BEADS AND CAPSULES AS A TOOL FOR DRUG DELIVERY AND BIOPROCESS OPTIMIZATION

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Introduction

The encapsulation of human, animal and plant cells, microbes, enzymes and drugs or other ingredients into microbeads have different reasons: the encapsulated item must be protected by environmental influences, e.g. immune system of a patient, or it has to resist share forces in a bioreactor or must be protected against oxidation. Following these demands, the encapsulation technology has its use in many different applications. A selection of known applications is presented below (Serp, 2000):

- Cell transplantation for the treatment of cancer, diabetes or liver diseases
- Test systems for screening experiments for new drugs
- Starter cultures within the food production
- Production of pharmaceutical drugs with encapsulated cell cultures
- Production of cosmetic products with encapsulated flavours or fragrance
- Controlled release of drugs or other ingredients
- In-situ extraction of organic compounds in a fermentation process

Methods

Beads and Capsules were produces with the Inotech Encapsulator Research Instrument (Inotech Encapsulation AG, Switzerland). All parts of the instrument which are in direct contact with the capsules can be sterilized by autoclaving. The product to be encapsulated (cells, microbes, yeast or other ingredients as e.g. hydrophobic or hydrophilic liquids) is put into the syringe or the product delivery bottle. The liquids are forced to the nozzle by either a syringe pump or by air pressure. The liquids then pass through a precisely drilled sapphire-nozzle and separate into equal sized droplets on exiting the nozzle. These droplets pass an electrical field between the nozzle and the electrode resulting in a surface charge. Electrostatic repulsion forces disperse the capsules as they drop to the hardening solution (Fig. 1).

Capsule size is controlled by several parameters including the vibration frequency, nozzle size, flow rate, and physical properties of the polymer-product mixture. Optimal parameters for capsule formation are indicated by visualization of real-time droplet formation in the light of a stroboscope lamp. When optimal parameters are reached, a standing chain of droplets is clearly visible.

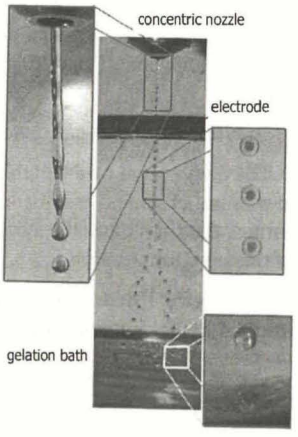

Fig. 1 Droplet formation at the concentric nozzle

Once established, the optimal parameters can be preset for subsequent capsule production runs with the same encapsulating polymer-product mixture. Poorly formed capsules, which occur at the beginning and end of production runs, are intercepted by the bypass-collection-cup.
Depending on several variables, 50 – 4000 capsules per second are generated and collected in a hardening solution within the reaction vessel. Solutions in the reaction vessel are continuously mixed by a magnetic stir bar to prevent capsules clumping. At the conclusion of the production run, the hardening solution is drained off (waste port), while the capsules are retained by a filtration grid. Washing solutions, or other reaction solutions, are added aseptically through a sterile membrane filter. The capsules can be further processed into microcapsules, or transferred to the capsules collection flask.
All experiments were performed with 1.5 % Na-alginate (IE-1010, Inotech Encapsulation AG, Switzerland) and Sunflower oil (Coop AG, Switzerland). The hardening bath was a 0.1 M solution of $CaCl_2$ (IE-1020, Inotech Encapsulation AG, Switzerland). Size distribution of the capsules were measured by using a coulter counter (Coulter Electronics GmbH, Germany). The source and methods of cell line handling is described elsewhere (Pernetti, 2001).

Results

Based on the described encapsulation technology we have successfully encapsulate CHO cells (Fig. 2) for the use in bioprocess optimization. Cells were encapsulated with a concentric nozzle having in the inner fluid the cells and a 0.5% alginate solution and in the outer fluid pure alginate solution. The capsules diameter was 750 µm and they had a very narrow size distribution (3.4 % standard deviation). Cells reached after 14 d of cultivation a cell density of 10^7 cells per ml and the capsules showed - compared to beads - a higher mechanical stability.

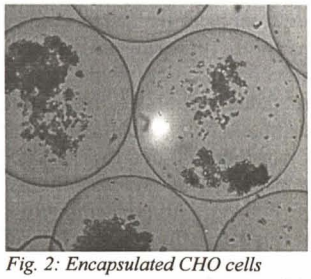

Fig. 2: Encapsulated CHO cells

Recently, the availability of capsules having a liquid core show a merging interest in biotechnology, food and cosmetic industry, e.g. hydrogel capsules with an organic core can be used as controlled release tool for flavors and fragrances. Fig. 3 shows capsules with sunflower oil (core) and alginate (shell). There capsules have an inner diameter of 475 µm and an outer diameter of 750 µm and they also show a narrow size distribution (3.8 % standard deviation). Such liquid core capsules can be made of any organic polymer or hydrogel and hydrophilic and hydrophobic compounds may be encapsulated.

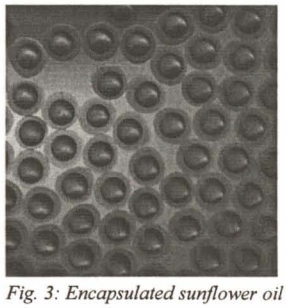

Fig. 3: Encapsulated sunflower oil

Conclusions

In this presentation the use of the Encapsulator Research with a concentric nozzle for the production of beads and capsules has been presented. Different systems of core and shell material were tested and the capsules show under reproducible production conditions very narrow size distributions. The possibility of encapsulation of cells in a liquid core lead to better cultivation conditions.

References

Serp, D. et al. 2000. *Characterization of an encapsulation device*. Biotechnol Bioeng 70: 41-53.
Pernetti, M. et al. 2001. *Animal cell encapsulation*. Proceedings BRG Meeting. Warsaw.