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Comparative Effects of *Ocimum tenuiflorum* and *Ocimum basilicum* on Isoniazid Microsphere Formulation Characteristics Prepared by Different Methods

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Abstract

Due to difficulties such as bacterial resistance, unpleasant effects, and low patient compliance with anti-tubercular drugs, tuberculosis treatment remains a challenge. Microspheres are a sort of multiparticulate drug delivery system that is used to distribute a drug for a longer period of time, boost bioavailability or stability, and target specific locations. Isoniazid is an important first-line anti-tubercular drug used in tuberculosis treatment in a fixed-dose combination. We attempted a unique way to address the above-mentioned obstacles of tuberculosis therapy by merging both these methodologies of microspheres and bioenhancers in the current work. Bioenhancers are 'bioavailability enhancers,' which have no therapeutic effect but enhance the action of drug molecules when administered in combination. The comparative effect of *Ocimum tenuiflorum* and *Ocimum basilicum* as a bioenhancer was studied and compared to a formulation without bioenhancers. Two processes are used to make microspheres: Complex Coacervation and Modified Emulsion Method. *In-vitro* release, drug entrapment efficiency, % bioadhesion, and permeability of the microspheres were all assessed using the intestinal sac method. *In-vitro* drug release from formulations containing *Ocimum tenuiflorum* and *Ocimum basilicum* as bioenhancers was reported to be around 56-84% in 12 hours. The microspheres were discovered to be smaller than 120 microns in diameter. The DEE was shown to be between 39-78%. The bioadhesion of the microsphere was determined to be 38-82% (increased in formulations where bioenhancers incorporated). The major findings of the USP paddle apparatus *in-vitro* release study concern the extraordinarily large increase in drug release due to the presence of bioenhancers.

Keywords: Microspheres, Double emulsion method, Complex coacervation method, Isoniazid, *Ocimum tenuiflorum*, *Ocimum basilicum*

Introduction

Tuberculosis is a leading source of illness and mortality around the world. Every year, over 2 million people are killed by the disease. Treatment regimens now offered are lengthy, putting excessive demands on destitute people. As a result, the tide of medication resistance is rising. The flexibility to adapt drug release rates to the needs of a specific application, as well as the capacity to dispense at a constant or pulsatile rate, are two potential advantages of the drug delivery system. It safeguards medications, particularly proteins, from being rapidly destroyed by the body. By substituting occasional (once a month or fewer) injections for regular (e.g., daily) injections, microspheres with controlled release systems might increase patient comfort and compliance (1).

As a result, using bioenhancers in combination therapy to improve release performance and, eventually, maximise their effectiveness while using microspheres as a drug

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delivery vehicle would be an appropriate formulation method for optimising anti-tubercular drugs' pharmacokinetic properties [2].

Bioenhancers are substances that, when taken with an active medicine, increase the drug's pharmacological activity while having no therapeutic benefit. The use of herbal ingredients to improve drug bioavailability has resulted in a major paradigm shift in therapeutics. Bioenhancers increase permeability and alter drug bioavailability for therapeutic efficacy, which may lead to a reduction in dose while maintaining therapeutic availability. Anti-TB drug therapy has a number of difficulties, including loss of efficacy due to bacterial resistance, unpleasant effects, and low patient compliance. C.K. Atal looked at a list of Ayurvedic compositions used to treat a variety of diseases in ancient India. He noticed that the majority of Ayurvedic formulations contained Trikatu or one of its ingredients, Piper longum (P. longum), which is used to cure a variety of diseases (210 formulations out of 370 tested). In the cited study effort, we aimed to address the aforementioned issues by merging the principles of microsphere drug delivery (enhanced bioavailability) with herbal bioenhancers with improved bioavailability [3,4].

Basils (Ocimum spp., Lamiaceae) have a variety of essential oils that are high in phenolic compounds, linalool, and other natural products. Basil leaves are dried and used to season stews, sauces, salads, soups, meat, and tea [5,6].

The objectives of cited research work are to:

- formulate isoniazid sustained-release microspheres using a variety of approaches.
- Study the effect of varying concentrations of herbal bioenhancer (extracts of Ocimum tenuiflorum and Ocimum basilicum) on *in-vitro* drug release from microspheres, and assess the impact of different processing parameters on microsphere characteristics.

Materials and Methods

Methods

Two methods were used to prepare microspheres including complex coacervation and a modified emulsion method.

Materials

Isoniazid was received as a gift sample from Lupin Pharmaceuticals Ltd., Aurangabad, Maharashtra, as a model anit-tubercular drug. Ocimum tenuiflorum (OT) and Ocimum basilicum (OB) were obtained from the herbal medicinal garden and authenticated by Department of Botany, SSVPS's Dr. P. R. Ghogrey College of Science in Dhule, Maharashtra. As a bioenhancer, hydro-alcoholic extracts of Ocimum tenuiflorum were used. Sodium alginate and Type B gelatin (bloom strength 220) were procured from Loba Chemie, Mumbai. Sigma Aldrich, Germany, provided the sodium tripolyphosphate and Chitosan. All of the other chemicals and polymers that were employed were of analytical quality.

Experimental Work

Extraction and Isolation of Ocimum Tenuiflorum (OT) and Ocimum Basilicum used as Bioenhancer:

Different components (leaves, stem, flower, root, seeds, and sometimes the entire

Fig 1A. Leaves and Dried Powder of A. *Ocimum Tenuiflorum*; B. *Ocimum Basilicum*

Ocimum Tenuiflorum and *Ocimum Basilicum*

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plant) of *Ocimum tenuiflorum* and *Ocimum basilicum* are used in traditional medicine in Fig. 1.

The leaves of *Ocimum tenuiflorum* and *Ocimum basilicum* were harvested between October and December and compared to a reference sample. The 50 % ethanolic extract was made by combining 500 g of dried, crushed, and powdered tulsi leaves with 1000 mL of 50 % ethanol in a round bottom flask and keeping it at room temperature for three days in the shade. After filtering the extract, the operation was performed twice more. The resulting extract filtrate was collected and evaporated on a water bath until dry. The yield of extract was approximately 5.00 % w/w [7].

***Phytochemical Evaluation of Ocimum Tenuiflorum and Ocimum Basilicum*:** The phytochemical examination of dried leaves of Holy basil was carried out according to Ayurvedic Pharmacopoeia [8] provisions for several criteria (Table 1).

***Preparation of Isoniazid Microspheres*:** Sustained release microspheres can be made using a variety of techniques, including emulsion cross-linking and multiple emulsions [9]. This microsphere was prepared using the double emulsification method and complex coacervation method in this investigation.

Method 1 - Complex Coacervation Method (CCM): In a 1:05 ratio, chitosan and gelatin were mixed in a dilute acetic acid solution (1 % v/v) at 3 % w/v and pH adjusted to 5.0. Isoniazid (150 mg) was dissolved in the polymeric mixture mentioned. At 40°C, the medicine in the polymeric mixture was emulsified with 1 mL Tween 80 (2 % w/v) in 100 mL liquid paraffin (1:1 ratio of light and heavy liquid paraffin). A mechanical stirrer was used to emulsify the mixture for 15 minutes at 1200 rpm (Remi Motors, India).

To induce gelatin coagulation, the resulting w/o emulsion was refrigerated to 4°C. Then, at 4°C, 50 ml Na-TPP (1.5 % w/v) with pH 5 was added drop by drop. Stirring was continued for another 30 minutes to obtain cross-linked microspheres. Centrifugation was used to collect microspheres, which were then washed three times with double distilled water, dried at room temperature under vacuum, and washed three times with acetone to remove water. The microspheres were created and stored in a desiccator for further study. Polymer concentration, polymer: copolymer ratio (Chitosan: Gelatin B), cross-linking period, and rpm were all factors in the formulation optimization [10,11]. Components of an optimal formulation comprising varied concentrations of hydroalcoholic extract of *Ocimum tenuiflorum and Ocimum basilicum* as a bioenhancer are shown in Table 2.

Method 2 - Double Emulsification Method (MEM): 150 mg isoniazid dissolved in a 3% sodium alginate aqueous solution (10 ml). The aqueous phase was emulsified in light liquid paraffin (in the ratio 1:10) containing 1% (v/v) Span 80 for 45 minutes using a

Table 1: Phytochemical Evaluation of *Ocimum Tenuiflorum and Ocimum Basilicum*

Parameter	Value (%w/w)	
	Ocimum Tenuiflorum	*Ocimum Basilicum*
Total Ash	8.5	8.9
Acid insoluble ash	1.1	1.5
Water soluble ash	3.9	4.4
Loss on drying	3.9	4.7
Swelling index	10 ml	12 ml
Water absorption capacity	10 ml	12.7 ml

Ocimum Tenuiflorum and *Ocimum Basilicum*

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mechanical stirrer (Remi Motors, India). 5 ml of 7.5 % calcium chloride dissolved in a 1:1 mixture of methanol and isopropyl alcohol was slowly added to the emulsion and agitated to ensure successful crosslinking. Microspheres were collected via vacuum filtration, then washed three times in isopropyl alcohol before being dried at ambient temperature. Variables like polymer concentration, drug-polymer ratio, cross-linking agent concentration, and cross-linking time were used to optimise the formulation [10,11]. Finally, different concentrations of hydro-alcoholic extracts of *Ocimum tenuiflorum* and *Ocimum basilicum* were added to the optimised formulation to investigate their effect on drug bioavailability (Table 3).

Characterization of Microspheres

***Compatibility Studies*:** Fourier Transform Infrared Spectroscopy was used to investigate any chemical interactions between the medication and the polymeric substance during the creation of the microspheres (FTIR). Pure drug INH, placebo microspheres, and INH microspheres (2-5 mg) manufactured with and without bioenhancer were weighed and combined appropriately with potassium

Table 2. Isoniazid Microsphere Formulations with Various Polymers and Hydroalcoholic Extract of Bioenhancer Ratios (Complex Coacervation Method)

Formulation Code	INH (mg)	Gelatin:Chitosan (ratio)	Sodium TPP (%)	Cross Linking Time (min.)	BE1 (mg)	BE2 (mg)
CI1	150	0.5:1	1	30	--	--
CI2	150	0.5:1	1	30	5	--
CI3	150	0.5:1	1	30	10	--
CI4	150	0.5:1	1	30	15	--
CI5	150	0.5:1	1	30	--	5
CI6	150	0.5:1	1	30	--	10
CI7	150	0.5:1	1	30	--	15

Table 3. Isoniazid Microsphere Formulations with Various Polymers and Hydroalcoholic Extract of Bioenhancer Ratios (Double Emulsification Method)

Formulation Code	INH (mg)	Sodium Alginate (%)	$CaCl_2$ (%)	Cross Linking Time (min.)	BE1 (mg)	BE2 (mg)
MI1	150	3	7.5	45	--	--
MI2	150	3	7.5	45	5	--
MI3	150	3	7.5	45	10	--
MI4	150	3	7.5	45	15	--
MI5	150	3	7.5	45	--	5
MI6	150	3	7.5	45	--	10
MI7	150	3	7.5	45	--	15
MI- Isoniazid microspheres by modified emulsion method, IHN- Isoniazid, BE_1- Bioenhancer 1 i.e., hydroalcoholic extract of *Ocimum tenuiflorum* (OT) BE_2- Bioenhancer 2 i.e., hydroalcoholic extract of *Ocimum basilicum* (OB)						

Ocimum Tenuiflorum and *Ocimum Basilicum*

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bromide to make a homogeneous mixture (0.1 to 0.2 g). A small amount of powder was crushed into a thin semi-transparent pellet by applying pressure to it. The pellet's IR spectrum was recorded with FTIR (Perkin Elmer, USA, Spectrum RX1 Model) with air as the reference, and the findings were compared to check if there was any drug-excipient interaction [12].

***Particle Size Analysis*:** The particle size of both plain medicine microspheres and microspheres with bioenhancer was evaluated using a Motic microscope at 40 X magnification. In each of the measurements, at least 100 particles were evaluated in each of the three fields [10,11].

***Percent Drug-Entrapment Eefficiency*:** The drug content of the microspheres was evaluated spectrophotometrically (max = 263 nm; Perkin Elmer, USA Lambda 25 model) to estimate the percentage drug entrapment. Sonication was used to dissolve isoniazid-loaded microspheres (10 mg) in 10 ml of isotonic phosphate buffer pH 6.8 for 20 minutes. After filtering the solutions with 0.22 µm Millipore filters, the amount of isoniazid was determined [13]. According to preliminary UV measurements, the presence of dissolved polymers had no effect on the drug's absorbance at 263 nm. The % drug entrapment was calculated using following formula:

$$\text{Percent drug entrapment} = \frac{\text{Mass of drug present in microparticles}}{\text{Mass of drug used in the formulation}} \text{ X } 100$$

***Percentage Yield*:** The yield of microspheres was calculated by comparing the total weight of microspheres produced to the total weight of the polymer, drug, and bioenhancers employed in the formulation [14]. The microsphere % yield was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of microspheres obtained}}{\text{Total weight of drug, polymer used in formulation}} \text{ X } 100$$

***Measurement of Bioadhesion*:** The in-vitro bioadhesion of microspheres (in triplicate) was determined using the falling liquid film method. Microspheres (50 mg) were spreaded over small intestine of albino rat (area 2cm^2) and detained for 20-30 minutes in a humidity temperature-controlled cabinet (Thermolab, India) at 75%RH and 25^{o}C to allow the microspheres to hydrate. After that, the mucosal lumen was thoroughly washed with isotonic phosphate buffer pH 6.8 and dried at 70^{o}C in a hot air oven [15].

The following formula was used to calculate the percentage of bioadhesion:

$$\text{Percentage bioadhesion} = \frac{\text{Weight of adhered microspheres}}{\text{Weight of applied microspheres}} \text{X } 100$$

***In-vitro Drug Release*:** The release characteristics of isoniazid from microspheres were examined in simulated gastric fluid (SGF pH 1.2) and simulated intestinal fluid (SIF pH 6.8). The drug-loaded microspheres (equivalent to 10 mg isoniazid) were put in empty capsule shells and spun at 50 rpm in 500 ml of 37°C dissolving fluid. At regular intervals, the aliquots (2 ml) were extracted and replaced with fresh media [16]. The drug content was measured spectrophotometrically at 263 nm after the samples were diluted and filtered.

***Statistical Analysis*:** The results were statistically analysed using the Student's t-test. The zero order, first order, and Higuchi's matrix models were used to compare the *in vitro* release profile [17].

Result and Discussion

***FTIR Study*:** INH had a significant C=O stretch band (Amide I) around 1650 cm1 and an Amide II due to N–H bend around 1620 cm1 in its FT-IR spectrum. The FT-IR spectrum of the drug-loaded microspheres, on the other hand, entirely obscured these peaks. Isoniazid drug release *in-vitro* from formulations containing bioenhancer extract was reported to be 85-90% in 12 hours. In formulations without bioenhancers, the similar percentage was

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around 45-50%. Other characteristics were investigated as well, such as % bioadhesion and permeability testing utilising the intestinal sac method.

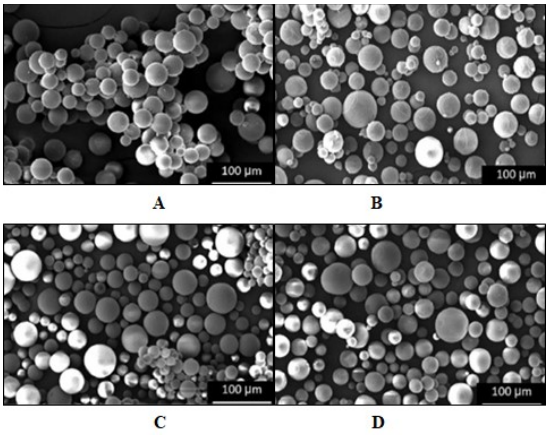

Fig 2. Microspheres by: A, B Complex Coacervation and C, D. Modified Emulsification Method using OT and OB as a Bioenhancer Respectively

***Differential Scanning Calorimetry*:** An empty aluminium pan was utilised as a reference and an isoniazid powder sample (2-8 mg) was weighed into an aluminium pan and assessed as sealed with pinholes. The heat-cool-heat cycle was also utilised to assess the thermodynamic relationship between two forms. At 70°C, the DSC endotherm exhibited a strong melting endotherm. The DSC curve of INH revealed an endothermic event between 50 and 80°C which was connected to the material's dehydration.

***Particle Size*:** The microspheres were found to have a smoother surface and were distinct and spherical in shape; the morphology of drug-loaded microspheres should not alter as shown in Fig. 2. As indicated in Table 4, the mean particle size of the microspheres generated by the complicated coacervation process was 100-120ɱ.

***Percentage Yield*:** To estimate the yield of microspheres, the total weight of microspheres collected was compared to the weight of the drug, polymers, and bioenhancer.

Table 4. Isoniazid Microspheres Evaluation Parameters Using Modified Emulsion and Complex Coacervation Method

Formulation Code	Mean Particle Size (ɱ)	Yield (%) ±SD	Drug Entrapment (%) ±SD	Bioadhesion (%) ±SD	Drug Release (at 12th hr.) % ±SD
CI1	113-118	35.25±1.01	40.07±0.73	38.17±0.19	42.15±1.11
CI2	112-116	46.25±1.11	60.13±0.47	53.87±1.02	56.81±0.94
CI3	103-108	57.42±1.01	62.21±1.07	66.41±0.98	68.96±1.21
CI4	104-110	69.11±0.79	77.10±0.31	81.91±0.54	82.16±0.81
CI5	111-118	49.17±1.07	51.96±0.72	58.47±0.29	62.64±0.87
CI6	103-108	62.66±0.42	66.18±0.49	66.94±1.07	72.71±0.68
CI7	106-112	69.18±1.01	76.11±0.87	80.41±0.91	81.11±0.53
MI1	116-120	34.25±0.25	39.11±0.37	38.01±0.49	40.17±0.33
MI2	111-114	49.25±1.07	59.87±0.74	53.11±1.22	57.15±1.04
MI3	101-108	71.50±0.19	78.01±0.13	82.50±0.45	83.61±0.18
MI4	108-113	59.24±1.17	64.12±1.11	67.14±0.89	69.71±1.07
MI5	109-114	51.24±0.97	53.69±1.27	59.14±0.92	61.46±0.99
MI6	100-105	71.11±0.39	76.89±1.17	81.14±1.11	82.17±0.33
MI7	106-112	63.16±1.24	67.01±0.94	67.49±1.17	73.17±0.86

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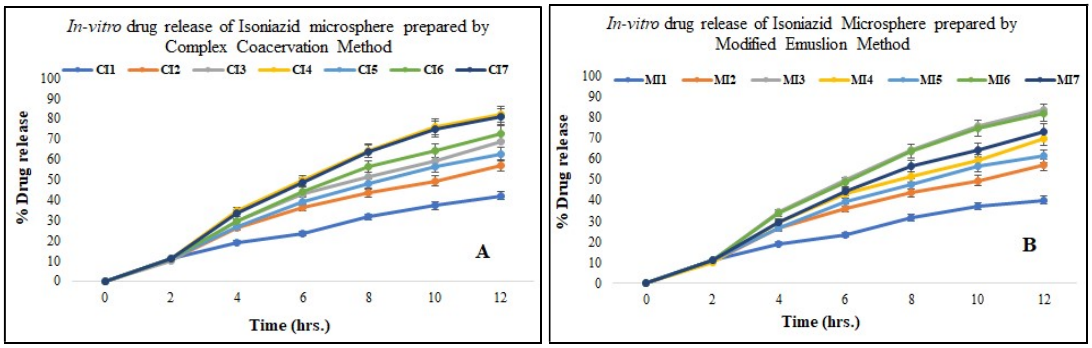

Coacervation and Modified Emulsion Method

Fig 3. *In vitro D*rug Release from Isoniazid Microspheres Prepared by Complex

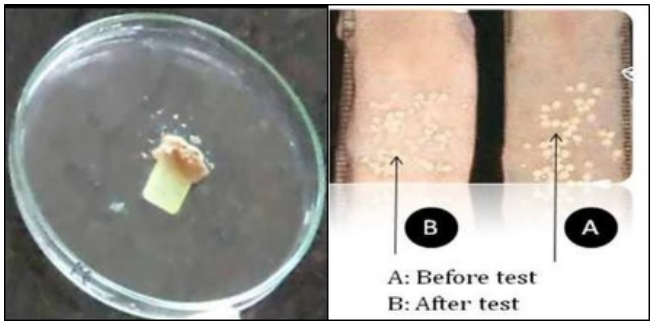

Fig 4. Bioadhesion Study of Isoniazid Microspheres

The percentage yield of the optimised formulations, which spans from 34.50 to 71.50%, is shown in Table 4. Losses accounted for throughout the microsphere hardening, washing, and filtering procedures could account for the loss of medicine in the strategy.

***Percent Entrapment Efficiency*:** The entrapment efficiency was determined to be between 39.50 to 78.14%. The loss of the medication in these approaches could be attributed to the hardening, washing, and filtration processes. As demonstrated in Table 4, during microsphere formulation optimization, polymer concentration, cross-linker concentration, and cross-linking time may have an impact on microsphere entrapment efficiency. The maximum entrapment efficiency values obtained are higher, or at least comparable, to the highest value reported in prior microsphere formation studies using sodium alginate. The discrepancy is attributable to high water solubility of isoniazid, which results in high drug concentrations in the preparation medium in this approach, as well as the usage of bioenhancer in formulations.

***Percent Bioadhesion*:** The presence and amount of bioenhancer had a substantial impact on the bioadhesion of the microspheres in the optimised formulations. The bioadhesion investigation was carried out using a previously documented method (in triplicate). The percentage bioadhesion ranged from 38.50 to 82.95% in Table 4. Microspheres with bioenhancers have a higher bioadhesive property (Fig. 3) than microspheres without bioenhancers (Fig. 4). The bioadhesive

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characteristics of microspheres resulted in long-term retention in the small intestine. It was revealed that microspheres that included a higher concentration of bioenhancer had a 45% increase in bioadhesion. Furthermore, as seen in Table 4, as the amount of bioenhancer increases, the percentage bioadhesion increases. Because of their bioadhesive properties, these particles were able to stay in the small intestine for a long time.

***In vitro Drug Release*:** Figures 4A and 4B show the in-vitro release behaviour of isoniazid microspheres generated by a modified emulsification and complicated coacervation approach using Ocimum tenuiflorum and Ocimum basilicum as a bioenhancer in simulated gastric fluid (SGF), pH 1.2, and simulated intestinal fluid (SIF), pH 6.8. Approximately 10-15% of the medicine was released over a 2-hour period in the SGF, pH 1.2, and 30-70% over a 12-hour period in the SIF, pH 6.8. Microspheres containing bioenhancer have a very high increase in microsphere release when compared to microspheres without bioenhancer (from 40.50% to 83.79% and 82.50% in case of modified emulsification method and complex coacervation method, respectively). CI1 and MI1 are bioenhancer-free formulations, whereas CI2, CI3, CI4, MI2, MI3, and MI4 are microsphere formulations containing Ocimum tenuiflorum, and CI5, CI6, CI7, MI5, MI6, and MI7 are microsphere formulations containing Ocimum basilicum as a bioenhancer in 5, 10, and 15 mg concentrations, respectively.

Conclusion

The particle size of microspheres made by both complex coacervation and modified emulsion methods using *Ocimum tenuiflorum* and *Ocimum basilicum* as a bioenhancer was consistent and less than 120 microns in size; however, the particle size may change when bioenhancer extract is added to the formulations. The efficiency of drug encapsulation was determined to be between 39.50 to 78.14% (increased on addition of bioenhancer in the formulations). When bioenhancers were utilised, the percentage bioadhesion of the microsphere increased by 40% above the baseline value (82.95% from 38.50% from formulation, where no bioenhancer were used. The most important findings from the in-vitro release study were the significant increases in drug release (from 40.50% to 83.79% and 82.50%, respectively) due to the presence of bioenhancers alone and in combination (from 40.50% to 83.79% and 82.50% in case of modified emulsification method and complex coacervation method).

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Conflict of Interest

Authors declare no conflict of interest.

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