Determination of the molecular weight cut off of microcapsules with a fluorescent labelled dextran

Demont A[a, b], Cole H.[a], Bones J.[b], Marison I[* a].
[a]School of Biotechnology, DCU, Glasnevin, Dublin 9, Ireland (aurelie.demont2@mail.dcu.ie)

INTRODUCTION AND OBJECTIVE

Bioencapsulation has potential application in many fields, such as biotechnology, pharmacy, food, cosmetics and medicine. Depending on the field of application, the permeability of the capsule membrane is extremely important. In the case of bioartificial organs, the proteins synthesized by the cells will have to be able to leave the capsules while the antibodies and other components of the immune system should not enter the capsules. In bioprocessing, however, allowing the produced molecules to stay in the capsules or to go out of the capsules will make the downstream processing easier.

In the literature, different methods are described to measure the permeability of the membrane.

Protein standard The spectrophotometric measurement of the diffusion of a known protein such as vitamin B12 (Lewiska 2002) is a simple way to obtain a study of the diffusion properties of the capsule membrane. To measure the MWCO, standards of different defined proteins may also be used.

Dextran or pullulan standards Dextran or pullulan standards of different known molecular weights may be mixed with microcapsules. Small molecular mass molecules are able to permeate through the capsule membrane and their concentration in the supernatant decreases, while molecules with a molecular mass higher than the cut-off are not able to diffuse through the membrane. After a pre-determined amount of time, samples of the supernatent are removed and analysed by size exclusion chromatography (Rosinski 2002) (Rossinski 2005). It is important to consider that dextrans and pullulans are polysaccharides and that they interact differently with the capsule membrane compared with proteins. However, the type of molecule is less important than the diffusional Stoke's radius (size of particle in solution) to allow or exclude the diffusion of molecules in or out of the capsules. (Wang 1997)

The protein method and the Dextran method seem to be complimentary. None of the methods, however, allow a quick and simple visualization of the ingress of molecules into the capsules. The developed method using a labeled dextran will allow a quick and simple visualization of the ingress of molecules into the capsules by microscopy and a rapid quantification by spectrophotometry.

MATERIALS AND METHODS

Method development
The spectrum of absorbance of FITC was measured with a 0.1% FITC Dextran 70kDa solution labelled with 0.003-0.020 mol FITC per mol glucose (Sigma) to determine if an absorbance can be measured and at which wavelength. In order to determine the volume of solution in each well, 3 standard curves from 0.0% to 1.0% were prepared and the absorbance was measured with 100µl, 200µl and 300µl of solution in each well. Following these first steps, 3 standards curves from 0.0% to 1.0% FITC-Dextran were prepared for each molecular weight of labelled dextran in order to determined the Limit of Detection, the Limit of Quantification and the linear range of the method. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as followed :

$$LOD = \frac{3.3 \cdot \sigma_{intercept}}{\bar{s}} \qquad LOQ = \frac{10 \cdot \sigma_{intercept}}{\bar{s}}$$

with $\sigma_{intercept}$ the standard deviation of the intercepts (y) and $\bar{s}$ the average of the slopes. The linearity was first evaluated by visual inspection of the plot of the signal. The linear range was then estimated and the linear relationship was confirmed using two statistical methods. A regression line was determined by the method of the least squares and the coefficient of determination r^2 was calculated. The linearity was also investigated with a F-test

Capsules preparation
A 1.5% Na-alginate solution in 10mM MOPS (Sigma) and 0.85% NaCl (Fluka) buffer was extruded through a 300µm diameter nozzle into a gelling bath of 100mM $CaCl_2$ and 10mM MOPS with an Encapsulator Biotech (Inotech, Switzerland). The microcapsules were then washed with a 10mM MOPS, 0.85% NaCl solution and coated with poly-L-lysine hydrobromide mw 30,000-70,000 in a 0.1% PLL, 100mM $CaCl_2$ and 10mM MOPS solution for 30min. The solution of PLL was then removed and the capsules coated in a second 0.05% PLL, 100mM $CaCl_2$ and 10mM (Sigma) MOPS solution for 30min. After coating, the beads were washed with 10 mM MOPS and 0.85% NaCl buffer, and coated with a second layer of Na-alginate in a 0.03% (w/v) Na-alginate, 10mM MOPS, 0.5% NaCl for 10min. The beads were washed in MOPS-NaCl buffer and the inner core solubilized by addition of a 50mM Na-citrate solution for 20min. The capsules were washed once again with MOPS-NaCl buffer and transferred to MOPS-NaCl buffer for storage or placed

in culture media.

Molecular weight cut off measurement

300µl of a solution of 0.1% FITC-labelled-dextran of mw 70kDa, 150kDa, 250kDa and 500kDa (Sigma) was mixed with 300µl of capsules for 2hours. 200µl of the solution was then transferred in a 96 well plate and the absorbance was measured with a VersaMax plate reader. To confirm the presence or absence of dextran in the capsules, a picture was also taken by confocal microscopy

RESULTS AND DISCUSSION

The spectrum of absorbance of FITC was measured with a 0.1% FITC Dextran 70kDa solution labelled with 0.003-0.020 mol FITC per mol glucose to determine if an absorbance can be measured and at which wavelength.

According to the spectrum, FITC-dextran has an absorbance between 400nm and 500nm, with a maximum between 440nm and 490nm. The chosen absorbance for the standard curves and the analysis will then be 450nm.

In order to determine the volume of solution in each well, 3 standard curves from 0.0% to 1.0% were prepared and the absorbance was measured with 100µl, 200µl and 300µl of solution in each well. Both 200µl and 300µl of solution had a good linearity between 0.5% and 0.005% of FITC dextran, while 100µl was slightly less linear. In order to use an amount of capsules as low as possible, the chosen volume of solution will be 200µl.

The linear range, the limit of detection (LOD) and the limit of quantification (LOQ) were determined for each molecular weight of FITC dextran. By observation of the curves the linear range was determined between 0.005% and 0.1% of FITC-dextran for each molecular weight of dextran. A regression line was determined by the method of the least squares and the coefficient of determination r^2 was calculated for each sample of each molecular weight dextran. The linearity was also investigated with a F-test and gave a cumulative probability P of 99.9% linearity.

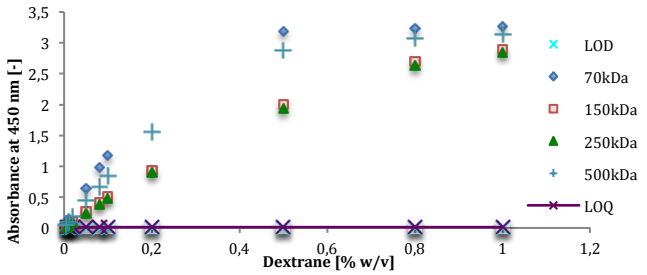

Figure 1 : Standard curves with different molecular weight FITC-Dextran, LOD and LOQ.

The limit of detection LOD and the limit of quantification LOQ were calculated in the previously determined linear range, to be between 0.005% and 0.1% of FITC-dextran. The LOD and the LOQ were calculated and the lowest concentration of FITC-dextran that could be measured in this method was 0.005% of FITC-dextran.

In order to test the method, the molecular weight cut off of alginate-poly-l-lysine-alginate microcapsules was determined. 300µl of microcapsules were added to 300µl of FITC-dextran, the solution was agitated and 200µl of liquid was then transferred to a 96 well plate. The measured absorbance was compared to a standard curve previously made in order to determine the presence or absence of dextran in the capsules. Confocal pictures were taken to confirm the spectrophotometer result.

CONCLUSION

The developed method using a labeled dextran allows a quick and simple visualization of the ingress of molecules into the capsules by microscopy and a rapid quantification by spectrophotometry. This method enables a fast screening of the molecule ingress, a simple comparison between different materials and a quantification of the dextran in the capsules.

REFERENCES

- D. Lewiska, S. Rosiskia, D. Hunkeler, D. Poncelet, and A. Weryski. *Mass transfer coefficient in characterization of gel beads and microcapsules*. Journal of Membrane Science, 209:533–540, 2002
- S. Rosinski, D. Lewinska, M. Wojcik, G. Orive, J. L. Pedraz, and A. Werynski. Mass transfer characteristics of poly-lysine, poly-ornithine and poly-methylene-co- guanidine membrane coated alginate microcapsules, 2005.
- S. Rosinski, G. Grigorescu, et al. Characterization of microcapsules: recommended methods based on round-robin testing. *Journal of Microencapsulation,* 19(5):641–659, 2002.
- T. Wang, I. Lacik, M. Brissova, A.V. Anilkumar, A. Prokop, D Hunkeler, R. Green, K. Shahrokhi, and A.C. Powers. *An encapsulation system for the immunoisolation of pancreatic islets*. Nature Biotechnology, 15:358–362, 1997

[b] Fosters Avenue, Mount Merrion,, Blackrock, Co. Dublin, Ireland.