RESEARCH ARTICLE

Process Optimization of Carvacrol Nanobeads: DoE Approach

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Received: 15th November, 2022; Revised: 03rd February, 2023; Accepted: 24th April, 2023; Available Online: 25th June, 2023

ABSTRACT

Present study aims optimization of critical process parameters of carvacrol nanobeads by DoE-based approach. Two factorial design was applied in which critical process parameters – mixing time of magnetic stirrer, mixing speed of magnetic stirrer and speed of homogenizer were chosen as factors against entrapment efficiency as responses. The entrapment efficiency of different trials of carvacrol-loaded nanobeads as per two factorial design was estimated as their responses. Carvacrolis is a phenolic monoterpenoid present in many essential oils of Labitae family. Carvacrol has antimicrobial action, so it kills many types of pathogens. For entrapment efficiency, UV spectrophotometric method was used using methanol as solvent. Two factorial design was successfully applied, and results were analyzed using Design Expert software. From the two-factorial design, it is concluded that there are significant changes occurs due to critical process parameters as the results of three factorial design shows significant data also calculated F-value is also more than tabulated F-value which indicates model is compatible and significant for mixing time and the speed of homogenizer. Optimized mixing speed and homogenizer speed are predicted by design of expert software. Coefficient of correlation was found to be 0.9820, indicating the model's linearity. The *p-value* is more than 0.05 for mixing time which shows that the effect of mixing speed is not significant on entrapment efficiency, while mixing speed and speed of homogenizer have significant and positive effect on entrapment efficiency. So as we increases the mixing speed and speed of the homogenizer, entrapment efficiency increases. Optimize mixing time is 9.99 (~10) minutes, and mixing speed is 2000 rpm.

Keywords: Carvacrol, Entrapment efficiency, Two factorial design, UV spectrophotometric method, Quality by Design

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.2.26

How to cite this article: Tandel FB, Pandya AJ, Prajapati BR. Process Optimization of Carvacrol Nanobeads: DoE Approach. International Journal of Drug Delivery Technology. 2023;13(2):644-648.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The study focuses on the nano formulation of extended-release targeted delivery of carvacrol for better wound healing management.

A wound is defined as a break in the epithelial tissue of the skin. The wound on skin can occur in all ages of persons and causes harm in number of ways. The correct antimicrobial treatment for wounds requires the proper identification of microorganisms; hence, wound healing activity can be enhanced in less time.[1] Wound allows enter the organisms in tissues to break the skin continuously. Wounds create some type of complication like infections. Wound infection presents with pus drainage, fever, foul odor, dull throbbing pain, mild swelling and heat at wound site.[2]

Oregano oil is largely effective medicinally because of the presence of the correct amount of this active ingredient. Carvacrol oil of oregano useful because of its efficacy in healing a wide variety of diseases. Carvacrol gives an antimicrobial activity because of its structural arrangement and functional properties. The hydroxyl (-OH) group present in the structure is responsible for its antimicrobial activity. Its antimicrobial activity can damage particular bacteria's cell membrane and inhibit their proliferation. It interacts with the bacterial membrane and changes permeability for cations like H^+& K^+.[3] It is an effective medicinal substance for many reasons like, it can prevent the growth of bacteria such as *Escherichia coli* and *Bacillus cereus*. For instance, this substance destroys the cell membrane of *Pseudomonas aeruginosa* preventing them from multiplying. It inhibits COX-2 (cyclooxygenase-2) inflammation and activates PPAR (peroxisome proliferator-activated receptor). The enzyme COX-2 causes pain and inflammation while PPARs are a group of nuclear receptorproteins which work as transcription factors regulating gene expression. These substances are important in the development and differentiation of cells and in the metabolism of carbohydrates, proteins and lipids.[3] It is very effective in controlling and managing pain and inflammation. Carvacrol activates V_3, our human channels receptor potential and TRPA1, the genes that helps in the efficient functioning of our pain-sensing neurons.It is useful in common cold, flu and

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fever, gastroenteritis, diarrhea, acne, nail fungus, bacteremia or presence of bacteria in the blood, cholangitis or inflammation of the bile duct, arthritis pain and inflammation, inflammation of the gall bladder (cholecystitis).[4]

Carvacrol is naturally available drug in high abundance with low toxicity. It is lipophilic in nature and gets easy diffusion through the skin. It is poorly water-soluble drug.[5] This property can create problems while conventional formulating emulsion, microemulsion, cream, etc. It does not give sustained release. By incorporating carvacrol in polymeric beads, sustained release can be achieved and reduction in the dosing frequency is possible. On preparing ointment containing carvacrol, it gets stuck on to the site of infection and becomes difficult to remove, found as the limitation of the ointment and emulsion. In order to avoid this limitation nano beads were prepared. The nanobeads are a hydrogel-like semisolid preparation used topically on the body part.[6] The formulation reduces the rate of dosing frequency. The formulation is prepared by emulsion extrusion method to incorporate drug easily into the beads. The nanobeads formulation requires a very small amount of surfactant compared to micro emulsion.[7] It is a thermodynamically or kinetically stable formulation. Because formulations preparation was based on emulsion extrusion method, so emulsion is thermodynamically stable, and beads are kinetically stable. Hence, the hydrogel nanobeads are found better formulation as compared to microemulsion.[8] Carvacrol loaded extended release nanobeads-based powder formulation hasbeen developed to increase the drug release rate and to reduce the local irritation by carvacrol with the incorporation of alginate and calcium chloride by achieving best cross-linking property. Optimization of process parameters is the leading requisite.

MATERIALS AND METHODS

The drug was purchased from Sigma Aldrich. Calcium chlorides, isopropyl alcohol, poly vinyl alcohol 124, methanol were purchased from merck. Tween 80, isopropyl myristate, sodium hydroxide, potassium dihydrogen phosphate, mannitol was purchased from SD fine chem limited.

Preparation of Carvacrol Loaded Nanobeads

Carvacrol loaded nanobeads were prepared as per the procedure shown in following flow chart (Figure 1).

Drug entrapment Efficiency[9-11]

The entrapped drug of nanobeads is estimated after separation of free drugs and bound drugs. Entrapment efficiency of drug is defined as the ratio of entrapped mass of active pharmaceutical ingredients (API) to the total mass of theoretical drug content. 1st order UV spectrophotometric method was used to quantify entrapped carvacrol in formulation.[12]

Optimization of Critical Process Parameters (CPP) of Carvacrol Nanobead Formulation Using Two Factorial Designs

CPP like speed of magnetic stirrer, speed of high-speed homogenizer and mixing time for preparation of beads were identified as independent factors. For the optimize CPP of all batches 2 level factorial design was used because involves less experimental run and time.

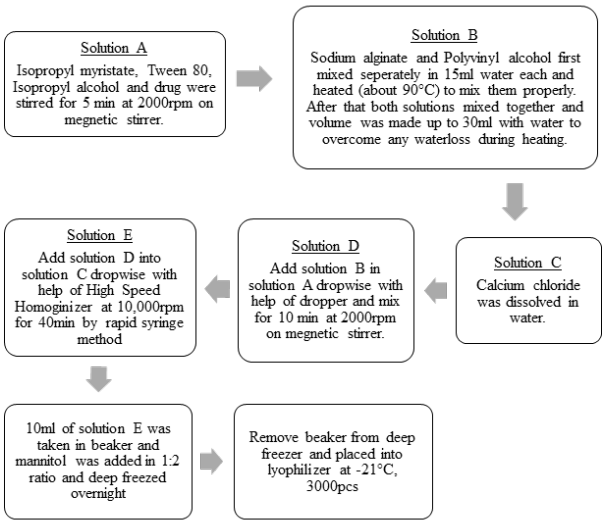

Figure 1: Process of formulating carvacol loaded nanobeads

Design Expert 7 software was used to apply DOE &Batches were prepared according to design (Table 1 & 2).

Results of entrapment efficiency for different trialswere found and feeded in software for determining influence of CPP on selected response (Table 3).

RESULTS AND DISCUSSION

Screening of CPP by DoE[13]

The results of particle size and entrapment efficiency for different trials are depicted in Table 3

The %entrapment efficiency was important factor for nanobeads formulation. As per Table 3 the entrapment efficiency of bead was found to be in range of 27.8 to 79.36%. From the above data, it was concluded that batch no. 8 was considered an optimized batch, which showed the maximum entrapment efficiency i. e. 79.36%.

Effect of Factors on %Entrapment Efficiency

Data of effect of mixing time, mixing speed and speed of homogenizer are as follows.

Full Polynomial Equation

Y1(%EE) = +54.71+4.50 * A+9.23 * B+11.73 * C-0.65 * A* C-2.60 * B * C

Reduced Equation

Y1 (%EE) = -495.68750 + 16.82750 * mixing time + 0.11741* Mixing speed + 0.025379* Speed of homo genizer- 6.48750E - 004 * mixing time * Speed of homogenizer- 5.20750E - 006 * Mixing speed * Speed of homogenizer

Table 4 describe the calculation of Analysis of Variance. It isrevealed that the *p-value* of the performed model was less than 0.05, indicating that the model was compatible and significant for mixing speed and speed of homogenizer.R2 value was found to be 0.9820, which indicates the model's

Table 1: Selection of independent and dependent variable

Independent variable		*Levels*	
Code	Name of Factors	Low (-1)	High (+1)
X1	Speed of homogenizer (RPM)	18000	20000
X2	Speed of magnetic stirrer (RPM)	1000	2000
X3	Mixing time (Minutes)	8	10
Dependent variable		Constraints	
Y1 (Entrapment Efficiency in %)		Maximize	

Table 2: Preparation of nanobeads by two factorial design

Run	*Coded value*			*Actual value*		
	X1	*X2*	*X3*	*X1 (rpm)*	*X2 (rpm)*	*X3 (Minutes)*
1	-1	-1	-1	18000	1000	8
2	1	-1	-1	20000	1000	8
3	-1	1	-1	18000	2000	8
4	1	1	-1	20000	2000	8
5	-1	-1	1	18000	1000	10
6	1	-1	1	20000	1000	10
7	-1	1	1	18000	2000	10
8	1	1	1	20000	2000	10

linearity. The *p-value* more than 0.05 for mixing time shows that the effect of mixing time is not significant on entrapment efficiency, while mixing speed and speed of homogenizer have significant and positive effect on entrapment efficiency. So as we increase the mixing speed and speed of homogenizer, entrapment efficiency increases.

Figure 2 shows the interaction between mixing speed and homogenizer speed, which does not show any interactions as graph have parallel lines for both factors. Figure 3 showed the optimal region of the formulation by considering the maximum entrapment efficiency. Figure 4 describes response counter plot of effect of mixing speed and speed homogenizer on entrapment efficiency. Figure 5 describe the 3- D surface plot. It shows that the combine effect of mixing speed and speed of homogenizer is very major. It indicates that with increase in speed of both mixing and homogenizer entrapment efficiency increases.

Figure 6 shows the effect of all three factors: mixing speed, mixing time and speed of homogenizer on entrapment efficiency in one cubic form. Cube form is helpful to just summarize all the factor and response interactions.

Figure 7 Yellow portion of overlay plot provides optimum desired result. Optimized mixing speed and homogenizer speed are predicted by design of expert software. Predicted optimize mixing time is 9.99 (~10) minutes, and mixing speed is 2000 rpm.

Table 3: Data of entrapment efficiency as per two level factorials (3FI)

Run	*Coded Value*			*Actual Value*			*Y1 (%Entrapment efficiency)*
	X1	*X2*	*X3*	*Mixing time (RPM)*	*Speed of magnetic stirrer (RPM)*	*Speed of homogenizer (Minutes)*	
1	-1	-1	-1	8	1000	18000	27.8
2	1	-1	-1	10	1000	20000	34.5
3	-1	1	-1	8	2000	18000	47.87
4	1	1	-1	10	2000	20000	61.77
5	-1	-1	1	8	1000	18000	58.4
6	1	-1	1	10	1000	20000	61.23
7	-1	1	1	8	2000	18000	66.78
8	1	1	1	10	2000	20000	79.36

Table 4: Analysis of variance table

Source	*Sum of Squares*	*DF*	*Mean Square*	*F value*	*p-value prob > F*	*Significant*
Model	2001.93	5	400.3859125	21.80417147	0.0444	Significant
A-Mixing time	162.09	1	162.0900125	8.827079866	0.0971	Not significant
B-Mixing speed on magnetic stirrer	681.73	1	681.7278125	37.12545736	0.0259	Significant
C-Speed of homogenizer	1100.51	1	1100.508613	59.93137557	0.0163	Significant
AC	3.37	1	3.3670125	0.183360392	0.7102	
BC	54.24	1	54.2361125	2.95358418	0.2278	
Residual	36.73	2	18.3628125			
Cor Total	2038.66	7				
Std. Dev. = 4.29						
R- Squared = 0.9820						
Adj. R- Squared = 0.9363						

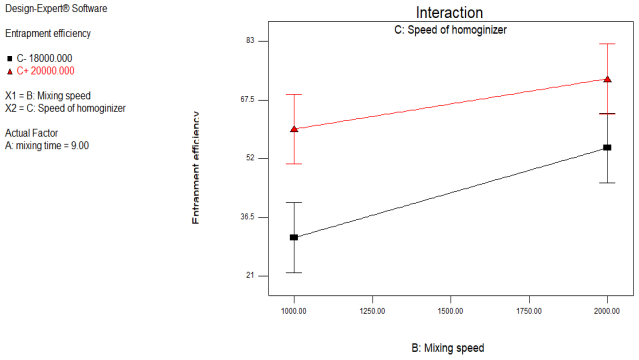

Figure 2: Interaction graph for mixing speed and homogenizer speed

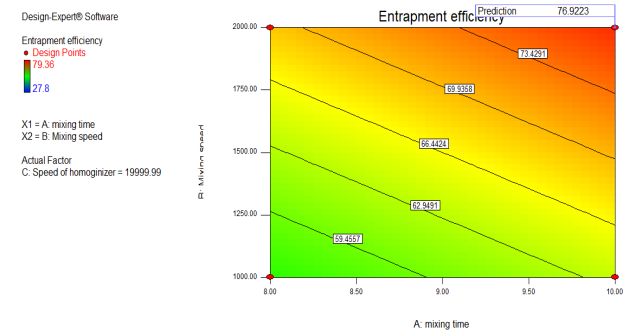

Figure 3: Numerical optimization for entrapment efficiency

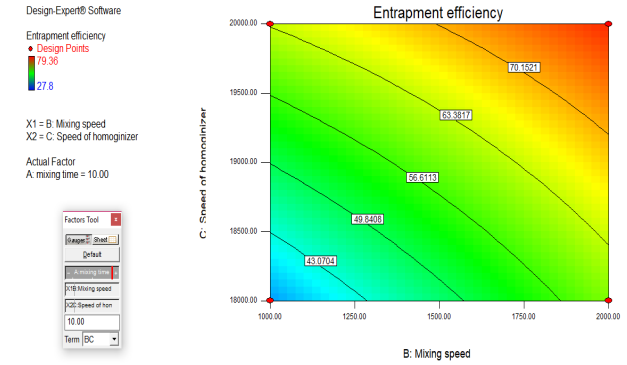

Figure 4: Response Counter Plot of effect of mixing speed and speed homogenizer on %EE

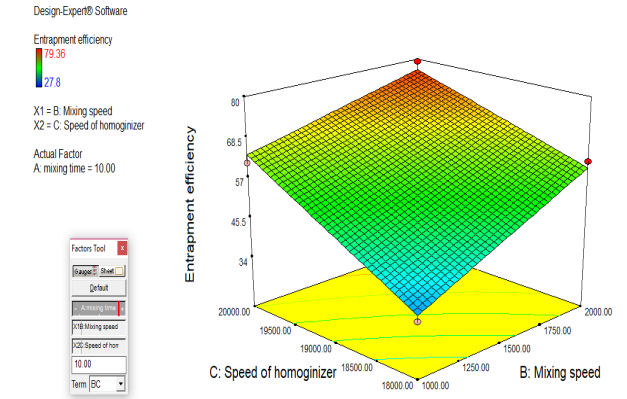

Figure 5: 3D Plot of effect of mixing speed and speed homogenizer on %EE

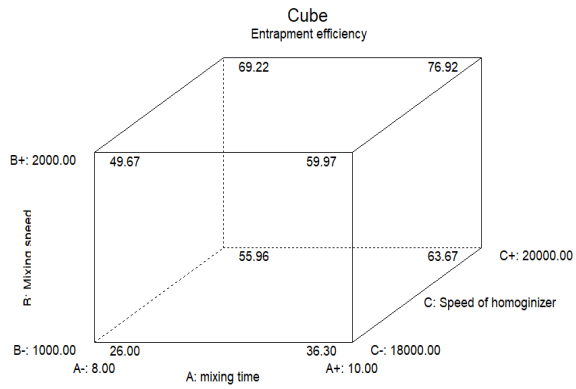

Figure 6: Entrapment efficiency in cube effect against all factors

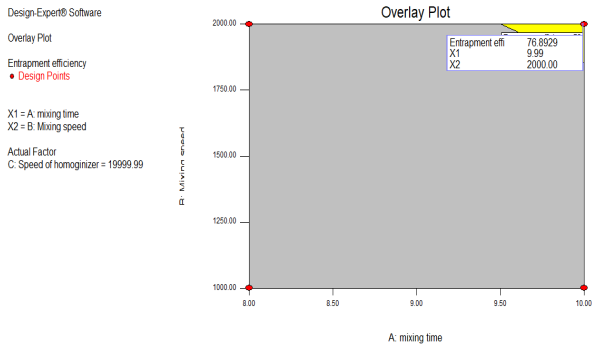

Figure 7: Overlay plot

CONCLUSION

From the three factorial designs applied to optimize CPP of nanobeads formulation, it was concluded that mixing time is not significant on entrapment efficiency; while mixing speed and speed of homogenizer have significant and positive effect on entrapment efficiency. R^2 value was found to be 0.9820 which indicate the linearity of model. The interaction between mixing speed and homogenizer speed does not show any interactions as graph have parallel lines for both factors. Model shows that the combine effect of mixing speed and speed of homogenizer is very major. It indicates that with increase in speed of both mixing and homogenizer entrapment efficiency increases.

ACKNOWLEDGEMENTS

The author would like to thank GUJCOST, Gujrat for providing economical assistance to conduct the research. Authors also thank Parul Institute of Pharmacy, Parul University for providing necessary resources for research work.

REFERENCES

1. Solanki R.and Lachoo N.A Review on Microorganisms Causing Wound Infections on Skin. Asian Journal of Pharmacy and Technology2013;3(3):119-122.
2. Kaushar H. Development of Carvacrol loaded hydrogel beads (M. Pharm Thesis), Gujarat Technological University,2016,51-66.
3. Ultee A., Leon G.M. and Smid E.J.Bactericidal activity of carvacrol towards thefood-borne pathogen Bacillus cereus.

Journal of AppliedMicrobiology1998;85(2):211-218.
4. The Benefits of Carvacrol and Oregano Oil, Available at http://www.carvacrol.co/
5. Profile of Carvacrol. Available at https://pubchem.ncbi.nlm.gov/compound/carvacrol
6. Gulrez H., Al-Assaf S.and Phillips G.Hydrogels: Methods of Preparation, Characterization and Applications, Progress in Molecular and Environmental Bioengineering. Anal. Model. Technol. Appl.2011;(5): 655-660.
7. Rana P., Ganarajan G. and Kothiyal P. Review on Preparation and properties of Hydrogel formulation. World Journal of Pharmacy and Pharmaceutical Sciences2015;4(12):1069-1087.
8. Enrica C. and Khutoryanskiy V.V.Biomedical applications of hydrogel: A review patents and commercial product. European Polymer Journal 2014;65(2015): 252-267.
9. Difference between drug entrapment efficiency and drug loading [Internet]. Difference between entrapment efficiency and drug Loading efficiency of microspheres [cited 2019 March 19]. Available from: http://www.answeres.com/Q/Difference_between_entrapmentment_efficiency_and_drug_Loading_efficiency_of_microspheres?
10. Chapter 4 Experimental methodology [Internet]. Chapter 4. [Cited 2019 March 16]. Available from: Shodhganga.inflibnet.ac.in/…/11_
11. Akhtar F. Drug entrapment efficiency and sustained drug release of chloramphenicol loaded polymeric iron nanoparticle. USA Patent US 2016/0101182A1, 2016.
12. Backett AH., Stenlake JB. Ultraviolet- visible absorption spectrophotometry. In: Practical Pharmaceutical Chemistry,CBS Publications and Distributors, New Delhi,2001,282-296-298.
13. Design of Experiments Guide. [Internet] JMP statistical discovery. [Cited 2019 March 23]. Available from: https://www.jmp.com/support/help/Design_of_Experiments_Guide.shtml#329680