Preparation of Gamma Polyglutamic Acid (Γ-PGA)/Gelation Composite Nanoparticle and Application on Osmanthus Fragrance Slow-Release

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Abstract. In order to extend the incense period, to improve the stability and processability of osmanthus fragrance. It has not reported to use γ-PGA/Gelatin nanoparticle prepare coated fragrance. This research used γ-PGA/Gelatin nanoparticles after embedding essence, observed its property in dex. γ-PGA/Gelatin nanoparticle was investigated with respect to its optimum experiment process and microencapsulation technology. Through coagulation precipitation, which is an ionic self-assembly process, a large amount obtained of non-toxic and edible. Thermogravimetric analysis uncovered that fragrance-γ-PGA/Gelatin nanoparticles were surprisingly well conserved at rapidly increasing temperature. E-nose measurement revealed fine stability of scent. The yield was obtained as 41.6g/L which increased by 32.9% under optimized conditions and less waste was created. As the temperature increased from 0°C to 200°C, the coated fragrance lost only by 11% while bare fragrance molecules almost fully evaporated. The unchanged and stable scent and a superior ability of heat resistant were attributed to the nano-size effect of fine structure and good characteristics of the incorporation of γ-PGA and gelatin.

1. Introduction
A growing demand for fragranced products is shown up but barriers exists due to poor aqueous solubility and volatility of fragrance molecules. In order to extend the incense period, to improve the stability and processability of osmanthus fragrance. Nanoparticle technology can be used to coat solid, liquid or gas molecules for enabling sustainability of core materials and improving the apparent characteristics of coated materials [1].

For nanoparticle technology, developing new wall materials to encapsulate fragrance has been a hot issue. Vegetable gum (gum Arabic, sodium alginate and carrageenan), starch and its derivatives (various types of dextrin, oligosaccharides, starch derivatives, etc.), and protein (gelatin, casein and soy protein, whey protein, etc.) are widely applied on foods. For industrial fields, a variety of cellulose derivatives (ethyl cellulose, methyl cellulose material, etc.) and wax (insect wax, paraffin wax, wax, etc.) are being used. Fine emulsification and film-formation are basic requirements of all these materials. Yi, Fan and so on [1] had created a heat-resistant nanoparticle via gelatin/gum arabic
of jasmine essential. Baharak, Chris and so on [2] had produced stabilized poly-l-lactic acid nano capsules through nanoprecipitation to coat fragrance molecules to enable its slow-release.

Using biocompatible nanocontainer material to encapsulate fragrance becomes novel [3]. γ-PGA is a biodegradable anionic polymer with the outstanding traits of film forming, sintering and moisturizing and also according to its non-toxicity and edibility [4], it has been widely used as drug and vaccine delivery system [5]. As an outstanding moisturizer, it can be compared with hyaluronic acid. γ-PGA/gelatin nanoparticle would be versatile in cosmetic industry both as fragrance carrier and moisturizer [6-7].

Considering its massive prospects and environmental impact of organic solvent extraction process [8], it would necessary to be worked on optimization of production process with lower cost and amounts of chemicals [9-10]. The first content of this paper is the optimization γ-PGA/gelatin nanoparticles preparation process. Nanocapsules made from γ-PGA crosslinked with gelatin through condensation method were used as fragrance carrier and the effect was further assessed. Then determination the size of osmanthus fragrance γ-PGA/gelatin nanoparticles. Using Infrared spectrum analysis and Thermogravimetric analysis to determine osmanthus fragrance, γ-PGA/gelatin nanoparticles, osmanthus fragrance -γ-PGA/gelatin nanoparticles diffientiate. Finally, e-nose was used to test the perfumes.

2. Materials and methods

2.1. Preparation of nanoparticle
γ-PGA and gelatin were dissolved in distilled water and adjusted pH 5.0 with 1mol/L HCl/NaOH. According to the self-assembly of polyelectrolyte, the intercoagulation of anionic and cationic polymers occurred. The process was slowly dropping γ-PGA solution into gelatin solution and keeping stirring for a while. Frozen drying all the mixture to obtain γ-PGA /gelatin nanoparticles used by SHZ-IIID and low speed centrifuge

2.2. Optimization process of nanoparticle
Response Surface Methodology was used to determine to optimum conditions. It was widely used in biochemical experiment [11]. First, single factor design determined best levels of each factor. Nanoparticle size laser analyzer (British Malvem instrument co., LTD) used to calculate intensity, PDI value and Zeta-potential with each factor changed. Then Placket-Burman design screened out three factors of most significant. Box-Behnken design of three levels (low, medium and high) was conducted according to the aforementioned three factors.

2.3. Preparation of fragrance nanocapsule
Two separate ways were conducted: add core materials (fragrance) and γ-PGA into gelatin solution and the other way around to compare which one was better. Single factor design was used to determine the final conditions. Then frozen centrifuging the mixture at 2000r/min for 10min and kept the liquid supernatant at -55°C (used the constant temperature magnetic stirrer). Frozen drying for 24h. TGA Analysis (used thermos-electron corporation from HERAEUS and TGA-Q5000IR from TA company in USA) uncovered its superior stability and we also used e-nose (E-nose Fox-4000, AlphaM.O.S co., LTD in France) to testify the scent change.

3. Results and Discussion

3.1. Data analysis
Within the Box-Behnken design, there are five groups of central composite design (group 4, 9, 10, 12, 15) and twelve groups for factorial experiment as in Table 1. The concentration of γ-PGA (A), the concentration of gelatin (B) and pH of gelatin (C) were three most significant factors. Y1 represented Z-Ave/nm (diameter of nanoparticle) and Y2 represented Zeta-potential value.
Table 1. Box-Behnken design of three variables and the experimentally observed responses

| No. | A   | B   | C   | Y₁ (nm) | Y₂ (mV) |
|-----|-----|-----|-----|---------|---------|
| 1   | 0.80| 0.20| 3.00| 309.50  | 15.20   |
| 2   | 0.80| 0.80| 3.00| 289.30  | 23.40   |
| 3   | 0.20| 0.80| 3.00| 276.90  | 16.10   |
| 4   | 0.50| 0.50| 3.00| 173.80  | 37.50   |
| 5   | 0.50| 0.20| 4.00| 243.30  | 15.60   |
| 6   | 0.50| 0.20| 2.00| 262.00  | 13.90   |
| 7   | 0.80| 0.50| 2.00| 226.60  | 15.00   |
| 8   | 0.80| 0.50| 4.00| 211.40  | 18.20   |
| 9   | 0.50| 0.50| 3.00| 176.80  | 39.90   |
| 10  | 0.50| 0.50| 3.00| 168.30  | 38.80   |
| 11  | 0.20| 0.20| 3.00| 307.20  | 9.40    |
| 12  | 0.50| 0.50| 3.00| 155.60  | 36.30   |
| 13  | 0.50| 0.80| 4.00| 247.80  | 15.40   |
| 14  | 0.20| 0.50| 2.00| 319.40  | 23.30   |
| 15  | 0.50| 0.50| 3.00| 152.30  | 38.20   |
| 16  | 0.50| 0.80| 2.00| 227.90  | 27.80   |
| 17  | 0.20| 0.50| 4.00| 234.10  | 12.60   |

Y₁=165.36-12.60A-10.01B-12.41C+2.52AB+17.53AC+9.65BC+66.49A²+63.87B²+16.2C²  
(1)

Y₂=38.14+1.30A+3.58B-2.28C+0.37AB+3.48AC-3.52BC-11.51A²-10.61B²-9.36C²  
(2)

Analysis of variance (ANOVA) for the responses indicated that three factors were significant and valid for each of the responses (p<0.01) shown in Table 2 and Table 3.

Table 2. ANOVA of Z-Ave.

| Source          | Sum of squares | df  | MS    | F value | P    |
|-----------------|----------------|-----|-------|---------|------|
| model           | 45003.56       | 9   | 5000.40| 8.88    | 0.0044|
| C (γ-PGA)       | 1270.08        | 1   | 1270.08| 2.25    | 0.1769|
| C (Gelatin)     | 802.00         | 1   | 802.00 | 1.42    | 0.2717|
| pH of gelatin   | 1232.56        | 1   | 1232.56| 2.19    | 0.1826|
| AB              | 25.50          | 1   | 25.50  | 0.045   | 0.8376|
| BC              | 1228.50        | 1   | 1228.50| 2.18    | 0.1832|
| A²              | 18617.20       | 1   | 18617.20| 33.05  | 0.0007|
| B²              | 17176.32       | 1   | 17176.32| 30.49  | 0.0009|
| C²              | 1080.59        | 1   | 1080.59| 1.92    | 0.2086|
| Residual        | 3943.04        | 7   | 563.29 |         |       |
| lack of fit     | 3466.47        | 3   | 1155.49| 9.70    | 0.2263|
| error           | 476.57         | 4   | 119.14 |         |       |
| Total           | 48946.60       | 16  |       |         |       |
| Std.Dev         | 23.73          |     | R-Squared | 0.9194 |       |
| Mean            | 234.25         |     | Adj R-Squared | 0.9059 |       |
| C.V.%           | 1.13           |     | Pred R-Square | 0.9484 |       |
| PRESS           | 3.50E-0.4      |     | Adep Precision | 38.543 |       |

df: degree of freedom f: fisher value p: probability
Table 3. ANOVA of Zeta-potential

| Source          | Sum of squares | df | MS  | F value | P value  |
|-----------------|----------------|----|-----|---------|----------|
| Model           | 1818.82        | 9  | 202.9 | 35.37   | <0.0001  |
| C(γ-PGA)        | 13.52          | 1  | 13.52 | 2.37    | 0.0167   |
| C(gelatin)      | 102.25         | 1  | 102.25 | 17.89   | 0.0039   |
| pH of gelatin   | 41.40          | 1  | 41.40 | 7.25    | 0.0310   |
| AB              | 0.56           | 1  | 0.56  | 0.098   | 0.7628   |
| AC              | 48.30          | 1  | 48.30 | 8.45    | 0.0227   |
| BC              | 49.70          | 1  | 49.70 | 8.70    | 0.0214   |
| A2              | 557.57         | 1  | 557.57| 97.58   | <0.0001  |
| B2              | 437.76         | 1  | 437.76| 82.91   | <0.0001  |
| C2              | 368.69         | 1  | 368.69| 64.52   | <0.0001  |
| Residual        | 40.00          | 7  | 5.71  |          |          |
| Lack offit      | 32.67          | 3  | 10.89 | 5.94    |          |
| Error           | 7.33           | 4  | 1.83  |          |          |
| Total           |                | 16 |      |         |          |
| Std.Dev         | 2.39           |    |       | R-Squared | 0.9785   |
| Mean            | 23.3           |    |       | Adj R-Squared | 0.9508   |
| C.V.%           | 3.25           |    |       | Pred R-Square | 0.9127   |
| PRESS           | 5.10           |    |       | Adep Precisor | 45.226   |

df: degree of freedom f: fisher value p: probability

Response surface plots allow graphical visual observation that the regression analysis equations are significant. The regression Eqs. (1) (2) were presented as response surface plots in Figure 1.

To validate the regression analysis equation, three groups parallel experiments were conducted as C(γ-PGA) of 0.7g/L, C(gelatin) of 0.5g/L, gelatin pH of 3.0, γ-PGA pH of 8.0, γ-PGA: gelatin = 1: 1, adding speed as 5.0 mL/h, stirring speed as 300r/min, 40℃ for 0.5h. The average Z-Ave was obtained as 189.2nm and Zeta-potential was 36.3Mv. This result is very close to predicted value as 187.6nm and 35.8Mv which indicated a fine predictability of the model.

The final conditions were set as follows: Confet γ-PGA concentration of 0.5g/L and pH of 8 and gelatin concentration of 0.5g/L and pH of 3. Vγ-PGA: Vgelatin=1:2, adding speed of 5mL/h, stirring speed of 300r/min, temperature of 40℃ and stirring time of 30min. For preparing fragrance nanocapsules, emulsifiers were chosen as tween 80 for 0.15g and additive of fragrance was 0.15g/L. Emulsify for 30min at the stirring speed of 400r/min.
3.2. TGA analysis

The dry samples of fragrance nanoparticles were precisely weighed as 0.0050g and measured by TGA. The result was as Figure 2.

The reduction proportion of samples during TGA is shown as in Table 4:

| Sample                  | Reduction of weight (%) |
|-------------------------|-------------------------|
|                         | 0-100°C    | 0-200°C | 0-550°C | 100-200°C | 100-550°C |
| osmanthus fragrance     | 2.76       | 99.54   | /       | 96.79     | /         |
| Empty nanoparticle      | 4.55       | 9.93    | 78.27   | 5.38      | 72.89     |
| Fragrance nanoparticle  | 4.26       | 11.06   | 65.56   | 6.80      | 54.50     |

**Figure. 1** Response surface plots of Z-Ave (a-f) and Zeta-potential (g-i).
It was shown that nude fragrance was barely residual when the temperature increased to 150°C which indicated that fragrance was extremely easy to volatilize away without encapsulated (Fig 2-1). The dried empty nanoparticles lost by 9.93% from 0°C to 150°C (Fig 2-2). That was because the water can hardly be completely removed and there were some reabsorbed water during the process of storage. At 500°C, the weight decreased by 78.27% due to the degradation of wall materials caused by heat (Fig 2-2). After that, the weight stopped changing along with the increasing temperature. With temperature changed from 200°C to 600°C, the weight decreased by 65.56% which was a great progress compared to 99.55% lost in Fig 2-1. Most of fragrance are heat-labile and easily volatilized away. Due to the stability and heat resistance of wall materials, coated by γ-PGA/gelatin nanoparticle endured the core materials. From Fig 2-1 and Fig 2-2, the amount of residue increases by 33.99% with increasing temperature which revealed a great slow-release effect could be imposed by using γ-PGA/gelatin nanoparticle as wall material. [13].

3.3. E-nose results

Electronic nose technology response time is short, fast detection. The tests were conducted 7 times en 3 days. In Figure 3, 1 to 7 represented the flavor fingerprints of nude fragrance and coated nanoparticles solution at room temperature for 12h, 24h, 36h, 48h, 60h and 72h. It could be seen that graph outline merely changed which substantiated the fragrance nanoparticle technology overcomed the disadvantage of different volatilization rates of various components. During the process of storage, the top note and middle note usually tend to volatilize quickly while the base note volatilizes relatively slowlier to create the changes of odor[14]. With the increase of storage period, the outline of flavor fingerprint had no obvious change so producing fragrance nanoparticles through this method did promise the stability.
Figure. 3 sustained release result of fragrance nanoparticle

4. Conclusion
This paper aimed to throw some light on preparation technology and γ-PGA application on fragrance products. As a biocompatible nanocontainer material, γ-PGA used as wall material for fragrance capsule proved to be efficient and effective. This experiment fully took the advantage of its features of moisturizing and non-toxicity, it is foreseeable that γ-PGA/gelatin nanoparticle would be versatile in cosmetic industry both as fragrance carrier and moisturizer. For different types of fragrance including water soluble, oil soluble etc., it is worth to screen out to best emulsifiers to increase encapsulation efficiency. For γ-PGA/gelatin nanoparticle, it can be produced as multilayer structure with ceramic scaffold. It could have superior properties for wider applications which could be the next stage of this research.

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