Powder Technology 253 (2014) 444–452

Contents lists available at ScienceDirect

Powder Technology

journal homepage: www.elsevier.com/locate/powtec

Preparation of uniform and large sized agarose microspheres by an improved membrane emulsification technique

Xi Zhao [a,b], Jie Wu [a,*], Fang-Ling Gong [a], Jin-Mei Cui [a], Jan-Christer Janson [c], Guang-Hui Ma [a,*], Zhi-Guo Su [a]

[a] National Key Lab of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, People's Republic of China
[b] University of Chinese Academy of Sciences, Beijing 100190, People's Republic of China
[c] Department of Physical and Analytical Chemistry, Uppsala University, SE-75123 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 26 February 2013
Received in revised form 3 October 2013
Accepted 4 December 2013
Available online 12 December 2013

Keywords:
SPG membrane emulsification technique
Agarose microspheres
Large size
Uniformity

ABSTRACT

The SPG (Shirasu porous-glass) membrane emulsification technique has been subject to much attention for the preparation of uniform emulsions. However, so far primarily used for the production of droplets with sizes below approximately 60 μm. A production bottleneck occurred if the desired size was further increased, especially when highly viscous dispersed phases were involved. To this end, an improved membrane emulsification technique was proposed and has been applied to the preparation of large agarose microspheres, with a size of around 90 μm and with a narrow size distribution. The effects of important emulsification parameters, including the pore size of the SPG membrane, the operating pressure, the stirring rate of the continuous phase, the composition of the continuous oil phase, and the concentration of agarose in the dispersed water phase, have been extensively studied. Under optimum conditions, uniform-size agarose microspheres with an average diameter of 93 μm and a size distribution index of 0.65 were successfully prepared. The average particle size of the home-made agarose microspheres was almost identical to that of the commercial product Sepharose 4 Fast Flow (4FF), which is produced by mechanical stirring and an additional sieving process. However, the size distribution of the former was much narrower than that of the latter. Therefore, the improved membrane emulsification technique presented here is promising for the application of high viscosity systems such as agarose solutions, and the production scale can be further enhanced by increasing the number of membrane units attached to the experimental apparatus.

1. Introduction

Agarose is a gel forming polysaccharide obtained from red sea weeds [1,2]. And agarose gel microspheres are widely used as a column packing material for chromatographic separation and purification of biological macromolecules such as proteins. The main advantages of agarose gel as a support matrix of chromatographic media for macromolecules are its inherent neutral, hydrophilicity and macroporosity. By covalent cross-linking, the microspheres can be rendered adequately rigid making possible the production of functional column packing material based on particle diameters down to approximately 10 μm. Chromatographic performance is to a large extent dependent on the particle size and size distribution of the column packing material. The more uniform the particle size distribution, the less zone spreading is obtained, hence leads to higher chromatographic resolution. In addition, less pressure drop and thus higher operational flow-rates are expected. In traditional agarose microsphere manufacturing using emulsification techniques based on mechanical stirring [3] or spraying [4], the particle size distributions are inherently broad causing band broadening and thus less than optimal resolution [5].

Membrane emulsification is a technique that has recently proven useful for the production of uniform emulsions of agarose solutions for the preparation of chromatographic separation media. It was reported by Zhou et al. [6] that uniform-sized agarose microspheres with an average particle diameter in the range of 15 to 60 μm can be prepared using the SPG membrane emulsification technique. As for all agarose gel media, these particles have sufficiently large surface area for ligand coupling and large enough pores to allow high mass transfer also of macromolecules such as proteins. However, when produced with too small particle diameters, inconveniently high back-pressures are obtained in packed beds [7,8]. To this end, we have found that uniform agarose microspheres with average particle diameters around 90 μm show low flow resistance at relatively high flow rates, which is beneficial especially for industrial scale application [9]. Despite the industrial importance of large agarose gel particles, there is little information available regarding the preparation of uniform-sized microspheres with average diameter around 90 μm using the SPG membrane emulsification technique.

Previous reports have shown that there is a linear relationship between the average diameter of droplets obtained and the pore size of the SPG membranes employed. Thus SPG membranes with large pores

* Corresponding authors. Tel.: +86 10 82544993; fax: +86 10 82627072.
E-mail addresses: wujie@mail.ipe.ac.cn (J. Wu), ghma@home.ipe.ac.cn (G.-H. Ma).

0032-5910/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.powtec.2013.12.012

are required for the preparation of large particles [10]. However, with increasing pore size, the optimum pressure drops to such a low level that even a subtle pressure fluctuation would significantly affect the size and size distribution of the resulting emulsion. Furthermore, the fast droplet generation induces coalescence on the surface of the SPG membrane caused by the high penetrability of the large pores. Therefore, certain obstacles have to be overcome when preparing uniform agarose microspheres using SPG membranes with average pore sizes larger than 20 μm. To the best of our knowledge, this is the first report published on this subject.

In this report, we presented an improved membrane emulsification technique for the preparation of uniform sized agarose microspheres with diameters of around 90 μm. The investigated process parameters, such as the membrane pore size, the operating pressure, the stirring rate of the continuous phase, the composition of the oil phase, and the viscosity of the water phase, were optimized to ascertain microsphere uniformity. Furthermore, the theoretical mechanism of droplet formation from a micropore was discussed in detail based on the membrane emulsification apparatus presented in this study. As a result, agarose microspheres with an average diameter of 93 μm and a narrow size distribution could be successfully prepared.

2. Experimental details

2.1. Reagents and material

Agarose was purchased from Promega Corporation Co., Ltd. (USA) with an average molecular weight of 120 kDa, a gelling point between 36 and 39 °C and a melting point around 88 °C. The Shirasu porous glass (SPG) membrane was obtained from SPG Technology Co. (Japan). A hydrophobic silane coupling agent, KP-18C, used to modify the membrane was purchased from Shin-Etsu Chemical Co. Ltd. (Japan). Hexaglycerin penta ester (PO-500) used as emulsifier in the continuous phase was kindly provided by Sakamoto Yakuhin Kogyo Co., Ltd. (Japan). Sepharose 4FF was from GE Healthcare Bio-Sciences (Sweden). Liquid paraffin and petroleum ether used as the continuous phase were purchased from Beijing Chemical Reagent Company (China). Other reagents were of analytical grade and supplied by Beijing Chemical Reagent Company (China).

2.2. Experimental apparatus

The schematic diagram of membrane emulsification apparatus ThermMEM-500 (National Engineering Research Center for Biotechnology, Beijing, China) is shown in Fig. 1. It is composed of a mechanical stirrer, a pressurized (N_2) steel tank, an emulsion container, and several tubular SPG membranes, all of which are enclosed in a thermostatted tank in order to prevent gelation during the emulsification process. The agarose solution is stored in a steel tank (500 mL), and is allowed to pass through the uniform pores of the SPG membrane into the surrounding continuous phase. The resulting emulsion is gently agitated by a mechanical stirrer so as to avoid undesirable creaming. 1–12 SPG membrane tubes with an individual membrane size of 5 cm (L) × 1 cm (Φ) are arranged radially inside the emulsion container, as depicted in Fig. 1b with 9 tubes. When multiple membranes are employed, the available specific surface area increases significantly, thus the throughput enhanced substantially.

2.3. Preparation of agarose microspheres

The process of agarose microsphere preparation was as follows. The agarose powder was carefully dissolved in boiling water, and the solution was allowed to pass through the membrane pores into the stirred oil phase continuously to form uniform droplets. The obtained emulsion was allowed to slowly cool to room temperature forming solid microspheres under gentle stirring. Finally, the agarose microsphere suspension was washed successively with petroleum ether, ethanol and water, in order to remove the oil phase.

Unless otherwise specified, the following standard operating procedure was followed:

1. The SPG membrane had an average pore size of 25.9 μm.
2. 4% agarose solution (w/w) as dispersed phase (water phase).
3. Mixture of liquid paraffin and petroleum ether 11:1 (v/v) as continuous phase (oil phase).
4. Emulsifier: 4 wt.% Hexaglycerin penta ester (PO-500) in the oil phase.
5. Operating temperature: 65 °C.
6. Operating pressure: 0 kPa.
7. Volume ratio water phase to oil phase, 1: 8.
8. Stirring rate of continuous phase: 200 rpm.

Relative standard deviation (RSD) represented for the reproducibility of the above process is calculated from the following equation:

$$S = \sqrt{\sum_{i=1}^{n} (X_i - \overline{X})^2 \Big/ (n-1)}$$

$$RSD = S/\overline{X} \times 100\%$$

where S is the standard deviation of the production batches, X_i is the average diameter of the ith batch of microspheres, $\overline{X}$ is the average diameter of the microspheres of all the batches, n is the analyzed number of batches.

2.4. Characterization of water and oil phases

2.4.1. Determination of the interfacial tension between the oil and water phase

The interfacial tension between oil and water phase was measured by pendant drop method [6]. In brief, this method consists of determining the volume, V_{max}, of the largest drop hanging at the end of a vertical capillary of radius, r, which can be held by the interfacial tension. To be specific, the water phase was injected slowly into the oil phase by the tip of a stainless steel blunted needle under a constant pressure provided by a micro-flow pump. The elongation of water drop was imaged and digitized by a CCD recording system coupled to computer, which then calculated the values of interfacial tension. Data presented were the average value of at least three measurements. Considering that the needle was easily blocked by the viscous agarose solution, pure water was used substitute for agarose solution to measure the interfacial tension against oil phase.

2.4.2. Viscosity measurement

The viscosity measurement was carried out using a Bohlin Gemini 2 Rheometer (Malvern Instruments, United Kingdom) with a cone plate as measuring unit (cone diameter 20 mm, angle 4°). A constant shear rate of 3 s^{-1} was selected to evaluate the viscosity of water phase and oil phase, respectively. A solvent trap was used to avoid drying out of the samples during measurement. Results from three measurements were averaged to obtain the final viscosity value.

2.4.3. Density calculation

The density of the oil phase was determined by calculating the weight to volume ratio. The apparent density (ρ) in gram per cubic centimeter was calculated using the following equation:

$$\rho = (m - m_0)/25 \times 1000$$

where m and m_0 represent the weight of the pycnometer with and without oil phase, respectively, and the volume of the oil phase set at 25 mL.