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Research Paper

Standardization of microencapsulation process of volatile oil of *Azadirachta indica*

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■ **ABSTRACT :** *Neem* oil microcapsule gel was successfully prepared with wall material including gelatin and gum Arabic using coacervation technique by the researcher as an outcome of standardization of microencapsulation process of volatile oil of *Azadirachta indica*. An essential oil is the volatile lipophilic component extracted from plants. The use of medicinal plants is a universal phenomenon. The plant *Neem* belongs to the big mahogany family of Meliaceae and the genus Azadirachta of *A. indica*. The diverse biological activities of *Neem* essential oils can be applied on a large scale as antioxidant, antimicrobial agents comprising many important benefits including their volatility, lower level of risk to the environment than with synthetic ones. Findings of the study revealed that best results of microcapsules formation using *Neem* oil were obtained when the ratio of oil:gum:gelatin was kept 2:4:4, at optimized 50ºC temperature having initial pH 4.5 and final pH 9.5.

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■ **KEY WORDS:** Standardization, Micro-encapsulation, *Azadirachta indica*, Essential, Oil

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An essential oil is the volatile lipophilic component extracted from plants. Microencapsulation systems protect the essential oil from degradation and evaporation, and, at the same time, allow a sustained release (Marcela *et al.,* 2015). Microencapsulation is an effective and important tool to prepare oil-based high-quality and health-beneficial products in various industries in order to enhance their chemical, oxidative, and thermal stability (Bakry *et al.,* 2016). The use of medicinal plants is a universal phenomenon. Natural products from plants are rich source to identify, select and process new drugs for medicinal use. The diverse biological activities of *Neem* essential oils can be applied on a large scale as antioxidant, antimicrobial and larvicidal agents comprising many important benefits including their volatility, lower level of risk to the environment than with synthetic ones (El-Hawary *et al.,* 2013).

Neem (*Azadirachta indica*) is one of the plants that have been identified in curing various infections. The plant *Neem* belongs to the big mahogany family of Meliaceae and the genus Azadirachta of A indica. This plant had origin from India but now it has wide distribution in huge numbers in tropical, subtropical, semi-arid and wet-tropical regions of the world (Dua *et al.,* 2009). The roots, stems, barks, leaves, seeds, flowers and fruits of *A. indica* have chemically bioactive substances such as peptides, alkaloids, tannins, phenols, sterols, flavonoids and glycosides that contributes in fighting against the bacteria. Among all the parts of *A. indica*, the seeds are listed as one of the most popular source of medicaments in antibacterial activity as the oil contains extensive spectrum against antibacterial infections (El-Mahmood *et al.,* 2010).

Among many well-known medicinal plants, *Neem* (*Azadirachta indica*) is one of the plants that have been identified in curing various infections (Sandanasamy *et al.*, 2013). *Neem* seed oil is a commercialized product which is used in treatment of various skin disorders, and promotion of wound healing due to its antibacterial, antifungal and anti parasitic activities (Abdel-Ghaffar and Semmler, 2007; Yang *et al.*, 2009b and Mandal and Mandal, 2011). Microencapsulation is a method in which tiny particles or droplets are surrounded by a coating wall, or are embedded in a homogeneous or heterogeneous matrix, to form small capsules (Gharsallaoui *et al.,* 2007; Calvo *et al.,* 2011). It can envelop a solid, liquid, or gaseous substance within another substance in a very small sealed capsule. The core material is gradually diffused through the capsule walls, thereby offering controlled release properties under desired conditions (Fang and Bhandari, 2010).

Present research is focused on standardization of microencapsulation process of volatile oil of *Neem* seeds to protect and maintain its stability by coacervation technique for its effective application as antibacterial, antifungal and anti mosquito finishing in developing various health care textile products.

■ RESEARCH METHODS

The raw material used in this study was the essential *Azadirachta indica*, *Neem* oil and coating materials such as gum arabic, gelatin. Other materials used were dilute acetic acid, 0.4 N sodium hydroxide solution, gluteraldehyde aqueous solution and sodium sulphate solution.

Selection of essential oil :

Essential oils are a complex liquid mixture of volatile, lipophilic and odoriferous compounds biosynthesized by living organisms, predominantly aromatic plants. Berger, (2007). Researcher explored the availability of essential oil-clove oil in the market, for the sake of purity and maintaining uniformity in quality parameter, It was procured form.

Selection of wall material:

Gelatine was used as an encapsulating agent, as wall material to encapsulate the inner core material (oil). Preparation of microcapsules by coacervation method usually involves a combination of gelatine and a carboxyl bearing polymer. Gum Arabic, a polymer most widely used for complex coacervation with a gelatine was used in this process with other naturally occurring polymer and synthetic polymer compatible with cotton fabric and other ingredients of microencapsulation process based on the past studies.

Selection of microencapsulation technique :

Out of the many physical and chemical techniques of microencapsulation *i.e.* solvent evaporation, polymerization, spray drying, pan coating, phase separation centrifugal extrusion etc. The phase separation -complex coacervation technique was selected for present study by the researcher on the basis of review and the suitability of the process to be carried out in the laboratory of the department.

Complex coacervation technique :

Gum acacia was taken as the wall material and essential oil as the core material. Gelatin is the common ingredient in all the processes of complex coacervation. The basic recipe (Teli *et al.*, 2005) was followed to optimized the various concentrations of the raw material used in microencapsulation process.

Standardization of microencapsulation :

The researcher conducted laboratory experiments for standardization of microencapsulation process of *Neem* oil. The resultant precipitate obtained after each process was analyzed under inverted microscope to ensure the formation of microcapsules and images were captured. The combination of ratio of oil, gum and gelatin which produced best results was further subjected to optimization of the other variables. At a time, the ratio of only one variable was varied and other variables were kept constant.

Optimization of ratio of essential oil:

To optimize ratio of essential oil, five different ratio of oil *i.e.* 0.5, 1.0, 1.5. 2.0, 2.5 were taken while other variables *i.e.* gum, gelatin, temperature and pH were kept constant to carry out microencapsulation process.

The resultant precipitate obtained was analyzed under inverted microscope to ensure the formation of microcapsules and optimization was done on the basis of the visual assessment of the microcapsules size, uniformity in size and distribution and wall of the microcapsules on comparative basis. The ratio giving best results was selected for next stage of optimization.

Optimization of ratio of gum:

For determination of optimum ratio of gum, five different ratio of gum *i.e.* 1, 2, 3, 4 and 5 were taken with optimized ratio of essential oils whereas ratio of gelatin was kept constant along with all other variables. Microencapsulation was carried out and optimum ratio of gum was optimized on the basis of visual assessment.

Optimization of ratio of gelatin:

For determination of optimum ratio of gelatin, five different ratio of gelatin *i.e.* 1, 2, 3, 4 and 5 were taken with optimized proportion of oil and gum and all other variables were kept constant. Microencapsulation was carried out and optimum ratio of gelatin was optimized on the basis of visual assessment.

Optimization of temperature:

For determination of optimum temperature for microencapsulation, the process was carried out at six different temperatures *i.e.* 30, 40, 50, 60 and 70^{0}C with optimized ratio of oils, gum and gelatin and other variables were kept constant. Optimized temperature was selected on the basis of visual assessment.

Optimization of initial and final pH:

pH plays very important role in microencapsulation as it is responsible for phase separation which leads to capsule formation. For optimization of pH, the optimized ratio of essential oil, gum and gelatin at optimized temperature was set to initial pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. The microencapsulation process was carried out till gel formation took place and then final pH was maintained at 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 with each initial pH. The initial and final pH was optimized on the basis of visual assessment of microcapsule gel.

■ RESEARCH FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Preparation of microcapsule gel :

Microcapsule gel was prepared by mixing selected essential oil, gum acacia and gelatin using the complex coacervation technique and optimization of various variables *i.e.* ratio of essential oil:gum:gelatin, temperature and pH was carried out to obtain the best results.

Optimization of the ratio of essential oils in microcapsule gel:

Essential oils form the core material of the microcapsules and is basically responsible for multifunctional properties. Microcapsule gel was prepared using different ratio of selected clove oil *i.e.* 0.5, 1.0, 1.5, 2.0 and 2.5 keeping other variables constant. The gel was observed under inverted microscope to ensure the presence of microcapsules. The ratio of essential oil was optimized on the basis of visual assessment on three parameters *i.e.* size of microcapsules, uniformity in size and distribution and wall of microcapsules.

It is evident from Table 1 and visual assessment of microcapsule gel (Image- 1 to 5) that in case of *Neem* oil microcapsules were formed in all five ratios of oil, gum and gelatin *i.e.* 0.5:4:4, 1:4:4, 1.5:4:4, 2:4:4 and 2.5:4:4. Best capsules were formed at 2:4:4 ratios of oil, gum and gelatin. Hence, ranked 1st indicating medium sized microcapsules with good uniformity in size having thick

Table 1 : Optimization of ratio of *Neem* oil in microcapsule gel

Ratio of Oil: gum: gelatin	Formation of microcapsules	Parameters			
		Size of microcapsules	Uniformity in size and distribution	Wall of microcapsules	Rank
0.5:4:4	Yes	Small	Poor	Very thin	IV
1:4:4	Yes	Very small	Very poor	Very thick	V
1.5:4:4	Yes	Medium + Small	Average	Thick	II
2:4:4	Yes	Medium	Good	Thick and sharp	I
2.5:4:4	yes	Medium +Large	Average	Thick	III

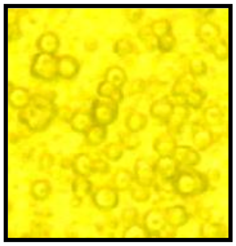
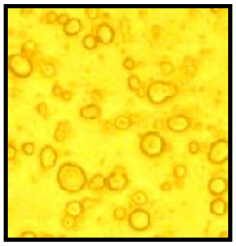


Image-1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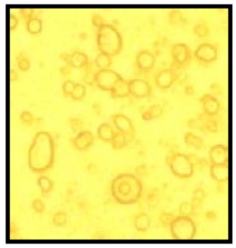
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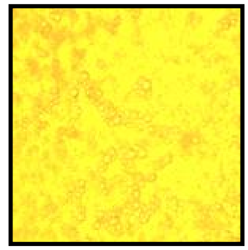
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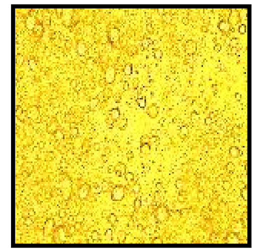


Image-5

and sharp walls of capsules. Thus, the ratio four of *Neem* oil was used for further optimization to achieve the best results.

Optimization of ratio of gum acacia in microcapsule gel:

Gum acacia forms the wall/outer core of the microcapsule and protects the oil from abrasion, sunlight and biodegradation thus provides a controlled release to the oil. Microcapsule gel was prepared using different ratios of gum acacia *i.e.* 1, 2, 3, 4, and 5.

The data presented in Table-2 and visual evaluation of microcapsule gel (Image-5 to 7) indicates that microcapsules were formed in only in three ratios *i.e.* 2:3:4, 2:4:4 and 2:5:4.

The microcapsules formed in the ratio of 2:4:4 were medium sized, having good uniformity in size and distribution and the walls were also sharp and thick as compared to the capsules formed in the other ratios. Therefore, the ratio 4 was optimized for *Neem* oil.

Optimization of ratio of gelatin in microcapsule gel:

Gelatin is a common ingredient of complex coacervation process and gives best results with gum acacia and essential oils. Microcapsule gel was prepared using different ratios of gelatin *i.e.* 1, 2, 3, 4 and 5.

The data presented in Table 3 and visual assessment of microcapsule gel indicates that microcapsules were formed in the three ratios of oil, gum and gelatin *i.e.* 2:4:3, 2:4:4 and 2:4:5 (Image- 9 to11) with *Neem* oil. Microcapsules were not formed at ratio 2:4:1 and 2:4:2, respectively. The best microcapsules formed at the ratio of 2:4:4 which were characterized as medium sized having good uniformity in size and distribution and the thick walls as compared to the capsules formed at other ratios. Therefore, the ratio 4 of gelatin was optimized for further experimentation.

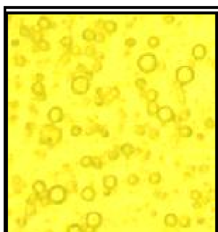
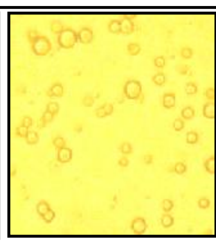


Image-6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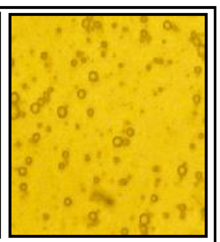
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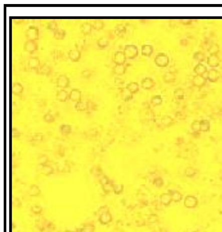
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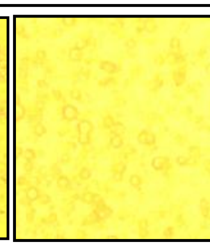
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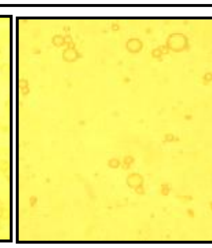
Image-11

Table 2 : Optimization of ratio of Gum acacia in microcapsule gel

Ratio of *Neem* Oil: gum: gelatin	Formation of microcapsules	Parameters			
		Size of microcapsules	Uniformity in size and distribution	Wall of microcapsules	Rank
2:1:4	No	-	-	-	-
2:2:4	No	-	-	-	-
2:3:4	Yes	Very Small	Average	Thick	III
2:4:4	Yes	Medium	Good	Thick and sharp	I
2:5:4	Yes	Medium+Small	Average	Very thick	II

Table 3 : Optimization of ratio of Gelatin in microcapsule gel

Ratio of *Neem* Oil: gum: gelatin	Formation of microcapsules	Parameters			
		Size of microcapsules	Uniformity in size and distribution	Wall of microcapsules	Rank
2:4:1	No	-	-	-	-
2:4:1	No	-	-	-	-
2:4:3	No	Small +Large	Poor	Thick	III
2:4:4	Yes	Medium	Good	Thick and sharp	I
2:4:5	Yes	Medium	Average	Thick	II

Table 4 : Optimization of temperature for microencapsulation of *Neem* oil

Temperature (ºC)	Formation of microcapsules	Parameters			Rank
		Size of microcapsules	Uniformity in size and distribution	Wall of microcapsules	
30	No	-	-	-	-
40	Yes	Small + Medium	Good	Thick	II
50	Yes	Medium + Large	Good	Thick and Sharp	I
60	Yes	Medium+Large	Average	Thick	III
70	No	-	-	-	-

Gum:oil:gelatine- 2:2:4

Optimization of temperature for microencapsulation process:

For optimization of temperature, microencapsulation process was carried out at five different temperatures *i.e*. 30, 40, 50, 60 and 70°C.

The data presented in Table 4 and visual evaluation (Image-12 to14) reveals that the microcapsules formed at 50°C were medium sized, had good uniformity and distribution and the walls were sharp and thick for *Neem* oil.

Microcapsules were not formed at temperature 30° and 70°C. Hence, 50°C temperature was optimized and used for development of essential oil microcapsules.

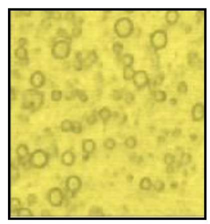

Image-12

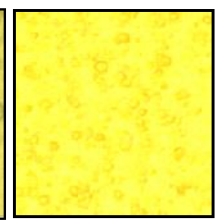

Image-13

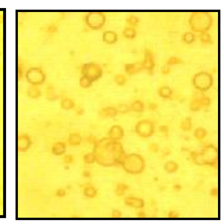

Image-14

Table 5 : Optimization of pH for microencapsulation of *Neem* oil

pH (initial / final)	Formation of microcapsules	Parameters			Rank
		Size of microcapsules	Uniformity in size and distribution	Wall of microcapsules	
4.0/8.5	Yes	Very Small	Very Poor	Thin	XII
4.0/9.0	Yes	Small	Good	Thin	VIII
4.0/9.5	Yes	Small	Average	Thick	IX
4.0/10.0	Yes	Medium + Small	Good	Thick	VI
4.5/8.5	Yes	Medium + Large	Average	Very Thick	VII
4.5/9.0	Yes	Medium	Average	Thick and sharp	II
4.5/9.5	Yes	Medium	Good	Thick and Sharp	I
4.5/10.0	Yes	Medium	Average	Thick	III
5.0/8.5	Yes	Large	Average	Very Thick	X
5.0/9	Yes	Medium	Average	Very Thick	IV
5.0/9.5	Yes	Medium	Average	Very Thin	V
5.0/10.0	Yes	Large	Poor	Very thick	XI

Gum:oil:gelatin-2:2:4, temperature- 50ºC

Optimization of pH for microencapsulation process:

To optimize initial pH and the final pH, microencapsulation was carried out with optimized ratio of oil:gum:gelatin and temperature. Microcapsule gel was initially started at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. After the completion of microencapsulation process, the final pH of the gel was set at 7.0, 7.5, 8.0, 8.5, 9.0 9.5 and 10.

As apparent from Table 5 and Image-15, microcapsules formed at initial pH 4.5 and final pH 9.5 were medium sized with uniform distribution having thick and sharp walls in case of clove oil Hence, these pH values were used for further experimental work as optimum initial and final pH for microencapsulation process of selected essential oil.

Image-15 : Optimization of pH for microencapsulation

Conclusion :

Micro encapsulation is the cost effective and long lasting method in storing volatile substances over a long period of time. Findings of the present study revealed that in standardization process of microencapsulation with *Neem* oil, best microcapsules were formed at the optimized ratio 2:4:4 of oil: gum: gelatin , at optimized 50ºC temperature having initial pH 4.5 and final pH 9.5. The microcapsules formed with these optimized conditions were observed medium having good uniformity in size and distribution with sharp and thick walls of capsules.

The standardized microencapsulation process can be effectively used in developing microcapsules gel with *Neem* oil for its wider applications per end uses to derive long term sustained benefit.

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