Methods of encapsulation efficiency determination

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Abstract

In this article selected methods of encapsulation efficiency determination are presented. Five analytical methods were compared: (i) with the use of the gas chromatography coupled with mass detector, (ii) by staining oil with rhodamine B, (iii) using Raman spectroscopy (iv) spectrophotometry with and without the addition of a contrasting substance, (v) by analyzing DSC thermograms normalized for oil and powders containing this oil. In order to compare the above-mentioned methods, spray drying microencapsulation experiment was carried out. The results of the comparison allow to determine the accuracy as well as strengths and weaknesses of each of the proposed methods for determining the efficiency of microencapsulation in spray drying.

Keywords: mikroencapsulation, spray drying, process efficiency, material properties.
1. Introduction

Microencapsulation performed by the spray drying is one of the common methods used in chemical, pharmaceutical and food industry to give new unique properties to loose materials by closing inside the sold shell liquid core [1]. This process is mostly performed by spraying specially prepared emulsion of oil material dispersed in water solution. The amount of liquid enclosed inside the particles depends on the conditions of the spray drying process. Incorrectly selected process parameters can cause in cracks of microcapsules crust and release of core material [2]. Choosing the conditions for the spray drying microencapsulation process the efficiency of the encapsulation must be considered. This parameter can only be determined experimentally from the analysis of the obtained powders. The following article presents methods for determining the efficiency of microencapsulation and comparing their accuracy based on the conducted experiment of microencapsulation of sunflower oil in maltodextrin the spray drying.

2. Spray drying microencapsulation experiment

In order to test selected from the literature and proprietary methods to determine the efficiency of microencapsulation, a spray drying experiment was carried out, in which oil was encapsulated in a maltodextrin shell. Collected samples were analyzed by these methods mentioned in later.

2.1. Materials

The materials used in the microencapsulation process were the spray drying method:

- Sunflower oil (ZT Kruszwica S.A, Poland) - non-volatile core material;
- Maltodextrin (MDX) DE 16 (Nowamyl S.A, Pland) - a coating material widely used in various industries;
- Rhodamine B (Merck KGaA, Germany) is an industrial dye for dyeing lipid substances such as oils, waxes, plastics. Rhodamine B dissolves in organic solvents such as benzene, acetone and ethanol; Used to visualize the core material in optical methods for determining the degree of microencapsulation.

Maltodextrin and distilled water were weighed and then mixed using a thermostated magnetic stirrer. The concentration of the prepared solution was 30%. The process of preparation of the maltodextrin aqueous solution took place at a constant temperature of 55 °C. In the next stage, the 30% MDX solution was divided into two samples, to which sunflower oil (without and with the dye Rhodamine B) was added (3%). The obtained mixtures were subjected to the process of emulsification using an ultrasonic homogenizer (U200s, IKA POL, Poland). The homogenization time of the solution was 15 min. Microscopic analysis of the emulsion showed a homogeneous distribution of the oil phase.

2.2. Spray drying process

The spray drying process of the 30% MDX aqueous emulsion solution was divided into three stages.

In the first stage, distilled water was sprayed into the dryer chamber in order to achieve stable process parameters. The stabilization time was about 50 min. After the outlet air temperature
stabilized, the second stage began, consisting of drying the aqueous emulsion solution without the addition of dye. Drying time was about 35 minutes. The last third step was drying the prepared emulsion of an aqueous MDX solution containing the colored oil fraction with Rhodamian B dye. The concentration of dye in oil was 0.076 g / 100g oil.

2.2.1. Process parameters

The spray drying process was carried out in a Bushi 190 laboratory dryer. Initial air temperature $T_{in, 0} = 175 ^\circ C$. The sprayed emulsion flow rate was 10.5 ml / min, and the atomizing air flow rate was $Q_{air} = 0.011$ m$^3$/min. Initial temperature of the solution 45 $^\circ C$.

2.2.2. Powder properties

The obtained oil microcapsules were characterized by a density of 1335 kg / m$^3$ (measured using an AccuPyc 1330 helium pycnometer from Micromeritics) and a moisture content of 0.06 kg / kg. Then the samples were divided into smaller fractions in order to carry out tests of the efficiency of the microencapsulation process.

3. Methods for determining the efficiency of microencapsulation

3.1 UV-VIS spectrophotometric method

The most accurate method to determine the efficiency of the microencapsulation process [3]. Uses the sunflower oil calibration curve determined with an Evolution ™ 300 UV-Vis spectrophotometer for wavelength of 287 nm. The method of analysis consisted of three stages:

In the first stage, a calibration curve was determined for pure sunflower oil determining the amount of oil mass in the sample based on the measured absorbance. In the second stage, the amount of surface oil by washing the sample for 300 s with isopropyl alcohol. The sample was then subjected to centrifugation and filtration. The obtained clear filtrate is placed in a spectrophotometer measuring cuvette. The analysis was carried out for the spectrum range 210 - 600 nm. Sunflower oil has been identified for a wavelength of 287 nm. In the last stage, the mass of total oil found on the surface and inside of the microcapsules was determined. For this purpose, the conical flask was weighed 1000 mg of material was then distilled water was added to dissolve the material and release the closed sunflower oil microcapsules. Then the solution was subjected to centrifugation separation of the oil phase of a solution of maltodextrin. The separated oil phase was removed with an automatic pipette and then 10 ml of isopropyl alcohol was added. The prepared sample was given to spectrophotometric analysis. The calculation of the encapsulation efficiency ($E_E$) was determined as the proportion of the mass of closed oil in the microcapsule structure to the mass of total oil, i.e. inside and on the surface of the particles. This relationship is described by the equation:

$$E_E = \frac{X_{cor}}{X_{all}} \cdot 100\%$$

(1)

where the ratio of $X_{cor}$ and $X_{all}$ oil was calculated according to the equations:

$$X_{cor} = \frac{m_{all\_oil}}{m_{powder}} - \frac{m_{surf\_oil}}{m_{powder}}$$

(2)

$$X_{all} = \frac{m_{all\_oil}}{m_{powder}}$$

(3)
**Methods of encapsulation efficiency determination**

Where: \( m_{\text{all, oil}} \) – overall mass of oil in sample, \( m_{\text{sur, oil}} \) – mass of surface oil, \( m_{\text{powder}} \) – overall mass of the sample.

The above equations were used to determine the efficiency of microencapsulation in all the analysis methods presented in this article.

### 3.2 Raman spectrophotometry

Raman spectroscopy is one of the most versatile analytical techniques capable of identifying the chemical composition of the test material. For the analysis of the obtained microcapsules, a Raman spectrometer (WITec alpha300 RSA) was used, equipped with an EMCCD detector and a laser light with a wavelength of 532 nm and a power of 10mW, coupled with an optical microscope. Material samples were tested using a 40x lens and the spectrum registration speed was 0.1 sec.

The analysis of the tested samples consisted of three stages:

1. In the first stage, it was necessary to remove the residual surface oil phase in order to show the location of the oil phase inside the microcapsules by washing the powder with isopropanol alcohol. In the second stage, individual materials were identified by performing spectral analysis for maltodextrin DE16 (MDX) and sunflower oil (OS). The analysis allowed determination of the characteristic wavelength from the whole spectrum range of \( 200 \div 3600 \text{ cm}^{-1} \) and was used as a reference point during the analysis of microcapsules. This procedure allowed the determination of standard curves for pure capsule components. In the last step, spectrum signal analysis was performed for sunflower oil microcapsules obtained during spray drying. Based on the measurement of the signal intensity, the location of the presence of sunflower oil in the structure of microcapsules was determined.

**Fig. 1** Raman spectra for (A) microcapsule components: sunflower oil (blue line), maltodextrin (red line) and silicon; (B) of a microcapsule containing a mixture of MDX and sunflower oil, **Raman spectrum in the range 200-1800 cm\(^{-1}\).**

Fig. 1A presents a Raman spectrum in the spectral range from 200 to 1800 cm\(^{-1}\) for individual materials forming the structure of the microcapsules: maltodextrin DE16 which is a matrix material (red), core material: sunflower oil (blue) and silicon plate (black) on which sunflower oil was measured. Spectrum analysis reveals that pure sunflower oil was characterized by Raman bands ranging from 1390 to 1490 cm\(^{-1}\) and from 1625 to 1686 cm\(^{-1}\) with a maximum of 1445 and 1656 cm\(^{-1}\), which corresponds to signals of lipid structures and...
fatty acids. For the measurement of pure maltodextrin showing a peak having a frequency of 482 cm\(^{-1}\) having a signal intensity level 2300 a.u. Figure 1B shows the measurement result of two samples of microcapsules containing an oil phase. The analysis of the obtained spectra showed characteristic curves derived from a mixture of MDX and sunflower oil. The Raman spectrum analysis is characterized by a strong signal at 482 cm\(^{-1}\), characteristic for maltodextrin. Based on the identification of the fat phase signal corresponding to peaks 1445 and 1656, it was shown that in both cases the material showed a sunflower oil content [4]. The distribution of sunflower oil in the structure of microcapsules was uneven, which is evidenced by a change in the signal level of the spectrum intensity.

Research on Raman spectra allowed for a more accurate analysis of morphological structures and the chemical composition of microcapsules. Images obtained by Raman microscopy (Figure 2) confirmed the presence of the oil phase in the formed microcapsules in the form of oil droplets/spots of varying diameter in the walls forming the matrix structure of the microcapsules. This technique allows assessing the locations of the oil phase as well as estimating the degree of retention.

![Figure 2 Images of the microcapsule structure obtained from optical and confocal-Raman microscope with sunflower oil: (a), light microscopy of particles suspended in isopropanol (b) light microscopy of particles without isopropanol; (c, d) Raman confocal microscopy microcapsules - maltodextrin distribution (482 cm\(^{-1}\)) (c), oil distribution (1656 cm\(^{-1}\)) (d).](image)

### 3.3 Optical microscopy method

In order to analyze the distribution of closed sunflower oil colored with Rhodamine B in microcapsules by optical analysis, the oil phase was first extracted from the surface of the particles to remove fat from the surface of the material. Isopropyl alcohol was used for this purpose. The material was rinsed three times and placed in a chamber dryer to remove residual alcohol. In the second stage, the dried material was placed on a glass plate and placed under an optical microscope. Then alcohol was added to the material to contrast the analyzed area and a series of photos was taken. In a next step, a mixture of distilled water and alcohol was added to the material to start the dissolution process of the MDX coating forming the microcapsules. This allowed for controlled release of the closed sunflower oil. On the basis of inflated particle structure before and after dissolution, the average diameter of oil droplets and the location of the oil phase were determined. The individual steps of the procedure are shown in Figure 3.
Methods of encapsulation efficiency determination

Microscopic method of oil phase dyeing allows to locate the sunflower oil inside the microcapsules (Figure 4). The visible red color is due to the dye (Rothamine B) used. The addition of solvent caused the maltodextrin coating to dissolve, releasing the closed sunflower oil. The obtained oil drop size was in the range from 1.2µm to 11.5µm. Such a large variation may result from the coalescence process of the finely dispersed phase during spray drying.

The staining microscopic method allowed to evaluate the structure of fat-containing microcapsules for hollow particles naturally formed when using pneumatic nozzle dryers. It was noted that the fat phase is in the wall and inside of the microcapsules, forming a thin film on the inner surface of the microcapsules. This phenomenon is associated with the natural separation of the oil phase and the aqueous solution of maltodextrin and the retained gas bubble. During evaporation, the greatest forces are oriented on the interface, leading to an increase in the coalescence effect. The increase in MDX concentration due to moisture diffusion leads to an arrest of the internal migration of sunflower oil forming a multi-matrix system. The accuracy of the method is limited by the accuracy of the program analyzing the size and distribution of oil droplets.

3.4 Gas chromatography method

The lipid fraction was obtained according to the modified methodology described by Śpitalniak-Bajerska et al [5]. In brief, around 200 mg of maltodextrin was washed out 3 times with 2 mL of cold pentane (HPLC grade, UQF Wroclaw, Poland) for removing the non-encapsulated oil. Solution was shaken 3 minutes and finally centrifuged. Extraction of
inorganic phase was repeated 3 times and collected n-pentane fractions were dried over anhydrous sodium sulphate. In the next step the extracted unpolar fraction, after evaporation on vacuum, was saponified (10 min at 75°C) with a 2 mL 0.5 M solution of KOH/MeOH and subjected to methylation (10 min at 75°C) in 2 mL 14% (v/v) BF3/MeOH. Then, water was added to reaction and the methyl esters of fatty acids were extracted with 5 mL of n-pentane, washed with 10 mL 10% sodium bicarbonate and dried over anhydrous sodium sulphate. The organic phase was evaporated under reduced pressure and stored in ~27°C until chromatographical analysis. FAME profile was assessed using gas chromatograph coupled with a mass spectrometer (Shimadzu GCMS QP 2020, Shimadzu, Kyoto, Japan). Separation was achieved using Zebron ZB-WAX capillary column with a length of 30 m, inner diameter of 0.25 mm, and film thickness of 0.25 μm (Phenomenex, Torrance, CA, USA). The GC-MS analysis was according to the following parameters: Scanning was performed from 50 to 400 m/z in electronic impact (EI) at 70 eV, mode at 5 scan s \(^{-1}\) mode. Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL min \(^{-1}\) in a split ratio of 1:10 and the following program: (a) 40 °C for 3 min; (b) rate of 5.0 °C min \(^{-1}\) from 40 to 160 °C; (c) rate of 30 °C min \(^{-1}\) from 160 to 280 °C. Injector was held at 220 °C, respectively. Compounds were identified by using three different analytical methods that compare: (i) Retention times with authentic chemicals (Supelco 37 Component FAME Mix); (ii) obtained mass spectra, with available library (Willey NIST 17, match index > 90%). The gas chromatography method is mainly used for the analysis of essential oils. In przyypadku sunflower oil obtained result may be affected by large errors.

### 3.5 Differential scanning calorimetry (DSC) method

The oil content in the microcapsules was determined using an innovative procedure using differential scanning calorimetry (DSC) using a Mettler Toledo DSC821 apparatus, in the temperature range from -40 to 200°C, with a scanning speed of 10°C/min. N5 nitrogen, flowing at a rate of 60 ml / min, was used as the drying gas for the measuring chamber. Dry samples were placed in aluminum crucibles with a capacity of 60 μL or steel, pressure crucibles with a capacity of 120 μL. The normalization of the thermogram was used, in which the heat of transformation was related to the sample mass. By analyzing changes in thermograph course, you can determine the differences in sample heat capacity [6]. After comparing with reference curves, the composition of the tested material can be calculated. It is a method that allows determining only the composition of the tested material.

### 4. Results

The results of the analysis of microcapsule samples obtained in the spray drying experiment carried out using the methods described in the article are presented in Table 1. It can be seen that the result obtained by gas chromatography with parallel mass measurement gave a significantly lower result. This is due to the low level of sunflower oil. The efficiency of microencapsulation determined by other methods show similar values which allows us to state that they can be used for quality control purposes in the production of microcapsules.
Methods of encapsulation efficiency determination

<table>
<thead>
<tr>
<th>Method</th>
<th>Measured process efficiency</th>
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<tbody>
<tr>
<td>UV-VIS spectrophotometric method</td>
<td>85.9 %</td>
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<tr>
<td>Raman spectrophotometry</td>
<td>82.6%</td>
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<tr>
<td>Optical microscopy method</td>
<td>78.1%</td>
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<tr>
<td>Gas chromatography</td>
<td>26.4%</td>
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<tr>
<td>Differential scanning calorimetry</td>
<td>81.1%</td>
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5. Conclusions
The above article presents five methods for determining the efficiency of microencapsulation carried out by spray drying. The methods were tested during analyzes of microcapsules obtained experimentally. The methods allow not only for quantitative but also qualitative analysis, allowing the structure and distribution of the oil phase to be determined in the interior of the particles.

Aa experiment of sunflower oil in maltodextrin crust microencapsulation by spray drying was carried out. The efficiency of the samples obtained was determined for each method and compared.

The result of the comparison allows to state that all methods can be used to determine the degree of oil encapsulation inside the microcapsules quite accurately. Only gas chromatography can be used to determine the content of volatile oils, generating a large error in the case of sunflower oil.

6. References