CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com Research Article

FORMULATION, OPTIMIZATION AND IN-VITRO CHARACTERIZATION OF ABACAVIR MUCOADHESIVE MICROSPHERES BY BOX BEHNKEN DESIGN

Kishore Bandarapalle*, Pasupulati Mounika [1]

*, [1] Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, 517503.

Abstract:

The purpose of this study was to formulate and optimize mucoadhesive microspheres of Abacavir within Carbopol 934, Moringa oleifera, and Tamarindus indica as a carrier polymer and mucoadhesive polymer for controlling the release of Abacavir. The ionic gelation technique was used for preparation of microspheres of Abacavir and the Box-Behnken design was employed. Results: Fourier transform infrared spectroscopy studies indicate that no interaction or minor at molecular level suggest the polymers added were compatible with the drug. The EE of ABC and were found to be in the range of 86.14 ± 0.88 to 91.83 ± 0.99 for A1 toA 3. and the average particle size 805.73±10.16 to 896.73±10.16 µm for A1 to A3: The analysis of variance showed a significant effect of independent variables. The scaning electron microscopy (SEM) analysis showed that the microspheres were spherical and free-flowing. The microspheres of Abacavir were stable after thee month stability study at accelerated condition..

Key words: Carbopol 934, ionic gelation technique, Box-Behnken design & Fourier transform infrared spectroscopy.

Corresponding author:

Kishore Bandarapalle,
Sri Padmavathi School of Pharmacy

QR CODE

Please cite this article in** press Kishore Bandarapalle **et al., Formulation, Optimization And In-Vitro Characterization Of Abacavir Mucoadhesive Microspheres By Box Behnken Design., Indo Am. J. P. Sci, 2026; 13(04).

INTRODUCTION:

Drug delivery systems [DDS], accurately control the rate of release or target to a requisite site of body have a tremendous impact on health human being. Microspheres are integral part of these DDS because of size and characteristics; the success is restricted due to short time availability at absorption site[1-3]. Therefore intimate contact with membranes is advantageous; this paved the development of mucoadhesive microspheres. Mucoadhesive microspheres are of 1-1000µm in size and constitute either entire or mixture of mucoadhesive polymer or having an outer layer16.Microspheres general purpose is targeted and controlled release, but coupling mucoadhesiveness to microspheres is advantageous, e.g. effective absorption and enhanced drug bioavailability due to more intimate contact with mucus, high surface to volume ratio, target specificity of the drug at absorption site[4-9]. Mucoadhesive microspheres can adhere any mucosal tissue viz., eye, nasal cavity, urinary and gastrointestinal tract, results in localised and systemic controlled drug release. Microspheres designed with mucoadhesive and biodegradable polymers makes selective uptake of M cells of gastrointestinal (GI) mucosa17.Such uptake mechanism used for the delivery of various drugs (antiretroviral), protein-peptides, vaccines and plasmid DNA for gene therapy.

MATERIALS AND METHODS:

Materials

Abacavir (ABC) was received from Hetero dugs Pvt ltd., Hyderabad, India, Carbopol 934P (CP934P) was purchased from Loba Chemie Pvt. Limited, Mumbai. Sodium alginate(SA) were obtained from LNR chemicals, Mumbai. *Moringa oleifera, Tamarindus indica,*were from local market.

Methods

SA and carbopol 934 were dissolved separately in 10ml distilled water. Homogeneous polymer solutions were prepared by mixing both solutions with continuous stirring, care should be taken to avoid entrapment of air. Measured weight of drug under the study was added to the polymer dispersion and thoroughly mixed in mortar using pestle to form viscous dispersion[10-12]. The resulting polymer dispersion was injected through 21No needle drop wise into cross linking agent CaCl2 solution with continuous stirring at 300 rpm. The formed droplets were retained in the CaCl2 solution for 15 min to produce rigid spherical microspheres138. The rigid microspheres formed were separated from cross linking solution by surface filtration or decantation, and washed for 2-3 times with distilled water, dried 12h and stored for their characterization study in desiccators. The trial batches of mucoadhesive microspheres loaded with ABC were formulated according to the study designs. Similarly same method is adapted for the fabrication of ABC loaded microspheres using mucilage/gum obtained from the natural source. The formulated mucoadhesive microspheres were evaluated for percent DEE, drug release at 6h and 12h for each trial and compute the data in DoE software to generate the possible statistics including ANOVA and point prediction optimization. Further the optimized ABC loaded mucoadhesive microspheres were subjected for following evaluation parameters.[13-14].

Optimization

DoE was used for optimization of ABC mucoadhesive microsphere selecting two surface response designs viz., Box-Behnken Designs (BBD) and using Design Expert 11Stat-Ease software. For BBD three factors viz., SA, mucoadhesive polymer, CaCl2; three level viz., low, medium, high; three response viz., DEE, release at 6h and 12 h133 as shown in tables 5 and 6 and second order polynomial equation is,

$$Y1= b0 + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X12 + b22X22 + b33X32 ,$$

Where Y1- response; b0-intercept. b1 to b33-regression coefficients.

X1, X2, X3- independent variables.

Drug Entrapment Efficiency (DEE)

100 mg of ABC mucoadhesive microspheres was powdered and encapsulated drug is extracted for 1h using 100 ml of methanol by continuous shaking. Strain the contents, dilute appropriately with pH 7.4 phosphate buffer and determine the absorbance at 267 nm. Same procedure was adapted to find the drug content of from the mucoadhesive microspheres by measuring the absorbance at 272 nm. The DC was measured from the std. graph. The studies were carried for 3 times. The percent EE was determined by using the formula.[15].

$$\text{DEE (\%)} = \frac{\text{Actual amount of drug encapsulated x100}}{\text{Theoretical drug content}}$$

In vitro dissolution:

The amount of drug release from mucoadhesive microspheres was investigated by using USP type I basket apparatus and 900ml phosphate buffer pH 7.4 used as dissolution medium. In each case mucoadhesive microspheres equivalent to 50 mg of drug ABC filled in hard gelatin capsules were used. A temperature of 37 ± 0.50C with 50 rpm maintained throughout the experiment. At different intervals 5ml dissolution medium was withdrawn and replaced with fresh dissolution medium to maintain the sink condition. The concentration was calculated by measuring the absorbance against blank. The studies were carried

out in triplicate. The data were computed by using PCP DISSO V3.0a dissolution software[16-17].

Fourier transforms infra-red spectroscpy (FT-IR)

The FT-IR spectrum of ABC, SA, carbopol, mucilages isolated from moringa oleifera, and tamarindus indica were measured at 4000 cm-1 to 500 cm-1 using BRUKER-FTIR spectrophotometer. Small amount of finely ground solid samples under the study were added to 100 times of its weight of KBr and compressed using hydraulic press to get a thin transparent pellet. These pellets are transferred to FT-IR instrument to determine the spectra.

Particle size

These parameters were determined by sieving using mechanical sieve shaker. A set of sieves were arranged in descending order with respect to their size viz., coarser at the top and finer at the bottom. Accurately weighed microspheres were subjected for shaking for optimized period and microspheres retained on each sieve was weighed and average particles size was determined.[18-19].

Scanning Electron Microscpy (SEM)

The surface of optimized mucoadhesive microspheres was analysed by SEM. Mucoadhesive microspheres were adhered on aluminium studs and coated with gold using a sputter coater SC 502, using vacuum (0.1 mm Hg) and then analysed by SEM.[17].

RESULTS & DISCUSSION:

BBD design and data of ABC: carbopol mucoadhesive microspheres.

Trials	Factors/levels			EE %	R 12h %
	SA mg	bopol mg	CaCl2 %		
1	1500	750	10	93.75	78.04
2	1250	750	7.5	85.91	84.68
3	1250	1000	10	95.73	81.71
4	1250	500	5	81.82	95.79
5	1250	750	7.5	87.38	83.98
6	1000	1000	7.5	88.60	86.35
7	1250	1000	5	84.06	91.72
8	1500	1000	7.5	89.47	85.56
9	1000	500	7.5	79.09	88.16
10	1250	750	7.5	83.75	84.07
11	1000	750	10	93.78	80.15
12	1250	750	7.5	84.92	84.48
13	1000	750	5	81.04	88.78
14	1500	750	5	84.76	86.43
15	1500	500	7.5	82.93	87.43
16	1250	500	10	90.15	83.56
17	1250	750	7.5	86.75	83.37

BBD design and data of ABC: *moringa oleifera* mucoadhesive microspheres

Trials	Factors/Levels			EE %	R12h %
	SA mg	*Moringa oleifera* mg	$CaCl_2$ %		
1	1250	750	7.5	86.29	86.16
2	1500	500	7.5	85.28	87.58
3	1000	750	5	87.28	88.16
4	1250	750	7.5	87.28	85.92
5	1250	1000	5	83.57	94.25
6	1250	750	7.5	89.77	86.59
7	1250	500	10	90.15	85.37
8	1250	750	7.5	85.86	84.59
9	1000	500	7.5	81.34	95.36
10	1250	750	7.5	86.46	85.77
11	1500	750	5	85.86	96.69
12	1250	1000	10	92.68	78.56
13	1000	1000	7.5	89.59	89.37
14	1500	750	10	93.73	86.53
15	1500	1000	7.5	89.73	79.34
16	1000	750	10	92.26	88.28
17	1250	500	5	80.28	96.56

BBD design and data ABC: *tamarindus indica* mucoadhesive microspheres.

Trials	Factors/Levels			EE %	R12h %
	SA mg	*amarindus indica* mg	$CaCl_2$ %		
1	1000	500	7.5	81.59	89.24
2	1000	1000	7.5	89.37	86.86
3	1250	750	7.5	86.47	86.89
4	1000	750	10	93.89	83.89
5	1250	750	7.5	83.48	85.26
6	1250	750	7.5	85.86	85.08
7	1250	1000	10	85.77	79.74
8	1250	750	7.5	85.37	86.18
9	1250	500	10	83.59	82.27
10	1500	750	5	78.45	92.06
11	1250	750	7.5	84.28	85.64
12	1500	500	7.5	84.17	88.47
13	1500	750	10	95.65	81.66
14	1500	1000	7.5	89.87	84.58
15	1250	500	5	79.59	91.59
16	1000	750	5	79.49	93.59
17	1250	1000	5	85.79	89.76

The response surface design was used for systematic optimization of ABC loaded mucoadhesive microspheres to identify influential factors by factor screening method. BBD with three factors, three levels; for optimization study. For BBD total 17 trial formulations, were characterized for EE, R 12h.

From the BBD design studies the optimized ABC (A1 to A3) mucoadhesive microspheres formula were generated for SA: carbopol/ moringa oleifera / tamarindus indica $CaCl_2$.

Table 5.19: Comparative EE data of experimental and optimized designs

Batches	ABC/ (mg)	SA (mg)	rbopol (mg)	Moringa oleifera (mg)	Tamarindus indica (mg)
ABC Batches					
A1	1000	1127.64	809.24	-----	-----
A2	1000	1593.30	-----	881.57	-----
A3	1000	1438.86	-----	-----	688.490

Encapsulation efficiency

The ABC loaded mucoadhesive microspheres were designed as per BBD and were prepared using SA in combination with novel mucoadhesive polymer carbopol and natural polymers under the study viz., Moringa oleifera, Tamarindus Indica The EE of ABC and were found to be in the range of 86.14 ± 0.88 to 91.83 ± 0.99 for A1 to A3.

Table 5.23: Comparative EE data of experimental and optimized designs.

Batches	Optimized values	Experimental values
	% EE*±SD	% EE*±SD
BBD-ABC batches		
A1	91.83 ± 0.99	88.33 ± 0.32
A2	89.40 ± 1.20	88.32 ± 0.23
A3	89.26 ± 0.93	86.91± 0.69

***In vitro* dissolution data of optimized ABC mucoadhesive microspheres as per BBD.**

Time in h	Cumulative percent drug released* ± SD		
	A1	A2	A3
0.25	6.12	5.18	6.21
0.50	9.58	9.58	10.16
0.75	16.96	14.61	15.83
1	17.84	19.54	17.77
2	21.57	23.86	22.37
3	26.99	25.87	25.67
4	33.31	31.94	29.67
5	37.25	35.78	34.58
6	45.63	43.46	39.88
8	53.57	53.55	52.53
10	73.44	70.18	69.10
12	86.21	86.95	84.23

The optimized ABC mucoadhesive microspheres were subjected for in vitro drug release rate pattern using USP-type I equipment. In each case equivalent to 100mg of ABC loaded microspheres filled in hard gelatin capsules were used

Fourier transforms infra-red spectroscpy (FT-IR)

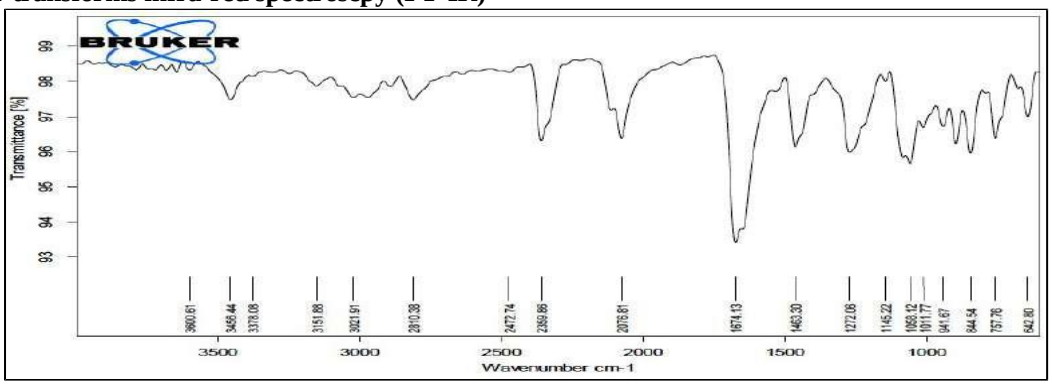

Fig. 5.3: ABC FTIR spectra.

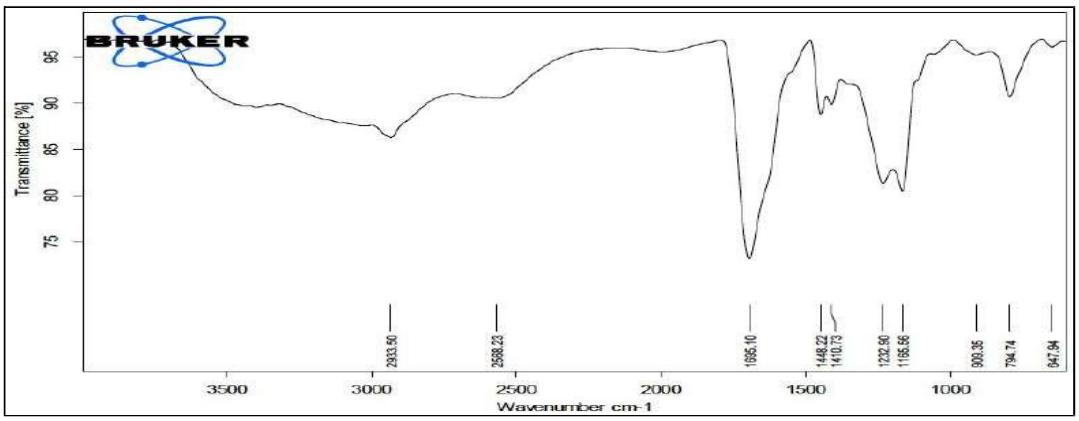

Fig. 5.4: SA FTIR spectra.

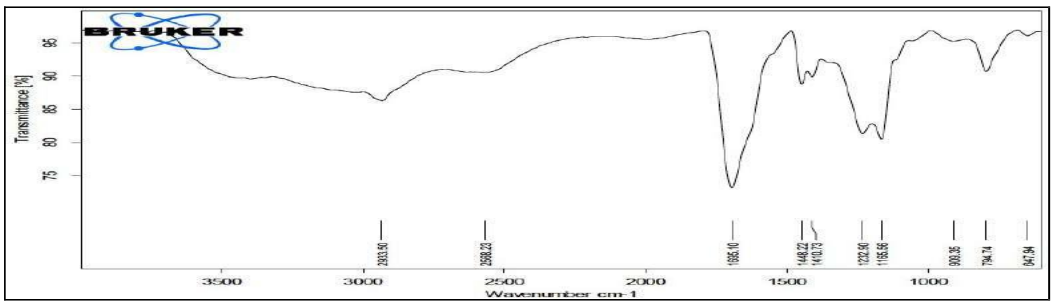

Fig. 5.5: Carbopol FTIR spectra.

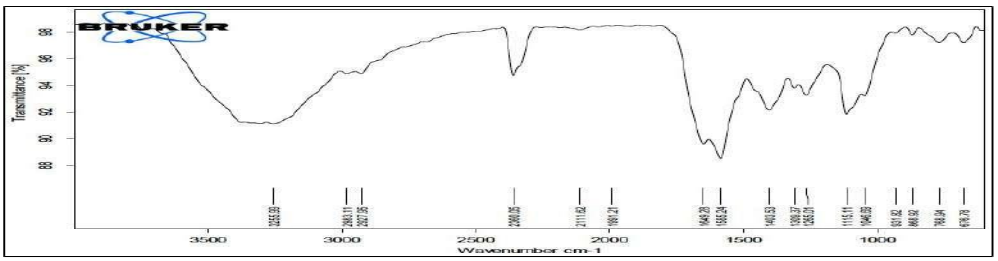

Fig. 5.6: *Moringa oleifera* FTIR spectra.

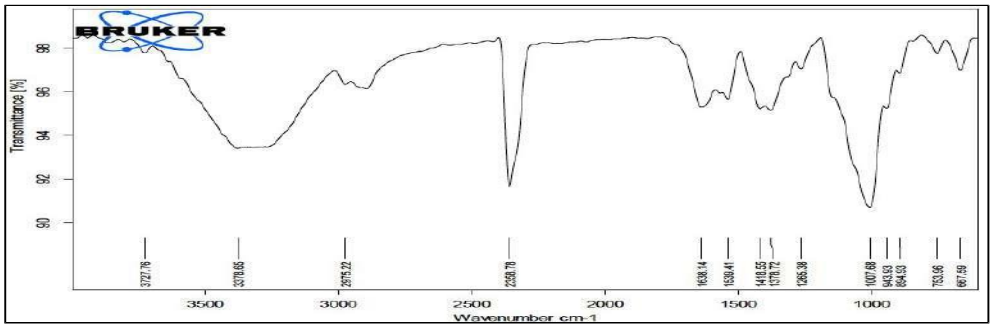

Fig. 5.7: *Tamarindus indica* FTIR spectra.

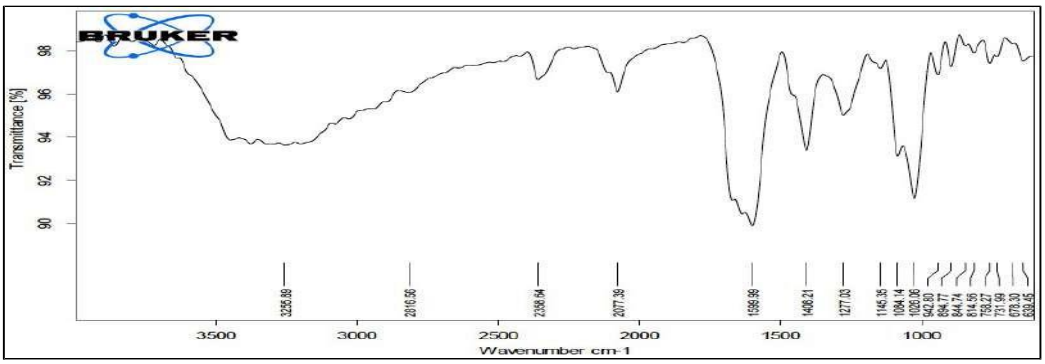

Fig. 5.7: A1 FTIR spectra

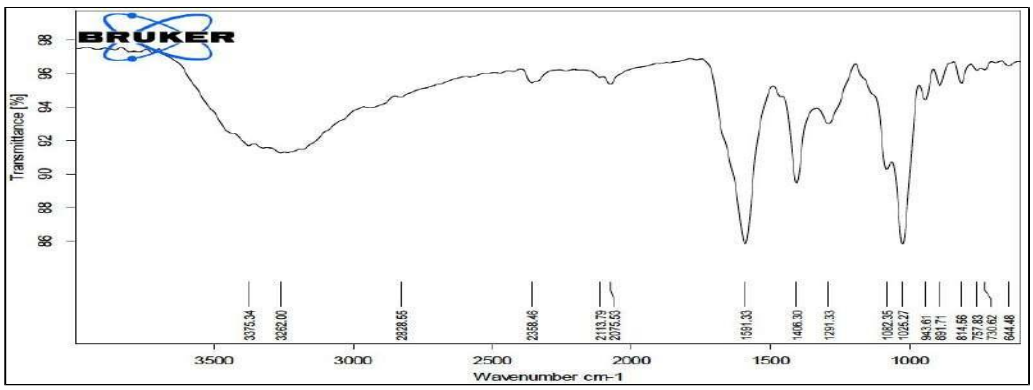

Fig. 5.8: A2 FTIR spectra.

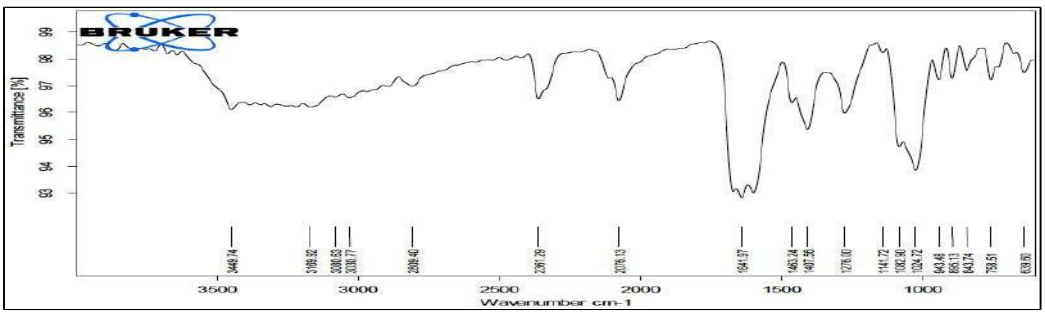

Fig. 5.9: A3 FTIR spectra.

From the FTIR spectra it was confirmed that ABC and formulation components were compatible with each other.

Particle size

Table5. 24: Particle size/distribution data of optimized ABC loaded mucoadhesive microspheres.

Batches	Percent* ained (Fi) 10/22 (1700-710) 1205(µm)	Weight Size (XiFi)	Percent* ained (Fi) 22/44 (710-355) 532.5(µm)	Weight Size (XiFi)	D.avg(µm)
Box Behnken design					
A1	40.63	48959.1	59.37	31614.5	805.73±10.16
A2	46.01	55442.0	53.99	28749.6	841.91±11.45
A3	48.24	58129.2	53.86	28680.4	868.09±12.29

***Average of three determinations**

The average particle size 805.73±10.16 to 896.73±10.16 µm for A1 to A3; 813.67±15.28 to 900.00±12.54 µm. In all the cases the microspheres were distributed within ♯10/22(1700µm-710µm),♯22/44 (710µm-355µm).The size of microspheres is directly proportional to concentration of SA in the formulation. The larger in size of microspheres was observed with increased SA concentration irrespective of polymer used in combination. This could be due to increase in viscosity as the concentration SA increases in polymeric dispersion which has direct influence on bigger particle during ionic gelation.

Surface characterization by SEM

The SEM study of ABC loaded batches reveals microspheres were spherical, discrete smooth to rough texture and completely covered with coat materials. A crystalline particle scattered on the surface of the microspheres was found, also through inner part of the microspheres it contain crystals, which was dense and porous, responsible for controlled drug release..

Figure 5.7: SEM micrographs.

CONCLUSION:

This research work will give importance or potential use of natural mucoadhesive mucilage coat polymer as alternate for microspheres formulation.

Microspheres formulation with SA as coat and carbopol 934, and natural mucoadhesive copolymers viz., *Moringa oleifera, and Tamarindus indica* (NP) mucilages as coating materials by novel ionic gelation method successfully synthesized using *Box-Behnken design and Central composite designs.*

The percent production yield for prepared formulation was not uniform, which may be due to minimum loss of product ingredients during the production process.

Drug distribution was uniform and the novel method adapted for preparation of ABC loaded mucoadhesive microspheres were good and reproducible.

A natural mucoadhesive polymer under the study shows promising results to encapsulate the drug with statistically similar to that of established mucoadhesive polymer.

The microspheres prepared with carbopol and *tamarindus indica* mucilage shows better mucoadhesion than *moringa oleifera*. Among the batches the microspheres loaded with ABC prepared using ABC: carbopol shows better controlled release when compared to other polymers. Experimental results obtained with the optimized formula are relatively nearer to the value predicted *via* experimental design. The differences are only in the dissolution profile of microspheres with respect to type and nature of polymer.

The stability studies indicate no variation in the parameters under the study and the data shows negligible loss in the drug and EE. The *in vitro* drug release for two time intervals suggest the release of drug was stable during work period of time.

This research work decisively manifested that DoE has been powerful, elegant and cost-effective statistical technique which yields more information from fewest runs, ($p > 0.05$) "lack of fit, *F*-value" for response variables accuracy of data. Adjusted R- square and predicted R-square indicated rational agreement between regression coefficients. Contour plot or response surface plot showed the effect of various operating conditions on response parameters in 2-D and 3-D, respectively.
These findings show that ABC amucoadhesive microspheres can be fabricated successfully using NP for controlled release formulations for better patient compliance.

ACKNOWLEDGEMENT
The corresponding author desires to explicit utmost gratitude to the Management and Prof. Dr. D. Ranganayakulu, M. Pharm., Ph. D., Principal, Sri Padmavati school of pharmacy, Tiruchanoor, Andhra Pradesh, India, for presenting all the necessary laboratory demands of the review and constant support.

Conflict of Interest
The authors declare no conflict of interest, financial or otherwise.

Funding Support
The authors declare that they have no funding for this study

REFERENCES:

1. Chen LF, Hoy J, Lewin SR. Ten years of highly active antiretroviral therapy for HIV infection. Med J Aust 186, 2007: 146-151.
2. Simon V, Ho DD, Abdool KQ. HIV/AIDS epidemiology, pathogenesis prevention and treatment. Lancet 368, 2006:489- 504.
3. Lassen K, The multi factorial nature of HIV-1 latency, Trends Mol Med 10, 2004:525-531.
4. Gonzalez AG. (1993). Optimization of pharmaceutical formulations based on response surface experimental designs. Int. J. Pharm., 97: 149-159.
5. Gonzalez-Rodriguez ML, Holgado MA, Sanchez L, RabasCo AM, Fini A. (2002). Alginate/chitosan particulate systems for sodium diclofenac release. Int. J. Pharm., 232:225-234.
6. Jelvehgari M, Montazam SH. (2012). Comparison of microencapsulation by emulsion-solvent extraction/evaportion technique using derivative Cellulose and acrylate-methacrylate copolymer as carriers. Jundishapur J. Nat. Pharm. Prod., 7(4):144-152.
7. Jelvehgari M, Milani PZ, Khonsari F. (2014). Comparative study of in vitro release and mucoacesivity of gastric-compacts Composed of multiple unit system/bilayered discs using direct Compression of metformin hydrochloride. BioImpaCts., 4(1):29–38.
8. Jimenez-Castellanos MR, Zia H, Rhodes CT. (1994). Design and testing in vitro of a bioadhesive and floating drug delivery system for oral application. Int. J. Pharm., 105:65-70.
9. Patil JS, Kamalapur MV, Marapur SC, Kadam DV. Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: A review. Digest J Nanomater Biostr 5, 2010:241-248.
10. Racovita S, Vasilu S, Popa M, Luca C. Polysaccharides based micro- and nanoparticles obtained by ionic gelation and their applications as drug delivery systems. Rev Rom De Chim 54, 2009:709-718.
11. Patil P, Chavanke D, Wagh M. A review on ionotropic gelation method: Novel approach for controlled gastro retentive spheres. Int J Pharm Pharma Sci 4, 2012:27-32.
12. Panel KH, Wancheng S. Use of seed gums from Tamarindus indica and Cassia fistula as controlled-release agents. Asian J. Pharm. Sci 13, 2018: 398-408.
13. Shukla AK, Bishnoi RS, Kumar M, Fenin V, Jain CP. Applications of Tamarind seeds Polysaccharide-based copolymers in Controlled Drug Delivery: An overview. Asian J Pharm Pharmacol 4,2018: 28-30.
14. Sethi S, Khurana RK, Kamboj S, Sharma R, Singh A, Rana V. Investigating the potential of

Tamarindus indica pectin–chitosan conjugate for reducing recovery period in TNBS induced colitis. Int J Biol Macromol 98, 2017:739-47.
15. Pal DK, Nayak AN. Novel tamarind seed polysaccharide-alginate mucoadhesive microspheres for oral gliclaABCe delivery: in vitro-in vivo evaluation. Drug Deli 3, 2012:123-131.
16. Meghana GM, Yogesh G, Yadav A. Formulation and evaluation of mucoadhesive buccal tablets of propranolol prepared using natural polymer. Int J Pharm Sci Res 9, 2018:2905-2913.
17. Yadav IK, Jaiswal D, Singh HP, Chandra D, Jain DA. Evaluation of seed mucilage of vigna mungo(L.) as a binder in tablet formulations. J Pharm Res 2, 2009:1281-1283.
18. Sravani B, Deveswaran R, Bharath S, Basavaraj BV, Madhavan. Studies on vigna mungo mucilage as a pharmaceutical excipient. J Chem Pharm Res 3, 2011:118-125.
19. Vijaya KS, Srinivas D, Kumar ACH, Ravishanker D. Effect of natural polymer on conventional and sustained release properties of indomethacin tablets. Ann Phytomedicine 1, 2012: 20-28.