RESEARCH ARTICLE

Formulation and Optimisation of Microspheres of Leaves extract of *Lantana camara* 3^2 Design Approach

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ABSTRACT

The leaf extracts of plant *Lantana camara* shows significant antithrombin and thrombolytic activity. Modified release form of extract will show better therapeutics efficiency. In this work the extracts of plant *L. camara*-loaded polycaprolactone microspheres were prepared using the double emulsion method. 3^2 design approaches were used for the optimization of polycaprolactone and PVP concentration for response entrapment efficiency (Y1) and drug release (Y2). The microspheres of *L. camara* were analyzed for EE, FTIR, DSC, XRD and drug release. The entrapment efficiency of *L. camara* microspheres was in ranges of 62.25 to 84.04%. The particle size of optimized batch of microspheres was 789.6 (d. nm) with PDI 0.838. It shows pH dependant swelling with no interaction between drug and polymer was observed in DSC studies. The % drug release of all thirteen batches was in ranges of 87.91 to 99.04% for 12 hours duration. From the study we conclude the prepared *L. camara* loaded polycaprolactone microspheres show sustained release action for 12 hours.

KEYWORDS: *Lantana camara;* Microspheres; 3^2 Design; Optimisation; Drug Release.

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INTRODUCTION

The oral route of administration is frequently referred to be very convenient way to administer medications. As a result, during the drug discovery process, a great deal of effort is placed into developing orally active compounds that will provide predictable and useful plasma concentrations *in-vivo*.[1] Lead optimization commonly addresses these problems during a discovery programme, but locating a drug with the required "perfect" physicochemical and/or pharmacokinetic properties is frequently impossible. Always remember the potential for achieving more optimum blood drug concentration-time profiles to enhance the clinical pharmacology profile of drug candidates in clinical research or medicines that have already been approved for sale.[2,3]

Microspheres are now recognized as dependable and effective release technology that reduces the dosing frequency and the risk of dose dumping. They have good patient compliance.[4,5]

Sustained release behavior is provided by microspheres made from natural or manmade polymers. The amount of medicine that is released from the microsphere is directly influenced by its physicochemical properties, excipients, and other factors. In the late 1970s, systems for injecting microspheres were developed.[6] The polymer of choice for producing microspheres that contain APIs has been PLGA, which is used in a w/o or o/w emulsion/solvent evaporation process.[7-9] Poly-caprolactone is the second most common polymer used to make microspheres that contain drugs. The release profiles of the drugs are influenced by the microspheres' chemical makeups and rates of degradation. For instance, PGA > PLA >> PCL are the three compounds with the highest hydrolytic breakdown rates. In contrast to PLA and PGA, which had superior steroidal pharmaceutical permeability but displayed uniform biodegradation, PCL had excellent steroidal pharmaceutical permeability but displays sluggish biodegradation.[10-12]

It has been discovered that the hydrophilic glycolide component of the system affects how rapidly PLGA microspheres degrade and release API. The chirality of the polymer, the density of cross-linking, and the loading of medications in microspheres all have an impact on the release patterns of API. When the amount of cross-linking is considerably increased, it creates an increase in the barrier density for drug diffusion and a longer release of the API in

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casein and chitosan microspheres. There is a higher initial release in microspheres when the unencapsulated drug is more easily accessible on the surface in larger quantities. The size of the microsphere can be determined using the surface area to volume ratio, which estimates the amount of surface accessible for releasing the APIs during diffusion.[13,14] Because of a better surface area and a shorter diffusion path, smaller microspheres provide greater drug release and shorter duration. The larger the microsphere, the more difficult it is to inject it with a needle. The effectiveness of the microsphere's injectability is inversely connected with its size. The polymer concentration used to make the microspheres control their average size. Increased polymer concentration causes emulsion droplets to have a higher organic phase viscosity and, as a result, a bigger average size, enabling more medicines to be encapsulated into the microspheres.[15,16] In this work we have prepared the microsphere of extract and evaluated the same using entrapment efficiency, size, EE, FTIR, XRD and drug release etc., The prepared *Lantana camara* loaded polycaprolactone microspheres show delayed release behavior for 12 hours.

MATERIAL AND METHOD

Materials

The polycaprolactone was received as a gift sample from Aurobindopharma lab, Hyderabad, India. Chloroform and polyvinylpyrrolidone (PVP) were purchased from Avantor Lab, India. All other substances, including reagents, were of analytical grade and were applied as directed.

Crude Plant Extract

L. camara Leaves methylene chloride extract used for the preparations of microspheres after toxicity study.

Methods

Preparation of Microspheres

As per the quantity given in Table 1, the plant extract was dissolved in polycaprolactone (PCL), which were earlier solubilized in chloroform and the mixture was then added to an aqueous phase containing polyvinylpyrrolidone (PVP). The aforementioned combination was stirred at 500 rpm, and the medication and polymer were then converted into tiny droplets. These droplets then evaporated into hard microspheres, which were collected by filtering, washed with demineralized water.[17-19]

Experimental design for optimization

For optimization, 3^2 (three level-two factor) methodologies were used. Independent variables were polycaprolactone concentration (X_1) and concentration of PVP (X_2%). The responses were selected as %EE (Y_1) and %DR (Y_2). It was modeled by using the following equation.

$$Y = \beta_0 + \beta_1 x_1 + \beta_1 x_1 + \beta_3 x_1 x_2 + \beta_4 x_1^2 + \beta_5 x_2^2 \quad (1)$$

Where,

Y is the response, β_0 is the intercept and $\beta_1 - \beta_5$ is regression coefficients. x_1, x_2 are individual effects. x_1, x_2 is the interaction effect and , x_1^2, x_2^2 are the quadratic effects.[20,21]

Table 1: Formulation composition for microspheres

	Independent variable		*Dependent variables*	
Experimental run	*Polycaprolactone concentration X1 (mg)*	*PVP Concent-ration X2 (%)*	*EE (Y1) (%)*	*DR (Y2)(%)*
F1	0	-1	65.2	99.04
F2	1	-1	84.02	97.87
F3	-1	0	80.25	91.29
F4	0	0	62.25	87.91
F5	1	0	78.11	97.99
F6	0	0	76.99	98.65
F7	-1	1	77	95.09
F8	0	0	77.5	98.28
F9	0	0	65.22	90.85
F10	0	1	79.25	94.67
F11	1	1	73.25	98.16
F12	0	0	69.02	97.14
F13	-1	-1	71.12	91.68

Coded levels

Independent variable	*Low level (-1)*	*Medium level (0)*	*High level (+1)*
X1=Polycaprolactone concentration	500	750	1000
X2 = PVP Concentration	0.3	0.6	0.9

Characterization

The following characterization were carried out

Microsphere recovery/yield

$$\% \text{ yield} = \frac{\text{Amount of encapsulated drug}}{\text{Amount of added drug}} \times 100$$

Drug Entrapment Efficiency

The content was calculated when 100 mg of microspheres were fully dissolved after 30 minutes of sonication in phosphate buffer with a pH of 7.4. The samples were then filtered and subjected to spectrophotometric analysis[22] It were carried by

$$\textbf{Drug EE (\%)} = \frac{\text{Obtained drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Surface Morphology

It gives important details regarding the microstructure and porosity of these drug delivery systems. The most popular method was employed, electron scanning microscopy (SEM).

Particle Size Analysis

The microscopy method was done for determination of the shape and surface structure of microparticles.

Swelling Index Study

The microsphere was subjected to swelling research by being submerged in 50 mL of three different media: simulated gastric fluid with a pH of 1.2, simulated intestinal fluid with a pH of 6.8 and pH of 7.4. The microsphere's weight increased at predetermined intervals until a steady weight was noticed using an electronic balance.[23]

$$\text{Swelling index} = \frac{\text{Mass of swollen microspheres} - \text{mass of dry microspheres}}{\text{Mass of dried microspheres}} \times 100$$

Differential scanning calorimetry

A DSC thermogram of extract and extract loaded formulation microspheres was conducted to study the thermal characteristics of the extract and formulations. The thermogram was heated from 32 to 40°C at a rate of 12°C per minute.

In-vitro active constituent release studies

Drug release profiles from microspheres containing extract were investigated in a USP dissolving type II equipment. The dissolving media volume was set at 500 mL at 37°C, with a stirring speed of 75 rpm. pH values of 1.2 (0.1 N HCl), 6.8 (phosphate buffer), and 7.4 (phosphate buffer) were used as dissolution medium. The samples will undergo filtering and spectrophotometric analysis. A percentage of the drug release will be used to express the triplicate results.[24]

RESULTS AND DISCUSSION

Optimisation

Thirteen runs were completed for the optimization of microspheres using a 3^2 factorial design. Both models display the quadratic model, as shown in Table 2. Figures 1 (a,b) and 2 (a,b) are the 2D contour plot and 3D response surface plot for EE and DR, respectively. The software Design Expert was also used to conduct all statistical analyses, including analysis of variance (ANOVA). The experiment's findings were used to calculate the coefficient for the interaction (A, B) and the linear effects. This study demonstrated that the formulation parameters (drug release and encapsulation efficiency) influenced the nanoparticles' properties. The ensuing equations represent the polynomial model, which also show the correlation between the response and formulation variables.

$$\mathbf{EE} = 76.8324 + 2.68 + 2.65333 + 4.2875 + 4.14655 + -10.7534 \ldots\ldots(2)$$

$$\mathbf{DR} = 96.339 + 0.203333 + 0.108333 + -3.3025 + 3.16862 + -5.46638 \ldots..(3)$$

The preceding equation illustrates how factors (A and B) quantitatively impact the answers. Table 2 displays model-level response statistics. The direction of a coefficient, which is presented along with all of its estimated values for each response, indicates the influence of a factor on a response. On the other hand, if the coefficient is negative, the opposite relationship is seen. Table 3 shows the model's F-value of 10.89

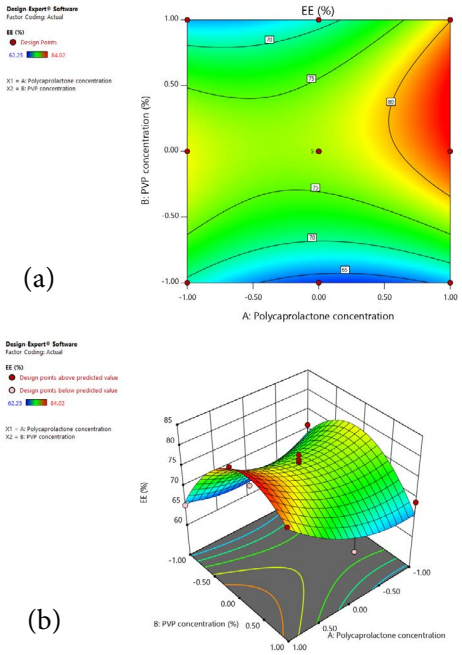

Figure 1: 2D contour plot (a) and 3D response surface plot (b) for EE

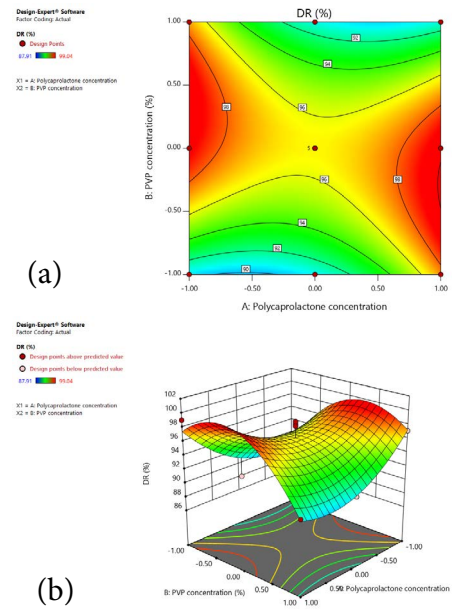

Figure 2: 2D contour plot (a) and 3D response surface plot (b) for DR.

suggests that it is significant for the response Y1. Only 0.01% of the time could noise account for an F-value. The model F-value of 5.49 for the response Y2 indicates that the model is significant. As shown in Table 4, the difference between the actual and predicted values is also less.

Microsphere Recovery/Yield

Microsphere recovery of all 13 batches is given in Table 5.

Drug Entrapment Efficiency

The microsphere yield for all of the experimental runs was in the range of 62.25 to 84.02%, as shown in Table 1.

Table 2: Model summary statistics of responses

Source	*Std dev*	*R²*	*Adjusted R²*	*Predicted R²*	*PRESS*	*Remarks*
Y1 Response						
Linear	6.74	0.1581	-0.0103	-0.6024	864.87	
2FI	6.51	0.2943	0.0591	-0.5479	835.47	
Quadratic	2.96	0.8861	0.8047	0.0966	487.61	Suggested
Cubic	2.66	0.9343	0.8424	-5.8702	3708.13	
Y2 Response						
Linear	4.04	0.0019	-0.1977	-0.9666	321.61	
2FI	3.65	0.2687	0.0250	-0.3036	213.19	
Quadratic	2.18	0.7967	0.6515	-0.0361	169.43	Suggested
Cubic	2.34	0.8329	0.5991	-8.3102	1522.50	

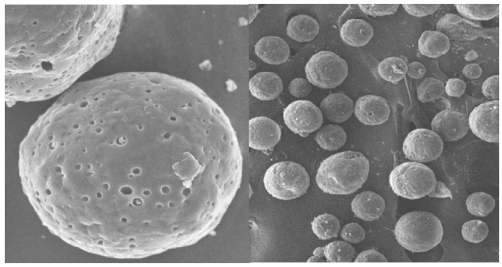

Figure 3: Field emission scanning electron microscopic image of microspheres

Surface Morphology

Figure 3 shows representative FESEM microphotographs of prepared microspheres with smooth surfaces and spherical in shapes.

Particle Size Analysis

The average particle size of obtained of optimized run microspheres was 789.6 (d. nm) with PDI 0.838 (Figure 4).

Swelling Index Study

Figure 5 illustrates how the prepared microspheres swell in different pH media. The swelling index in 0.1 N HCL

Table 3: ANOVA of the models for responses Y1 and Y2

Source	*Sum of Squares*	*df*	*Mean square*	*F-value*	*p-value*	*Remarks*
Y1 Response						
Model	478.25	5	95.65	10.89	0.0034	Significant
	43.09	1	43.09	4.91	0.0623	
	42.24	1	42.24	4.81	0.0644	
	73.53	1	73.53	8.37	0.0232	
	47.49	1	47.49	5.41	0.0530	
	319.38	1	319.38	36.36	0.0005	
Y2 Response						
Model	130.29	5	26.06	5.49	0.0227	Significant
	0.2481	1	0.2481	0.0522	0.8258	
	0.0704	1	0.0704	0.0148	0.9065	
	43.63	1	43.63	9.19	0.0191	
	27.73	1	27.73	5.84	0.0463	
	82.53	1	82.53	17.38	0.0042	

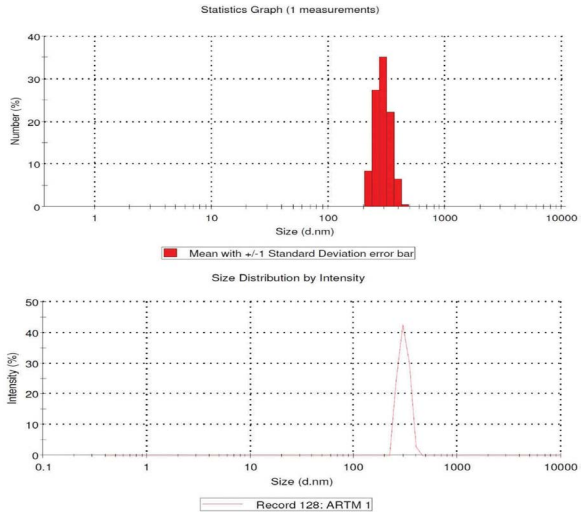

Figure 4: Average particle size (d. nm) and PDI of microspheres

Table 4: Difference between actual and predicted values

Run Order	Actual Value	Predicted Value	Residual	Run Order	Actual Value	Predicted Value	Residual
	Y1 Response				Y2 Response		
1	62.25	63.43	-1.18	1	87.91	90.76	-2.85
2	65.20	65.96	-0.7647	2	99.04	97.44	1.60
3	73.25	78.30	-5.05	3	98.16	99.30	-1.14
4	78.11	76.83	1.28	4	97.99	96.34	1.65
5	84.02	83.66	0.3610	5	97.87	99.71	-1.84
6	76.99	76.83	0.1576	6	98.65	96.34	2.31
7	69.02	65.91	3.11	7	97.14	97.25	-0.1087
8	77.00	76.83	0.1676	8	95.09	96.34	-1.25
9	77.50	76.83	0.6676	9	98.28	96.34	1.94
10	65.22	68.73	-3.51	10	90.85	90.98	-0.1309
11	80.25	79.85	0.4036	11	91.29	91.05	0.2396
12	79.25	76.83	2.42	12	94.67	96.34	-1.67
13	71.12	69.18	1.94	13	91.68	90.43	1.25

Table 5: %Microsphere recovery of all prepared batches

Batches Number	*Microsphere recovery/yield (%)*
F1	87.98
F2	79.25
F3	80.01
F4	76.17
F5	85.99
F6	90.12
F7	84.64
F8	89.55
F9	90.28
F10	87.69
F11	89.03
F12	86.11
F13	90.43

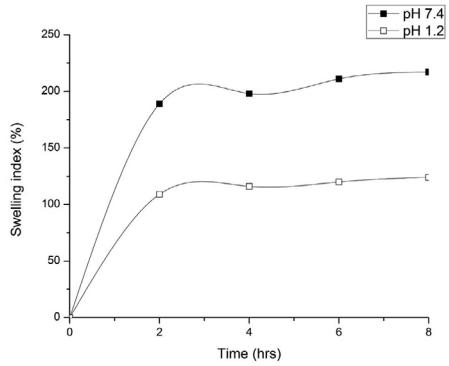

Figure 5: Swelling behaviour of microspheres in 0.1 N HCl (pH 1.2), and phosphate buffer (pH 7.4)

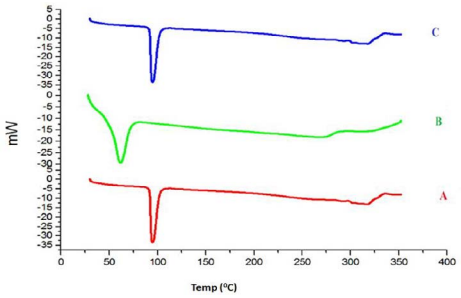

Figure 6: (A) DSC spectra of extract, (B) PCL, (C) Microspheres

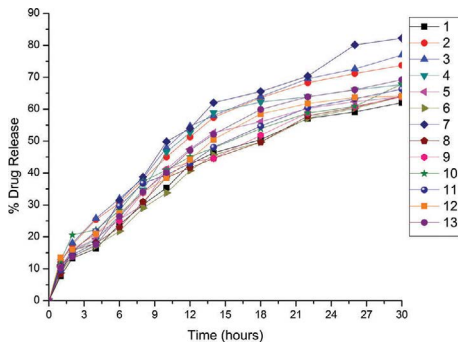

Figure 7: Drug release behaviours of different batches of microspheres

(acidic media) was lower than that in phosphate buffer pH-7.4 (alkaline media). In pH-7.4, the swelling happens quickly, followed by erosion and dissolution.

Differential Scanning Calorimetry

Using a DSC, this was done for pure extract (A), pure polymer (B), and extract-loaded microspheres (C) (DSC- 60, Shimadzu). Figure 6 demonstrates that there was no drug-polymer interaction.

In-vitro Active Constituent Release Studies

The drug release profile of microspheres and pure extract are given in Figure 7. The %drug releases from microspheres were ranged from 87.91 to 99.04% for all batches.

CONCLUSION

Natural or synthetic polymers prepared microspheres shows sustained release behavior. In this work, we have prepared microspheres of extract using polycaprolactone as a polymer. The statistical data shows significant results with *p-value* less than 0.5. The optimized formulations were analyzed using FTIR, DSC, swelling behavior, particle size analysis, and drug release etc. DSC confirms that there was no interaction between drug and the polymer. In an alkaline pH, the microspheres swell rapidly. Cumulative %drug releases from microspheres were in ranges from 87.91 to 99.04% for all batches. These results conclude that the prepared microspheres showed delay release action for long duration.

REFERENCES

1. Ajazuddin, Saraf S. Applications of novel drug delivery system for herbal formulations. Fitoterapia. 2010;81(7):680-689. Available from: DOI: 10.1016/j.fitote.2010.05.001
2. Kumar K, Dhawan N, Sharma H, Vaidya S, Vaidya B. Bioadhesive polymers: Novel tool for drug delivery. Artificial cells, nanomedicine, and biotechnology. 2014;42(4):274-83. Available from: PMID: 23859698 DOI: 10.3109/21691401.2013.815194
3. Mahajan A, Aggarwal G. Smart polymers: innovations in novel drug delivery. International Journal of Drug Development and Research. 2011;3(3):16-30.
4. Venkatesan P, Manavalan R, Valliappan K. Microencapsulation: a vital technique in novel drug delivery system. Journal of Pharmaceutical Sciences and Research. 2009;1(4):26-35.
5. Ajazuddin, Saraf S. Applications of novel drug delivery system for herbal formulations. Fitoterapia. 2010;81(7):680-689. Available from: DOI: 10.1016/j.fitote.2010.05.001
6. Ramteke KH, Jadhav VB, Dhole SN. Microspheres: As carriers used for novel drug delivery system. IOSR Journal of Pharmacy. 2012;2(4):44-48.
7. Patel NR, Patel DA, Bharadia PD, Pandya V, Modi D. Microsphere as a novel drug delivery. International Journal of Pharmacy & Life Sciences. 2011;2(8):992-997.
8. Shirsath NR, Goswami AK. Nanocarriers based novel drug delivery as effective drug delivery: a review. Current Nanomaterials. 2019;4(2):71-83. Available from: DOI: 10.2174/2405461504666190527101436
9. Kakkar V, Wani SU, Gautam SP, Qadrie ZL. Role of microspheres in novel drug delivery systems: preparation methods and applications. International Journal of Current Pharmaceutical Research. 2020;13(3):10-15. Available from: DOI: http://dx.doi.

org/10.22159/ijcpr.2020v12i3.38326.
10. Metkari VB, Kulkarni LV, Patil PS, Jadhav PA, Bamane GS, Kumbhar CM. Microspheres-a new drug delivery system: A review. Journal of Current Pharma Research. 2014;4(2):1128-1133.
11. Mondal D, Griffith M, Venkatraman SS. Polycaprolactone-based biomaterials for tissue engineering and drug delivery: Current scenario and challenges. International Journal of Polymeric Materials and Polymeric Biomaterials. 2016;65(5):255-65. Available from: DOI:10.1080/00914037.2015.1103241
12. Byeon JC, Ahn JB, Jang WS, Lee SE, Choi JS, Park JS. Recent formulation approaches to oral delivery of herbal medicines. Journal of pharmaceutical investigation.2018;49(1):17-26. Available from: DOI:10.1007/s40005-018-0394-4
13. Koren E, Torchilin VP. Drug carriers for vascular drug delivery.IUBMB life. 2011;63(8):586-595. Available from: PMID: 21766415 DOI: 10.1002/iub.496
14. Shen M, Wang Y, Hu F, Lv L, Chen K, Xing G. Thrombolytic agents: Nanocarriers in targeted release. Molecules. 2021;26(22):6776. Available from: https://doi.org/10.3390/molecules26226776
15. Ma H, Jiang Z, Xu J, Liu J, Guo ZN. Targeted nano-delivery strategies for facilitating thrombolysis treatment in ischemic stroke. Drug Delivery. 2021; 28(1):357-371. Available from: doi.org/10.1080/10717544.2021.1879315
16. Keziah EA, Nukenine EN, Danga SP, Younoussa L, Esimone CO. Creams formulated with Ocimumgratissimum L. and *L. camara* L. crude extracts and fractions as mosquito repellents against Aedesaegypti L.(Diptera: Culicidae). Journal of Insect Science. 2015;15(1):15. Available from: doi: 10.1093/jisesa/iev025
17. Sharma HK, Lahkar S, Nath LK. Formulation and in vitro evaluation of metformin hydrochloride loaded microspheres prepared with polysaccharide extracted from natural sources. ActaPharmaceutica. 2013; 63(2):209-222. Available from: PMID: 23846143 DOI: 10.2478/acph-2013-0019
18. Muthukumar T, Sankari D, Tamil Selvi A, Sastry TP. Preparation, characterization, and in vitro bioactivity of Bixa Orellana extract-impregnated collagen microspheres. Journal of Materials Science. 2014;49(16):5730-5737. Available from: DOI:10.1007/s10853-014-8291-3
19. Shirsath NR, Goswami AK. Vildagliptin-loaded gellan gum mucoadhesive beads for sustained drug delivery: design, optimisation and evaluation. Materials Technology. 2020;36(2):1-13. Available from: DOI:10.1080/10667857.2020.1786783
20. Kosaraju SL, D'ath L, Lawrence A. Preparation and characterisation of chitosan microspheres for antioxidant delivery. Carbohydrate polymers. 2006;64(2):163-167. Available from: DOI:10.1016/j.carbpol.2005.11.027
21. Liu Z, Lu W, Qian L, Zhang X, Zeng P, Pan J. In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. Journal of controlled release. 2005;102(1):135-144. Available from: PMID: 15653140 DOI: 10.1016/j.jconrel.2004.06.022
22. Tao Y, Lu Y, Sun Y, Gu B, Lu W, Pan J. Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability. International journal of pharmaceutics. 2009; 378(1-2):30-36. Available from: PMID: 19465102 DOI: 10.1016/j.ijpharm.2009.05.025
23. Shirsath NR, Goswami AK. Vildagliptin-loaded gellan gum mucoadhesive beads for sustained drug delivery: design, optimisation and evaluation. Materials Technology. 2020;36(2):1-13. Available from: DOI:10.1080/10667857.2020.1786783
24. Kumar YG. Formulation Development and Evaluation of Sustained Release Matrix Tablets of Vildagliptin-Synthetic and Natural Polymers. Asian Journal of Pharmaceutics. 2015;9(4):S26-S33. Available from: DOI: https://doi.org/10.22377/ajp.v9i4.482