ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201709.00104

Preparation of Uniform Small-Particle-Size, High-Concentration Agarose Biochemical Separation Media by Rapid Membrane Emulsification Technology and Its Application for High-Efficiency Separation and Purification of Antibiotics Post-Print

Authors: Zhu Kai, Zhao Lan, Huang Yongdong, Ma Guanghui

Date: 2017-09-20T00:00:00+00:00

Abstract

Agarose microspheres are the most widely used matrix for separation media in the field of biochemical separation, yet they still suffer from issues such as non-uniform particle size, low mechanical strength, and low raw material conversion rate, which result in low efficiency and complicated processes for the separation and purification of biomacromolecules as well as large-scale chromatography production, becoming a developmental bottleneck in the field of biochemical separation. Agarose microspheres prepared by conventional methods such as stirring mostly contain less than 6 wt% agarose; when the agarose content exceeds 6 wt%, the aqueous phase viscosity becomes too high, making it difficult for traditional preparation methods to uniformly disperse the high-viscosity aqueous phase into the oil phase to form a homogeneous emulsion, leading to non-uniform emulsion droplet sizes. For biochemical separation media, small particle size can provide a large specific surface area and high resolution; high agarose content can not only enhance media strength but also enrich effective functional groups within the microspheres, providing a foundation for subsequent derivatization applications.

This study employs a novel rapid membrane emulsification technique, where agarose is heated and dissolved to prepare an agarose solution of the desired concentration as the aqueous phase. A certain amount of oil-soluble emulsifier is dissolved in a water-immiscible organic phase and preheated to a certain temperature as the oil phase. The aqueous and oil phases are mixed using a stirring method to prepare a W/O type primary emulsion. Through rapid membrane emulsification technology and by regulating pressure, the primary

emulsion is rapidly passed through a hydrophobically modified SPG membrane to obtain a W/O type emulsion with uniform particle size, and finally, under slow stirring conditions, the emulsion is cooled and solidified to gel, yielding agarose microspheres with uniform particle size.

Taking microspheres with 6% agarose concentration as an example, using petroleum ether and liquid paraffin as the oil phase at a volume ratio of 1:6 to the external aqueous phase, under a membrane pressure of 0.3 kgf/cm^2, the obtained microspheres have an average particle size of 30 m, a Span value of 0.62, and a CV value of 25%. Compared with commercial Sepharose 6FF media, the particle size decreased from 90 m to 30 m, while the CV value improved by 47%. Moreover, the entire microsphere preparation process takes no more than 10 minutes, and the conversion rate of agarose raw material into microspheres reached 100%. The research results demonstrate that when agarose concentrations reach 8%, 10%, 12%, and 16% respectively, microspheres prepared by the rapid membrane emulsification method can achieve particle sizes of 28-32 m, with Span values <0.9 and CV values <18%.

The 8% agarose microspheres prepared by rapid membrane emulsification were crosslinked, then activated with allyl glycidyl ether and coupled with sulfonic acid groups to prepare cation exchange media. The aforementioned media were used to purify antibiotics (vancomycin and spiramycin). After crude products were separated by column chromatography, the eluate was collected and the purity of the product was analyzed by HPLC. The research results show that vancomycin purity increased from 88.7% to 95.1%, and spiramycin purity increased to 99.9%, representing a substantial improvement in purity.

Rapid membrane emulsification offers advantages such as simplicity and high efficiency, demonstrating outstanding potential in the preparation of separation media with uniform small particle size and high agarose content. Simultaneously, this method is also applicable to the preparation of other drug-loaded nano-/microspheres and holds promising application prospects.

Full Text

Preamble

Preparation of Uniform Small-Particle-Size, High-Concentration Agarose Biochemical Separation Media by Rapid Membrane Emulsification Technology for Efficient Antibiotic Separation and Purification

Authors: Zhu Kai, Zhao Lan, Huang Yongdong, Ma Guanghui
Affiliation: State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190

Agarose microspheres represent the most widely utilized matrix for separation media in biochemical separation processes, yet they are plagued by several crit-

ical limitations including non-uniform particle size distribution, low mechanical strength, and poor raw material conversion efficiency. These deficiencies lead to low separation efficiency and complicated protocols for biomacromolecule purification and large-scale chromatographic production, creating a significant bottleneck in the advancement of biochemical separation technologies. Conventional stirring methods typically yield agarose microspheres with agarose content below 6 wt%. When agarose concentration exceeds 6 wt%, the aqueous phase viscosity becomes prohibitively high, preventing traditional preparation methods from achieving uniform dispersion of the viscous aqueous phase into the oil phase to form a homogeneous emulsion, which results in polydisperse droplet sizes. For biochemical separation applications, small particle sizes offer a large specific surface area and high resolution, while high agarose content not only enhances mechanical strength but also provides abundant functional groups for subsequent derivatization and applications.

This study employed a novel rapid membrane emulsification technique to address these challenges. Agarose was dissolved by heating to prepare aqueous solutions of desired concentrations as the water phase. A predetermined amount of oil-soluble emulsifier was dissolved in a water-immiscible organic phase and preheated to an appropriate temperature as the oil phase. The water and oil phases were mixed via stirring to prepare a W/O primary emulsion. Using rapid membrane emulsification technology and controlled pressure, the primary emulsion was rapidly passed through a hydrophobically modified SPG membrane to obtain a uniform W/O emulsion. Subsequently, under gentle stirring conditions, the emulsion was cooled and solidified through gelation to produce uniform agarose microspheres.

Using 6% agarose microspheres as a representative example, with petroleum ether and liquid paraffin as the oil phase at a volume ratio of 1:6 to the external aqueous phase and under a membrane pressure of 0.3 kgf/cm^2, the resulting microspheres exhibited an average particle size of 30 m, a Span value of 0.62, and a CV value of 25%. Compared with commercial Sepharose 6FF media, the particle size was reduced from 90 m to 30 m, while the CV value improved by 47%. The entire microsphere preparation process required less than 10 minutes, achieving a 100% conversion rate of agarose raw material. Furthermore, when agarose concentrations were increased to 8%, 10%, 12%, and 16%, the rapid membrane emulsification method successfully produced microspheres with particle sizes ranging from 28-32 m, Span values below 0.9, and CV values below 18%.

The 8% agarose microspheres prepared by this method were cross-linked, activated with allyl glycidyl ether, and functionalized with sulfonic acid groups to create cation exchange media. This media was applied to the purification of antibiotics (vancomycin and spiramycin). After separating crude products via column chromatography, the eluent was collected and analyzed by HPLC to determine product purity. The results demonstrated that vancomycin purity increased from 88.7% to 95.1%, while spiramycin purity reached 99.9%, repre-

senting a substantial improvement in purity.

Rapid membrane emulsification offers notable advantages in simplicity and efficiency, demonstrating exceptional potential for preparing uniform small-particle-size, high-agarose-content separation media. This versatile method is also applicable to the preparation of other drug-loaded nano/microparticles and shows promising prospects for broad application.

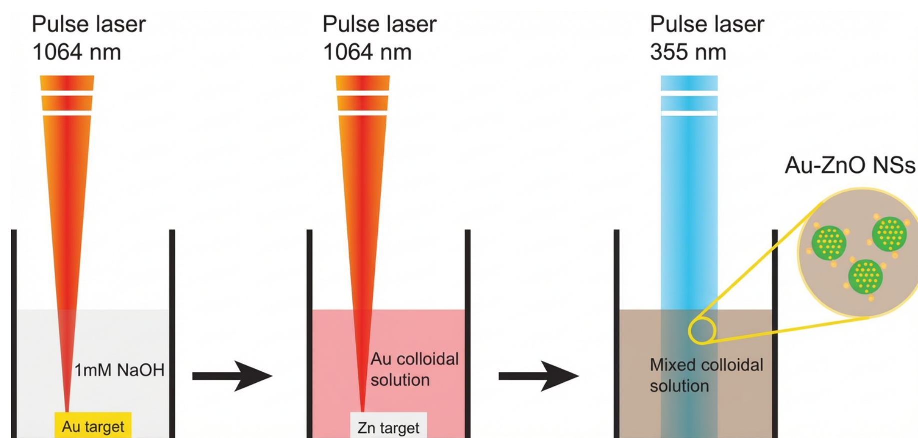

Figure 1: Figure 1

Agarose microspheres: (a) optical microscope image; (b) particle size distribution

Acknowledgments: This work was supported by the National Key R&D Program of China (No. 2016YFF0202304), the National Natural Science Foundation of China (No. 21306206, 21476241), the National Major Scientific Instrument Development Project (No. 2013YQ14040502), and the Beijing Natural Science Foundation (No. 2172054).

Author Biography: Zhu Kai (1990-), male, from Huainan City, Anhui Province, master' s degree candidate, works at the Institute of Process Engineering, Chinese Academy of Sciences as an assistant engineer. Research direction: separation media and purification. Email: zhukai@ipe.ac.cn

Source: ChinaXiv —Machine translation. Verify with original.