Does size matter? The bioencapsulation adventure from milli to micro to nano, served dry or on the rocks.

Ronald J. Neufeld
Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada, K7L 3N6
ron.neufeld@chee.queensu.ca

Introduction

The Bioencapsulation Research Group was first formed by Denis Poncelet in 1990, and the first workshop was held in Montreal in 1991. This year in Dublin, we are participating in the 16th annual workshop.

My first memory of being aware of bioencapsulation work was at the International Fermentation Symposium in 1980, where I recall being intrigued by the ability of researchers to immobilize active cells and enzymes in the form of gel beads. My earliest paper in bioencapsulation was published in 1983.

In the 1980's, we formed beads, and they were good... wet, round, shiny and big. We didn't need a microscope to see the beads, and new bioreactors appeared (eg. New Brunswick Scientific) with multiple needles, designed to aseptically extrude polymer droplets (typically alginate) into a gelling bath (typically $CaCl_2$). The 1990's brought a trend toward larger scale formulation methods such as emulsion polymerization, and toward smaller diameters (microspheres). I recall the excitement of formulating 30, then 20, then 10 μm diameter cell-loaded gellan microspheres, with controlled diameter and size distribution. Dry but bioactive spherical granules followed with the new millennium, but this decade is increasingly asking that we go nano, and that we apply multiple coatings.

We now formulate a nanoplex (nanoparticulate complex) with up to 5 different polymers, plus the bioactive material. Others have taught the importance of considering other shapes (lenticats, rods, filaments, slabs, films), and others yet have taught alternative and novel formulation methodologies (microfluidics, JetCutter, electrostatics, vibration, coaxial extrusion), we have seen the emergence of alternative or modified polymers (chemoenzymatically engineered alginates) and increasingly see the need for extreme imaging (AFM, confocal laser scanning).

What will the next decade ask of us?

One person's path from milli to micro to nano (beads r us)

Early on, I was fortunate to have as colleague, Thomas Chang, Director of the Artificial Cells and Organs Research Centre at McGill University. Collaboration resulted in the formulation of solvent based collodion and subsequently nylon membrane microcapsules. There were challenges. One is that the largest batch produced to that point was 3 mL, with no control over capsule size or size distribution. The second was that the solvents, reagents and reactive formulation conditions were toxic to cells, and damaging to enzymes.

A rising star within the immobilization community... a chemist by the name of Dr. Denis Poncelet, assisted by a young, dynamic, engaging and determined chemist by the name of Brigitte Poncelet De Smet brought engineering and chemical skills to the team. Our mission was to formulate beads and capules on a large scale, with controlled diameter and size distribution, while maintaining high activity of the bioactive. These goals required several changes to the way that capsules and beads were formulated:

- abandoned the use of solvents other than water, or polymers which required highly reactive conditions;

- designed reactors with controlled shear to preselect particle/bead size and control size distribution;

- while the desire to formulate monodisperse beads/capsules on large scale became an exercise in futility, significant improvements were possible and achieved;

- avoided single droplet extrusion systems, moving toward emulsion/dispersion technologies;

- embraced biopolymers such as alginate as a desirable matrix, but had to devise method to trigger the gelation of the dispersed beads/spheres by instantaneous and *in situ* release of soluble calcium;

- dealt with undesirable release of active from "leaky" gel by applying coating materials (poly-L-lysine, chitosan, co-guanidine) or using compressed gels.

Other events had an impact on the ability to advance our work. Through the BRG, Todd Becker from Genencor taught industrial priorities, such as the need for dry particles containing stable active (Figure 1), the need for triggered release of active, the desire to reduce cost and reduce processing steps, the desire to reduce/eliminate/recyle extraneous reactants or materials so as to minimize environmental impact and/or need for remediation, and finally the importance of thinking about the appearance of the granules from a consumer standpoint, such as color and visual appearance.

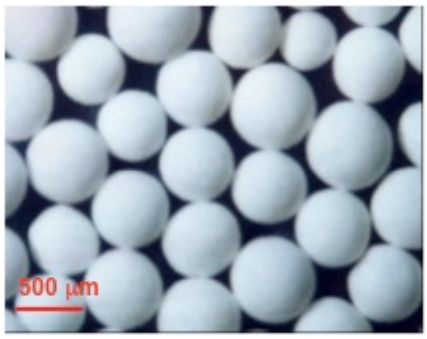

Figure 1: Subtilisin granules of alginate plus excipients

Another turning point in the last several years was an approach by Portuguese colleagues, to extend our microcapsule/microsphere/microparticle formulation methodologies, into the nano-range. To date, we have developed three formulation methodologies (Table 1), typically using alginate as a core material, forming nanoparticles containing a therapeutic peptide (see Figure 2).

Nanospray drying has the advantage of producing dry by active particles, but is limited by the ability to apply polymer coatings. Dispersion and ionotropic pre-gelation methodologies allow particle suspension based coating methodologies, but the washing and handling of nanoparticles can be time consuming and thus problematic. The formulations tested thus far on diabetic rats are summarized in table 2. The most effective formulation are particles entrapping insulin in an alginate-dextran core, coated with chitosan-polyethylene glycol, then albumin. The albumin coat provides a sacrificial target to gut proteases, the chitosan and alginate are mucoadhesive polymers and chitosan is known to transiently open tight junctions facilitating nanoparticle translocation, and the dextran and PEG help to stabilize insulin within the nanoparticle. Pharmacological availablity levels approaching 50% of a comparable injected dose of free insulin have been demonstrated.

Nanoemulsion dispersion/triggered *in situ* polymer gelation

Ionotropic pre-gel/polyelectrolyte complex coating

Nanospray drying

Table 1 :Methods developed to formulate insulin nanoparticles for oral delivery

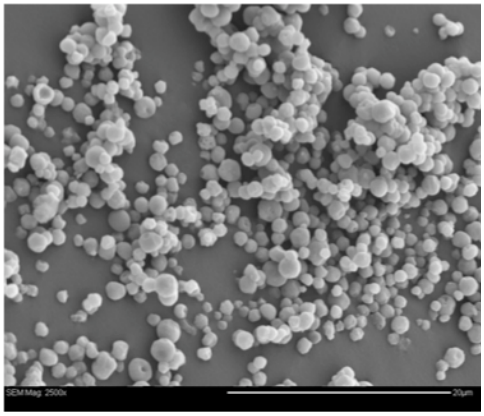

Figure 2: SEM of insulin nanoplex

Key questions on my mind as we move forward

The challenges in bioencapsulation research and development, toward application include:

- can you formulate the particles on large scale?
- is the active, still active, and how active is it?
- what control do you have over mean size and size distribution?
- can you reduce the number of processing steps to one or fewer?
- do you require solvents, or reagents that must be treated before disposing?
- is the active fully retained, or do you need to control or trigger release?
- how will you reduce the cost of formulation?
- what are the steps toward process and materials validation?
- is the polymer biodegradable? and how biodegradable is it?
- do your particles or beads swell/contract and under what conditions?
- are you able to modify matrix polymer properties?
- how permeable/strong are your capsules?
- what is the chemistry/purity of the polymer
- are there toxic break down products of the polymer?
- what formulation excipients are available for your particles?

CORE	COAT
alginate	uncoated
alginate	chitosan-pectin
alginate	chitosan-casein
alginate	chitosan-albumin
alginate-dextran	chitosan-PEG/albumin
dextran	chitosan

Table 2: Nanoparticulate formulations tested on diabetic rats. In all cases, insulin is contained within the particle core.

Conclusions

While it has been therapeutic (for me at least) to reflect on where we have come from as a community, it is also important to look ahead as to where we might or should be going. I have often wondered where we might have been as a research community, had it not been for the BRG and for the tireless and effective leadership of its President. I believe that as a well established BRG, we owe Denis Poncelet a huge vote of thanks and a hearty pat of the back, for guiding us as a community, for promoting and planning our annual workshops, for raising funds to support our network and for tirelessly promoting our field of bioencapsulation. I believe that he has put our field of research on the international map. Thank you Denis.

References

Dr. Denis Poncelet, President, Bioencapsulation Research Group, 1990 to 2008 and beyond (Fig. 3).

Figure 3: Denis Poncelet with girlfriends