Spray-dried Prodigiosin from *Serratia marcescens* As A colorant

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**Encapsulation technique via spray drying can be employed to enhance the natural pigment’s properties. In this study, spray-dried microparticles containing red pigment (prodigiosin) extracted from *Serratia marcescens* was produced using κ-carrageenan and maltodextrin as encapsulation agents. The effect of spray-drying parameters on the encapsulation yield (EY), particle size and color intensity of the prodigiosin microcapsules were studied. The most intense color was obtained at a 1:1 ratio (volume ratio of prodigiosin in ethyl acetate to κ-carrageenan solution) with a 200°C inlet temperature, a feed flow rate of 60 m³/h, a pressure of 1.5 bars and a feed rate of 3 mL/min. The characterization of spray-dried prodigiosin using FTIR and FESEM confirmed that the particles were properly coated with encapsulating agents and pigment morphology showed that the particles were regular shaped spheres. The results suggest that the spray-dried prodigiosin can be useful as a colorant under the above optimum operating conditions.**

**Key words:** Encapsulation; pigment; spray drying; prodigiosin; colorant

Prodigiosin is a non-diffusible tripyrrole that occurs naturally as a secondary metabolite and is a member of a group of compounds that contain pyrrolyl pyrromethene (PPM) skeletons produced by *Serratia marcescens*, a Gram negative bacterium. The rapid production of its red flashy pigment, chemical nature, and the properties of prodigiosin has made it the subject of many studies into its possible industrial applications, especially as a food additive. However, the usage of prodigiosin pigment is limited because its color quickly fades or changes upon exposure to light and it is insoluble in water.

The process of entrapping one substance (active agents) within another substance (carrier material) may improve some properties of sensitive compounds, such as stability and solubility. A widely-used technique for converting suspensions and liquids to powder form materials is called encapsulation. Therefore, encapsulation is an effective method to protect substances ingredients against deterioration and volatile losses. Spray-drying is one of the well-established one-step processes of encapsulation technique to forming microparticles.

The choice of carrier materials (encapsulating agent) depends on the type of microparticles desired. Some pseudoplastic properties of carrageenan allow this carrier act as a plasticiser and make the formation of spherical and smooth-surfaced microparticles more desirable. Hydrolysed starches (maltodextrins) are often used as carrier materials due to similar properties and their low cost, high water solubility and low viscosity.

The goal of this study was to produce spray-dried prodigiosin particles extracted from *Serratia marcescens* and to investigate the effect of spray dryer parameters i.e. inlet temperature, feed flow rate, and atomization pressure on the properties of encapsulated prodigiosin.
MATERIALS AND METHODS

Chemicals

The pigment (prodigiosin) used in this study was kindly provided by Wastewater Treatment Facilities Unit, Universiti Technologi Malaysia, Johor, Malaysia, from bacterium (*Serratia marcescens*). The kappa-carrageenan (Fluka) and maltodextrin (13-17DE, Sigma Aldrich) were obtained commercially. All reagents and chemicals used were of analytical grade (AnalaR).

Method

Procedure for Spray drying Prodigiosin

The preparation of the feed solution started with dissolving \(\kappa\)-carrageenan (1% w/v) and maltodextrin (5% w/v) in 100 mL of distilled water. The viscous liquids were mixed with 100 mL of diluted prodigiosin (150 ppm) separately and stirred at 70°C until the ethyl acetate was completely evaporated. The suspension (1:1 ratio of prodigiosin/ \(\kappa\)-carrageenan and maltodextrin) was homogenized using a sonicator (Elmasonic, S100/H) for 10 minutes. Different core to carrier ratios of 1:2, 1:3 and 2:1 were prepared using a similar procedure and then subjected to a pilot scale spray dryer (LU-222, SD-05, Labultima, Japan) for further processing. In all spray drying experiments, the airflow was set to 60 m\(^3\)/h, the atomizing air and temperature feed rate were kept constant at 1.5 bar and at 30°C. Feed rates spanned from 3, 5 and 7 mL/min. The inlet temperatures varied from 150°C, 170°C, and 200°C. Based on previous studies the outlet temperature was kept constant at 100°C [21].

Encapsulation efficiency (EY)

Encapsulation efficiency (EY) is a measure of the effectiveness of the applied parameters on the formation of particles. The encapsulation yield (EY) is the mass ratio of the final microparticles obtained over the mass of the initial substances added including the coating materials and dried prodigiosin, as shown below [9].

\[
\text{EY}\% = \frac{\text{Particles weight after spray dry} \times 100}{\text{Total weight of coating material and prodigiosin added initially (in 100 mL)}}
\]

Determination of Solubility

To determine solubility, the spray-dried particles were dissolved in different types of solutions, including water, HCl (0.1M), NaOH (0.1M), acetone, \(n\)-hexane, ethyl acetate, methanol, and ethyl alcohol. A small portion of the sample (0.005g) was added to 5mL of each solvent. The changes were recorded.

Field Emission Scanning Electron Microscopy (FESEM)

Morphology was observed using an electron microscope (FESEM, JSM-6701F) operating at 15kV. Prior to scanning, samples in a powder form were attached to double sided adhesive tape which was made electrically conductive (10 mm stubs). Digital images were obtained with an excitation voltage of 5kV and magnification varied from 2.50 to 25.00KX.

Fourier Transform Infrared (FTIR)

The FTIR analysis was carried out to evaluate the effects of encapsulating agents on prodigiosin functional groups. The samples were prepared as follows; about 25 mg of each sample was mixed with 300 mg of dry potassium bromide and ground to obtain a fine powder. It was then pressed and analyzed using an FTIR spectrometer ( Spectrum One, Perkin Elmer) in the range of 4000 - 450 cm\(^{-1}\).

RESULTS AND DISCUSSION

Formation of Prodigiosin Microparticles via Spray drying Process

The hydrophobic nature of prodigiosin, which is due to the resonance of its functional group electrons and non-polar dipole moment, means that prodigiosin molecules can be used as a core material in an encapsulation process and they can be surrounded with hydrophilic coating materials. In the first spray dry trial, prodigiosin was used without a carrier material and the result was sticky dried pigment that was not in a powder form. This explains the requirement of \(\kappa\)-carrageenan and maltodextrin as coating materials and confirmed the importance of coating material when attempting to form powders. A fine microcapsule pink powder of spray-dried prodigiosin was created using coating materials (Figure 1).

Tables 1 and 2 summarized the effects of different spray drying parameters such as inlet temperatures, core to carrier ratios, and feed rate on color intensity and the moisture content of the encapsulated prodigiosin using \(\kappa\)-carrageenan or maltodextrin as coating materials. The most intense
color and lowest moisture content was obtained when the air inlet was 200°C, the feed rate was 3 mL/min and (1:1) core/carrier ratio. Other parameters such as outlet temperature, flow rate and atomizing air pressure were kept constant at 100°C, 60 m³/h and 1.5 bar, respectively.

In the formation of encapsulated particles, spray drying parameters play an important role. Inlet air temperature is one of the most important parameters in any microencapsulation system. Unsuitably high inlet temperatures may lead to the breakdown of the particle crust by upsetting the balance of the water evaporation rate and film formation. In other words, unsuitably high inlet temperatures cause a decrease in encapsulation efficiency by permeation of the core material. In some cases, high air inlet temperatures contributed to heat damage, surface imperfections and excessive bubble growth.

Prodigiosin particles dried at 200°C inlet air temperature gave the most intense colored particle compared to other temperatures used. At higher temperature, the outer surface of the droplets dry faster formed particles with thin crust [8]. Therefore, the particle color seems more obvious. In addition, the size of the particles formed at higher temperature is bigger and more prodigiosin are being entrapped inside. Additionally, the high viscosity of κ-carrageenan (in comparison to maltodextrin) led to the formation of large droplets that contained more feed solution. This led to more intense color in the case of prodigiosin powder coated with κ-carrageenan formed with air inlet temperatures of 200°C when

Table 1. The effects of spray drying parameters on encapsulation of prodigiosin using κ-carrageenan 1% w/v

<table>
<thead>
<tr>
<th>No.</th>
<th>Ratio core/wall</th>
<th>Inlet (°C)</th>
<th>Outlet (°C)</th>
<th>Atomization pressure (bar)</th>
<th>Feed rate (mL/min)</th>
<th>Air-Flow rate (m³/h)</th>
<th>OD λ: 540</th>
<th>EY %</th>
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<tbody>
<tr>
<td>1</td>
<td>1:1</td>
<td>200</td>
<td>100</td>
<td>1.5</td>
<td>3</td>
<td>60</td>
<td>0.087</td>
<td>40</td>
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<td>0.064</td>
<td>30</td>
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<td>60</td>
<td>0.052</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>1:1</td>
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<td>100</td>
<td>1.5</td>
<td>3</td>
<td>60</td>
<td>0.054</td>
<td>45</td>
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<td>100</td>
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<td>0.027</td>
<td>65</td>
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<tr>
<td>8</td>
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<td>200</td>
<td>100</td>
<td>1.5</td>
<td>3</td>
<td>60</td>
<td>0.052</td>
<td>30</td>
</tr>
</tbody>
</table>

OD: optical density, EY: encapsulation yield

Table 2. The effects of spray drying parameters on encapsulation of prodigiosin using maltodextrin 5% w/v

<table>
<thead>
<tr>
<th>No.</th>
<th>Ratio core/wall</th>
<th>Inlet (°C)</th>
<th>Outlet (°C)</th>
<th>Atomization pressure (bar)</th>
<th>Feed rate (mL/min)</th>
<th>Air-Flow rate (m³/h)</th>
<th>OD λ: 540</th>
<th>EY %</th>
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<td>60</td>
<td>0.028</td>
<td>40</td>
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</table>

OD: optical density, EY: encapsulation yield
compared to the same amount of powder formed under other conditions and dissolved in water.

In this study, the encapsulation efficiency increases with increasing encapsulation agents used. The highest encapsulation yield was obtained when 1:3 core/ carrier ratio was used with using either κ-carrageenan or maltodextrin as the encapsulating agent. However, the concentration of maltodextrin used was 5 times higher compared to κ-carrageenan. Increasing the amount of core material (prodigiosin) to the amount of encapsulation agent used will lead to decrease in encapsulation efficiency. The EY was at minimum when 2:1 core/ carrier ratio used. This is due to insufficient amount of the binding material proportion compared to core materials.

Another factor which has a significant effect on the encapsulation process is the feed rate. The optimum feed rate obtained from the drying process is 3 mL/min for prodigiosin particles coated with κ-carrageenan or maltodextrin. The feed rate is an important factor in order for the particles to form spherical shape and to ensure the retention of the core ingredient. High feed rate leads to fast drying, resulted in insufficient time for dehydration to take place and therefore, the particles will not shrink properly.

**Solubility of the Encapsulated Prodigiosin**

Encapsulated prodigiosin coated with either coating agent was soluble in water at any volume ratio while non-encapsulated prodigiosin was not soluble in water (Fig. 2). Spray-dried

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**Fig. 1.** Spray-dried prodigiosin coated with κ-carrageenan (a) and maltodextrin (b) with the same prodigiosin concentration (150 ppm) and same spray dry operation conditions

**Fig. 2.** Effect of different solvents on encapsulated and non-encapsulated prodigiosin (a) non-encapsulated prodigiosin in water, (b) encapsulated prodigiosin in water, (c) encapsulated prodigiosin in (1) acetone (2) n- hexane (3) ethyl acetate , (d) encapsulated prodigiosin in (1) HCl (2) NaOH
Prodigiosin particles were also soluble in acidic (HCl, 0.1 M) and alkaline (NaOH, 0.1 M) solutions and insoluble in acetone, n-hexane, ethyl acetate, methanol, and ethyl alcohol. The color changed from pink to orange when an alkaline solution was added because of the delocalization of electron resonance in the carbonyl group which features of a double bond structure. No significant changes in color were observed when other solvents were added to the particles. The spray drying method has been reported to increase hydrophilic groups of coating material because they are more exposed and therefore have increased levels of the solubility in water.

Characterization of Encapsulated Prodigiosin

FTIR Spectroscopy

In this study, FTIR spectroscopy provided information for comparing the spectra of functional groups of prodigiosin in the presence and absence of coating materials. The FTIR spectrum results for the non-encapsulated prodigiosin showed bands at 2909 cm\(^{-1}\) (methylene group), 1565 cm\(^{-1}\) (pyrrole group) and 3463.65 cm\(^{-1}\) (amide group). Peaks that occurred at 3400-3445 cm\(^{-1}\) were due to aliphatic alcohols, primary amines and amides, 1655 cm\(^{-1}\) (C=C) stretching vibrations. From the spectrum, the main functional groups that resulted in red pigments were pyrrole, methylene,
alkane and alkene. The spectrum of pure κ-carrageenan showed bands at 847, 928, 1050, 1263 and 3446 cm⁻¹, which were attributed to D-galactose-4-sulfate, 3, 6-anhydro-D-galactose, glycoside linkage, ester sulfate, and O-H stretching of κ-carrageenan, respectively. The FTIR bands observed for pure maltodextrin were 3400, 2926, 1642, 1418, 1370, 1154, 1079, 931, 856, and 763 cm⁻¹. O-H stretching peaked at 3400 cm⁻¹ and C-H stretching (sp³) at 2926 cm⁻¹. The characteristic peak at 1642 was due to C=O stretching and the peak at 1418 was due to α-CH₂ bending. Another peak at 1370 was caused by O-H bending, and the peaks at 1154 and 1079 were attributed to C-O stretching. CH and CH₂ bending occurred at 931 cm⁻¹, C-H bending and ring puckering caused a spectra at 856 and 763 cm⁻¹. All FTIR bands corresponding to pure maltodextrin and κ-carrageenan were found in spray-dried prodigiosin spectra, which indicated sufficient coating with corresponding carrier materials during the spray drying process. The characteristic peaks of non-encapsulated prodigiosin disappeared in the spray-dried spectra due to the encapsulation process.

**Morphology**

FESEM images confirmed that a wide range of particles that had a mean diameters ranging from 0.5µm to 5µm, were observed in all samples and most of the particles were spherical (Fig. 3). In addition, the electron micrographs showed that smooth spheres of spray-dried particles containing prodigiosin coated with κ-carrageenan or maltodextrin were formed.

Various factors influence particle size and droplet diameter making it difficult to control these variables. Particle size may vary depending on inlet temperature, feed rate, and the viscosity of the feed. Large size particles form in high viscous solutions with high surface tension. The nozzle used in this study was 0.7mm and caused a large surface to volume ratio of the atomized droplets; as a result, droplets dried very rapidly at high temperatures. When there was an increase in the inlet temperature, larger particles with either type of coating material, were observed (Fig. 3, (a) and (d)). The reason is that the fast drying rates do not allow the particles to shrink during the initial drying stages.

Figure 4 shows the effect of three inlet temperatures on particle size distribution of spray-dried prodigiosin coated with κ-carrageenan and maltodextrin.

The results revealed that all samples exhibited similar particle size distribution, regardless of the inlet air temperature or the ratio of prodigiosin to κ-carrageenan or maltodextrin.

![UV-Vis spectra](image_url)
However, the particle size distribution (Fig. 4) shows that the mean particle size diameter was slightly lower when maltodextrin was used. The results are in agreement with the study done by Krishnaiah et al. (2012).

**UV-Visible spectroscopy**

Non-encapsulated and encapsulated prodigiosin pigments were analyzed for maximum UV-Vis absorption. Figure 5 shows that the non-encapsulated pigment in ethyl acetate is red and exhibits a sharp spectral peak at 535 nm. Prodigiosin spectra also showed a persistent shoulder at about 510 nm. The spectrum shifted to 545 nm for encapsulated prodigiosin (0.005 g in 5 mL water). Encapsulated prodigiosin dried at an inlet temperature of 200°C showed the highest level of absorptions. The color intensity of spray-dried prodigiosin coated with κ-carrageenan was much higher than the maltodextrin coated prodigiosin due to the low uniformity of prodigiosin and the coating agent in the initial solution prior to the spray drying process. The spectral properties of non-encapsulated prodigiosin are in accordance with the result obtained by Song et al. (2006).

**CONCLUSION**

It is possible to obtain water-dispersed microencapsulated products of water-insoluble prodigiosin extracted from *Serratia marcescens* by using a spray drying technique and applying κ-carrageenan or maltodextrin as encapsulation agents. κ-carrageenan showed the best encapsulating agent giving the most intense color after spray drying using the optimum parameters: inlet temperature of 200°C, feed rate of 3 mL/min, and flow rate of 60 m³/h. By converting the prodigiosin into spray-dried form, some properties such as solubility is improved and therefore, it is suggested that the spray-dried prodigiosin can be used as a colorant.

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