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Preparation of large-sized highly uniform agarose beads by novel rotating membrane emulsification

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ABSTRACT

A novel rotating membrane emulsification setup incorporating a 100 μm pore diameter stainless steel hydrophobic membrane is used to produce W/O emulsions consisting of 4 wt% hexaglycerin penta ester (PO-500) as emulsifier, the mixture of liquid paraffin (LP) and petroleum ether (PE) in 11:1 (v/v) as continuous oil phase and agarose solution as the dispersed phase. The agarose beads of average sizes between 108.4 and 385.4 μm can be obtained after the emulsion solidification process. The membrane rotational speed (500–2000 rpm), agarose concentration (2–4 wt%) and oil phase composition are all investigated as to their effect on emulsion droplet size and size uniformity of agarose beads. The optimal conditions for producing uniform agarose beads are an agarose solution of 3 wt%, a mixture of liquid paraffin and petroleum ether in 11:1 (v/v) and a membrane rotational speed of 900 rpm, under which the average bead diameter is 220 μm with a very narrow size distribution value of 0.76 (Span value). A model describing the operation is presented to predict droplet size under a certain condition and the prediction results fit with the experimental data fairly well. The emulsification reproducibility and stable beads properties demonstrate that the rotating membrane emulsification is a manufacturing protocol for uniform droplets and beads of controlled size.

Contents

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1. Introduction

Biocompatible agarose with abundant hydroxyl groups is a natural polysaccharide extracted from red seaweeds, and it has a distinguishing feature of changing into heat-reversible macroporous gel from cooling aqueous solution [1–4]. Due to the characteristics of hydrophilic, macroporous and electrically neutral, agarose beads are widely used in the fields of functionalized beads to sort bacteria [5], chromatographic separation and purification of macromolecules [6]. As column packing material, the size and uniform of beads have a profound impact on the evaluation of chromatographic performance. Since large-sized beads are superior to small-sized beads in flow rate and treatment capacity, large-sized beads are extensively used in pilot and industrial scale, such as desalting, buffer exchange and the capture step of the purification process. Presently, large-sized agarose beads can be prepared by some typical methods, such as mechanical stirring and spraying methods. However, these methods suffer from poor control of droplet size and size distribution, low energy efficiency, and time consuming characteristics [4,7]. So developing an effective method for preparation of uniform large-sized agarose beads is highly desired.

Compared with these conventional methods, a membrane emulsification technology is an effective route to prepare uniform sized beads. Crossflow membrane emulsification, which utilizes forced crossflow of the re-circulating continuous phase over the membrane surface to initiate droplet detachment, plays an important role in membrane emulsification technology with many advantages as controllability of droplets size, narrow size distribution and easy scale-up [8–11]. Previous investigations of our research group have produced agarose beads with a size less than 60 μm using a crossflow membrane emulsification system [1,4,5], as well as agarose beads with a much smaller size using a premix membrane emulsification system [12,13]. It is difficult to prepare agarose beads with size up to several hundreds of micrometers using crossflow membrane emulsification because large droplets are apt to breakup and coalescence during recirculation of continuous phase with a pump [14]. Meanwhile, the relatively low emulsion production rate as determined by the dispersed phase flux is one of the limitations.

Rotating membrane emulsification system is capable of overcoming problems described above [9,14], since the detachment of droplet is initiated by the centrifugal force provided by rotating the membrane rather than reticulating the continuous phase. Williams et al. prepared liquid paraffin in water emulsions with a stainless steel membrane [15–17], and the influence of pore shape on the throughput had been studied [18,19]. Schadler et al. prepared water in sunflower oil emulsions using a nickel membrane and found that the gap width between the membrane tube and outside vessel had a remarkable influence on the droplet size [20]. In addition, some food grade double emulsions were prepared with SPG (Shirasu Porous Glass) rotating membrane [21,22]. Compared with cross membrane emulsification methods [4,5,14] this can be particularly advantageous to the production of coarse emulsions and fragile structured products, in which the droplets and/or particles are subject to breakage during the pump circulation. To the best of our knowledge, preparation of large-sized agarose beads using rotating membrane emulsification has not been reported.

The aim of this work is to gain insight into the practical application of the innovative rotating membrane emulsification for preparing W/O emulsion and large-sized uniform agarose beads. Here, the effects of membrane rotational speed, agarose concentration and oil phase composition were investigated via experiments and statistical analysis by a response surface method. The major forces acted on droplet were analyzed and a droplet formation model was built. Moreover, some properties of the obtained agarose beads were tested for their chromatography behavior.

2. Experimental

2.1. Materials

The water/oil (W/O) emulsions were prepared using 2 wt%, 3 wt% and 4 wt% agarose (with an average molecular weight of 120 kDa, purchased from Promega Corporation Co., Ltd., USA) solution as the dispersed phase, respectively. The continuous phase was the mixture of liquid paraffin (LP) and petroleum ether (PE, from Beijing Chemical Reagent Company, China), respectively in volume ratio of 10:2, 11:1 and 12:0 (v/v). 4 wt% hexaglycerinpenta ester (PO-500, purchased from Sakamoto Yakuhin Kogyo Co., Ltd., Japan) was used as oil phase emulsifier. Crosslinking agents including epoxy chloropropane (ECH) and 1, 4-butanediol diglycidyl ether (BDE) and other agents used were of analytical purity and purchased from Beijing Chemical Reagent Company. IPE agarose big beads, which have an average diameter of 212.4 μm and a Span value of 1.54, were purchased from National Engineering Research Center for Biotechnology (NERCB, Beijing, China) and used as control beads in Section 2.3.6.

2.2. Experimental setup and procedure

2.2.1. Experimental apparatus and procedure

A rotating membrane emulsification system with a stainless steel membrane tube was set-up in this work. The experiments had been carried out using a tubular stainless steel membrane with laser-drilled pores. The geometric characteristics of the membrane are shown in Fig. 1(a). The membrane pores with a mean size of approximately 100 μm were arranged in a cubic array, with an average pitch of 500 μm. 10 mm diameter × 40 mm length membrane tube is concentrically positioned in a stationary cylindrical glass container (34 mm diameter × 100 mm length). The wall thickness of the membrane was approximately 1 mm. The membrane tube was mounted on an IKA Eurostar digital overhead stirrer and situated in a stationary glass cylinder with an inner diameter of 30 mm. A 2 mL agarose aqueous solution was