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Antibacterial efficacy of 2-citronellyl benzimidazole nanoencapsulation with chitosan-tripolyphosphate and casein micellar coatings

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Antibacterial efficacy of 2-citronellyl benzimidazole nanoencapsulation with chitosan-tripolyphosphate and casein micellar coatings

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Abstract. Benzimidazole shows antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. Nanoencapsulation of 2-citronellyl benzimidazole is a technique that packages the drug's active ingredient, offering the advantage of its controlled release. The 2-citronellyl benzimidazole nanoencapsulation was constructed from casein micelles and chitosan-tripolyphosphate using an ionic cross-linking method. The nanocapsules were characterized using a particle size analyzer (PSA), a Fourier transform infrared (FT-IR) spectrophotometer, and scanning electron microscopy (SEM), while the release profile and amount of the encapsulated active compound were determined using an ultraviolet (UV)-Vis spectrophotometer. The 2-citronellyl benzimidazole encapsulated by chitosan-tripolyphosphate showed particle diameters of 10.700 nm, 81.110 nm, and 159.410 nm at 10%, 50%, and 90% magnification, respectively. The casein micelles had an average particle size of 1017 nm. The nanocapsule diameters with and without freeze drying were 3.293 nm and 1017 nm, respectively. The presence of an imine group with a wave number of 1651.72 cm⁻¹ on the FT-IR spectra of this band indicated that the active compound was coated. The nanocapsule surface morphology was trapezoid with a rough and uneven surface. The nanocapsular efficiencies using the casein micellar and chitosan-tripolyphosphate coatings were 56.763% and 79.97%, respectively. The kinetics release of 2-citronellyl benzimidazole showed a half-life of 86.63 minutes with a 60-minute stirring time. The nanocapsule's antibacterial activity showed the greatest inhibition zones at 2.3 mm and 10 mm for *S. aureus*, 4.7 mm and 8.3 mm for *E. coli*, and at 6.7 mm for *S. typhi*.

1. Introduction

The effectiveness of a drug's active ingredients can be determined from the molecular structure as well as the drug packaging model. One such model is encapsulation, which is one of the most efficient methods for formulating essential oils, and various encapsulation approaches have been developed [1].

Some recent encapsulation approaches include packaging in microencapsulation and nanoencapsulation [2], both of which have extensive applications, and nanoencapsulation shows great potential [3]. Nanoencapsulation enables releasing drugs and carrying active substances, thus enabling the drugs to be highly efficient because they have optimal solubility [4].



Nanoencapsulation coatings can include synthetic or natural materials according to the desired characteristics of the final delivery system [5]. Some advantages of using natural materials are that they lack side effects and are adaptable.

This research compared the effectiveness of chitosan-tripolyphosphate and casein micelle coating materials to overlay the active material, 2-citronellyl benzimidazole, which has antibacterial efficacy.

2. Method

The 2-citronellyl benzimidazole compound was identified using gas chromatography-mass spectrometry (GC-MS; Shimadzu QP2010S). The final result was obtained from each component's total ionic chromatogram (TIC) and mass spectra [6].

2.1. Preparing the 2-Citronellyl Benzimidazole Nanoencapsulation

The 2-citronellyl benzimidazole nanoencapsulation with casein micellar coating was prepared with 3 g, 4 g, and 5 g of casein, each of which was added with 50 mL phosphate buffer at pH 8.7, then homogenized using a basic IKA T25 homogenizer at 9500 rpm. Next, 1.5 mg of 2-citronellyl benzimidazole in 5 mL of methanol was added, and 1 mL of 10% CaCl₂ solution was added six times every 5 minutes during the homogenization process. The pH was neutralized using 0.1 M NaOH and homogenized at 9500 rpm for 5 minutes, then the mixture was filtered [7,8]. The obtained nanoencapsulation was freeze-dried for 48 hours (15 Pa pressure, -54°C chamber temperature, -20°C sample temperature) to obtain the dry nanoencapsulation.

The 2-citronellyl benzimidazole nanoencapsulation with chitosan-tripolyphosphate coating material was prepared using 1 g, 2 g, and 3 g chitosan dissolved in 200 mL acetic acid. Next, 0.05 g of 2-citronellyl benzimidazole was added, followed by slowly adding tripolyphosphate solution (2 g of tripolyphosphate dissolved in 8 mL of distilled water) while stirring with the homogenizer at 9,500 rpm for 30 minutes. The solution was then centrifuged at 10,000 rpm for 20 minutes, the supernatant was discarded, and the lower layer was used. Finally, the solution was freeze-dried for 48 hours to obtain dry nanoencapsulation (free of water) [9].

2.2. Characterizing the 2-Citronellyl Benzimidazole Nanoencapsulation

The 2-citronellyl benzimidazole nanoencapsulation's efficiency was determined using casein micelle and chitosan-tripolyphosphate coating material using a Shimadzu 1601 UV-Vis spectrophotometer. The absorbance data were interpolated with the line equation of the standard curve of the relationship between the concentration and absorbance to determine how much 2-citronellyl benzimidazole was coated.

The nanoencapsulation was characterized to determine the particle size distribution using a particle size analyzer (PSA) and Zetasizer Nano. Functional groups were characterized using the Shimadzu 8400S Fourier transform infrared (FT-IR) spectroscopy. Scanning electron microscopy (SEM) using a Hitachi TM-3000 was performed to characterize the surface morphology of the nanoencapsulation formed with the casein micelle.

The release profile of 2-citronellyl benzimidazole from the micelle casein coating was determined by adding 10 mL of methanol to 0.25 g of the 2-citronellyl benzimidazole nanoencapsulation and stirring for 15, 30, 45 and 60 minutes. The filtrate absorption at each time was measured at a maximum wavelength with an ultraviolet (UV)-Vis spectrophotometer (Shimadzu 1601) to analyze the concentration of 2-citronellyl benzimidazole released from the coating material. The same treatment was also performed for 0.1 g of the nanoencapsulation using the chitosan-tripolyphosphate coating material in 20 mL of methanol.

2.3. Antibacterial Activity Testing of the 2-Citronellyl Benzimidazole

The bacteria were revived and inoculated by dissolving 4 g of nutrients in 200 mL of distilled water while heating, then sterilized. Five milliliters of sterile nutrient agar was put into a test tube and placed

at a slope of 30–45° until it solidified. Swabs of the cultured *E. coli* and *S. aureus* bacteria were streaked on the surface of tilted media agar and incubated at 37°C for 24 hours [10].

The bacterial suspensions were prepared using 1.6 g of nutrient broth dissolved in 200 mL of distilled water, sterilized, and put in a test tube. Bacteria that grew in the media were used as 1 dose, dipped in nutrient broth media until no bacteria were attached to the needle and incubated for 1 day (24 hours). The *S. aureus* bacteria from the suspensions were then counted under a microscope using a hemacytometer to obtain the colony numbers of 105–108 colony-forming units (CFU)/mL [10]. This procedure was repeated on the suspensions of all gram-positive and gram-negative bacteria.

The inhibitory zones for the 2-citronellyl benzimidazole nanoencapsulation were determined using the well-diffusion method with 0.25 g of benzimidazole. For this, 0.25 g of the 2-citronellyl benzimidazole nanoencapsulation dissolved in 10 mL of methanol was stirred for 17.46, 32.46, and 47.46 minutes for the casein micellar coating and 26.6, 57.8, and 86.6 minutes for the chitosan-tripolyphosphate coating. Methanol was used as a positive control and distilled water as a negative control for the bacteria. The nanoencapsulation using the casein micellar coating material was observed after 24 hours, and the nanoencapsulation using the chitosan-tripolyphosphate coating material was observed after 18 hours. The sterilized nutrients were poured into a petri dish aseptically and allowed to solidify. Fifty to 100 µL of the bacterial suspension was injected onto the agar surface using a micropipette and leveled using a spreader. The well was made using a coke drill to load 5 well pits, 3 of which were filled with a test solution with time variation. One was filled with methanol as a positive control, and one was filled with distilled water as a negative control using 50–100 µL. Finally, the plate with the nanoencapsulation of the micellar casein coating was incubated at 37°C for 24 hours, and the plate with the nanoencapsulation of the chitosan-tripolyphosphate coating was incubated for 18 hours, after which time the clear inhibitory zones were observed.

3. Result and Discussion

3.1. Nanoencapsulation of 2- Citronellyl Benzimidazole

The 2-citronellyl benzimidazole is a white crystal with a melting point of 140–142°C and *m/z* of 242 (GC-MS), obtained from the reaction of citronellal (kaffir lime oil) with 1,2-phenyl diamine [6]. Figure 1 shows the different nanoencapsulation forms using the casein micellar and chitosan-tripolyphosphate coatings.



Figure 1. Nanoencapsulation of the two coating materials: Casein Micelle Coating (A); Chitosan-Tripolyphosphate Coating (B).

The efficiencies of the 2-citronellyl benzimidazole nanoencapsulation using the casein micellar and chitosan-tripolyphosphate coatings were 56.76% (2 g) and 79.97% (5 g), respectively (Figure 2).

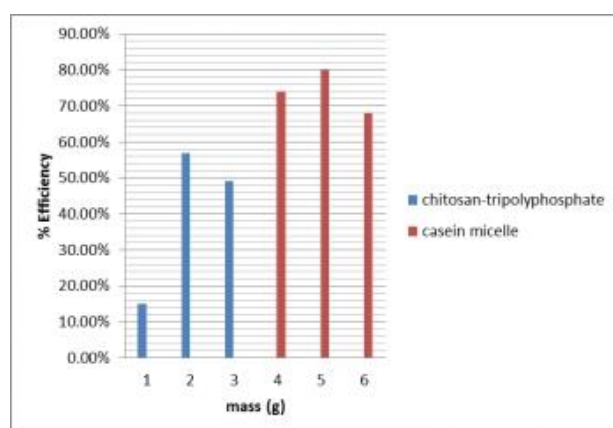


Figure 2. Percent efficiency of the 2-citronellyl benzimidazole nanoencapsulation.

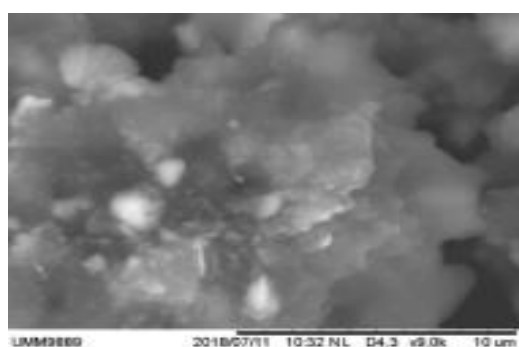
3.2. Characterization of the 2-Citronellyl Benzimidazole Nanoencapsulation

Per the Zetasizer Nano, the particle size of the nanochitosan-tripolyphosphate without the active ingredient was 223.47 nm, and the formed nanochitosan-tripolyphosphate had a good level of uniformity [11]. The particle size of the nanochitosan-tripolyphosphate with the active ingredient was 3.293 nm. Statistical analysis using Tukey's test revealed that the nanocytosan-tripolyphosphate had a larger than 2-citronellyl benzimidazole nanoencapsulation. Table 1 compares the particle sizes based on the PSA.

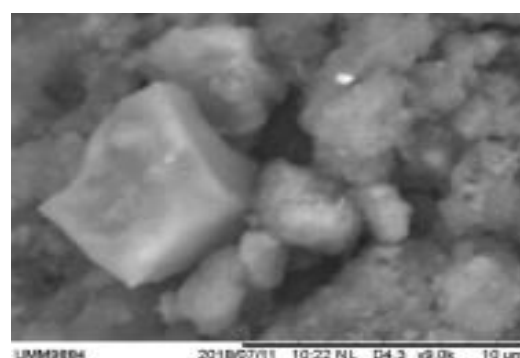
Table 1. Particle size comparison between the casein micellar and chitosan-tripolyphosphate coating materials.

Coating material	Particle size (nm)	
	Without 2-citronellyl benzimidazole	With 2-citronellyl benzimidazole
Casein micelles	1178 nm	1017 nm
Chitosan-tripolyphosphate	95.180 nm	159.410 nm

SEM revealed that the surface morphology of the benzimidazole nanoencapsulation was trapezoidal with a rough and uneven surface, while the blank nanoencapsulation formed agglomerates with a finer surface (Figure 3).



(A)



(B)

Figure 3. Surface Morphology via SEM: Casein Micelle Coating (A); Chitosan-Tripolyphosphate Coating (B).

Table 2 shows the spectra produced via FT-IR characterization. The new absorption at wave number 1651.72 cm^{-1} , which is an imine group from benzimidazole, shows that the benzimidazole was coated in casein micelles.

Table 2. Functional groups and wave numbers of benzimidazole nanoencapsulation, casein isolates and blank solution.

Functional Group	Wave Number (Cm^{-1})		
	Casein	Blank	Nanoencapsulation benzimidazole
N-H amine	3420.32	3343.17	3202.37
C-H	2924.65	2926.57	2924.65
	2853.29	2855.21	2853.29
C=O	1746.22	1746.22	1746.22
C=C	1520.56	1516.71	1541.78
C=N	-	-	1651.72
OH	2400–3600	2400–3300	2410–3400

Figures 4 and 5 show the relationship between concentration and time: as the stirring time increased, the concentration of 2-citronellyl released increased.

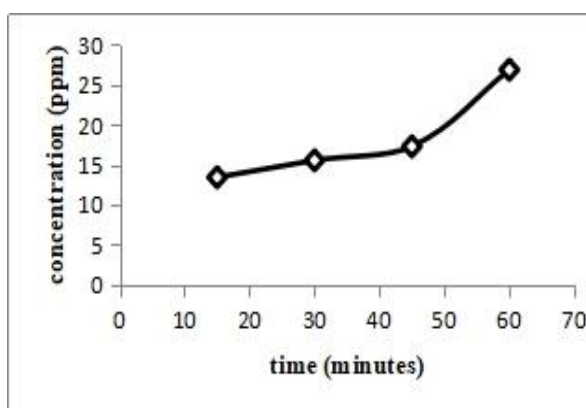


Figure 4. The release profile of the 2-citronellyl benzimidazole with the casein micellar coating.

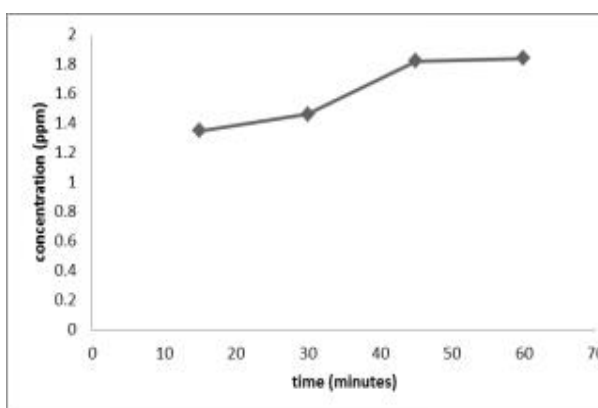


Figure 5. The release profile of the 2-citronellyl benzimidazole with the chitosan-tripolyphosphate coating.

3.3. Antibacterial Activity Testing of 2-Citronellyl Benzimidazole

After 32.46 minutes of stirring, the inhibitory zone diameter of the 2-citronellyl benzimidazole with the micellar casein coating was the largest (10 mm) around *S. aureus*, followed by that around *E. coli*, which was 8.3 mm. According to Davis and Stout [12], criteria for determining the inhibitory power include the medium used. The largest inhibitory zone diameters for the 2-citronellyl benzimidazole nanoencapsulation using the chitosan-tripolyphosphate coating material against *S. aureus*, *E. coli*, and *S. thypi* were found after stirring for 86.6 minutes (2.3 mm), 26.6 minutes (4.7 mm), and 86.6 minutes (6.7 mm), respectively.

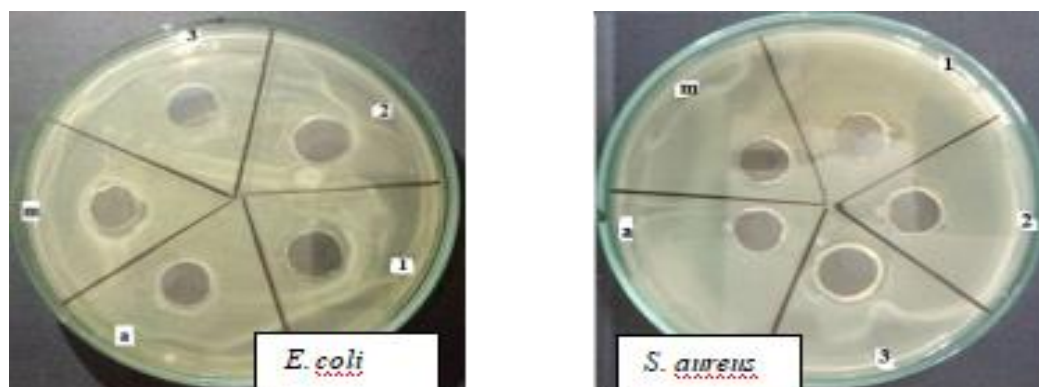


Figure 6. Inhibitory zones of the 2-citronellyl benzimidazole nanoencapsulation using casein micellar coating material.

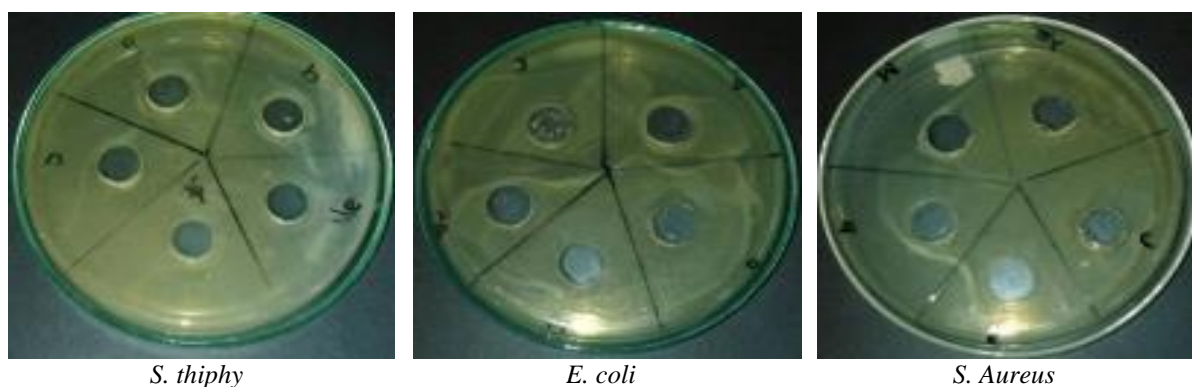


Figure 7. Inhibitory zones of the 2-citronellyl benzimidazole nanoencapsulation using chitosan-tripolyphosphate coating material.

Table 3. Antibacterial activity of the 2-citronellyl benzimidazole nanoencapsulation.

Bacteria	Coating material	Number of bacterial cells (CFU/mL)	Stirring time (minutes)	Inhibitory diameter (mm)
<i>S. aureus</i>	Casein micelle	8.4×10^7	17.46	5.3
			32.46	10
			47.46	8
			17.46	5
<i>E. coli</i>	Casein micelle	7.8×10^7	32.46	8.3
			47.46	4
			26.6	2
			57.8	2
<i>S. aureus</i>	Chitosan-tripolyphosphate	16.88×10^6	86.6	2.3
			26.6	4.7
			57.8	2
			86.6	1.7
<i>E. coli</i>	Chitosan-tripolyphosphate	8.56×10^6	26.6	2
			57.8	3
			86.6	6.7
			26.6	2
<i>S. thypi</i>	Chitosan-tripolyphosphate	10.56×10^6	57.8	3
			86.6	6.7
			26.6	2
			57.8	3

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