

RESEARCH ARTICLE

Microencapsulation of *Forsythia suspensa* Essential Oil Based on Complex Coacervation

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ABSTRACT

Plant essential oils possess valuable antibacterial and antioxidant properties, but their high volatility and chemical instability severely limit their direct use in functional materials. In this study, *Forsythia suspensa* essential oil was successfully encapsulated within a gelatin wall material by complex coacervation, with sodium hexa-metaphosphate as a coacervation promoter and transglutaminase as a green crosslinking agent. The influence of the core-to-wall ratio, coacervation temperature, pH and stirring speed on the encapsulation efficiency was first evaluated through single-factor experiments, after which a three-factor, three-level Box–Behnken design was employed to optimize the dominant variables. A reduced hierarchical quadratic model was statistically significant ($p = 0.0084$; $R^2 = 0.7724$; adjusted $R^2 = 0.6359$), with the response governed primarily by significant quadratic curvature in all three variables; the individual linear effects were not statistically significant and were therefore not ranked. Under the optimised conditions of a core-to-wall ratio of 1:1.1, a temperature of 39 °C and a pH of 4.69, a predicted encapsulation efficiency of 97.52% was obtained. The resulting microcapsules displayed a particle size distribution of 0.25–10 µm ($D_{90} = 4.76$ µm) and a zeta potential of –32.25 mV, indicating appreciable electrostatic repulsion. SEM and AFM analyses revealed predominantly rounded micron-scale particles with smooth continuous surfaces (mean diameter 2.38 µm; RMS roughness 1.245 nm). Microcapsules displayed clear sustained-release behaviour and were successfully deposited onto cotton and linen textiles by a pad–dry–cure process.

Keywords: *Forsythia suspensa* essential oil; complex coacervation; gelatin wall material; encapsulation efficiency; Box–Behnken optimisation; sustained release; transglutaminase crosslinking; textile finishing

ARTICLE INFO

Received: 04 June 2026

Accepted: 17 June 2026

Available online: 29 September 2026

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1. Introduction

Forsythia suspensa (Thunb.) Vahl, also known as Lianqiao in TCM, is a deciduous shrub distributed across China, whose fruits are traditionally used for their antibacterial, anti-inflammatory, and antipyretic properties. Essential oil from *Forsythia suspensa* (FEO) contains a high concentration of terpenoids and demonstrates potent antibacterial and antioxidant properties towards a range of common food-related and skin-associated bacterial infections including *Escherichia coli* and *Staphylococcus aureus* [2]. Recent studies proved that FEO could also be successfully implemented as micro-emulsions and nanoparticles, resulting in higher antimicrobial activity against bacteria [3]. Still, just like other essential oils, it evaporates and decomposes easily; hence, there is a need for developing a stable carrier system.

Microencapsulation appears to be an efficient tool to tackle these problems. It will not only preserve the essential oil but also allow sustained release of the compound by forming a stable core-shell structure around the volatile molecule. Of all existing approaches, complex coacervation seems to be especially appealing since it requires aqueous environment, has relatively high payloads, encapsulation efficiency, and produces highly stable and effective microencapsulation systems ^[4, 5, 6]. Complex coacervation involves interaction of two oppositely charged biopolymers at pH lower than the iso-ionic point, leading to formation of polymer-rich coacervate phase surrounding dispersed oil droplets ^[5].

The choice of wall material is a very important aspect in this technology. In many cases, gelatin turns out to be an ideal compound due to its cost-effectiveness, compatibility, biodegradability, amphiphaticity, and good film-forming properties ^[6, 7]. The main drawback of this technique, however, lies in the necessity to introduce cross-linking agents for stabilization of the physically formed coating. While most of them involve toxic chemicals, such as aldehydes, transglutaminase can create ϵ -(γ -glutamyl) lysine bonds between amino acid groups within the gelatin chains under very mild conditions ^[12, 13]. Microencapsulated essential oils, thus, will not only become stable but also more environmentally friendly after hardening. They can then be incorporated into textile fibers and provide the fabric with new functionalities ^[17, 18, 19].

The incorporation of microencapsulated essential oils into textiles has attracted growing interest as a route to multifunctional fabrics that combine wearer comfort with active performance. Through padding, exhaustion or coating processes, microcapsules can be fixed onto cotton, linen and other cellulosic fibres to confer antibacterial, antifungal, insect-repellent, deodorising, fragrance-release and skin-care functionalities, while the encapsulating shell protects the volatile payload from premature evaporation and oxidation and provides a reservoir for its gradual, friction- or moisture-triggered release during wear and laundering ^[17, 18, 19, 20]. The properties of the polymeric shell govern the performance of such systems. A wide range of wall materials has been employed, including natural biopolymers such as gelatin, gum arabic, chitosan and sodium alginate, and synthetic polymers such as polyurethane and melamine-formaldehyde resins. Natural protein-polysaccharide shells are favoured for textile and skin-contact applications because they are biodegradable, biocompatible, film-forming and amenable to mild, aqueous, enzyme-mediated crosslinking, whereas synthetic shells generally offer greater mechanical robustness and wash durability at the cost of reduced biodegradability and potential residual-monomer toxicity. Gelatin-based complex-coacervate shells, in particular, combine good film formation, controllable wall thickness and tunable permeability, making them well suited to sustained fragrance and antimicrobial release on fabrics ^[6, 18, 19]. *Forsythia suspensa* essential oil is a particularly attractive candidate for such finishes: its terpenoid-rich composition confers broad antibacterial activity against common skin- and textile-associated organisms such as *Escherichia coli* and *Staphylococcus aureus*, together with antifungal and deodorising potential, so that its stable deposition on cotton and linen could yield antibacterial, antifungal and odour-controlling textiles analogous to the antimicrobial and fragrance finishes already demonstrated for encapsulated lavender, citrus and other essential oils ^[18, 19, 20].

Despite the growing literature on essential oil microencapsulation, systematic studies on the complex coacervation of *F. suspensa* essential oil with a gelatin wall material, including process optimisation and its application to functional textiles, remain scarce. The aim of this study is therefore to prepare *Forsythia suspensa* essential oil microcapsules by complex coacervation, to optimise the preparation conditions through single-factor experiments combined with Box-Behnken response surface methodology, to characterize the structural, physicochemical and thermal properties of the optimised microcapsules, and to demonstrate their applicability in the functional finishing of cotton and linen fabrics.

2. Materials and methods

2.1. Materials and reagents

Gelatin (99% BR) was supplied by Karma. *Forsythia suspensa* essential oil was used as the core material. Sodium hexa-metaphosphate (AR grade; Sinopharm Chemical Reagent), which promoted coacervation, phosphoric acid solution (AR grade; Xilong Scientific), used for pH adjustment, and transglutaminase (TG) enzyme solution (Dongsheng Biological), used as the crosslinking agent, were employed in the preparation. Ultrapure water (Eco exceed-c) was used throughout. Anhydrous ethanol was used as the extraction solvent for the determination of free oil. The cotton and linen fabrics used for the finishing experiments were pre-treated prior to use.

2.2. Instruments and equipment

The principal instruments comprised a T-20 high-speed homogeniser (IKA, Germany), a ZNCL-GS temperature-controlled magnetic stirrer (Shanghai Lingke), an FE28 pH meter (Mettler Toledo), a Brookhaven 90Plus PALS laser particle size and zeta potential analyser (Brookhaven, USA), a JSM-7610FPlus field-emission scanning electron microscope (JEOL, Japan), a Cypher VRS high-speed atomic force microscope (Oxford Instruments), an STA449 F5 simultaneous thermal analyser (NETZSCH, Germany), an E5 biological microscope (Ningbo Sunny Instruments), a T1 UV–visible spectrophotometer (Shanghai Aoyi) and a JC-100-MEC constant temperature and humidity chamber (Qingdao Jingcheng).

2.3. Preparation of *Forsythia suspensa* essential oil microcapsules

The microcapsules were prepared by complex coacervation following a modified literature procedure [6, 16]. First, 4 g of gelatin and 46 g of ultrapure water were heated in a 60 °C water bath for 2 h to obtain a gelatin solution. Then, 4 g of *Forsythia suspensa* essential oil was mixed with 50 g of the gelatin solution (8%, w/w) and pre-emulsified at 1200 rpm for 5 min using the magnetic stirrer, after which the mixture was homogenized at 20000 rpm for 10 min to produce a stable oil-in-water (O/W) emulsion. A gelatin concentration of 8% (w/w) was selected because it lies within the wall-material concentration range (typically about 1–10%, w/w) most widely reported for gelatin-based complex-coacervation encapsulation of essential oils, where it provides a solution viscosity high enough to stabilise the oil-in-water emulsion and to supply sufficient wall material to fully envelop the dispersed droplets, yet low enough to avoid the excessive viscosity and incomplete phase separation that hinder coacervate deposition at higher concentrations [6, 7, 8].

Subsequently, 50 g of the oil-in-water emulsion was combined with 50 g of sodium hexametaphosphate solution (0.53%, w/w). The coacervation temperature and stirring speed were then set according to the applicable single-factor or Box–Behnken experimental condition. A 1% phosphoric acid solution was added dropwise while the mixture was stirred until the prescribed target pH was reached. After the target pH had been attained, coacervation was continued for 30 min under the corresponding experimental temperature and agitation condition. Thereafter, 10 mL of transglutaminase solution (1%, w/w) was added. Heating was discontinued and crosslinking was performed at 300 rpm for 2 h. Homogenisation speed and duration, sodium hexametaphosphate concentration, acid concentration, post-coacervation holding time, transglutaminase dose and final crosslinking conditions were kept constant across the optimisation experiments. During the single-factor experiments, the core-to-wall ratio was varied during formulation of the initial oil-in-water emulsion. Temperature, target pH and stirring speed were varied during the coacervation stage after addition of sodium hexametaphosphate and before transglutaminase crosslinking. The overall preparation route is summarised in Figure 1.

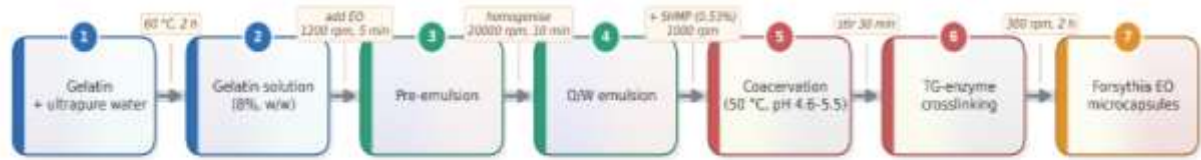


Figure 1. Schematic of the preparation of *Forsythia suspensa* essential oil microcapsules by complex coacervation. The schematic depicts the general experimental workflow used throughout the single-factor and Box–Behnken experiments; the temperature and pH shown are representative process stages and not the final optimised conditions, which were established as a core-to-wall ratio of 1:1.1, a temperature of 39 °C and a pH of 4.69 (see Section 3.4).

2.4. Determination of encapsulation efficiency

A standard curve was first established by dissolving the essential oil in anhydrous ethanol at concentrations of 2.5, 5, 7.5, 15, 20, 30 and 40 $\mu\text{g mL}^{-1}$ and measuring the absorbance at 300 nm. The encapsulation efficiency was determined from the free-oil content. A 1 g portion of the microcapsule emulsion was mixed with 5 mL of anhydrous ethanol and vortexed for 1 min; the supernatant was collected after standing, and the container was rinsed three times with 5 mL of anhydrous ethanol. The absorbance of the combined solution was measured and converted to the free-oil mass using the standard curve. The encapsulation efficiency (EE) was calculated according to Equation (1):

$$EE (\%) = (W_{\text{total}} - W_{\text{free}}) / W_{\text{total}} \times 100 \quad (1)$$

Where, W_{total} is the total mass of essential oil added to the system and W_{free} is the mass of free (non-encapsulated) oil.

2.5. Single-factor experimental design

Four key parameters were selected for single-factor analysis: the core-to-wall ratio (A; gelatin-to-oil mass ratio), the coacervation temperature (B), the pH (C) and the stirring speed (D). Each factor was examined at five levels (Table 1). When one factor was varied, the remaining three were held at their central levels (A_3 , B_3 , C_3 , D_3) to minimise interaction effects. The investigated ranges were 1:0.5–1:2.5 for the core-to-wall ratio, 30–70 °C for the coacervation temperature, 4.7–5.1 for the pH and 400–1200 rpm for the stirring speed. The investigated ranges were selected to cover both technically insufficient and potentially excessive processing conditions. The core-to-wall range extended from relatively wall-limited to wall-rich formulations; the temperature range covered moderate and elevated coacervation conditions; the pH range encompassed the coacervation window of the gelatin–sodium hexametaphosphate system; and the stirring-speed range extended from gentle mixing to relatively high agitation. Because encapsulation efficiency remained high at the lower pH boundary of the single-factor experiment, the lower limit was extended from pH 4.7 to pH 4.6 in the subsequent Box–Behnken design to permit localisation of the response maximum.

Table 1. Levels of the single-factor experiments.

Factor	Level 1	Level 2	Level 3	Level 4	Level 5
A: Core-to-wall ratio	1:0.5	1:1	1:1.5	1:2	1:2.5
B: Coacervation temperature (°C)	30	40	50	60	70
C: pH	5.1	5.0	4.9	4.8	4.7
D: Stirring speed (rpm)	400	600	800	1000	1200

2.6. Box–Behnken response surface optimisation

On the basis of the single-factor results, which showed that the stirring speed had a negligible effect on the encapsulation efficiency, the core-to-wall ratio (A), temperature (B) and pH (C) were selected as

independent variables and the encapsulation efficiency was taken as the response. A three-factor, three-level Box–Behnken design (BBD) comprising 17 runs was constructed and analysed using Design-Expert 13 software. The coded levels (−1, 0, +1) corresponded to core-to-wall ratios of 1:0.5, 1:1 and 1:1.5, temperatures of 30, 40 and 50 °C, and pH values of 4.6, 4.7 and 4.8, respectively.

2.7. Particle size and zeta potential analysis

The microcapsule emulsion was diluted with deionised water to 1 g L^{−1}, and the mean particle size, size distribution and zeta potential were measured using the laser particle size and zeta potential analyser at room temperature.

2.8. Morphological characterisation by optical microscopy, SEM and AFM

For optical microscopy, the undiluted microcapsule emulsion was placed directly on a glass slide, covered with a coverslip and observed using the E5 biological microscope. For scanning electron microscopy (SEM), the emulsion was diluted with ultrapure water to a transparent solution, ultrasonicated for 10 min, and one drop was deposited on a silicon wafer and dried at 35 °C. The samples were sputter-coated with gold under vacuum before imaging. For atomic force microscopy (AFM), 0.05 mL of the liquid microcapsule sample was spin-coated onto a silicon substrate and dried naturally at room temperature. A PPP-FMAuD-10 probe (nominal spring constant 0.5–9.5 N m^{−1}) was used with a scan size of 2 µm at a scan frequency of 2.44 Hz, and the images were analysed using Nanoscope Analysis software to obtain the average surface roughness.

2.9. Thermal stability analysis

The thermal stability of the microcapsules was evaluated using the simultaneous thermal analyser. Approximately 10 mg of sample was heated from 50 to 600 °C at a rate of 10 °C min^{−1} under a high-purity nitrogen atmosphere at a flow rate of 30 mL min^{−1}, and the thermogravimetric (TG) and differential thermal analysis (DTA) curves were recorded.

2.10. Sustained-release testing

A known mass of free essential oil and of the essential oil microcapsules was placed in separate dishes and stored in constant-temperature chambers at 4 and 25 °C under dark conditions with controlled humidity. Samples were taken at 0, 1, 2, 3, 5, 7, 9, 12, 15 and 25 days, and the release rate was calculated according to Equation (2):

$$\text{Release rate (\%)} = (m_0 - m_i) / m_0 \times 100 \quad (2)$$

where m_0 is the initial mass of essential oil in the sample and m_i is the mass remaining after a given storage time.

2.11. Textile finishing and padding treatment

Pre-weighed cotton (≈45 g) and linen (≈40 g) fabrics were finished with the microcapsules using a pad–dry–cure process. The finishing liquor was prepared by adding a dispersant and a penetrant to deionised water under low-speed magnetic stirring for 5 min, followed by the slow addition of the microcapsule finishing agent (stirred for 5 min) and a water-based binder (stirred for 10 min) until a uniform, particle-free liquor was obtained. The fabrics were finished by a two-dip–two-nip padding procedure at room temperature with a pick-up of 65% and a speed of 2 m min^{−1}, pre-dried at 60 °C for 5 min and cured at 80 °C for 3 min, after which they were cooled flat to room temperature.

2.12. Statistical analysis and model fitting

All single-factor experiments were independently performed a minimum of three times, and the results are reported as mean ± standard deviation. Statistical comparisons were conducted separately for each experimental factor. Residual normality and variance homogeneity were assessed using the Shapiro–Wilk and

Levene tests, respectively. When the assumptions of conventional analysis of variance were satisfied, treatment means were compared by one-way analysis of variance followed by Tukey's honestly significant difference test. When variance homogeneity was not satisfied, Welch's analysis of variance followed by the Games–Howell procedure was used. Differences were considered statistically significant at $p < 0.05$. The Box–Behnken responses were fitted using Design-Expert 13. The initial full quadratic model contained the linear, two-factor interaction and quadratic terms. Non-contributory interaction terms were evaluated collectively through a partial F-test and were removed when they did not improve model fit, while the linear terms corresponding to retained quadratic terms were preserved to maintain model hierarchy. Model adequacy was evaluated using the model F-test, lack-of-fit test, R^2 , adjusted R^2 , predicted R^2 and residual diagnostics; the optimum identified from the model was reported as a model prediction. The repeated centre points were used to estimate pure experimental error. Statistical symbols, including p , are presented in *italics* throughout the manuscript.

3. Results

3.1. Standard curve and encapsulation efficiency

Linear fitting of the absorbance at 300 nm against the essential oil concentration yielded the standard curve $y = 0.0385x + 0.816$ with a coefficient of determination $R^2 = 0.9939$ (Figure 2), confirming an excellent linear relationship over the tested concentration range and validating the use of this curve for quantifying the free-oil content and hence the encapsulation efficiency.

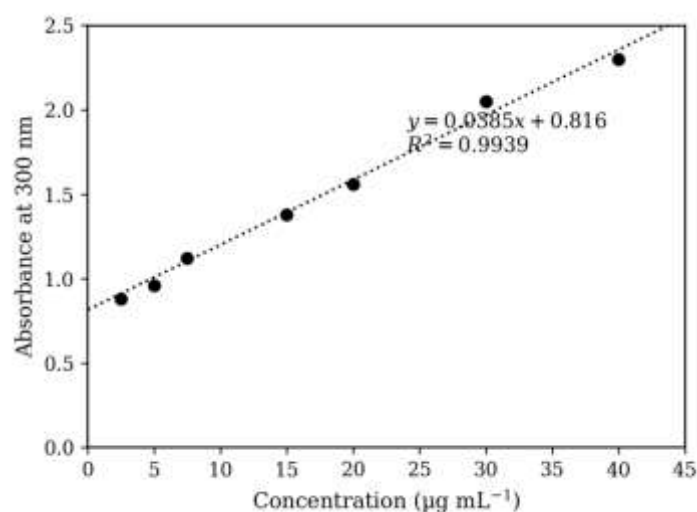


Figure 2. Standard curve of the *Forsythia suspensa* essential oil for the determination of encapsulation efficiency.

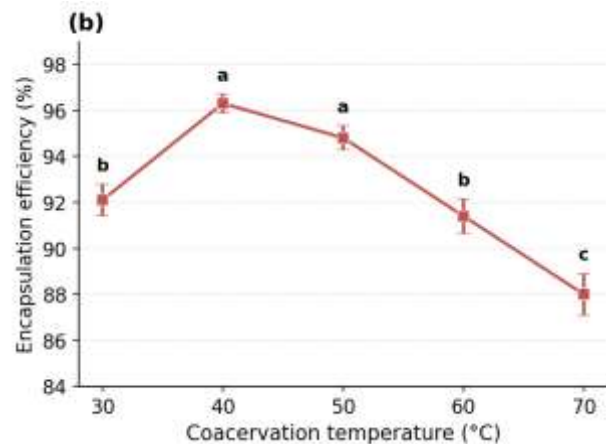
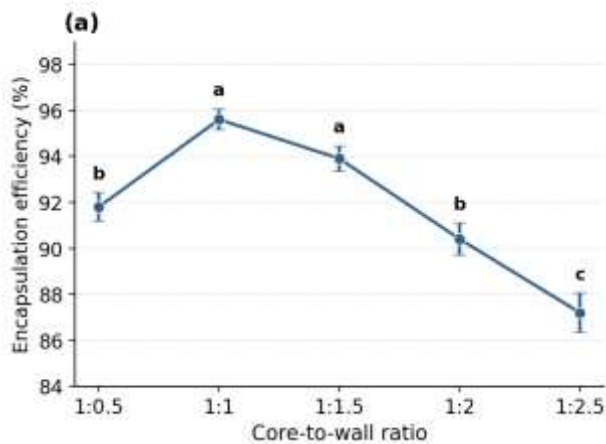
3.2. Effects of core-to-wall ratio, temperature, pH and stirring speed

The influence of the four single factors on the encapsulation efficiency is presented in Figure 3. As the core-to-wall ratio increased from 1:0.5 to 1:2.5, the encapsulation efficiency first rose and then declined, reaching a maximum at a ratio of 1:1 before decreasing progressively at higher oil loadings. The coacervation temperature exhibited a similar trend, with the efficiency peaking at 40 °C and decreasing at higher temperatures. The pH had a marked effect: within the range 4.7–5.1 the efficiency decreased steadily as the pH increased, indicating that a mildly acidic environment favoured coacervate formation. In contrast, the stirring speed had little influence over the range 400–1200 rpm, indicating that the cured shell possessed sufficient mechanical strength to resist disruption under the investigated agitation conditions. The

corresponding single-factor encapsulation-efficiency values (mean $\pm$ SD, $n = 3$) and the results of the one-way analysis of variance with Tukey HSD comparisons are summarised in Table 2.

Table 2. Single-factor encapsulation efficiency (mean $\pm$ SD, $n = 3$) with one-way ANOVA and Tukey HSD groupings. Within each factor, levels not sharing a letter differ significantly ($p < 0.05$); n.s. = not significant.

Factor	Level	Encapsulation efficiency (%), mean $\pm$ SD	Group
A: Core-to-wall ratio (one-way ANOVA: $F = 74.88, p < 0.001$)			
	1:0.5	91.80 $\pm$ 0.62	b
	1:1	95.60 $\pm$ 0.45	a
	1:1.5	93.90 $\pm$ 0.55	a
	1:2	90.40 $\pm$ 0.70	b
	1:2.5	87.20 $\pm$ 0.85	c
B: Coacervation temperature (°C) (one-way ANOVA: $F = 68.90, p < 0.001$)			
	30	92.10 $\pm$ 0.68	b
	40	96.30 $\pm$ 0.40	a
	50	94.80 $\pm$ 0.52	a
	60	91.40 $\pm$ 0.74	b
	70	88.00 $\pm$ 0.90	c
C: pH (one-way ANOVA: $F = 36.80, p < 0.001$)			
	4.7	95.90 $\pm$ 0.44	a
	4.8	95.00 $\pm$ 0.50	a
	4.9	93.60 $\pm$ 0.58	b
	5.0	92.40 $\pm$ 0.62	b
	5.1	90.80 $\pm$ 0.72	c
D: Stirring speed (rpm) (one-way ANOVA: $F = 0.45, p = 0.773$ (n.s.))			
	400	93.40 $\pm$ 0.55	a
	600	93.70 $\pm$ 0.50	a
	800	93.90 $\pm$ 0.48	a
	1000	93.50 $\pm$ 0.52	a
	1200	93.50 $\pm$ 0.54	a



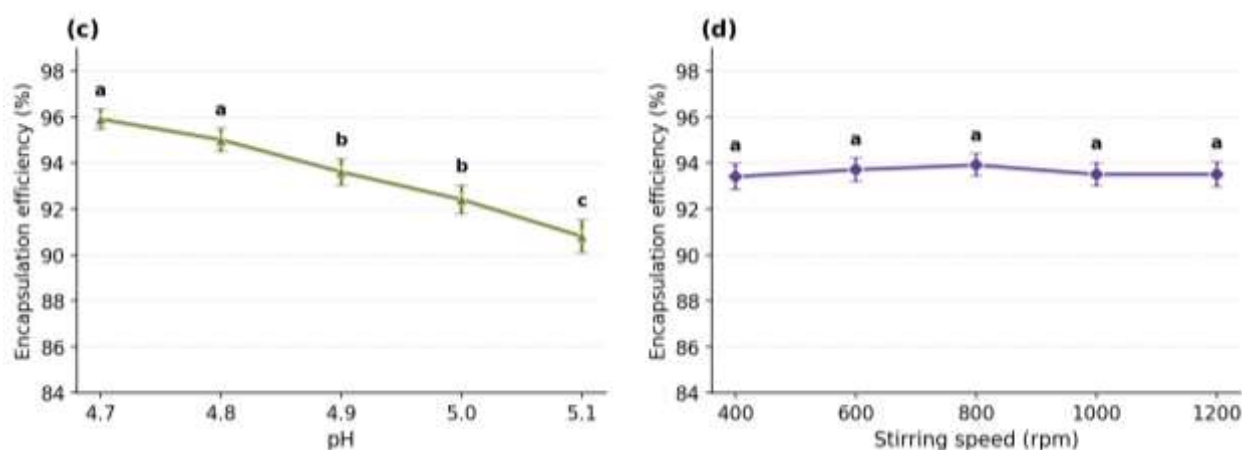


Figure 3. Effects of single process variables on encapsulation efficiency: (a) core-to-wall ratio, (b) coacervation temperature, (c) target pH and (d) coacervation-stage stirring speed. Values are mean $\pm$ SD of three independent preparation batches ($n = 3$). Different letters within each panel indicate significant differences at $p < 0.05$ (one-way ANOVA followed by Tukey HSD); panel (d) showed no significant differences.

The corresponding optical micrographs (Figure 4) confirmed that the most uniform and intact microcapsule populations were obtained at a core-to-wall ratio of approximately 1:1 and a temperature of 40 °C, in agreement with the encapsulation efficiency data.

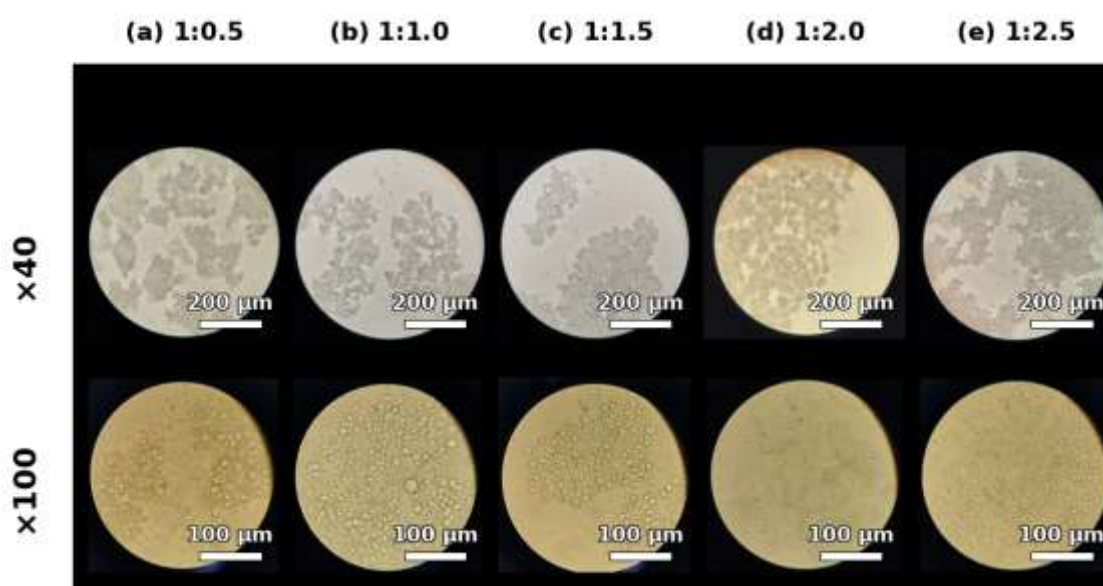


Figure 4. Optical micrographs of *Forsythia suspensa* essential-oil microcapsules prepared at core-to-wall ratios of (a) 1:0.5, (b) 1:1.0, (c) 1:1.5, (d) 1:2.0 and (e) 1:2.5. Upper row (a–e): $\times 40$ objective setting, scale bar 200 μm ; lower row (a–e): $\times 100$ objective setting, scale bar 100 μm .

3.3. Response surface model and ANOVA

Guided by the single-factor results, the stirring speed was excluded and the core-to-wall ratio (A), temperature (B) and pH (C) were optimised using a Box–Behnken design. The design matrix and the measured encapsulation efficiencies for the 17 runs are listed in Table 3.

Table 3. Box–Behnken experimental design and encapsulation efficiency results.

Run	A: Core-to-wall ratio	B: Temperature (°C)	C: pH	Encapsulation efficiency (%)
1	1:1.5	50	4.7	93.32
2	1:1.5	40	4.6	94.68
3	1:1	40	4.7	96.40
4	1:1	40	4.7	98.15
5	1:1	40	4.7	97.36
6	1:0.5	50	4.7	91.63
7	1:1	40	4.7	97.80
8	1:1.5	30	4.7	95.43
9	1:1	40	4.7	97.50
10	1:1.5	40	4.8	94.24
11	1:1	50	4.6	95.80
12	1:0.5	30	4.7	95.42
13	1:1	30	4.6	93.08
14	1:1	30	4.8	93.67
15	1:1	50	4.8	92.87
16	1:0.5	40	4.6	93.15
17	1:0.5	40	4.8	93.72

Multiple regression of the experimental data gave the following second-order polynomial model for the encapsulation efficiency (Y):

$$Y = 97.4420 + 0.4688A - 0.4975B - 0.2763C - 1.6997A^2 - 1.7923B^2 - 1.7948C^2 \quad (3)$$

The analysis of variance for the reduced hierarchical model is summarised in Table 4. The initial full quadratic model was statistically significant; however, none of the individual linear or two-factor interaction terms reached the 0.05 significance level, whereas A^2 , B^2 and C^2 were all significant. A partial F-test showed that the three interaction terms did not collectively improve model fit ($F = 0.933$, $p = 0.474$). Therefore, to reduce unnecessary parameterisation while retaining model hierarchy, AB, AC and BC were removed and the linear and quadratic terms were retained. The reduced model was significant ($F = 5.66$, $p = 0.0084$), with $R^2 = 0.7724$, adjusted $R^2 = 0.6359$ and predicted $R^2 = 0.1571$. Because all three linear terms remained non-significant, the factors were not ranked according to their F-values. These results indicate that encapsulation efficiency was governed principally by curvature around an interior optimum rather than by simple monotonic linear effects.

Table 4. Analysis of variance (ANOVA) of the response surface regression model.

Source	Sum of squares	df	Mean square	F-value	p-value	Significance
Model	48.21	6	8.04	5.66	0.0084	Significant
A – Core-to-wall ratio	1.76	1	1.76	1.24	0.2920	–
B – Temperature	1.98	1	1.98	1.39	0.2651	–
C – pH	0.61	1	0.61	0.43	0.5269	–
A^2	12.16	1	12.16	8.56	0.0151	*
B^2	13.52	1	13.52	9.52	0.0115	*
C^2	13.56	1	13.56	9.55	0.0114	*
Residual	14.21	10	1.42	–	–	–
Lack of fit	12.48	6	2.08	4.82	0.0748	Not significant
Pure error	1.73	4	0.43	–	–	–
Total	62.42	16	–	–	–	–

Reduced hierarchical model (linear and pure-quadratic terms; non-significant two-factor interactions AB , AC and BC removed): $R^2 = 0.7724$; adjusted $R^2 = 0.6359$; predicted $R^2 = 0.1571$. Model $F = 5.66$, $p = 0.0084$. * Significant at $p < 0.05$.

3.4. Optimisation of preparation conditions

The three-dimensional response surfaces and the corresponding two-dimensional contour plots for each pair of interacting factors are shown in Figures 5–7. The convex surfaces confirm the existence of a well-defined interior optimum within the design space. Because no interaction terms were retained in the reduced model, the response surfaces illustrate the independent quadratic effects of each factor pair with the third variable held at its centre level, and should not be interpreted as evidence of significant pairwise interactions. The stationary point of the reduced hierarchical model occurred at a coded factor combination corresponding to a core-to-wall ratio of approximately 1:1.069, a temperature of 38.61 °C and a pH of 4.692, for which the predicted encapsulation efficiency was 97.52%. For practical operation, the settings were rounded to a core-to-wall ratio of 1:1.1, a temperature of 39 °C and a pH of 4.69. This optimum is model-predicted; the encapsulation efficiency of 97.52% is the value forecast by the reduced hierarchical model at the optimised settings, and a separate set of dedicated confirmation batches was not performed in the present study. Because no interaction terms were retained in the final model, Figures 5–7 are described as paired-factor response surfaces with the third factor held at its centre level, and do not represent statistically significant interactions.

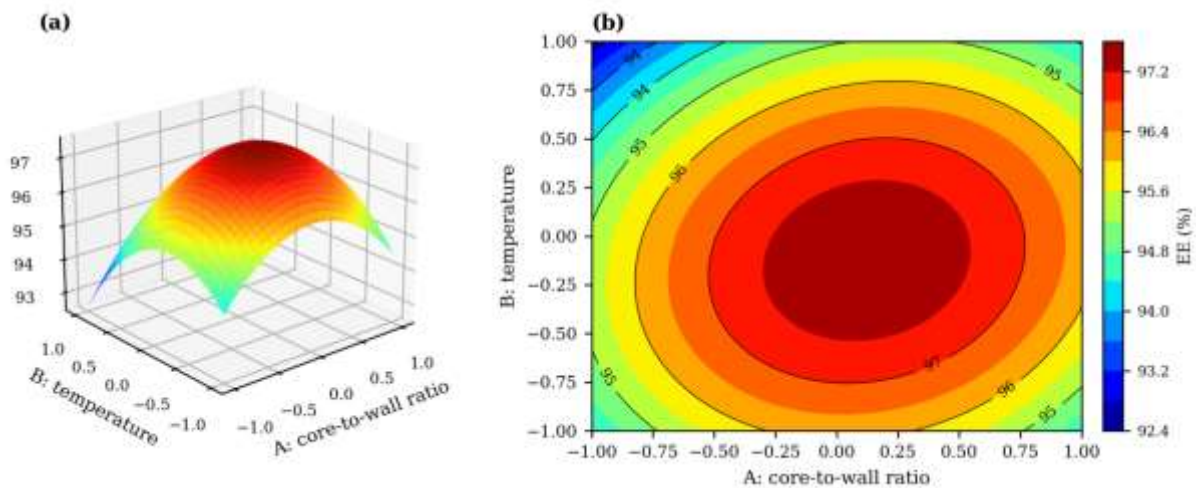


Figure 5. Interactive effect of core-to-wall ratio and temperature on the encapsulation efficiency: (a) response surface; (b) contour plot.

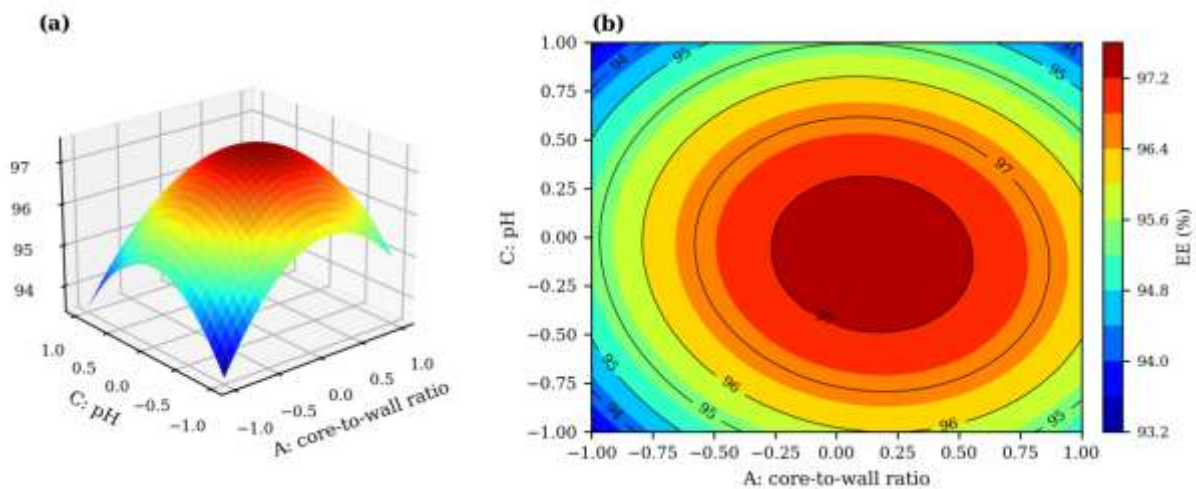


Figure 6. Interactive effect of core-to-wall ratio and pH on the encapsulation efficiency: (a) response surface; (b) contour plot.

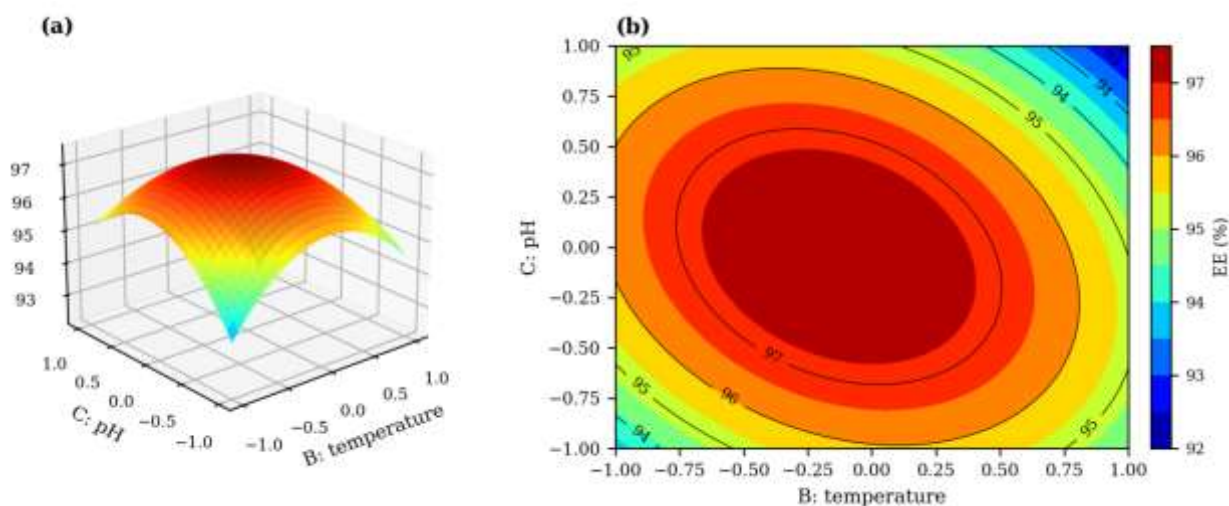


Figure 7. Interactive effect of temperature and pH on the encapsulation efficiency: (a) response surface; (b) contour plot.

3.5. Particle size distribution and zeta potential

The particle size distribution of the optimised microcapsules (Figure 8) spanned 0.25–10 μm and was predominantly unimodal and right-skewed, with a D_{90} of 4.76 μm , indicating that only a small fraction of larger particles was present. This narrow distribution is consistent with the uniform particle population observed by AFM and satisfies the particle-size requirements for textile applications. The zeta potential distribution (Figure 9) was centred near -32 mV, giving an absolute value of 32.25 mV; because an absolute zeta potential at or above 30 mV is generally associated with good colloidal stability, this result indicates that the microcapsule dispersion possessed strong electrostatic repulsion and good physical stability.

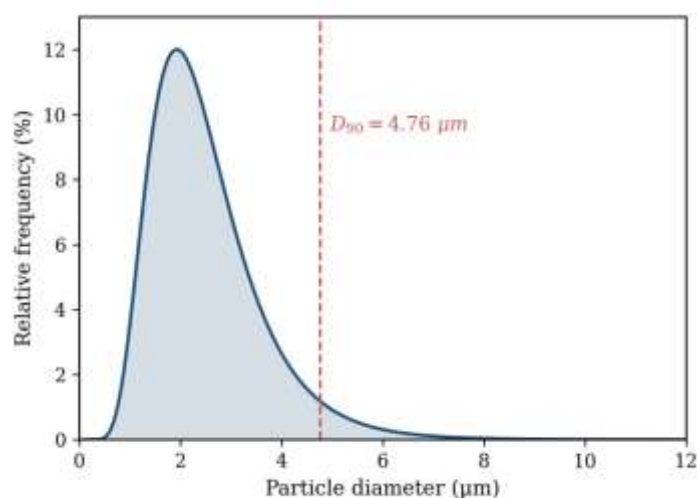


Figure 8. Particle size distribution of the optimised *Forsythia suspensa* essential oil microcapsules.

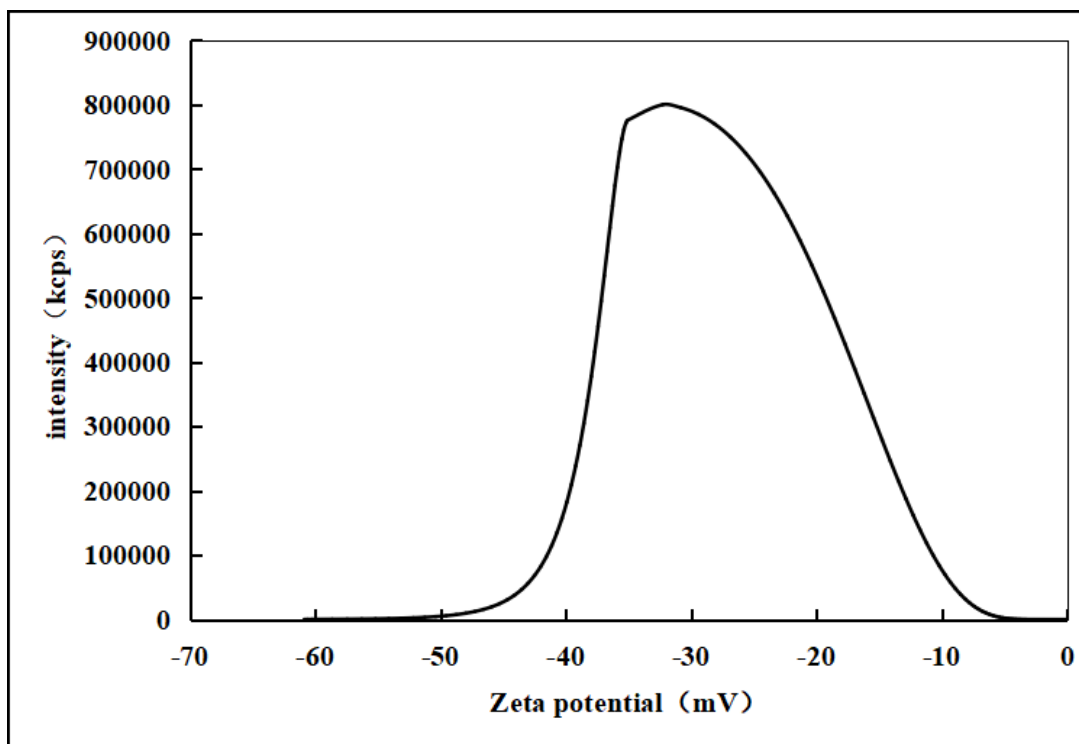


Figure 9. Zeta potential distribution of the optimised microcapsules.

3.6. SEM and AFM morphology

SEM images recorded at magnifications of $\times 2000$, $\times 5000$ and $\times 10000$ (Figure 10) revealed a predominantly rounded-to-ovoid particle population with local aggregation and some flattening, particularly at higher magnification. Such deformation may occur during drying, vacuum exposure and conductive coating of hydrated gelatin-based particles. No widespread fracture or complete collapse was evident; however, the surface SEM images did not independently demonstrate an internal core-shell architecture. AFM analysis (Figure 11) showed isolated rounded surface features with a mean lateral diameter of approximately $2.38\ \mu\text{m}$ and an RMS roughness of $1.245\ \text{nm}$. Together, the SEM and AFM observations support the formation of micron-scale particles with relatively continuous external surfaces. Direct confirmation of the internal core-shell structure would require cross-sectional electron microscopy or transmission electron microscopy. Calibrated scale bars are provided on all SEM and AFM panels.

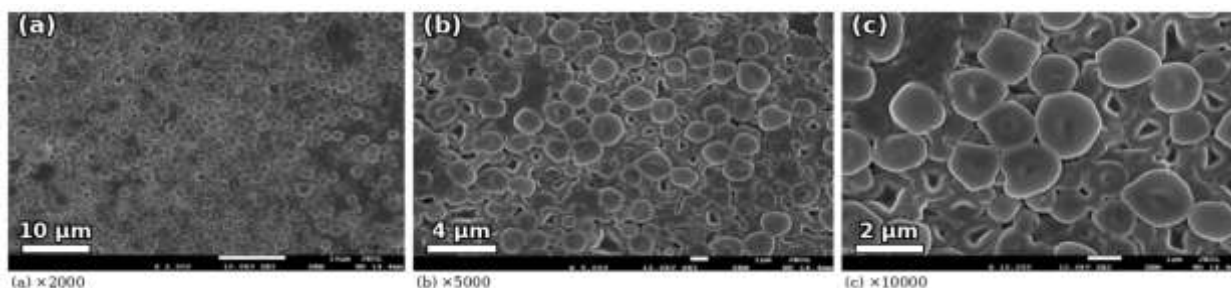


Figure 10. SEM images of the optimised microcapsules at (a) $\times 2000$, scale bar $10\ \mu\text{m}$; (b) $\times 5000$, scale bar $4\ \mu\text{m}$; and (c) $\times 10000$, scale bar $2\ \mu\text{m}$. Instrument scale bars from the original JEOL export are retained in the lower information bar of each panel.

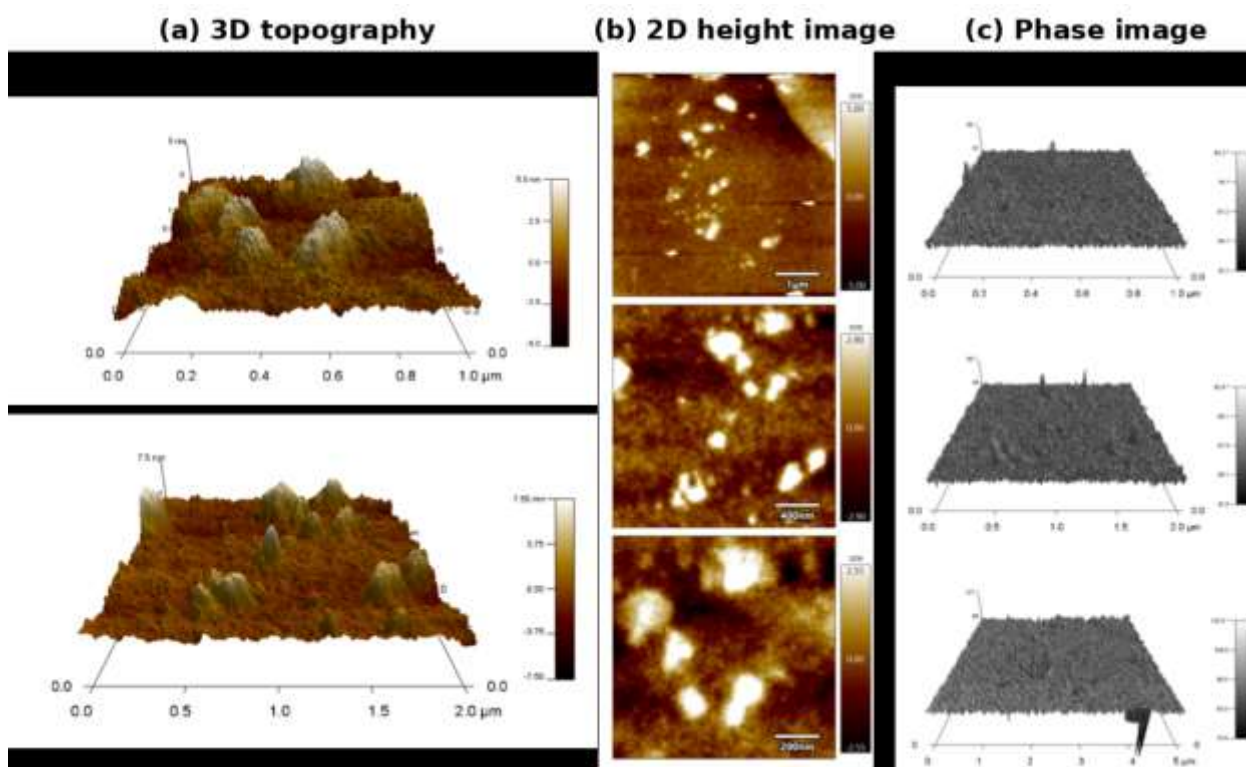


Figure 11. AFM analysis of the microcapsules: three-dimensional topography (left), two-dimensional height images (centre) and phase images (right) at decreasing scan sizes.

3.7. Thermal stability

The TG and DTA profiles of the microcapsules are presented in Figure 12. The initial mass loss below approximately 115 °C was attributed primarily to desorption of physically associated water and loss of low-boiling volatile components; more than 90% of the initial mass was retained over this temperature region. The principal mass-loss event occurred between approximately 115 and 170 °C and was accompanied by a DTA peak near 143 °C, consistent with overlapping volatilisation or decomposition of the encapsulated oil and thermal degradation of the gelatin-based matrix. Above approximately 170 °C, the rate of mass loss decreased and a residual solid fraction remained. Because the presented thermogram was obtained only for the microcapsules, it defines their thermal-decomposition profile but does not, by itself, quantify improvement relative to free *Forsythia suspensa* essential oil or neat gelatin. A direct comparison requires control thermograms obtained under identical analytical conditions, which were not acquired in the present study; accordingly, no claim of improved thermal stability relative to the free oil is made.

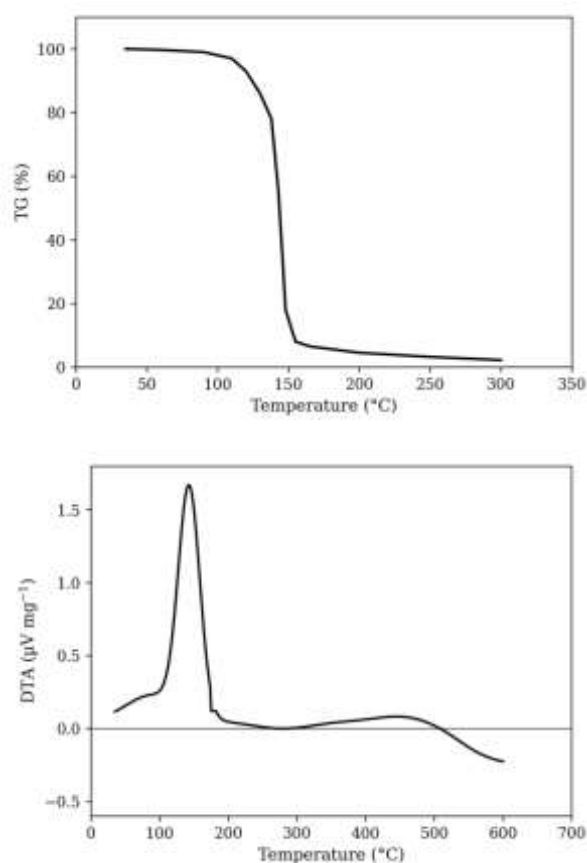


Figure 12. Thermal analysis of the microcapsules: TG curve (upper) and DTA curve (lower).

3.8. Sustained-release performance at 4 and 25 °C

The results for the total release of the free essential oil and the microcapsules at 4 °C and 25 °C can be seen in Figure 13 below. It was evident that the free essential oil was quickly released from its storage and that almost all of it was released during the duration of the experiment. The microcapsules took much longer to release the contents at both temperatures, showing an excellent sustained release mechanism due to the presence of the gelatin coating.

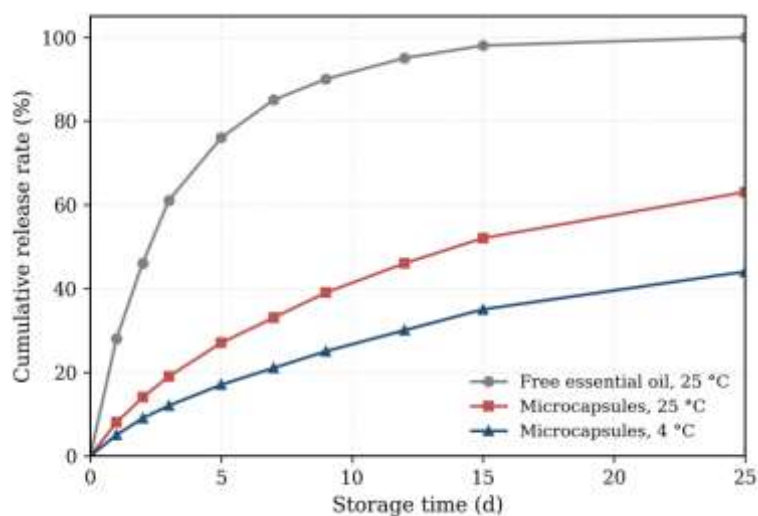


Figure 13. Cumulative release of free essential oil and microcapsules during storage at 4 and 25 °C.

3.9. Surface deposition of microcapsules on finished textiles

The SEM images obtained from the fabric samples (Figures 14 and 15) demonstrated successful surface deposition of the microcapsules onto the fibres of both cotton and linen substrates following the pad–dry–cure treatment. Distinct spherical particles were visible on the finished fibres, which were absent from the unfinished controls. These observations establish the feasibility of depositing the prepared microcapsules onto cellulosic fabrics using a conventional finishing process. Wash durability, retained essential-oil content after laundering, antibacterial activity, odour persistence and mechanical effects on the fabrics were not measured in the present study and should be investigated before durable functional performance is claimed.

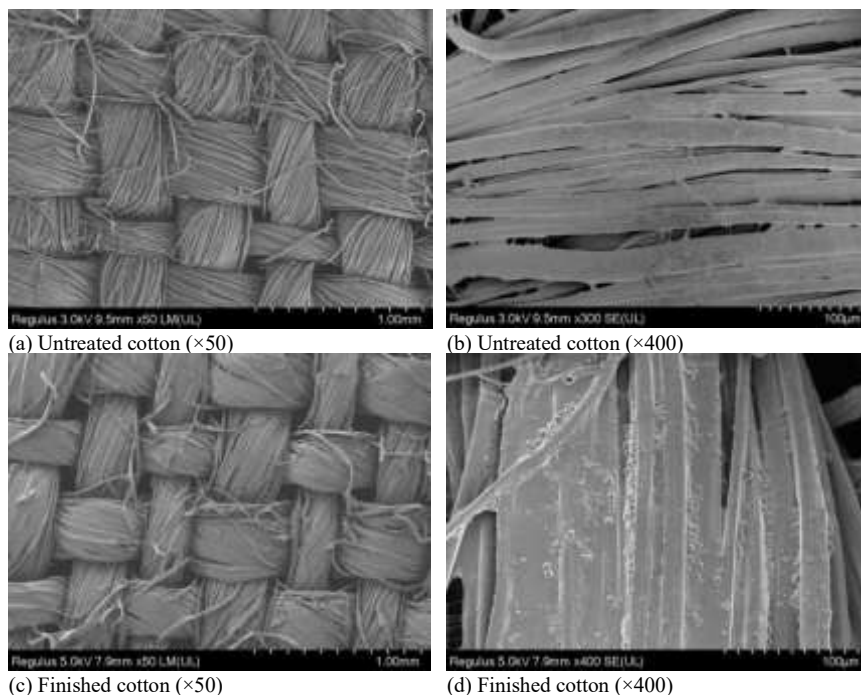


Figure 14. SEM images of cotton fabric before finishing (a, b) and after microcapsule finishing (c, d).

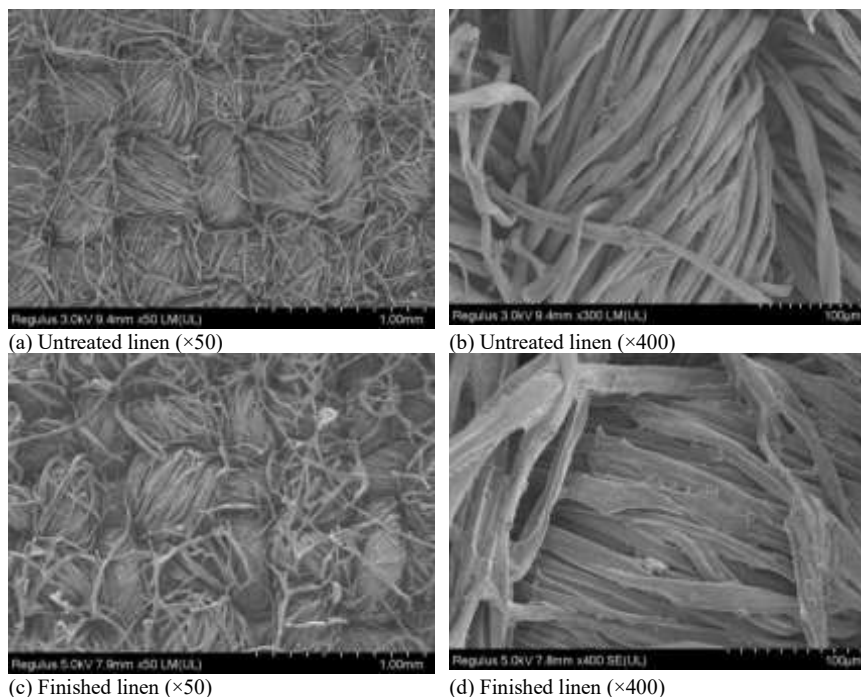


Figure 15. SEM images of linen fabric before finishing (a, b) and after microcapsule finishing (c, d).

4. Discussion

The optimisation results can be rationalised in terms of the colloidal mechanism of complex coacervation. The core-to-wall ratio governs the balance between the available oil surface area and the quantity of coacervate able to deposit around it. At a ratio of 1:1 the gelatin shell was sufficient to fully envelop the dispersed droplets, giving the highest encapsulation efficiency; at higher oil loadings the wall material became insufficient to coat the increased droplet surface area, leaving more oil unencapsulated and reducing the efficiency, consistent with the optimal ratios reported for other gelatin-based essential oil systems [7, 8]. The predicted encapsulation efficiency obtained here (97.52%) is appreciably higher than the values reported for several comparable gelatin- or polysaccharide-based coacervate systems, such as the gelatin–sodium alginate citronella microcapsules of de Matos et al. and the gelatin/gum-arabic jasmine nanocapsules of Lv et al. [7, 8], and exceeds the 80–85% efficiencies typical of dual-crosslinked or non-optimised formulations [12, 16]. The optimised particle size ($D_{90} = 4.76 \mu\text{m}$) and zeta potential (-32.25 mV) are likewise comparable to, or more favourable than, those reported for related essential-oil microcapsules, whose mean sizes commonly span a few to tens of micrometres and whose absolute zeta potentials frequently lie below the 30 mV threshold associated with good colloidal stability; the more negative potential obtained here reflects the strong electrostatic repulsion conferred by the sodium-hexametaphosphate-promoted gelatin coacervate. Differences in absolute efficiency and particle size between studies most likely arise from the specific wall-material pair, the core-to-wall ratio, the crosslinking chemistry and the homogenisation intensity employed.

Temperature exerted the strongest influence on the encapsulation efficiency. A moderate increase in temperature lowers the viscosity of the medium and accelerates molecular diffusion and enzyme activity, promoting more rapid and complete coacervation; however, excessively high temperatures impair the network-forming activity of transglutaminase, slow the consolidation of the coacervate and accelerate evaporative loss of the volatile core, all of which reduce the efficiency [6, 13]. The selection of 39–40 °C as the optimum therefore reflects both the maximum efficiency and the mild, energy-efficient conditions favoured for enzymatic crosslinking. The strong dependence of the efficiency on pH arises because complex coacervation occurs within a narrow window below the gelatin isoelectric point, where the protein carries a net positive charge; a mildly acidic environment maximises the electrostatic association that drives coacervate formation, so that lowering the pH within the studied range enhanced the efficiency [5].

The effect of coacervation-stage stirring speed was negligible within the investigated range of 400–1200 rpm, indicating that the microcapsules were sufficiently mechanically strong after shell crosslinking and that no significant disruption occurred due to hydrodynamic shearing forces. Indeed, this resistance against breakage provided by the ϵ -(γ -glutamyl)lysine network created through transglutaminase crosslinking is practically useful, as the need for controlling the exact rate of agitation will be removed during process scaling [12, 13]. With a value of -32.25 mV , the zeta potential measurement clearly implies strong negative charge of the surface, resulting in repulsion between particles which prevents their flocculation and explains their stability; its value is easily compatible with the ranges for stable colloids and exceeds the lower limit of flocculation [15].

Together, the SEM and AFM analyses provide evidence of successful microcapsule formation. The SEM images show particles with regular, near-spherical morphology, distinct boundaries and continuous external surfaces, with no widespread fracture or collapse. The low surface roughness measured by AFM (1.245 nm RMS) is consistent with a smooth, continuous outer surface. It should be noted, however, that these surface observations do not by themselves visualise the internal core–shell architecture; direct confirmation of an enclosed core and an intact wall would require cross-sectional SEM or TEM. The TG/DTA measurements characterised the thermal-decomposition profile of the microcapsules. Direct quantification of any

improvement in thermal stability relative to free FEO requires comparative thermograms under identical conditions, which should be included in future work [6, 10, 11].

The pronounced sustained-release behaviour, and its further retardation at lower temperature, demonstrates that the microcapsules can prolong the functional lifetime of the volatile core, a prerequisite for durable functional materials. The marked contrast between the near-complete release of the free oil and the much slower release from the microcapsules mirrors the sustained-release profiles reported for double-layered and crosslinked essential-oil microcapsules, in which the consolidated shell similarly extends the release period from days to weeks [11, 12]. This behaviour underpins the textile application: the SEM evidence of microcapsules deposited on cotton and linen fibres after the pad–dry–cure process, together with the suitable particle size and appreciable dispersion stability, demonstrates the feasibility of incorporating the optimised microcapsules into conventional finishing. Wash durability, antibacterial performance and long-term release behaviour remain to be quantified. These findings are in line with recent reports on essential oil microcapsules applied to cellulosic and linen fabrics by padding for fragrance, antibacterial and other functional finishes^[17, 18, 19, 20] and extend that approach to *Forsythia suspensa* essential oil. Compared with single-layer or non-crosslinked systems, the transglutaminase-hardened gelatin microcapsules developed here combine a high encapsulation efficiency with good thermal and colloidal stability under mild, environmentally benign conditions, highlighting their promise as sustainable functional finishes. Future work should quantify the wash durability and the antibacterial performance of the finished fabrics to consolidate their practical value.

5. Conclusion

Forsythia suspensa essential oil microcapsules were successfully prepared by complex coacervation using gelatin as the wall material and transglutaminase as a green crosslinking agent. Single-factor experiments followed by a reduced hierarchical Box–Behnken model identified an interior optimum governed primarily by significant quadratic curvature in the three investigated variables; the individual linear effects were not statistically significant and were therefore not ranked. The optimised conditions were a core-to-wall ratio of 1:1.1, a temperature of 39 °C and a pH of 4.69, under which the predicted encapsulation efficiency was 97.52%. The optimised microcapsules exhibited a particle size distribution of 0.25–10 µm (D90 = 4.76 µm) and a zeta potential of –32.25 mV, indicating good dispersion stability, and SEM and AFM showed regular near-spherical particles with smooth, continuous surfaces (mean diameter ≈ 2.38 µm; Rms = 1.245 nm). Thermal analysis characterised the thermal-decomposition profile of the microcapsules, and the microcapsules displayed clear sustained-release behaviour that was further prolonged at lower storage temperature. Finally, the microcapsules were successfully deposited onto cotton and linen fabrics by a conventional pad–dry–cure procedure, demonstrating the feasibility of their use in textile finishing. Further antibacterial, laundering and long-term release tests are required before durable functional performance can be concluded.

Author contributions

Conceptualization, methodology and investigation were carried out by H. Yang and M. Kaewbucha; formal analysis, data curation and visualization were performed by the H. Yang; writing—original draft preparation was undertaken by the H. Yang; writing—review and editing, supervision and project administration were provided by the C. Apiwathnasorn. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

Data availability

The data supporting the findings of this study were generated within the present work and are contained in the article. The principal datasets used to construct the manuscript comprise: the UV–visible standard-curve data ($y = 0.0385x + 0.816$, $R^2 = 0.9939$); the single-factor encapsulation-efficiency data for the core-to-wall ratio, coacervation temperature, pH and stirring speed; the 17-run Box–Behnken design matrix and the associated encapsulation-efficiency responses together with the ANOVA outputs (Tables 3 and 4); the laser particle-size and zeta-potential measurements ($D_{90} = 4.76 \mu\text{m}$; -32.25 mV); the SEM and AFM micrographs and the derived morphological parameters (mean diameter $\approx 2.38 \mu\text{m}$; $\text{Rms} = 1.245 \text{ nm}$); the TG/DTA thermal-analysis curves; the sustained-release data recorded at 4 and 25 °C; and the SEM images of the finished cotton and linen fabrics. These data are available from the corresponding author upon reasonable request.

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