JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/33927
RESEARCH ARTICLE

Formulation, Optimization and Evaluation of Nutraceutical Effervescent Granules Containing Curcumin and Ascorbic Acid by Fusion Technique Using Full Factorial Design

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Abstract

Recent studies suggest that curcumin has potent immunological activity in combination with ascorbic acid. Hence, research was carried out to formulate effervescent granules of curcumin and ascorbic acid as preventive measures for COVID-19. Thirteen formulas (F1 to F13) were prepared by applying 3^2 full factorial designs by applying the fusion method using Stat-Ease Design-Expert v7.0.0 software. Here, a complex of hydroxypropyl beta-cyclodextrin and curcumin was prepared by lyophilization technique to improve the solubility of curcumin. Citric acid, tartaric acid, sodium bicarbonate, polyethylene glycol, and sorbitol were used in the formulation of effervescent granules. Evaluation studies were carried out for all 13 formulas, including compatibility study, flowability study and effervescence time. The results show that granules have excellent flow property with an appropriate bulk density and tapped density for the uniting dose. The FTIR study also indicates that there is no interaction between excipients. The effervescence time is less than 3 minutes for all the batches. Formula 4 shows the best effervescence. This research article covers the development of effervescent granules containing curcumin, which has antiviral properties (both through direct movement on viruses and through modulating the immune system). With the pandemic of coronavirus that we are facing, curcumin and ascorbic acid can provide us protection against COVID-19 by keeping our immunity up and protecting against the onslaught of infections.

Keywords: Curcumin, Effervescence, Factorial Design, Fusion Method

1. Introduction

Curcumin has antioxidant, anti-inflammatory, antiviral, and antifungal effects. Literature and studies show curcumin is non-poisonous to humans[1]. Curcumin exerts an anti-inflammatory effect by inhibiting a variety of specific molecules that play a critical role in inflammation[2]. Curcumin is soluble in oil; practically, it is insoluble in water at neutral and acidic pH levels but soluble in alkali pH levels. So, to improve solubility, in this research, we make a complex of curcumin and 2-hydroxypropylbetacyclodextrin[3,4].

Ascorbic acid is a potent antioxidant which can be obtained from food and pharmaceutical product formulations because it is not synthesized by the human body. It keeps safe against upper and lower respiratory tract infections and lowers the risk of cardiac arrest and malignant cancer. Also, ascorbic acid enhances iron absorption and is required for collagen synthesis[5]. Various pharmaceutical products can be

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Article Received on: 01.06.2023 Revised on: 11.09.2023 Accepted on: 16.09.2023

administered orally, and oral administration is the common route for administration for different dosage forms[6,7]. Pharmaceutical technology states that oral medications, especially solid dosage forms, comprise a majority of produced medications worldwide[8,9].

A literature survey reveals that the combination of curcumin and ascorbic acid is useful for increasing the immune power of the body[10-16]. There is no formulation in the form of a nutraceutical's granule sachet available in the market. Therefore, an attempt is made to develop the formulation along with its method for the estimation of both the nutraceuticals simultaneously. The advantages of ascorbic acid and curcumin granules are no side effects, efficient as prophylactic agents, and they can easily be used in routine life as immunity enhancers and patient compliance.

Effervescent granules are the most widely used solid oral dosage form for oral delivery. The effervescent granules are soluble and dissolve fast, creating a tasty formulation that can reduce the unpleasant bitter taste of medications and boost the bioavailability of poorly absorbed medications. It is a reliable and convenient administration form. Prior to the patient's consumption, effervescent granules are intentionally added to a cup that has 250 millilitres of water. The granules quickly scatter in the water and dissolve with a release of carbon dioxide gas[17,18]. This happens as a result of the water-mediated reaction between the basic bicarbonates and the acidic components. The breakdown of the medicine can be accelerated, and the consequent bitter taste of the drug is covered up by the release of carbon dioxide. The objective of this study is to make effective and better use of curcumin and ascorbic acid[19].

Quality by Design (QbD), the trendiest approach employed for the design and development of pharmaceutical dosage forms (FbD), is the term used for statistical experiments that attempt to increase the quality of the product[20,21]. Design of experiment (DoE) is an optimisation approach created for analysing all potentially significant variables simultaneously, systematically, and promptly for goods and/or processes[22]. This study's goal was to develop curcumin and ascorbic acid effervescent granules with excellent quality and more patient compliance using the Formulation by Design (FbD) methodology in a time and cost-effective manner[23,24]. To investigate the granules' physical and chemical properties, a 3^2 Full factorial design was employed. The fusion granulation technique was used to produce the formulae[25].

2. Materials and Methods

Curcumin, ascorbic acid, tartaric acid, citric acid, sodium bicarbonate, propylene glycol, 2-hydroxylpropyl-beta-cyclodextrin, and d-sorbitol were purchased from Himedia Laboratories Pvt. Ltd. India. AR grade methanol was used as a solvent and procured from Chiti-Chem Corporation, Vadodara, Gujarat.

2.1 Preformulation Studies

2.1.1 Melting Point Determination

The melting point of curcumin, ascorbic acid and other excipients were determined by using a Thiele tube and liquid paraffin by manual method.

2.1.2 Identification of Active Pharmaceutical Ingredients and Formulation by Infrared Spectroscopy

Confirmation of the structure and functional group of curcumin and ascorbic acid in presence of the excipients by Fourier Transform Infrared Spectroscopy (FTIR) were done. The graphs of FTIR for curcumin, ascorbic acid and granules are depicted in Figures 1-3, respectively. It shows no interaction between excipients.

2.2 Preparation of Complex (Curcumin + HP-β-CD)

Freeze drying (lyophilization) method was used to make the complex. It is prepared by slowly and gradually adding 50 ml (50% v/v) ethanol to 1.38 gm HP -β- CD. To this mixture, 0.3680 g of curcumin is added and stirred on a magnetic stirrer until the desired consistency is obtained. The mixture is Lyophilized for 24 hrs. The freeze-dried mixture is passed through 80 mesh sieves.

2.3 Preparation of Granules by Fusion Method

Citric acid, tartaric acid, sodium bicarbonate, d-sorbitol, propylene glycol, ethanol, and 2-hydroxypropyl-beta-cyclodextrin were used to effectively produce

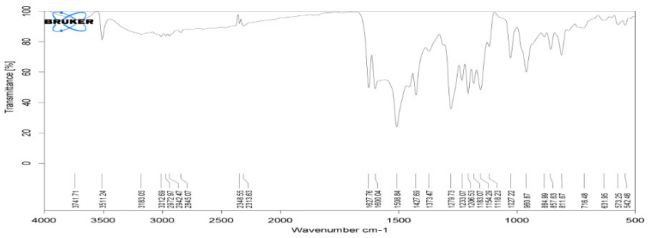

Figure 1. Fourier Transform Infrared spectrum of curcumin.

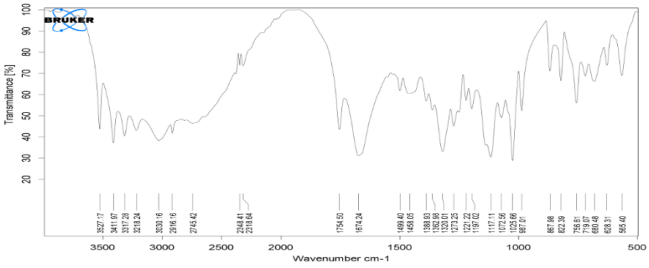

Figure 2. Fourier Transform Infrared spectrum of ascorbic acid.

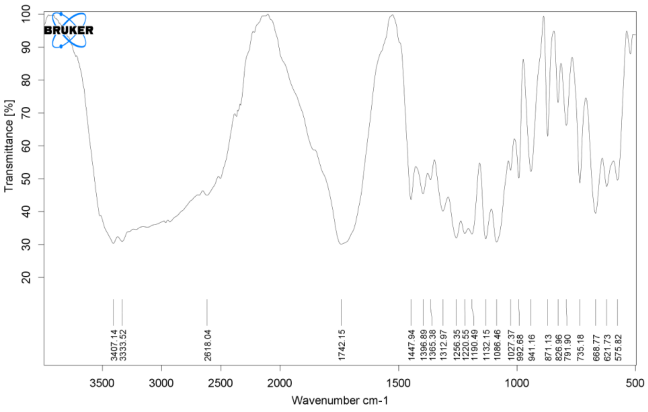

Figure 3. Fourier Transform Infrared spectrum of formulation**.**

effervescent curcumin and ascorbic acid granules. 150 mg of complex (curcumin + HP-β-CD), ascorbic acid 1000 mg, 600 mg of tartaric acid, 300 mg of citric acid, sodium bicarbonate 1032 mg, D-sorbitol 600 mg were accurately weighed and mixed. One drop of polyethylene glycol (90 mg) is added to the mixture. The final mixture is kept in a hot air oven for 7-8 mins at 40°C. Granules were produced by passing the dry material through sieve number 16.

2.4 FbD based Statistical Screening of Critical Factors Using 3^2 Full Factorial Designs

Stat-ease design-expert v7.0.0 was used to check the effect of independent variables on the responses, considered dependent variables, using 3^2 full factorial designs. Screening of effervescent granules of curcumin and ascorbic acid was done according to 3^2 full factorial designs. A total of 13 batches were prepared. An amount of drug complex concentration (X1) and an amount of sodium bicarbonate (X2) were selected as independent variables depicted in Table 1. The response evaluated was the effervescent time, i.e., the dependent variable. The optimized formulation was selected on the basis of specified effervesces time, and it is depicted in Table 4. The coded values for Factor X1 and X2 composition are listed in Table 2. The best-fitting mathematical model was selected for each response from Table 3. Through stepwise multiple regression analysis, the model predictor equations were calculated. The model was validated using a 0.05 significance level analysis of variance and the coefficient of determination (R^2). Using the stat-ease design-expert v7.0.0 programme, all statistical computations and graphic plots were made (Table 5).

Table 1. Variables selected for optimization of formulation

Independent Variables	Dependent Variable (Response)
X1: Drug Complex concentration (mg)	Y1: Effervescent time (s)
X2: Amount of Sodium Bicarbonate (mg)	-

Table 2. Coded value for Factor X1 and X2

Coded values for variables	Low (-1)	Medium (0)	High (+1)
X1: Drug Complex concentration (mg)	150	250	350
X2: Amount of Sodium Bicarbonate (mg)	500	1000	1500

Table 3. Formulation optimization using 3^2 full factorial design

Batch	Level of variables in coded form		Levels of variable in actual form		Response (dependent) variable
	X1	X2	Drug complex (Curcumin + HP-β- CD) mg	Amount of sodium bicarbonate (mg)	Effervescence time (seconds)
F1	-1	-1	150	500	120
F2	0	-1	250	500	125
F3	+1	-1	350	500	104
F4	**-1**	**0**	**150**	**1000**	**175**
F5	0	0	250	1000	124
F6	+1	0	350	1000	130
F7	-1	+1	150	1500	195
F8	0	+1	250	1500	99
F9	+1	+1	350	1500	120
F10	0	0	250	1000	125
F11	0	0	250	1000	118
F12	0	0	250	1000	127
F13	0	0	250	1000	122

Table 4. The optimized formula for Batch F4

Reagent	Amount
complex (curcumin + HP-β-CD)	150 mg
Ascorbic acid	1000 mg
Tartaric acid	600 mg
Citric acid	300 mg
Sodium bicarbonate	1032 mg
D- sorbitol	600 mg
Polyethylene glycol	1 drop (90 mg)

Effervescence time was selected as a response for optimization of the formulation. An optimization study was carried out by using Design Expert® 7 software. Results obtained are shown in Figure 4, the form responses surface plot, Figure 5, the contour plot, and Figure 6, overlay for the effervescence time of the drug complex. The p-value of the model was found to be 0.0261, which was less than 0.05, which shows that the model was significant. Additionally, it has been found that the quantity of drug complex and the amount of sodium bicarbonate, both independent factors, have a considerable impact on the response's effervescence time.

2.5 Polynomial Equation for Effervescence Time in Coded Value

Effervescence Time: +124.10 -22.67* amount of drug complex + 10.83* amount of sodium bicarbonate -14.75* concentration of drug complex** amount of sodium bicarbonate+ 26.14* concentration of drug complex^2 – 14.36* amount of sodium bicarbonate^2.

The equation represents the quantitative effect of the independent variables on the effervescence time, which indicates that the amount of sodium bicarbonate has a positive effect on effervescence time. The Polynomial equation also represents that the amount of drug complex has a negative effect on response effervescence time. The same can be depicted in the contour plot and Response Surface model.

Table 5. ANOVA table for effervescence time

Source	Sum of squares	Degree of freedom	MEAN SQUARE	F Value	p-value	Signification
Model	6606.68	5	1321.34	5.19	0.0261	Significant
A: Amount of drug complex	3082.67	1	3082.67	12.12	0.0102	Significant
B: Amount of sodium bicarbonate	704.17	1	704.17	2.77	0.0386	Significant
AB	870.25	1	870.25	3.42	0.1068	
A^2	1886.91	1	1886.91	7.42	0.0296	
B^2	569.70	1	569.70	2.24	0.1782	
Residual	1780.55	7	254.36			
Pure Error	46.80	4	11.70			
Corrected Total	8387.23	12				

2.6 Model Graphs

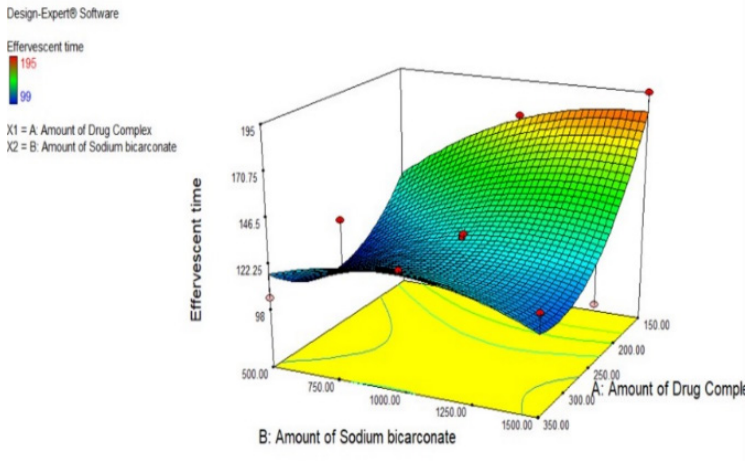

Figure 4. Response surface plot of effervescence time.

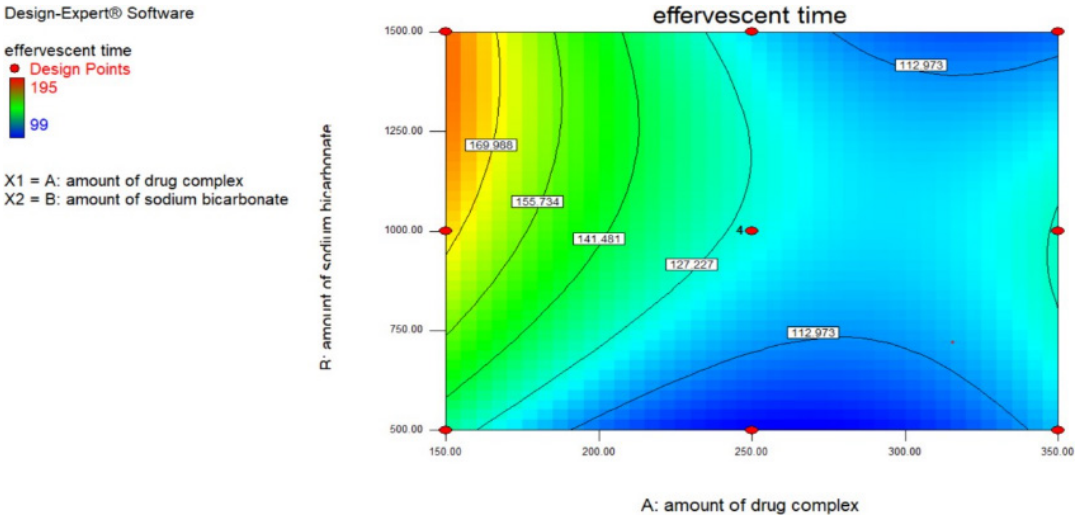

Figure 5. The counter plot of drug complex.

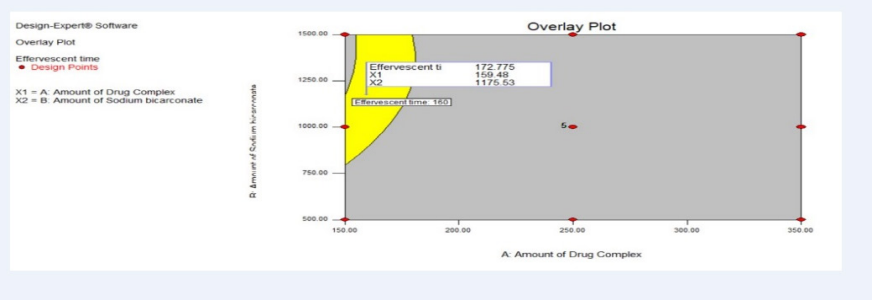

Figure 6. Overlay plot of drug complex.

2.7 Validation of Experimental Design

To validate the experimental design, a checkpoint batch was prepared by taking the amount of drug complex 150mg and sodium bicarbonate 1000mg as per the method given in the preparation of the granule method.

The actual checkpoint batch was based on an overlay plot in that the amount of drug complex was 159.48 mg, and Sodium Bicarbonate was 1176.63 mg.

Table 6. Comparison of the result of checkpoint batch with performed batch

Results of actual checkpoint batch	Results of performed checkpoint batch
Y1: Effervescence time Y1 = 172.77 s	Y1: Effervescence time Y1 = 174.86 s

The checkpoint batch result is shown in Table 6, along with the actual research that was done on the formulation created using the checkpoint approach. Effervescence time values were discovered to be 172.77 s and 174.86 s, respectively, which were equivalent without significantly differing. The outcomes confirmed the equation calculated using the dependent variable's data analysis. Therefore, the degree of similarity between the experimental and predicted response values evaluated the prediction's reliability.

3.Results and Discussion

Table 7. Summary of evaluation parameters

Bulk density (gm/ml)	Tapped density (gm/ml)	Angle of repose (degree°)	Carr's Index	Hausner's Ratio
0.840 ± 0.07	1.05 ± 0.12	15.6° ± 0.10	20 ± 0.13	0.8 ± 0.09

The outcomes of the flowability study of granules can be seen in Table 7. Bulk density was determined to be 0.840g/ml. The produced Granules tapped density values were discovered to be 1.05gm/ml. It was determined that the angle of repose was 15.6°. It indicates an excellent flow characteristic. While the produced Granules' Carr's Index result fell inside the 20 range. It indicates the fair flow property of granules. Hausner's ratio is 0.8. These results imply that the formulation has reasonable flow characteristics.

4. Conclusion

The physicochemical characterization of the optimized formulation showed no interaction between the drug and excipients. The fusion technique was successfully utilised for the preparation of curcumin and ascorbic acid effervescent granules. Stat-ease design-expert v7.0.0 software demonstrated that factorial design is a useful method for the characterization of the effects of variables in the preparation of effervescent granules. A full factorial 3^2 design was employed as a screening technique to determine the significant factors for the preparation and screening of the effervescent granules. The drug complex (curcumin + HP-β-CD) and amount of sodium bicarbonate were selected to have the most significant effect on the quality attributes, i.e., effervescent time. Using the factorial design feature of the stat-ease design-expert v7.0.0 programme, a very significant statistical model was produced that can accurately describe or forecast the formulation optimisation of effervescent granules for the specified quality.

Although curcumin is used to treat a number of chronic conditions, its low bioavailability has prevented it from being widely employed as a medicinal agent. Here, we tried to reduce these issues by incorporating

Hydroxypropyl β-Cyclodextrin. This approach is taken to develop a simple and effective immunity enhancer in the form of granules, which are easily taken by oral route. A simple and cost-effective approach to preparing the granules.

In conclusion, the effervescent granules had the proper mechanical and physical features and satisfied the pharmacopeial quality standards. The effervescent granules demonstrated promising characteristics, including simple administration, quick disintegration, acceptable flow characteristics, patient compliance, and low cost. By extrapolating from present studies, this formulation has a very high commercial potential.

5. Acknowledgement

The authors are grateful to the Gujarat Council on Science and Technology (GUJCOST) Department of Science and Technology, Gandhinagar, Gujarat, India, for providing financial support.

6. References

1. Shelat DY, Acharya SR. CUR-CA-THIONE: A novel curcumin concoction with enhanced water solubility and brain bio-availability. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8:265-70. https://doi.org/10.22159/ijpps.2016v8i12.15093
2. Li N, Wang N, Wu T, Qiu C, Wang X, Jiang S, *et al.* Preparation of curcumin-hydroxypropyl-β-cyclodextrin inclusion complex by cosolvency-lyophilization procedure to enhance oral bioavailability of the drug. Drug Development and Industrial Pharmacy. 2018; 44(12):1966-74. https://doi.org/10.1080/03639045.2018.1505904
3. Guo S. Encapsulation of curcumin into β-cyclodextrins inclusion: A review. 2nd International Conference on Biofilms (ChinaBiofilms 2019); 2019. p. 01100. https://doi.org/10.1051/e3sconf/201913101100
4. Stohs SJ, Chen O, Ray SD, Ji J, Bucci LR, Preuss HG. Highly bioavailable forms of curcumin and promising avenues for curcumin-based research and application: A review. Molecules. 2020; 25(6):1397. https://doi.org/10.3390/molecules25061397
5. Prajapati BG, Patel DV. Formulation and optimization of domperidone fast dissolving tablet by wet granulation techniques using factorial design. International Journal of PharmTech Research. 2010; 2(1):292-9.
6. Diyya AS, Thomas NV. Formulation and evaluation of metronidazole effervescent granules. International Journal of Pharmaceutical Sciences and Research. 2018; 9(6):2525-9.
7. Al-Mousawy JI, Al-Hussainy ZA, Alaayedi M. Formulation and evaluation of effervescent granules of ibuprofen. International Journal of Applied Pharmaceutics. 2019; p. 66-9. https://doi.org/10.22159/ijap.2019v11i6.34912
8. Kuswardhani N, Belgis M, Tiara A. Characterization of physical and chemical properties of effervescent tablets Temulawak (*Curcuma zanthorrhiza*) in the various proportion of sodium bicarbonate and tartaric acid. The 3rd International Conference on Agricultural and Life Sciences (ICALS 2019); 2020. p. 03006. https://doi.org/10.1051/e3sconf/202014203006
9. Husni P, Baitariza A, Fathurohman D. Formulation of nano curcuminoid effervescent tablet. Research Journal of Pharmaceutical Biological and Chemical Sciences. 2017; 8:179-84.
10. Jantarat C, Sirathanarun P, Ratanapongsai S, Watcharakan P, Sunyapong S, Wadu A. Curcumin-hydroxypropyl-β-cyclodextrin inclusion complex preparation methods: Effect of common solvent evaporation, freeze drying, and pH shift on solubility and stability of curcumin. Tropical Journal of Pharmaceutical Research. 2014; 13(8):1215-23. https://doi.org/10.4314/tjpr.v13i8.4
11. Parajuli-Baral K. Formulation and evaluation of quality parameters of effervescent granules from the potent antioxidant between two variants of the adaptogenic herb *Ocimum tenuiflorum* L. The Scientific World Journal. 2023. https://doi.org/10.1155/2023/2050846
12. Alemad A, Al-Absi M, Alagbarri S, Al-Nowihi M. Formulation by design approach for effervescent granules of vitamin C using statistical optimization methodologies. Journal of Applied Pharmaceutical Research. 2020; 8(4):62-9. https://doi.org/10.18231/j.joapr.2020.v.8.i.4.62.69
13. Khudhair DH, Al-Gareeb AI, Al-Kuraishy HM, El-Kadem AH, Elekhnawy E, Negm WA, *et al.* Combination of Vitamin C and curcumin safeguards against methotrexate-induced acute liver injury in mice by synergistic antioxidant effects. Frontiers in Medicine. 2022; p. 9. https://doi.org/10.3389/fmed.2022.866343
14. Lim DJ. 3-O-ethyl-l-ascorbic acid doped enteric-coated gelatin capsules towards the advanced oral curcumin delivery for cancers. Polymers. 2022; 14(11):2207. https://doi.org/10.3390/polym14112207
15. Mehany AB, Farrag IM, Diab M, Ghoneim MM, El-Sherbiny M, Al-Serwi RH, *et al.* Curcumin and Vitamin C improve immunity of kidney via gene expression against diethyl nitrosamine induced nephrotoxicity in rats: *In vivo* and molecular docking studies. Heliyon. 2023; 9(3):e14126. https://doi.org/10.1016/j.heliyon.2023.e14126
16. Pantwalawalkar J, More H, Bhange D, Patil U, Jadhav N. Novel curcumin ascorbic acid cocrystal for improved solubility. Journal of Drug Delivery Science and Technology. 2021; 61:1022-33. https://doi.org/10.1016/j.jddst.2020.102233

17. Bhagwat A, Pathan IB, Chishti NA. Design and optimization of pellets formulation containing curcumin ascorbic acid co-amorphous mixture for ulcerative colitis management. Particulate Science and Technology. 2021; 39(7):859-67. https://doi.org/10.1080/02726351.2020.1848946
18. El-Saadony MT, Yang T, Korma SA, Sitohy M, El-Mageed A, Taia A, *et al*. Impacts of turmeric and its principal bioactive curcumin on human health: Pharmaceutical, medicinal, and food applications: A comprehensive review. Frontiers in Nutrition. 2023; 9:1040-59. https://doi.org/10.3389/fnut.2022.1040259
19. Fuloria S, Mehta J, Chandel A, Sekar M, Rani NN, Begum MY, *et al*. A comprehensive review on the therapeutic potential of *Curcuma longa* Linn. in relation to its major active constituent curcumin. Frontiers in Pharmacology. 2022; 13:806-20. https://doi.org/10.3389/fphar.2022.820806
20. Kunnumakkara AB, Hegde M, Parama D, Girisa S, Kumar A, Daimary UD, *et al*. Role of turmeric and curcumin in prevention and treatment of chronic diseases: Lessons learned from clinical trials. ACS Pharmacology and Translational Science. 2023; 6(4):447-518. https://doi.org/10.1021/acsptsci.2c00012
21. Boosting Bioavailability of curcumin and Vit C with liposomal nanotechnology [Internet]. 2022. Available from: https://nuffoodsspectrum.in/2022/03/28/boosting-bioavailability-of-curcumin-and-vit-c-with-liposomal-nanotechnology.html
22. Jannat E, Al Arif A, Hasan MM, Zarziz AB, Rashid HA. Granulation techniques and its updated modules. The Pharma Innovation. 2016; 5(10, Part B).
23. Petchsomrit A, Sermkaew N, Wiwattanapatapee R. Self-microemulsifying, reconstituted granules for oral administration of curcumin: Development and *in vitro* characterization. Latin American Journal of Pharmacy. 2018; 37(4):740-7.
24. Avula PR, Veesam H. Influence of dependent variables on granule formulation using factorial design: Microwave irradiation as one of the factor. International Current Pharmaceutical Journal. 2013; 2(7):115-8. https://doi.org/10.3329/icpj.v2i7.15153
25. Alemad A, Al-Absi M, Alagbarri S, Al-Nowihi M. Formulation by design approach for effervescent granules of Vitamin C using statistical optimization methodologies. Journal of Applied Pharmaceutical Research. 2020; 8(4):62-9. https://doi.org/10.18231/j.joapr.2020.v.8.i.4.62.69