THE OPTIMUM CONDITION TO PREPARE AGAR NANO-PARTICLES BY EMULSIFICATION METHOD

Kwang-Hee Lim, Eun Ju Lee*, Tae-Jong Lee** and Saeed Ahmad Khan
Dept. of Chem. Eng., Daegu University, Gyungsan, Gyungbuk, Korea, 712-714
*Dept. of Chem. Eng., Kyungpook National University, Daegu, Korea, 702-701
**GBRIA, Gyeongbuk Techno. Park, Gyungsan, Gyungbuk, Korea, 712-210

Introduction

During gelation of agar the Polysaccharide chains wrap together so tightly that water can be trapped inside the helix. As more and more helices are formed and become cross-linked, a three-dimensional network of water-containing helices is created. The entire structure has no net charge [1]. The reported method for the preparation of agar particles are mainly based on water in oil emulsion, which may be due to the inert and uncharged nature of agar [1]. For instance, Tam et al.[2], Martins et al.[3], and Manjunatha et al.[4] prepared agar beads by emulsifying agar aqueous solution in paraffin oil. To the best of our knowledge no study is available on the formation of agar particles as small as in the nanometer range. However agarose nanoparticles of 500nm have been reported to be formed by emulsification of agarose aqueous solution in corn oil and subsequent homogenization [5]. Since agar is considerably less expensive than agarose, our focus in this paper is to design a method of optimum condition for preparation of small agar particles, preferentially in the nanometer range.

Materials and method

Preparation of agar particles and analysis

0.5, 1, or 2 gram of agar powder (gel strength 600-800g/cm^2, lot No 011320, DC chemicals Korea) was dissolved in boiling de-ionized water, the final volume was made up to 100ml and the solution was stored at 60℃. The particles were prepared by emulsion method; 1ml of agar solution (50-60℃) was added drop wise to 10 ml of the soybean oil at 50-60℃ (100%, CJ corporation Korea) under continuous stirring. The resultant w/o emulsion was homogenized (OPE.AM-001E Ace homogenizer, Nihonseiki kaishal Ltd Japan) at 13000rpm, 15000rpm, or 17000rpm for 10 min as per case at 50-60℃ (temperature maintained by putting hot water in the vessel around the homogenization cup). Subsequently, 5ml of the chilled soybean oil (5℃) was added to the emulsion, the hot water in the vessel around the homogenization cup was replaced with ice and the system was again homogenized (13000rpm, 15000rpm, or 17000 rpm, as per case) for five minutes. The final suspension was centrifuged at 1300×g for 10min, the pellets obtained were redispersed and re-centrifuged two times in 5ml each of toluene, ethanol and water consecutively, and finally redispersed in 5ml of water and frozen for freeze drying. The yield of the dried mass was determined after freeze drying. In order to see the effect of emulsifier on the particle formation, Sorbitan sisqueoleate (Sigma, batch#096K0161) was added to the oil phase (i.e. 5%v/v). The agar concentration was kept constant at 2%w/v, and the homogenization speed was varied as 13000rpm, 15000rpm and 17000rpm. The size of prepared agar particles was measured with size distribution analyzer (Nanotrac U2304ES, Microtrac Inc.).

Results and discussion

Size distribution of agar nano-particles

The agar nanoparticles were prepared at varied operating conditions and their size distributions were analyzed to obtain their volumetric mean particle size with standard deviation in Table 1.

Table 1. Size distribution of agar nano-particles

formed at varied homogenizer speeds and agar concentrations.

r.p.m.	Agar 0.5%	Agar 1%	Agar 2%	Agar 2% (emulsifier 5%)
13000	169* (±121.1)	112 (±33.4)	131 (±33.7)	159 (±52.3)
15000	123.2 (±47.2)	110 (±32.3).	125 (±33.7)	113 (±43.8)
17000	129 (±41.8)	118 (±35.5)	112 (±34.3)	141 (±59.0)

*: Double peaks

Optimum condition to prepare agar nanoparticles

The results of yield at various agar concentrations and homogenizer speeds are shown in Table 2. In terms of homogenizer speed the yields of agar nanoparticles were optimized at 15000 rpm for all the agar concentrations of 0.5%, 1% , 2% and 2% with the emulsifier (5%v/v). Besides in terms of agar concentration the yields of agar nanoparticles were also optimized at 1% of agar concentration for all the homogenizer speed of 13000 rpm, 15000 rpm and 17000 rpm. Accordingly the yield of 76.5% was the highest at 1% of agar concentration and 15000 rpm of homogenized speed.

Table 2. Yield of agar nano-particles formed at varied homogenizer speeds and agar concentrations.

r.p.m.	Agar 0.5%	Agar 1%	Agar 2%	Agar 2% (emulsifier 5%)
13000	53 (±7.1)	60.5 (±3.5)	54 (±5.3)	51.5 (±3.5)
15000	60 (±5.6)	76.5 (±9.2).	60.2 (±1.1)	51.5 (±5.7)
17000	58 (±11.3)	73.5 (±4.9)	51.2 (±0.3)	46.3 (±6.7)

Surprisingly the optimum condition of 1% of agar concentration and 15000 rpm of homogenized speed was exactly correspond to that to prepare the smallest agar nano-particles of 110nm as shown in Table 1. The size distribution of agar nano-particles prepared under the optimum condition of 1% of agar concentration and 15000 rpm of homogenized speed is shown in Fig. 1.

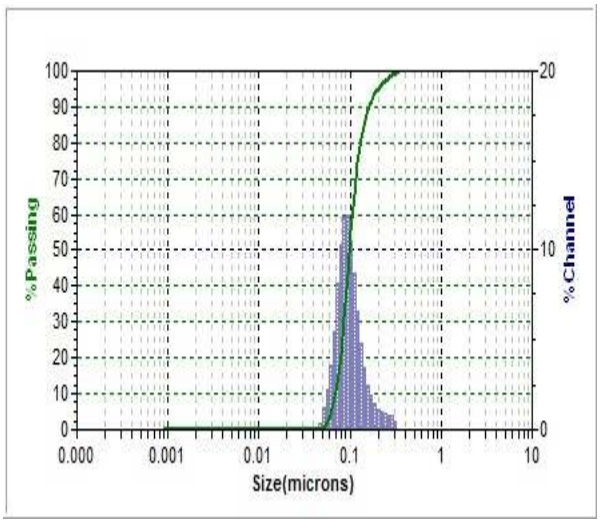

Fig. 1. Size distribution of agar nano-particles.

Conclusion

The yield of 76.5% was the highest at 1% of agar concentration and 15000 rpm of homogenized speed. Surprisingly the optimum condition of 1% of agar concentration and 15000 rpm of homogenized speed was exactly correspond to that to prepare the smallest agar nano-particles of 110nm.

References

1. Hashemi P, Rahmani Z, Kakanejadifard A, and Niknam E, Analytical Sci. 21(2005) 1297-1301.
2. Tam M, Snipes J, and Stevenson M, Am. J. Respir. Cell Mol. Biol. 20(1999) 710–719.
3. Martins dos Santos V A P, Leenen E J T M, Rippoll M M, der Sluis C V, van Vliet T, Tramper J, and Wijffels R H, Biotech. Bioeng. 56(5) (1997) 517-529.
4. Manjunatha K M, Ramana M V, and Satyanarayana D, Ind. J. Pharm. Sci, 69(3) (2007) 384-389.
5. Wang N and Wu XS, Pharm. Dev. Technol. 2(2) (1997) 135-42.