Production of crosslinked protein particles through membrane emulsification

L KOTZÉ-JACOBS, T MAHLANGU, N MOKONE
CSIR Polymers and Composites, PO Box 395, Pretoria, South Africa, 0001
Email: lkotze@csir.co.za – www.csir.co.za

INTRODUCTION

In recent years, the drive towards **cleaner technologies** has made us more aware of the need for green and sustainable methodologies for **chemical synthesis**. It is here that enzymes are beneficial due to mild reaction conditions, biodegradability and using mainly water as a solvent, which makes enzymes catalysis more environmentally friendly than organic synthesis routes[1,2,3]. Additionally, enzymes provide high activities and can be very substrate-specific leading to better yields. Enzymes applications in industry include the food and detergent industry, biomedical applications and the paper processing industry[4].

NEED

The current Spherezymes™ manufacturing process is through a stirred beaker emulsion reaction. Disadvantages include:

- wide particle size distribution resulting in a **polydisperse** product;
- **shear** is imparted from the stirrer tip to the reaction fluid reducing enzyme activity; and
- difficult **scale-up**.

Membrane emulsification has been identified as a production method that potentially addresses the disadvantages of stirred reactors for our application.

AIM

The purpose of this study was to investigate membrane emulsification as a scale-up method for crosslinked bovine serum albumin (BSA) particles. Factors investigated were crosslinker and surfactant concentration.

ENZYME IMMOBILISATION

Biocatalytic process economics can be positively influenced through the **recycling** and **reuse** of enzymes as well as the stability improvement obtained through enzyme immobilisation[5]. Immobilisation affords many advantages as opposed to the use of free enzymes for example, enhanced activity, specificity and selectivity, and reduction of enzyme inhibition[6]. In the past, these poor characteristics of free enzymes have hampered their efficient use in biocatalysis. Existing enzyme immobilisation techniques include attaching the enzymes to support structures as well as crosslinking of enzymes and entrapment.

SPHEREZYMES™

A CSIR patented technology called Spherezymes™ is an example of a crosslinked immobilisation technology. It has been developed to produce crosslinked, spherical protein particles (**Figure 1**) through an emulsion process that is more easily recoverable than free enzymes and show enhanced activity. The enzymes can be recycled at least nine times with an insignificant associated loss of activity.

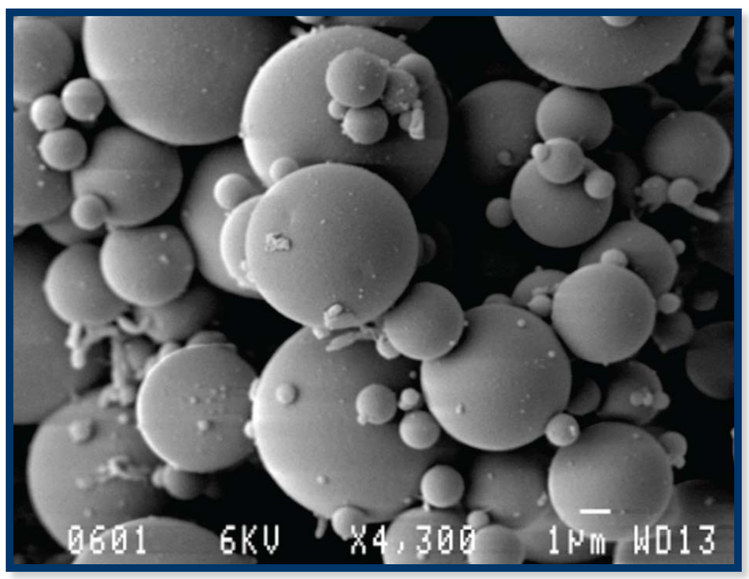


Figure 1: Scanning Electron Microscope (SEM) image of 20% lipase / 80% albumin Spherezymes™ manufactured through magnetic stirring

MEMBRANE EMULSION

Membrane emulsification is a technique that employs the use of a membrane to make emulsion droplets. There are two phases involved namely, the dispersed phase and the continuous phase. The dispersed phase permeates through the membrane at a low pressure into the continuous phase. The membrane has a uniform-pore size, therefore allowing uniform droplet size distribution. The method can be applied to both oil-in-water and water-in-oil emulsions[7,8] and is a suitable method for creating a protein emulsion due to the low shear of the process.

EXPERIMENTAL DESIGN AND SETUP

Initial studies showed that particles produced through membrane emulsion were much larger than the expected size of 2-3 times the mesh size (20 μm) of the membrane (i.e. much larger than 40-60 μm). Crosslinker and surfactant concentration were identified as two possible parameters that influence particle size (through inter-particle crosslinking and agglomeration, respectively) and an initial study was carried out to determine their effects (**Table 1**).The process is described in Diagram 1 with an image of the actual experimental set-up (**Figure 2**).

Table 1: Experimental design

Exp. No.	Crosslinker Volume (ml)	Surfactant Volume (ml)
1	1	1
2	1.5	1
3	1.25	1.5
4	1.5	1.5
5	2	2
6	1.25	2

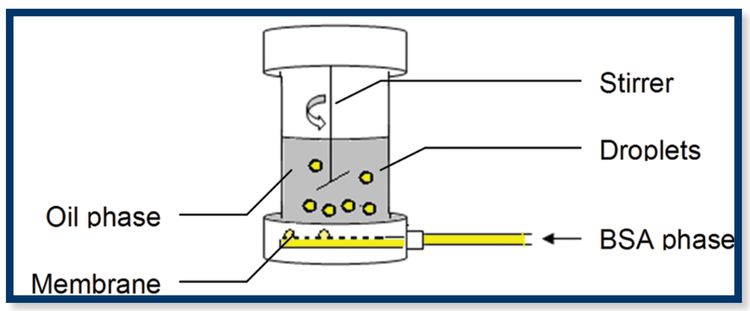


Diagram 1: schematic of membrane emulsification process

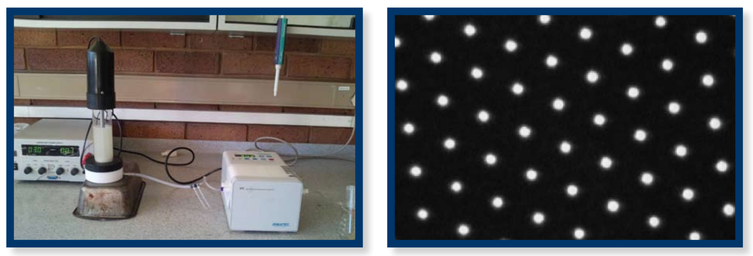


Figure 2: Experimental setup – membrane emulsification device and teflon membrane (pores from 2μm in diameter)

RESULTS AND DISCUSSION

An analyis of the experimental results in (**Table 2**) indicates that the expected particle size of between 40 μm and 60 μm is obtained with a crosslinker and surfactant volume of between 1 ml and 1.5 ml. An increase in crosslinker concentration from 1 ml to 2 ml resulted in an average particle size that ranged between 49.5 μm and 139.5 μm, while a surfactant increase from 1 ml to 1.5 ml resulted in a minimum particle size of 49.5 μm. Increasing crosslinker and surfactant concentrations also decreased the polydispersity index (PDI) which is indicative of a narrower size distribution. However, values above 0.1 are not deemed monodispersed.

Several factors may be responsible for particles that are larger than expected. One of these factors may be the method of external crosslinking where the crosslinker is present in the continuous or external phase causing crosslinking of individual particles into clumps that appear larger than the individual particle average size when analysed. Another factor may be that the individual particles attract each other when the emulsion is not stable enough. It would seem that the addition of surfactant in larger volumes reduces the particle average size and that low surfactant concentration is probably the more likely reason for larger average particle sizes. Interestingly, the particle size distributions obtained in the experiments were not monodispersed. This was unexpected as one of the major benefits of membrane emulsion is droplet monodispersity.

Table 2: Experimental results

Crosslinker Volume (ml)	Surfactant Volume (ml)	Average Particle size (μl)	St dev	Polydispersity index (PDI)	Comments
1	1	72	32.2	12.6	Base case or standard conditions
1.5	1	139.5	21.9	7.2	Polydispersity decreased*, perceived mass yield increased
1.25	1.5	49.5	9.2	5.8	Polydispersity decreased*, perceived mass yield increased
1.5	1.5	60	8.5	3.4	Polydispersity decreased*, perceived mass yield increased
2	2	101	**	4.4	Polydispersity decreased*, perceived mass yield increased
1.25	2	76	**	3.7	Polydispersity decreased*, perceived mass yield increased

*As opposed to the standard or base case
**Only one experiment conducted

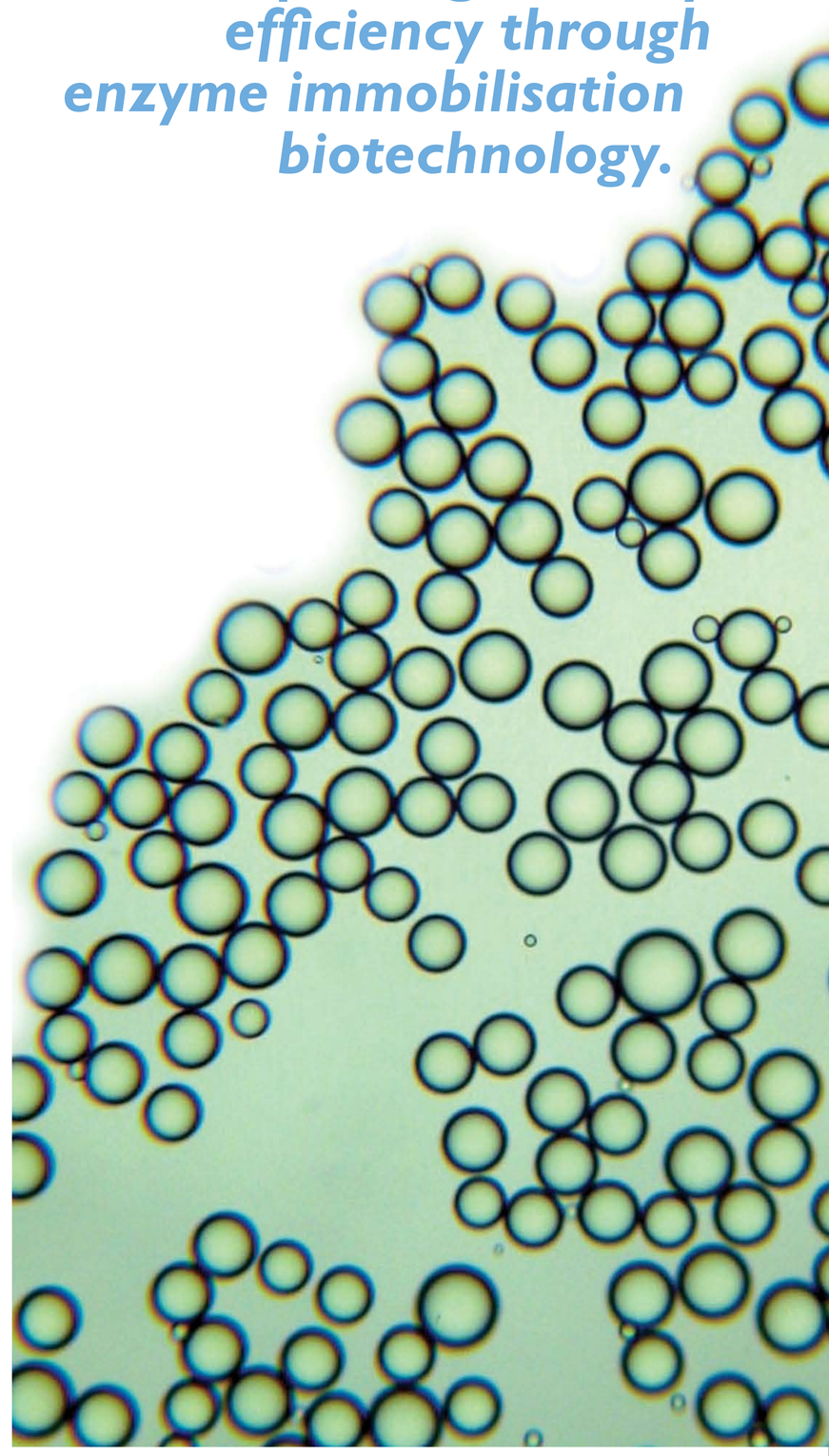
CONCLUSION

Through membrane emulsification, we were able to produce crosslinked protein particles. Although the experiments did shed some light on the effect of variations in crosslinker and surfactant volumes on average particle size and particle size distribution, more experiments are needed to obtain a method of producing particles of the correct size range and monodispersity.

FUTURE WORK

Planned future experiments include a statistically-designed trial in which parameters such as stirring rate, dispersed phase flow rate, protein concentration and membrane mesh size will be investigated as well as the interaction of these parameters. Subsequently, experiments will be repeated with lipase to determine the efficiency of the process in making enzyme particles that retain an enhanced activity.

Improving industry efficiency through enzyme immobilisation biotechnology.



Monodispersed oil droplets in water formed through membrane emulsion

ACKNOWLEDGEMENTS

The authors would like to thank the CSIR for the funding for this study and also their colleagues in Polymers and Composites for their valuable contributions.

REFERENCES

1. Alcalde, M., Ferrer, M., Plou, F.J., Ballesteros, A. 2006. Environmental biocatalysis: from remediation with enzymes to novel green processes. *Trends Biotechnol*. 24: 281-7.
2. Brady, D., Jordaan, J., Simpson, C., Chetty, A., Arumugam, C. and Moolman, F. 2008. SpherezymesTM: A novel structured self-immobilisation enzyme technology. *BMC Biotechnol*. 8(1):8.
3. Sheldon, R.A. 2007. Enzyme Immobilization: The Quest for Optimum Performance. Adv. *Synth. Catal*. 349:1289-1307.
4. Foose, L.L., Blanch, H.W., Radke, C.J., 2007. Immobilized protein films for assessing surface proteolysis kinetics. J. *Biotechnol*. 132(1): 32-7.
5. Brady, D and Jordaan, J. 2009. Advances in enzyme immobilisation. *Biotechnol Lett*. 31(11):1639-50.
6. Mateo, C., Palomo, J.M., Fernandez-Lorente, G., Guisan, J.M. and Fernandez-Lafuente, R. 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzym Microb Tech*. 40:1451-63.
7. Gijsbertsen-Abrahamse, A. 2003. *Membrane Emulsification: Process Principles*. Wagenigen University. Thesis.
8. Joscelyne, S.M. and Trägårdh, G. 2000. Membrane emulsification – a literature review. *J. Membr Sci*. 169(1): 107-17.