Open Access

Research Article

Pre-Formulation Study for Palatable Microbeads of Lycopene

Yasir Rahman[1], Saima Amin[2]*, Showkat R Mir[3], Kanchan Kohli[4]

[1] Research Scholar Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi.

[2] Associate Professor, Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi.

[3] Assistant Professor, Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi.

[4] Professor, Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi.

ABSTRACT

Lycopene is a widely used nutraceuticals for its antioxidant property but the molecule has poor aqueous solubility, high instability, and extremely low intestinal permeability leading to its poor bioavailability. In the present study, pre-formulation study was carried out to prepare sodium alginate microbeads with the intention to deliver an effective amount of lycopene for high absorption through oral route. A thorough physical characterization and spectral analysis were done to understand the characteristic of lycopene such as its melting point, UV spectrophotometric analysis, chromatography through reverse phase HPLC. Fourier transform infrared spectroscopy and differential scanning calorimetry were adopted to know the interaction of sodium alginate with lycopene. Box-Behnken design (version 9.0.2.0, Stat Ease Inc, USA) was used to analyse the effect of formulation variables such as sodium alginate (%), glutaraldehyde (%) and stirring speed on lycopene entrapment and its loading into microbeads. The adopted preformulation strategy revealed that lycopene was a crystalline powder with a sharp melting point at 155°C and the prominent functional groups were present in the sample. UV and HPLC analysis revealed precise quantitation and authenticity of lycopene. Excipient compatibility also revealed inertness of sodium alginate. Response surface morphology revealed significant effect of alginate, glutaraldehyde and stirring speed on formation of best composition. Therefore, it is concluded that lycopene can be formulated as microbeads for oral drug delivery.

Keywords: lycopene, sodium alginate, permeation, absorption, microbeads

INTRODUCTION

Oral drug delivery is considered as an attractive and most preferred route for drug administration as it provides some advantages for patients, such as the ease of drug administration and a high degree of flexibility on dosages. In oral administration, drugs should be protected under unstable biological environments including drug degradation induced by the GI tract and first-pass liver effects after oral administration before reaching the targeted sites [1].

Most of the drugs exhibit inconsistent gastrointestinal absorption, thereby resulting in unsatisfactory therapeutic efficacy which can be attributed to their inadequate solubility in vivo [2]. Besides poor solubilisation of drug in the gastrointestinal tract (GIT), there are multiple other factors which reduce the degree of absorption of poorly soluble drugs. Several lipophilic drugs are candidates for efflux transporters such as P-glycoprotein which further leads to their poor in vivo prospect [3,4]. Therefore, there is a substantial call for an ideal microencapsulation system which takes into accounts all these aspects and subsequently fills the loopholes for enhanced deliverance of lipophilic drugs. In this regard sodium alginate microbeads are a promising formulation approach as they have the potential of fixing these predicaments by increasing the stability and life of the drug being encapsulated, facilitate the manipulation of the drug and control its liberation in an adequate time and space further improving and normalizing the absorption of such drugs [4].

Lycopene, a phytonutrient belongs to the family of acyclic carotenoids [5,6] and is responsible for the red colour in tomato and its potent antioxidant [7]. This attributes to the

prevention of certain types of cancer, cardiovascular diseases, hyperlipidaemia, etc. [8.] A great interest has recently been focused on lycopene due to its preventive activity against several pathologies, such as cardiovascular disease[9,] hepatic fibrogenesis[10], solar light induced erythema [11,] human papillomavirus persistence [12] and some cancer types, such as prostate, gastrointestinal and epithelial[13.] Lycopene has also been recently reported to play a role in lung function [14] as well as in foetal growth [15.]

Despite the tremendous health benefits, poor aqueous solubility, low bioavailability, high instability and extremely low intestinal permeability are the frightening challenges for clinical utility of lycopene [16,17.] Besides, in certain health disorders, the demand of high therapeutic dose of lycopene is still remained a challenge for conventional dosage forms. Therefore, a well-organized delivery system is indeed warranted to enhance oral bioavailability and therapeutic effectiveness of lycopene. Therefore, in the present work we studied the pre-formulation aspects of sodium alginate microbeads.

MATERIAL AND METHODS

Material

Lycopene of purity 6% was obtained from Bio-ethical Pharmaceutical Ltd., Baddhi, India and sodium alginate (216.12 g/mol), glutaraldehyde (25% w/v) solution, acetonitrile (HPLC grade), tetrahydrofuran (HPLC grade), methanol (HPLC grade) and n-hexane (HPLC grade) were all purchased from s.d. Fine Chemicals, Mumbai, India.

Method

Physical characterization and identification of drug

Lycopene was evaluated for physical characteristics such as appearance, odour and melting point.

Spectral analysis

FT-IR spectral analysis

FTIR spectrum of lycopene was taken by Tensor II FTIR-Spectrophotometer (Bruker, USA). Lycopene was scanned at the rate of 4 mm/s and a resolution of 2 cm^{-1} between wave number ranging from 4000 cm^{-1} to 650 cm^{-1}.

Ultraviolet spectral analysis

For the purpose of quantification of lycopene at various stages of formulation development and characterization, UV-Visible spectrophotometry was used. For UV-Visible spectroscopic analysis of lycopene, the standard plot for lycopene in n-hexane was prepared using Shimadzu UV-1601 spectrophotometer (Shimadzu Corp, Kyoto, Japan). 2 mg lycopene was accurately weighed and dissolved in n-hexane and volume was made up to 10 ml to obtain 100 µg/ml stock solution. It was scanned with UV- Spectrometer between 200nm to 800nm. The calibration plot was prepared in the concentration range 10-90 µg/ml.

HPLC analysis

A simple and sensitive RP- HPLC method was developed to assure the purity of the lycopene sample. The HPLC system comprised of a binary pump having UV detector (Shimadzu, Japan), C-18 column (150 mm × 4.6 mm) particle size 5 µm (Agilent, Switzerland) as an analytical column. The mobile phase consisted of acetonitrile and tetrahydrofuran (50:50 % v/v). The flow rate was maintained at 1 mL min^{-1}. 5mg

lycopene was accurately weighed and dissolved in acetonitrile and volume was made up to 25 ml to obtain a stock 200 µg/ml concentration. From the stock different dilutions ranging from 10-200 µg/ml were prepared using acetonitrile as diluting medium. Samples were filtered through a 0.25 µm filter (Axiva Sichem Biotech, Delhi, India) and injected by a 20 µL loop injector. Detection was carried out at a wavelength of 479 nm. Data and chromatogram were obtained using Class-VP software.

Solubility study

2 mg of lycopene was dissolved in 10mL of simulated gastric fluid of pH 1.2 and simulated intestinal fluid of pH6.8 and was scanned through UV. The samples were then incubated with equal volume of n-hexane for a period 3h. The samples were scanned from 200-800 to observe the wavelength related to identity of lycopene. The amount of drug extracted in n-hexane was estimated through the calibration plot prepared in n-hexane.

Drug excipient compatibility study

The mixture of lycopene and sodium alginate in the ratio 1:1 was stored in glass vials for 7 days at 25°C and 60% relative humidity. The samples were analysed for any physical or chemical changes.

Statistical optimization of developed formulation by response surface methodology

In order to get optimized formulation with best % entrapment efficiency and % drug loading, a three factor three level Box-Behnken design (version 9.0.2.0, Stat Ease Inc, USA) was used. The response surface methodology consisted of 3 factors: sodium alginate (%) (X1), glutaraldehyde (%) (X2), and stirring speed (rpm) (X3) tested at 3 levels: low, medium and high levels represented as -1, 0,+1, respectively. Dependent variables for the study were % encapsulation efficiency (Y1) and % drug loading (Y2) respectively. 17 experimental runs were obtained and analysed for the influence of the variables (Table 1).

RESULTS AND DISCUSSION

Lycopene is a red colour component extracted from tomatoes, watermelon and other fruits. Till date, a lot of research has been done to prove its antioxidant property but a successful dosage form for such a health supplement is not available. Though solid lipid nanoparticles, protein nanoparticles *etc.* are available at research level but the problems related to its poor bioavailability and thorough elucidation of its mechanism of action through such dosage forms, is missing. In the present research work, sodium alginate microbeads were envisaged to deliver an effective amount of lycopene for high absorption per oral. To begin the research work, lycopene provided as gift sample was characterized for its physical attributes such as colour, nature of the powder and melting point. The provided sample showed presence of unsaturation bonds and straight chain configuration as supported by FTIR spectrum (Figure 1). Major peaks of the spectrum with characteristics peak at 2972.09, 2865.80, 1540.25 cm^{-1} corresponding to C-H bond, =C–CH$_3$ bond, and C=C bond respectively were interpreted to determine the respective functional groups present. To authenticate the spectrum, FTIR spectrum of the extracted lycopene reported by Lopez-Cervantes *et al* [18] was compared with lycopene sample. The spectrum revealed the true identity of lycopene as reported in literature [19]
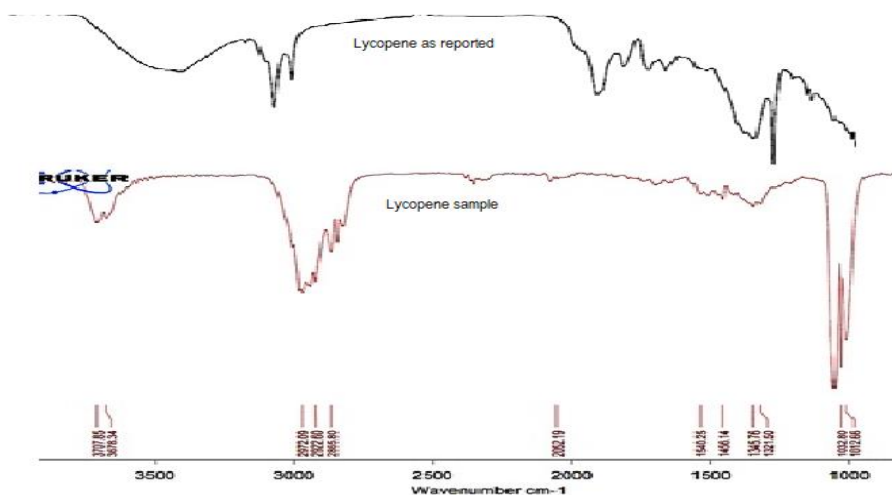
Figure 1: FTIR for lycopene as reported in literature and the sample procured.

Lycopene did not show solubility in solvents such as water, buffer of pH 1.2 and 6.8, methanol and ethanol. Therefore, it was decided to adopt incubation method for estimation of lycopene. The amount of lycopene (1mg) was placed in buffer pH 1.2 and 6.8. After thorough stirring and vortexing, the sample did not show solubility. It was then treated with equal volume of n-hexane. After vortexing, the sample (n-hexane) turned slight orange due to presence of lycopene. The sample in n-hexane was scanned for λmax at 471 nm

(Figure 2). Since the initial sample was transparent with lycopene granules floating on the surface, but with n-hexane the colour developed. Thus it was observed that lycopene partitioned to n-hexane phase completely and was estimated. Therefore, standard plot of lycopene was prepared in n-hexane. The component showed good linearity (R^2=0.995) between concentration of analyte and the absorbance at 471 nm (Figure 2)[20].
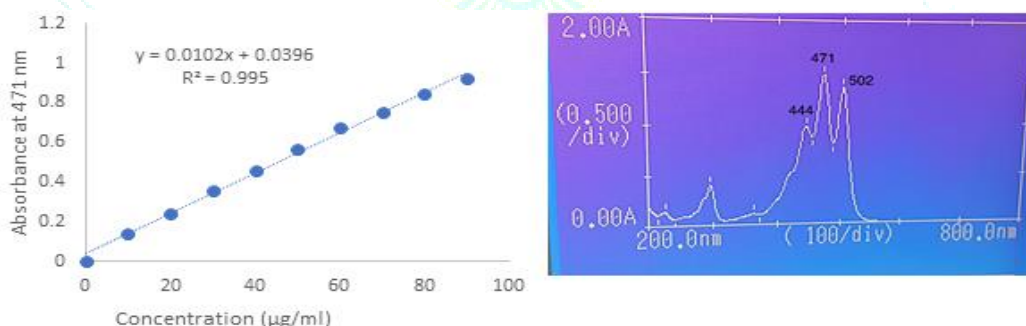

F**igure 2: UV Spectrum of lycopene and calibration plot prepared in n-hexane.**

HPLC method of analysis was also reproduced for estimation of lycopene [21]. HPLC analysis of the lycopene showed linearity in the range 10-200 µg/ml with retention time

3.683 ± 0.25 min at wavelength 479 nm. The chromatogram and the calibration plot for concentrations detected are shown in Figure 3.

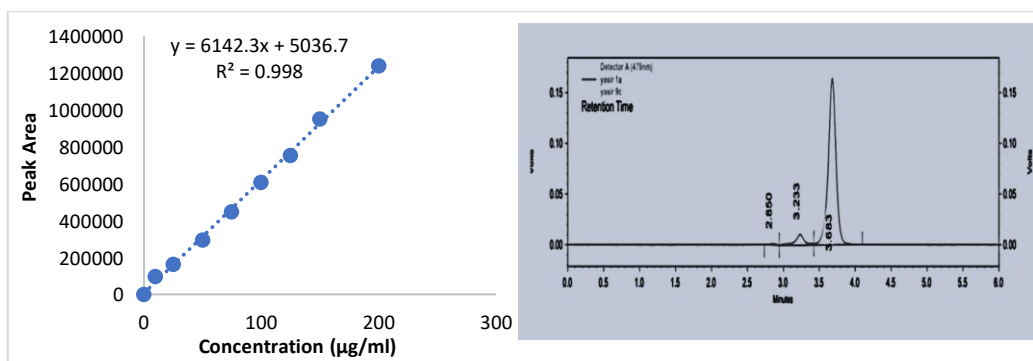**Figure 3: HPLC chromatogram and calibration plot of lycopene**

A compatibility study was done to examine the physical and chemical interaction between lycopene and sodium alginate used in the formulation. There was no notable change in the

samples when observed visually. No change in colour and odour was observed. Physical changes like liquefaction and change in weight were also not observed. Lycopene

exhibited an endothermic peak at 155.499 °C due to its melting. It revealed the crystalline nature of the provided small granules of lycopene [22] (Figure 4). Sodium alginate likely to be used in microbeads exhibited 3 sharp endothermic peaks i.e. at 100 °C, 250 °C and 340-410 °C (Figure 4) in DSC [23]. The first peak was due to release of water while the second and third were attributed to

decomposition of polymer to respective carbonates which melted at high temperature. DSC analysis of the mixture was also performed (Figure 4) which indicated absence of peak due to lycopene which was due released water that might have interacted with lycopene leading to its instability and absence of its endothermic peak (155 °C) [23].
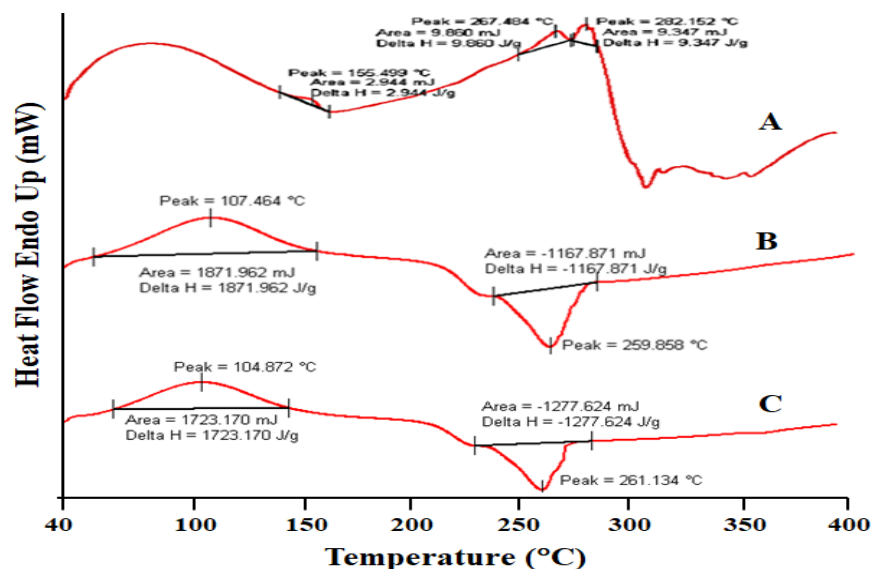

Figure 4: DSC thermogram of lycopene (A), sodium alginate (B) and physical mixture (C)

The selected independent variables for optimization of response influenced the % entrapment efficiency and % drug loading efficiency which is evident from the result reported in Table 1.

Table 1: Box-Behnken experimental design for optimization of lycopene sodium alginate beads

Exp. run	Sodium Alginate (%) (A)	Glutaraldehyde (%) (B)	Stirring speed (rpm) (C)	Encapsulation Efficiency %	Drug Loading %
R1	3.5	0.5	300	19.42	6.94
R2	3.5	0.75	250	27.54	6.03
R3	3	0.75	200	10.241	6.99
R4	4	0.5	250	31.287	5.58
R5	3	0.625	300	3.8	6.16
R6	3	0.5	250	2.87	6.1
R7	3.5	0.5	200	19.42	6.94
R8	4	0.625	200	6.49	5.53
R9	3.5	0.625	250	10.08	7.04
R10	3.5	0.625	250	10.08	7.04
R11	4	0.625	300	6.49	5.53
R12	3.5	0.625	250	10.08	7.04
R13	3.5	0.625	250	10.08	7.04
R14	4	0.75	250	5.245	5.49
R15	3.5	0.75	300	10.241	6.99
R16	3	0.625	200	3.8	6.16
R17	3.5	0.625	250	10.08	7.04

The ability of micrbeads to entrap the drug is the determining factor for selection of the method of preparation and composition of the beads. The effect of sodium alginate on the entrapment efficiency was optimized. The % drug entrapment of lycopene microbeads were obtained in the varying range from 3.8 to 31.287%. Different statistical tools such as sum of square value, mean square value, F-value, p-value, PRESS (prediction sum of squares) values and adequate precision value, test of ANOVA, R^2 value on linear, quadratic and cross product term effects of the above-mentioned variables were estimated in order to

generate a mathematical relationship on the basis of coefficient of each factor with best fitting to the polynomial equation [24]. The Model F-value of 9325.53 implied that quadratic model was significant. There was only a 0.01% chance that an F-value that large could occur due to noise. Values of "Prob > F" less than 0.0500 indicated model terms were significant. In this case A, B, AB, A^2, B^2, C^2, A^2B were significant model terms. Values greater than 0.1000 indicated the model terms were not significant. Summary of this observation is given in Table 2

Table 2: Statistical analysis for entrapment efficiency

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1069.65	10	106.96	9325.53	< 0.0001	significant
A-Sodium alginate	16.54	1	16.54	1441.75	< 0.0001	
B-Glutaraldehyde	84.25	1	84.25	7345.55	< 0.0001	
C-Stirring speed	0.000	1	0.000	0.000	1.0000	
AB	642.93	1	642.93	56052.49	< 0.0001	
AC	0.000	1	0.000	0.000	1.0000	
BC	0.000	1	0.000	0.000	1.0000	
A^2	9.66	1	9.66	842.55	< 0.0001	
B^2	281.08	1	281.08	24505.70	< 0.0001	
C^2	49.25	1	49.25	4293.60	< 0.0001	
A^2B	36.07	1	36.07	3144.31	< 0.0001	
Residual	0.069	6	0.011			
Lack of Fit	0.069	2	0.034			
Pure Error	0.000	4	0.000			
Corr Total	1069.72	16				

The "Pred R-Squared" of 0.9974 was in reasonable agreement with the "Adj R-Squared" of 0.9998; i.e. the difference was less than 0.0024. The model proposed the following polynomial equation for % drug entrapment

% Encapsulation efficiency = +10.08 +1.44A – 4.59B + 0.000C – 12.68AB + 7.448E- 016AC + 5.380E – 016BC – 1.52A² +8.17B² -3.42C² +4.25A²B

From the above equation it was inferred that % drug entrapment efficiency increased with increasing sodium alginate % (A) concentration. At higher alginate concentrations, viscosity increased leading to a less diffuse matrix structure that hindered the drug escape from the core. Entrapment efficiency decreased with increase in glutaraldehyde % (B) as it might have affected the swelling of the alginate. The decrease in stirring speed resulted in increase in drug entrapment due to greater time provided for polymer swelling (Figure 5).

For the effect of formulation variables on drug loading (%), again all statistical parameters were estimated in order to generate a mathematical relationship on the basis of coefficient of each factor with best fitting to the polynomial equation (Table 3). The Model F-value of 796.48 implied that the model was significant. There was only a 0.01% chance that an F-value that large could occur due to noise. Values of "Prob > F" less than 0.0500 indicated model terms were significant. In this case A, A^2, B^2, A^2B were significant model terms. Values greater than 0.1000 indicated the model terms were not significant. If there are many insignificant model terms, model reduction would improve the model. The summary of this observation is given in Table 3.

Table 3: Statistical analysis for % drug loading

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	6.64	10	0.66	796.48	< 0.0001	significant
A-Sodium alginate	0.67	1	0.67	807.36	< 0.0001	
B-Glutaraldehyde	2.500E-003	1	2.500E-003	3.00	0.1340	
C-Stirring speed	0.000	1	0.000	0.000	1.0000	
AB	1.000E-004	1	1.000E-004	0.12	0.7409	
AC	0.000	1	0.000	0.000	1.0000	
BC	0.000	1	0.000	0.000	1.0000	
A^2	5.86	1	5.86	7035.28	< 0.0001	
B^2	0.015	1	0.015	18.19	0.0053	
C^2	9.474E-004	1	9.474E-004	1.14	0.3273	
A^2B	8.450E-003	1	8.450E-003	10.14	0.0190	
Residual	5.000E-003	6	8.333E-004			
Lack of Fit	5.000E-003	2	2.500E-003			
Pure Error	0.000	4	0.000			
Cor Total	6.64	16				

The "Pred R-Square" of 0.9699 was in reasonable agreement with the "Adj R-Squared" of 0.9980; i.e. the difference was less than 0.0281. The model proposed the following polynomial equation for % drug entrapment

% drug loading = +7.04 - 0.29A + 0.025B + 0.000C - 5.000E -003AB + 1.911E- 017AC -5.270E – 017BC – 1.18A² -0.060B² -0.015C² -0.065A²B

From the above equation it was inferred that % drug loading increased with increasing sodium alginate % (A) as sodium alginate beads provided greater surface area for the loaded drug. Drug loading decreased with increase in glutaraldehyde % (B) concentration perhaps due to high polymer content that might have hindered the homogeneous distribution of glutaraldehyde leading to the formation of particles with reduced drug content [25.] Drug loading decreased with decrease in stirring speed. Since the viscosity of the polymeric solution increased with high polymer concentration, low stirring speed was incapable of loading the drug on micrbeads (Figure 6).
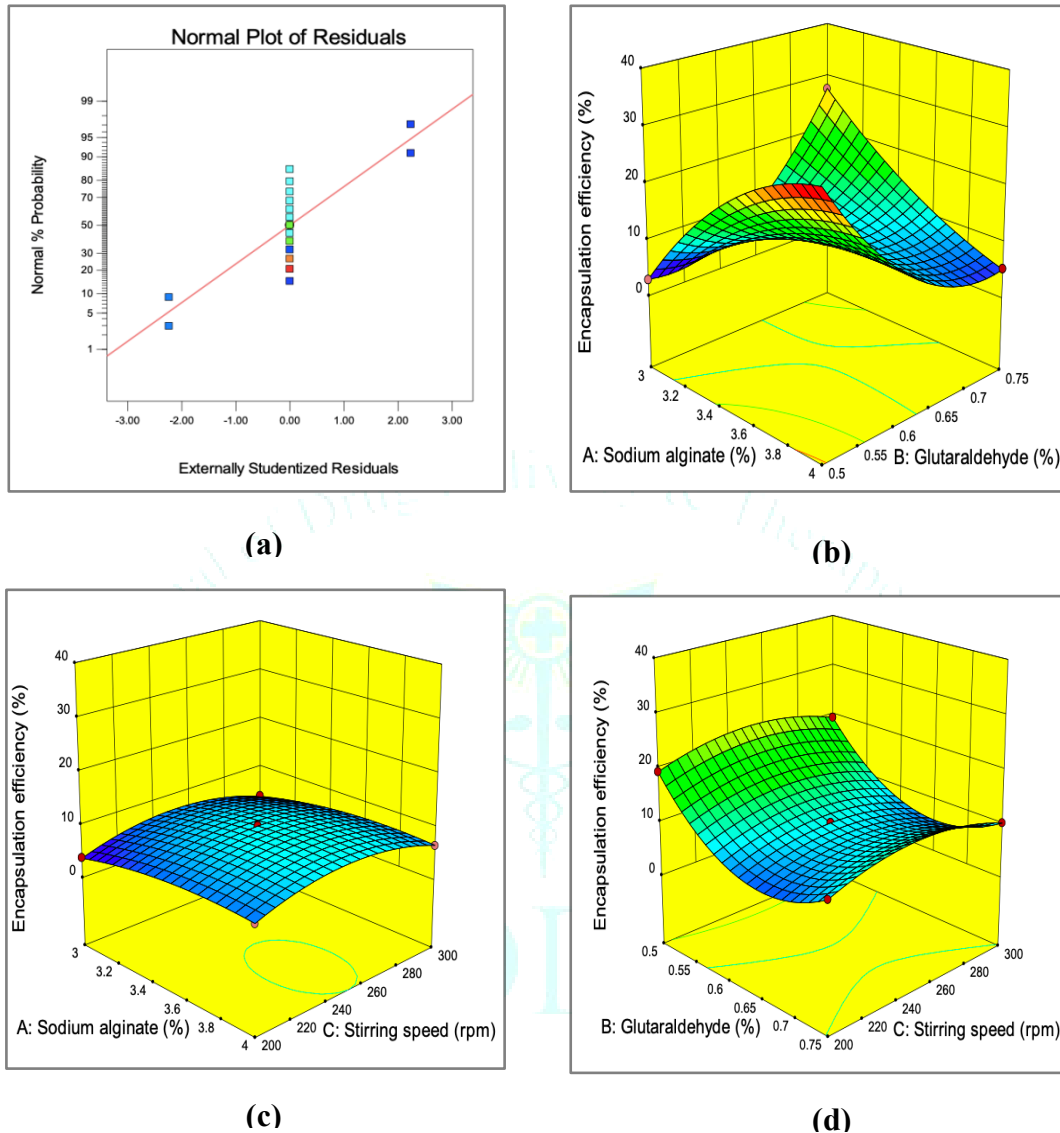

(a)

(b)

(c)

(d)

Figure 5: (a) Linear correlation plot between actual and predicted value and (b), (c), and (d) 3D response surface plot showing effect of independent variables on entrapment efficiency (%).
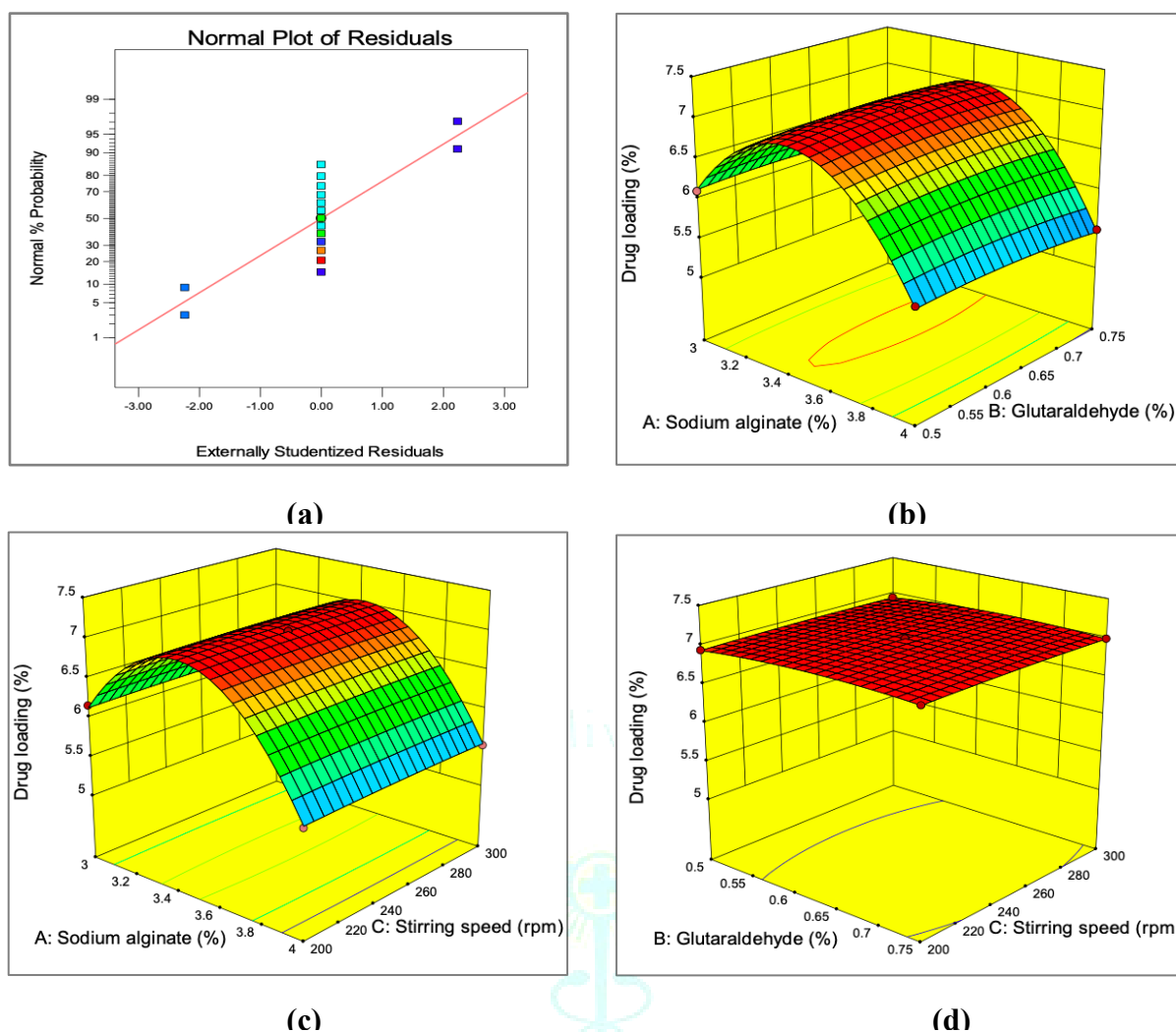
Figure 6: (a) Linear correlation plot between actual and predicted value and (b), (c), and (d) 3D response surface plot showing effect of independent variables on drug loading (%)

CONCLUSION

Lycopene is a lipophilic molecule which has poor bioavailability due to poor solubility. All its formulations are used as nutraceuticals which are not able to improve the bioavailability. In the present research, we carried out systematic pre-formulation study for preparation of sodium alginate microbeads that would result in improved bioavailability through prolonged adhesion to intestinal mucosa. Novel lycopene microbeads were prepared by precipitation method which showed higher drug entrapment and sufficient drug loading without incompatability with the polymer.

REFERENCES

1. Jain KK, Drug delivery systems—an overview. Springer, Totowa, NJ, 2008; 500-509.
2. Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G, Impact of excipients on the absorption of P-glycoprotein substrates *in vitro* and *in vivo*. Int. J. Pharm. 2004; 278 (1):119-131.
3. Gursoy RN, Benita S, Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharmacother.2004; 58(3): 173-82.
4. Wandel C, Kim RB, Stein CM, Inactive excipients such as Cremophor can affect in vivo drug disposition. Clin. Pharmacol, Ther. 2003; 73(5): 394-396.
5. Cefali LC, Cazedey ECL, Souza-Moreira TM, Correa MA, Salgado HRN, Isaac VLB, Antioxidant activity and validation of quantification method for lycopene extracted from tomato. J. AOAC Int. 2015a;98 (5): 1340–1345.
6. Cefali LC, Souza-Moreira TM, Corrêa MA, Salgado HRN, Isaac VLB, Development and evaluation of an emulsion containing lycopene for combating acceleration of skin aging. Brazil. J. Pharm. Sci. 2015b; 51 (3): 579–590.
7. Cheng HM, Koutsidis G, Lodge JK, Ashor A, Siervo M, Lara J, Tomato and lycopene supplementation and cardiovascular risk factors: a systematic review and meta-analysis. Atherosclerosis, 2017; 257: 100–108.
8. Sheriff SA, Ibrahim S, Devaki T, Chakraborty S, Agarwal S, Pérez-Sánchez H, Lycopene prevents mitochondrial dysfunction during d-galactosamine/ lipopolysaccharide - induced fulminant hepatic failure in albino rats. J. Proteome Res. 2017; 16 (9): 3190–3199.
9. Rao A V, Lycopene, tomatoes, and the prevention of coronary heart disease. Experimental Biology and Medicine, 2002; 227(10): 908–913.
10. Kitade Y, Watanabe S, Masaki T, Nishioka M, Nishino H, Inhibition of liver fibrosis in LEC rats by a carotenoid, lycopene, or herbal medicine, Sho-saiko-to. Hepatology Research, (2002); 22(3): 196–205.
11. Stahl W, Sies H. Carotenoids and protection against solar UV radiation. Skin Pharmacology and Applied Skin Physiology, 2002; 15(5): 291–296.

12. Sedjo R L, Roe D J, Abrahamsen M, Harris R B, Craft N, Baldwin, S. Vitamin A, carotenoids and risk of persistent oncogenic human papillomavirus infection. Cancer Epidemiology Biomarkers & Prevention, 2002; 11(9): 876–884.

13. Clinton S K, Lycopene: chemistry, biology, and implications for human health and disease. Nutrition Reviews, 1998; 56: 35–51.

14. Schunemann H J, McCann S, Grant B J B, Trevisan M, Muti P, Freudenheim J L, Lung function in relation to intake of carotenoid and other antioxidant vitamins in a population-based study. American Journal of Epidemiology, 2002; 155(5): 463–471.

15. Sharma J B, Kumar A, Kumar A, Malhotra M, Arora R, Prasad S, Effect of lycopene on pre-eclampsia and intra-uterine growth retardation in primigravidas. International Journal of Gynecology & Obstetrics, 2002; 81(3): 257–262.

16. Jain A, Thakur D, Ghoshal G, Katare OP, Singh, B, Shivhare US, Formation and functional attributes of electrostatic complexes involving casein and anionic polysaccharides: An approach to enhance oral absorption of lycopene in rats in vivo. Int. J. Biol. Macromol, 2016; 93: 746–756.

17. Rein MJ, Renouf M, Cruz-Hernandez C, Actis-Goretta L, Thakkar SK, Da Silva Pinto, M, Bioavailability of bioactive food compounds: a challenging journey to bioefficacy, Br J Clin Pharmacol, 2013; 75: 588-602.

18. Lopez-Cervantes J, Sanchez-Machado DI, Valenzuela-sanchez KP, Nunez-Gastelum JA, Escarcega-Galaz AA, Rodriguez-Ramirez R, Effects of solvents and methods of stirring in extraction of lycopene, oleoresin and fatty acids from overriped tomatoes. Int. J. Food Sci. Nutr, 2014; 65 (2): 187-193.

19. Kamil MM, Mohamed GF, Shaheen MS , Fourier transformer infrared spectroscopy for quality assurance of tomato products. J. Am. Sci. 2011; 7(6): 559-572.

20. Singh A, Neupane YR, Panda BP, Kohli K, Lipid based nanoformulation of lycopene improves oral delivery: formulation optimization, *ex-vivo* assessment and its efficacy against breast cancer, J. Microencapsul, 2017; 34(4): 416-429.

21. Sathish T, Udayakiran D, Himabindu K, HPLC method for determination of lycopene in crude oleoresin extracts. Asian J. Hem, 2009; 21(1):139-148

22. Pu C, Tang W, Encapsulation of lycopene in Chlorella pyrenoidosa: Loading properties and stability improvement. Food Chem, 2017; 235: 283-289.

23. Soares J P, Santos J E, Chierice, G O, Cavalheiro E T G, Thermal behavior of alginic acid and its sodium salt, 2004; 29(2): 536-556.

24. Javed N, Kohli K, Amin S, Risk assessment integrated QbD approach for development of optimized bicontinuous mucoadhesive limicubes for oral delivery of rosuvastatin. AAPS PharmSciTech, 2018: DOI: 10.1208/s12249-018-0951.

25. Ramachandran S, Nandhakumar S, Dhanaraju MD, Formulation and characterization of glutaraldehyde cross-linked chitosan biodegradable microspheres loaded with famotidine. Trop. J. Pharm. Res., 2011;10 (3): 309-316