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# ON PREPARATION OF MAGNETIC MICROBEADS BY TWO MICROFLUIDIC EMULSIFICATION TECHNIQUES AND POLYMERIZATION

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The size distribution of magnetic polymer microsphere carriers critically impacts the space and time control in upstream and downstream biomaterials processing. We have adopted two methods for producing microspheres of precisely controlled and/or monodisperse size distributions. First method is based on a capillary flow focusing emulsification technique and the second on a membrane emulsification technique. We used these methods to fabricate Ca–alginate microcapsules with various percentages of alginate-stabilized ferrofluid incorporated in the core. We obtained very uniform microspheres with average diameters from ~10 to > 400 μm in diameter in which ≥ 95% of them were within 1.5-2.0 μm of the average. Furthermore, by varying the experimental parameters, we obtained microsphere populations with predefined size distributions. In summary, our methods provide high control of magnetic microbeads size and may allow development of advanced controlled in space and time carrier systems.

## INTRODUCTION

Magnetic microbeads represent an increasingly important class of supports devices that provide enhanced control of biological entities. Comparatively with nonmagnetic polymer carriers, magnetic microbeads offer still an additional advantage: having embedded magnetic entities, they can be magnetically manipulated using permanent magnets or electromagnets. Magnetic microbeads realized from natural polymers have advantages of being used in very interesting areas such as biomedicine and biotechnology, because of their properties like very good biocompatibility, biodegradability and the feasibility to incorporate drugs/enzymes into their matrices. Amongst the natural polymers, alginate has been used for these purposes by many workers.[1,2] Alginate, commercially available as alginic acid sodium salt is a polysaccharide normally isolated from many strains of marine brown seaweed and algae. It is a copolymer consists of two uronic acids: D-mannuronic acid (M) and L-guluronic acid (G). With one carboxylate functional group in each M or G unit, alginate is highly negatively charged polyelectrolyte at neutral or basic pH, so the alginate can suffer a sol-gel transformation by cross-linking reaction with divalent cations such as $Ca^{2+}$. The gelation phenomenon can be explained by the fact that one divalent cation binds to two carboxyl groups on adjacent alginate molecules.

Calcium alginate beads with incorporated magnetic materials represent promising materials for various applications, being already used for affinity separation of enzymes.[3, 4] Magnetically modified beads easily manipulated with an external magnetic field can be used when working with difficult to handle samples, such as biological crude broths, which are often viscous suspensions. There are some techniques for obtaining alginate magnetic beads, each with its advantages and limitations; amongst the limitations are high dimensions and large size distribution of the particles.

We have adopted two methods for producing magnetic alginate microspheres of precisely controlled and/or monodisperse size distributions. First method is based on dripping microjets in a flow focusing system. The second method is based on a membrane emulsification process. The two methods are coupled with internal gelation of resulted microdrops.

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## MATERIALS AND METHODS

### 1. Materials

Sodium alginate (medium viscosity), calcium chloride dehydrate and isooctane were from Aldrich, Germany. Span 85, Tween 85, $FeCl_2$ hexahydrate and $FeCl_3$ tetrahydrate were purchased from Merck, Germany. The sodium hydroxide pure, NaCl and glacial acetic acid were procured from Fluka, Germany. The microporous glass membrane with average pore of 2.9 μm was purchased from Utsu Co. Ltd., Japan.

### 2. Methods

The nanoparticles of magnetite were prepared by co-precipitation in aqueous media of the iron (II) and iron (III) salts with a concentrated NaOH solution, at a temperature of $70^0$ C with vigorous mixing for 30 minutes. The magnetic precipitate was cooled at room temperature, washed five times with distillate water. 100 mL of 0.9 % w/v NaCl solution containing 1 % w/v sodium alginate and 0.2 % w/w magnetic material were mechanically mixed and deaerated. Then, a solution 0.25M $CaCO_3$ was added. The mixture was purified by centrifugation to remove insoluble residues and excess alginate. The supernatant was ultrasonated and filtered through a 0.25 μm Santorius Polycarbonate Membrane Filter, obtaining a ferrofluid with a destabilization time of more than 30 hours.

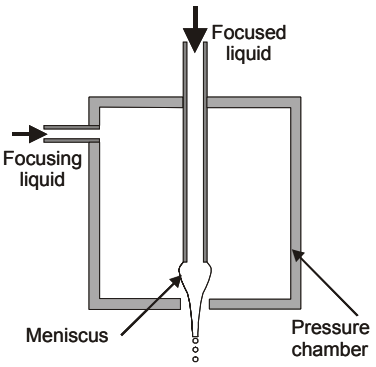


Fig. 1 – The dripping by flow focussing.

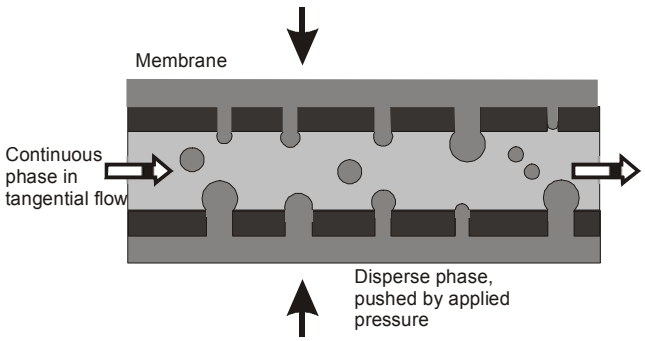


Fig. 2 – The membrane emulsification process.

The obtained ferrofluid was used as disperse phase in the two microfluidic systems, the continuous phase being isooctane (75 g) mixed with 2.8 g of Span 85. Flow-focusing consists on an atomization technique which relies on hydrodynamic forces to produce microjets.[5, 6] In this method, a flow rate Q is injected through a capillary needle. This needle is placed inside a pressure chamber as sketched in Fig. 1. The liquid disperse phase is injected through the needle, giving rise to a meniscus at the extremity of the needle. An orifice in the chamber wall facing the tip of the needle opens the camber to the outer ambient. The continuous phase is forced to escape the camber through the orifice by means of an extra pressure in the camber. When this reaches a certain value, the surface tension stress at the meniscus are overcome, so that it is pulled into a cusp-like shape from whose vertex a very slender micro-jet issues with diameter smaller than inner diameter of the needle. Once the micro-jet exits the orifice, the pressure gradient (the main axially accelerating force) vanishes and the jet evolves under the influence of the viscous shear stress produced by the continuous phase stream and the capillary stresses. Perturbations of the capillary jet grow downstream until the jet breaks up. Our experimental data were obtained with an atomizer for which the inner diameter of the needle was 60 μm, the diameter of the orifice in the wall chamber was 500 μm and the thickness of the wall chamber was 150 μm. Most experimental data were obtained varying the continuous phase flow rate between 0.67 and 1.67 mL/min while maintaining the disperse phase flow rate of 0.033 mL/min. In the membrane emulsification method, the aqueous sodium alginate solution containing magnetic material is passed under pressure through a porous membrane and the formed drops are dispersed in the immiscible continuum phase (Fig. 2).[7,8] The formed water-in-oil emulsions were treated with glacial acetic acid for releasing $Ca^{2+}$. After half an hour, the particles were separated by gravity in a 0.2 M $CaCl_2$

solution, and then the beds are rinsed with 1.5 % Tween 85 solution, distillate water and stored in distillate water.

## RESULTS AND DISCUSSION

The size distribution of magnetite nanoparticles in aqueous ferrofluid was obtained by dynamic light scattering, DLS (Nanotrac, Microtrac Inc., USA). The analysis shows a log-normal distribution (Fig. 3), with particles having sizes < 33 nm and an average diameter of $x_c$ = 15.6 nm. The volume magnetic susceptibility and the magnetization of ferrofluid were obtained using a home made Gouy Susceptibility Balance (Fig. 4). The ferrofluid behave as a superparamagnetic material, this character being important for biotechnological and biomedical applications were a remanent magnetization is undesirable. For such applications, the particles must rapidly relax their magnetic moment vectors to random directions when the applied magnetic field is removed.

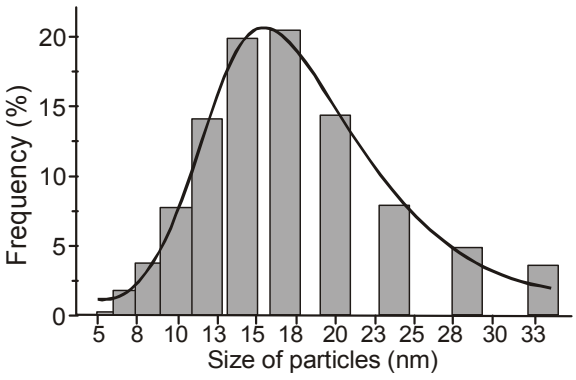


Fig. 3 – The size distribution of magnetite in the aqueous ferrofluid.

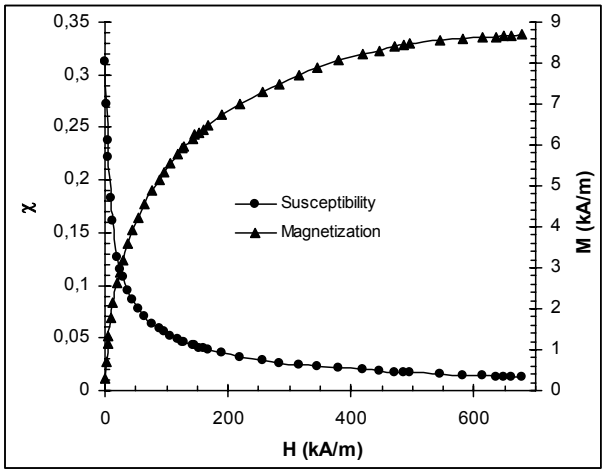


Fig. 4 – The magnetic properties of the aqueous ferrofluid.

In the flow-focusing apparatus the micro-jet, and therefore micro-droplet, diameters are determined by the size of the alginate ferrofluid needle orifice and the relative flow rates of the ferrofluid and the exterior stream. We have obtained by this method uniform microspheres from 900 to 30 μm in diameter, as well as more polydisperse spheres as small as ~ 1 μm from a 60 μm needle. The flow-focusing method allows production of microspheres as small as 1% of the needle inner diameter and reduced the formation of satellite droplets and other undesired spheres; the size distributions are very clean with no very large or very small spheres (Fig. 5). However, the microspheres polydispersity increased when producing microspheres less than ~ 15 μm. Distributions were 3-4 μm wide when fabricating the smallest (~ 1 μm) microspheres.

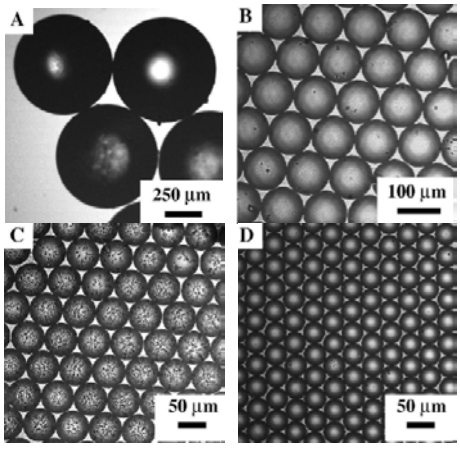


Fig. 5 – Light micrographs of magnetic calcium alginate microspheres produced by flow focusing emulsification from a 100 μm orifice decreasing the carrier stream flow rate from 1.67 to 0.67 mL/min. Sizes are approximately (A) 875 μm, (B) 95 μm, (C) 70 μm, and (D) 45 μm.

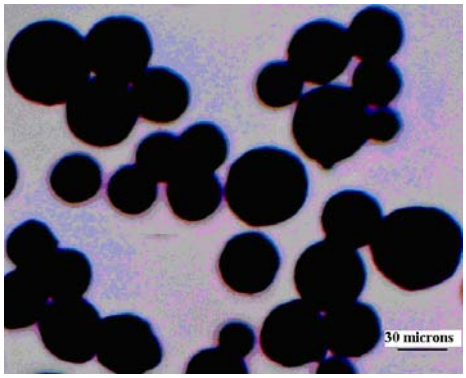


Fig. 6 – Optical microscopy images for magnetic calcium alginate microspheres obtained by membrane emulsification.

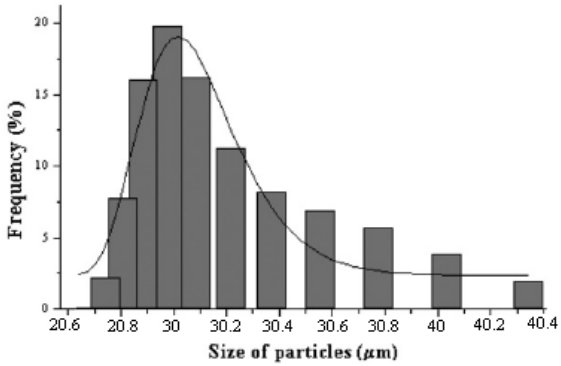


Fig. 7 – The size distribution of magnetic calcium alginate microspheres obtained by membrane emulsification.

In the membrane emulsification system, the best results were obtained with a trans-membrane pressure of 0.4 x $10^5$ Pa. In these conditions, it could be obtained a small size and a narrow size distribution of the magnetic alginate particles. The diameter and the size distribution of the obtained microspheres were determined by optical microscopy (Olympus, BX-RLA 2) (Fig.6) and dynamic light scattering (Fig.7). The optical microscopy and DLS measurements indicated that the medium size of the prepared magnetic calcium alginate particles was ~ 30 μm.

## CONCLUSIONS

Spherical magnetic Ca-alginate beads with small and uniform size were successfully prepared with flow focusing and membrane emulsification coupled with internal gelation technology. By adjusting process parameters, we could control the size and size distribution of emulsion droplets produced by the two microfluidic methods much narrower than comparable systems emulsified by stirring the component phases.

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