Microencapsulation of antimicrobial agents by three chemical methods

Boh B.[1*#], Sumiga B.[1,3], Salar-Behzadi S.[2], Viernstein H.[2]
[1] University of Ljubljana, Slovenia ([*#] bojana.boh@ntf.uni-lj.si), [2] University of Vienna, Austria, [3] Aero d.d., Slovenia

INTRODUCTION

The contribution presents microencapsulation of antimicrobial agents for technical applications by three chemical methods: (1) *in situ* polymerization of melamine-formaldehyde resins, (2) complex coacervation of gelatin and carboxymethyl cellulose, and (3) cyclodextrin molecular inclusion.

MATERIALS AND METHODS

In situ polymerization microencapsulation

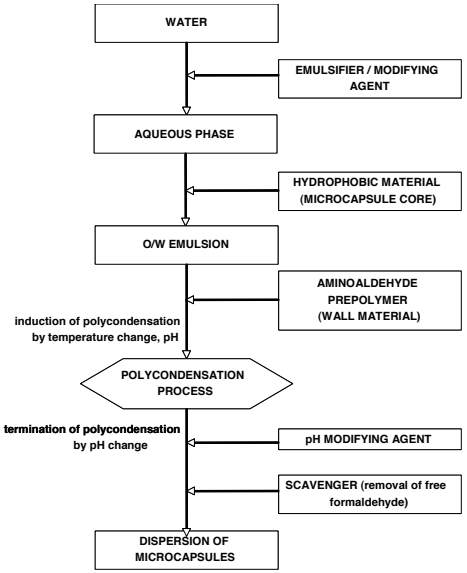

Figure 1: *In situ* polymerization microencapsulation procedure

Trimethylolmelamine and hexa-methoxymethylolmelamine resins (both Melamin, Slovenia) were used as amino-aldehyde prepolymers for microcapsule wall formation. Styrene-maleic acid anhydride copolymer with average mol. weight 350,000 (Hercules) was used as a modifying agent/emulsifier for *in situ* polymerisation. Analytical grade NaOH (Kemika Zagreb) and sodium metabisulphite $Na_2S_2O_5$ (BASF) were used for termination of the polycondensation reaction and removal of free formaldehyde from the suspension of microcapsules. As microcapsule core materials, two antimicrobial agents were used: (1) 5-chloro-2-(2,4-dichloro-phenoxy) phenol (Triclosan, Sigma-Aldrich), which is a commercial wide spectrum antibacterial and antifungal agent, and (2) a mixture of essential oils (Etol, Slovenia) of lavender (*Lavandula sp.)*, rosemary (*Rosmarinus officinalis)* and sage (*Salvia officinalis)* in isopropylmyristate as a solvent, in 10%, 25% and 40% essential oil concentrations.

Laboratory microencapsulation experiments were performed in a 1-L stainless steel reactor (Vollrath), diameter 150 mm, equipped with 5 exchangeable dissolver stirrers of different diameters (90, 70, 60, 55, 45 mm) with impeller speed 1200 to 6000 rpm, and a cooling/heating system. For process scaling-up, a 10 L stainless-steel pilot reactor was used, with a diameter of 300 mm, double outer walls for heating/cooling operations, equipped with a 0.25 KW electric motor for a dissolver

stirrer (diameter 75 mm, 0 - 2800 rpm, continuous adjustment), and a 0.25 KW electric motor for anchor stirrer with teflon scrapers (25 rpm).

Complex coacervation microencapsulation

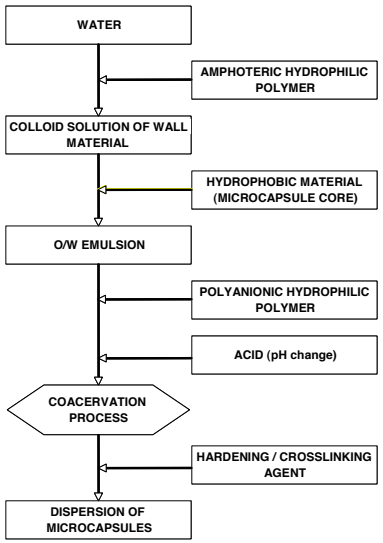

Figure 2: Complex coacervation microencapsulation procedure

10.00 g of acid treated gelatin with the isoelectric point at pH=8 (Pharmagel A, Rousselot S.A.) was left to swell in 80 ml water for 1 hour at room temperature, and was then heated to T=50°C to obtain a clear aqueous colloid solution. Two antimicrobial microcapsule core mixtures were used: (1) a 3 : 1 mixture of vegetable oil and eugenol (isolated from cloves, purified), and (2) a mixture of essential oils (Etol, Slovenia) of lavender, rosemary and sage in isopropylmyristate as a solvent. 50.00 g of the hydrophobic phase was emulsified into the aqueous phase at 1500 rpm (Heidolph), 3 min, to obtain an O/W emulsion. 3.33 g of carboxymethyl cellulose, low viscosity type 7L (Hercules) was dissolved in 80 ml of distilled water, heated to T=50°C, and added to the emulsion at 400 rpm. pH was adjusted to pH=6.0 with 10% aqueous NaOH solution, and 150 ml of distilled water preheated to T=50°C was added to the system while stirring continued at 400 rpm. pH was adjusted to pH=4.5 with 10% acetic acid (Riedel de Haen) to induce the complex coacervation process. Stirring at 400 rpm was continued for 1 hour, then the system was cooled to T=10°C at a cooling rate of 1°C/min. 1.75 ml of 37% aqueous solution of formaldehyde (Kemika) was added drop wise as a cross-linking agent. Stirring was continued at 300 rpm for 1 hour to obtain cross-linked coacervate microcapsules.

Cyclodextrin molecular inclusion

Complexation of antibacterial essential oils of lavender, rosemary and sage (Etol, Slovenia) with beta-cyclodextrin (β-CD, Cavamax W7 Pharma, Wacker-Chemie, Germany) was performed separately by the precipitation method. Molar ratios of β-CD: essential oil 1:1, 1:2 and 1:3 were prepared: Required amounts of β-CD were dissolved in 25 ml distilled water. After addition of the essential oil, the mixtures were stirred under closed conditions, at room T, and stirring rate of 100 rpm for 1h, 24h, 36h and 60h, respectively, in order to investigate the equilibrium time for each molar ratio. Afterwards, the mixtures were centrifuged and the supernatants were decanted. The precipitates were dried in desiccators over silica gel till achieving the constant weight.

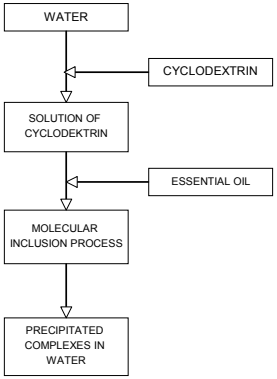

Figure 3: Cyclodextrin molecular inclusion procedure

Gas chromatography was applied for the analysis of encapsulated essential oils in the precipitate. Dimethylsulphoxide - DMSO (Sigma, Austria) was used as a solvent for β-CD. Equal amounts of dry precipitates were dissolved in 10 ml DMSO and extracted with n-hexane. After the phase separation, the DMSO phase was collected and again extracted with n-hexane. The n-hexane extracts were combined and filled up to 25 ml. The concentration of oil in precipitates was determined using a GC-MS system (Shimadzu GC-MS QP 2010, Japan), with a capillary colon Zebron ZB Wax® 60:0.25:0.25 and Ei detector, 70+V, 250°C. Menthol (Merck, Austria) was used as an internal standard. Operating conditions of GC-MS were as follows: injector temperature 180°C, detector temperature 250°C, carrier gas flow 1.5ml/min at 60°C, temperature program: hold for 3 min at 60°C, then heat from 60°C to 190°C at 3.5°C/min, hold at 190°C for 30 min.

RESULTS

In situ polymerization

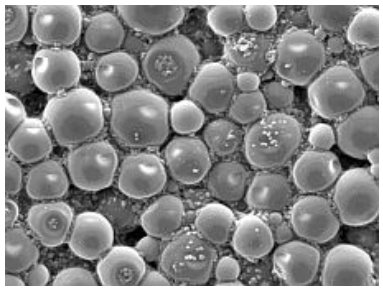

Figure 4: Aminoaldehyde microcapsules containing Triclosan, produced by *in situ* polymerization (scanning electron microscope)

Coacervation

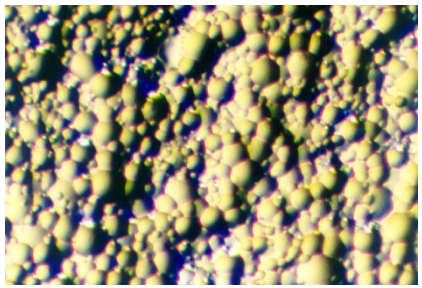

Figure 5: Dry film of a dispersion of coacervate microcapsules containing a mixture of vegetable oil and eugenol (stereomicroscope)

The experiments showed that the permeability of *in situ* polymerisation microcapsules could mainly be controlled by the selection of wall prepolymers, modifying agents, and the ratio between them. In the complex coacervate microcapsules, the permeability was controlled by (1) the addition of a cross-linking agent – stronger cross-linking reduced the permeability of microcapsule walls, and the (2) cooling rate after the induction of coacervation – rapid cooling enhanced the permeability, while slow cooling rates resulted in lesser permeability of microcapsule walls. Both types of microcapsules were pressure-sensitive, i.e. could release their content under the mechanical pressure.

Cyclodextrin molecular inclusion

The suitability of the precipitation method for encapsulation of essential oils was confirmed. High encapsulation rates of 85-90% [w/w] of each oil were achieved. Furthermore, using this method it was possible to scale up the production to gain gram quantities.

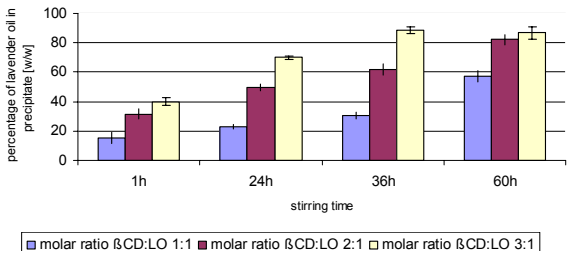

Figure 6: Percentage of encapsulated lavender oil in precipitates produced with different molar ratios of host:guest and different stirring times

The investigation of different host:guest molar ratios and different complexation times showed similar results for the encapsulation of lavender, rosemary and sage oil. As an example, the results of lavender oil encapsulation are presented in Figure 6.
For the quantitative and qualitative analysis of the precipitates, an analytical method based on gas chromatography was optimised.

The chromatograms showed that the main components of oils, e.g. for the lavender oil cineole, L-linalool, linalyl acetate, terpineol and borneol, have been encapsulated at all encapsulation conditions.

CONCLUSIONS

Microencapsulation is essential in the development of antimicrobial formulations for technical applications, especially to (1) reduce the volatility of volatile substances, (2) convert liquid components into a solid powder, (3) prolong the activity by slow/sustained release or by targeted pressure-sensitive release of active components. Different encapsulation techniques can be used for this purpose. In our work, after the optimization of process parameters, all three methods, namely the *in situ* polymerization, complex coacervation and cyclodextrin molecular inclusion, were proved to be suitable for the encapsulation of hydrophobic antimicrobial agents. Melamine-aldehyde resins in combination with the *in situ* polymerization microencapsulation produce pressure-sensitive microcapsules which can be totally impermeable or moderately permeable, they have good thermal resistance and are suitable for controlled release of volatile core materials. The drawback of this technique is the composition of the polymeric wall, which is a synthetic polymer with slow biodegradability, and is therefore only applicable in technical domains. Complex coacervation microcapsules are composed of natural wall materials, they have relatively good mechanical properties, the wall permeability can be controlled, but the encapsulation efficiency is slightly lower, compared to *in situ* polymerization. Molecular inclusion with cyclodextrins was proved to be a successful method for encapsulating antimicrobial agents at a molecular level.

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