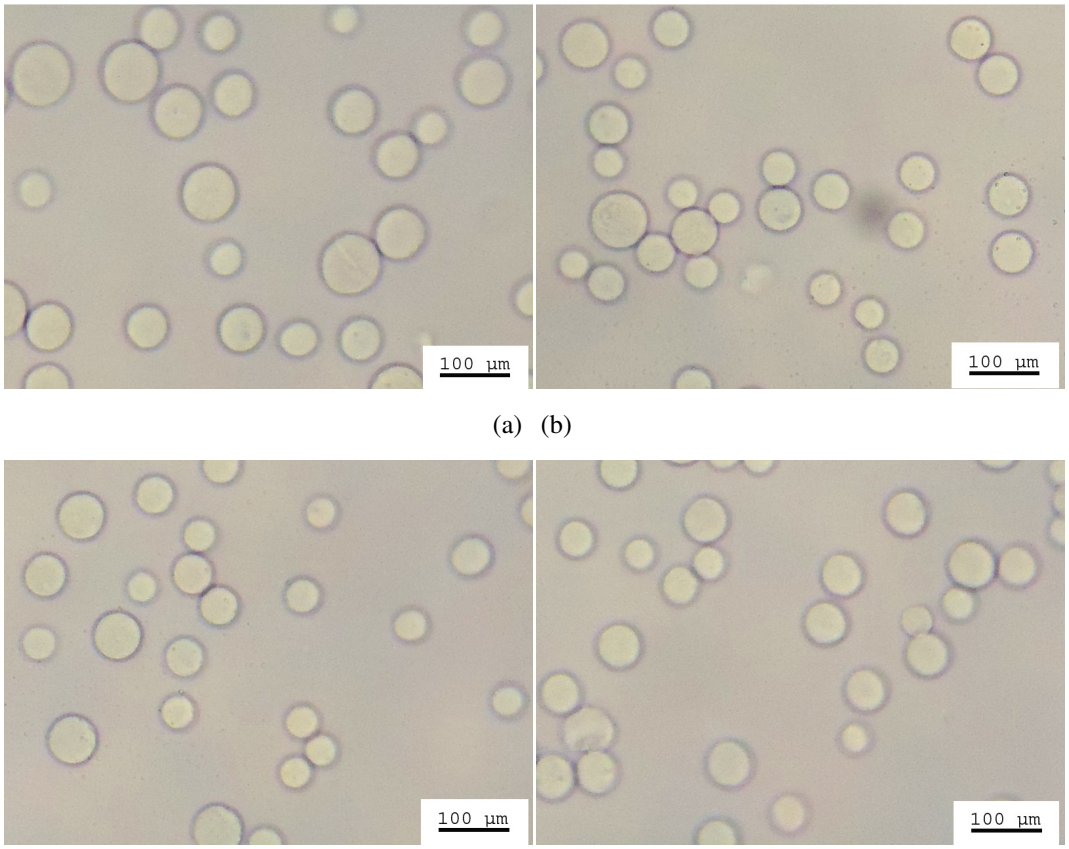
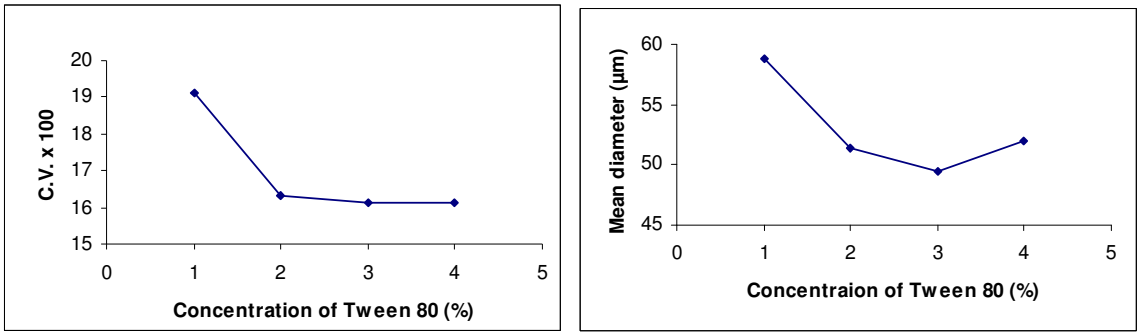


(a) (b)

(b) (d)

Figure 2: Optical microscopic photographs of agarose beads prepared using different concentration of tween 80: (a) 1 %; (b) 2%; (c) 3% and (d) 4%.



(a) (b)

Figure 3: The emulsification results of W/O emulsion prepared by emulsification technique with different concentration of tween 80: (a) C.V.; (b) Mean diameters of droplets.

CONCLUSION

In this paper, uniform-sized agarose gel beads were prepared with emulsification technique, which is suitable for all laboratories. Further studies are however needed to preparation of affinity chromatography matirx.

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