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Original Article

QUALITY BY DESIGN-DRIVEN FORMULATION DEVELOPMENT AND OPTIMIZATION OF ENALAPRIL MALEATE LOADED MUCOADHESIVE MICROSPHERES: *IN VITRO* AND *IN VIVO* CHARACTERIZATION

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ABSTRACT

Objective: The study is to formulate the enalapril maleate-loaded mucoadhesive microspheres with varied compositions of selected polymers for developing the oral controlled release formulations prepared by ionic gelation method and optimization through central composite design.

Methods: Systematic optimization of microspheres was accomplished by the central composite design and characterized for particle size, entrapment efficiency, *in vitro* drug release and ex vivo mucoadhesion strength, which indicated that microspheres were a consequence to be spherical and free-flowing in nature. The microspheres exhibited high drug entrapment efficiency and *in vitro* drug release in a sustained manner, which was considered to be dependent on the concentration of rate-controlling polymers. The microspheres are showed 389.2 to 850 µm particle size and 22.36 to 85.22 % encapsulation efficiency. *In vitro* studies indicated optimized formulation showed 89.26% drug release after 12h and reduced blood pressure effectively.

Results: The pharmacokinetic parameters were evaluated with C_{max} of 75.39 µg/ml, t_{max} of 8h, and AUC of 53.55 µg/hr/ml, elimination rate constant of 0.0392 and $t_{1/2}$ of 10h. The stability studies were conducted for 3 mo under various conditions and identified no significant deviations in selected key quality attributes.

Conclusion: The formulated mucoadhesive microspheres of enalapril maleate tend to reduce the blood pressure in the animal model, with the novel drug delivery approach in the efficient management of hypertension.

Keywords: Mucoadhesion, Ionic gelation, Central composite design, Loose surface crystal study, *In vitro* drug release, Pharmacokinetics parameters, Stability studies

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INTRODUCTION

Enalapril male ate is an ACE inhibitor to treat hypertension, high blood pressure, diabetic kidney disease, and heart failure [1]. It is typically administered by mouth or injection into a vein in tandem with a diuretic such as furosemide to manage heart failure. The manifestations usually start within an hour and extend for approximately a day when administered orally. Due to the apparent inexpensive therapy and administration availability, the oral route is extensively used to deliver therapeutic drugs to manage hypertension, leading to high patient adherence. Oral-controlled drug delivery techniques represent more than half of the market's drug delivery systems [2]. If taken numerous times per day, the traditional drug delivery method achieves and sustains the concentration of the drug within the optimum therapeutic range required for therapy [3]. As a result, medicine levels vary drastically; as a response, numerous technological advances, such as innovative drug delivery systems, have been introduced to tackle these issues. In addition, NDDS can improve the treatment strategy while presenting numerous therapeutic potentials [4]. The research has been focused on the development and monitoring of hypertension using a central composite design method for (angiotensin-converting enzyme inhibitor) enalapril maleate as the desired medication that assimilates as well as initiates the de-esterification process, culminating in the active form of a potential angiotensin-converting enzyme inhibitor, i.e., enalaprilat [5].

Design of Experiments (DoE) affords the remarkable and even more quantity of instructions as of the slightest number of experimental runs by the methodical distinction of the factors and simultaneous evaluation of the effects of multiple variables. But on the other hand, quality assurance (QA) has altered from the demand to elucidate that the ultimate product gets the predefined requirements and specifications to a novel circumstance where it needs to be confirmed that the product is controlled within a significant and organized design space [6, 7].

Administration of enalapril maleate in sustain release dosage mucoadhesive microspheres as once daily dose would be further enviable since this formulation is proposed to be given to the patients for the management of essential and reno-vascular hypertension and cardiac congestion. The objective of the study is to design a sustained release mucoadhesive microspheres using sodium alginate along with HPMC K4M mucoadhesive polymer to liberate the drug slowly over an extended period for the improvement of therapeutic efficacy and patient compliance [8, 9].

MATERIALS AND METHODS

Materials

The gifted sample of Enalapril maleate (ELM) was received from Dr. Reddy Labs, Telangana, Hyderabad, India (ELM), an active pharmaceutical ingredient. The polymers, such as sodium alginate polymer and HPMC K4M were purchased from Kemphasol India, Mumbai, India. The Sigma Aldrich Limited facilitated sodium chloride and acetonitrile (ACN). Analytical grade chemicals and solvents were utilised throughout the studies.

Methods

Analytical method development by UV spectrometry

Determination of λ_{max} of enalapril maleate in pH 7.4 phosphate buffer

100 mg of ELM was carefully weighed and wholly dissolved in 10 ml methanol, and the volume was then increased to the needed mark of

100 ml using pH 7.4 phosphate buffer to provide a stock solution of 1000 ppm. Then, employing pH 7.4 phosphate buffer, 10 ml of the standard working solution was diluted to 100 ml to obtain a 100 µg/ml solution. When 10 ml of the first dilution step is adjusted to 100 ml utilising phosphate buffer pH 7.4, it yields a concentrated solution of 10 ppm. These solutions were carefully scanned at λ_{max} 200-400 nm wavelength. The UV corresponding scan spectrum curve was recorded down the corresponding wavelength having the maximum absorbance marked for additional dilutions of 10 to 80 g/ml concentrated solutions. The maximum wavelength of λ_{max} was 221 nm [10].

Formulation development of mucoadhesive microspheres

Sodium alginate aqueous dispersions were separately generated using distilled water and a magnetic stirrer at 60 °C. On the other hand, polymeric dispersions were produced independently at room temperature using a magnetic stirrer. The sodium alginate dispersion was prepared; both dispersions were thoroughly mixed for about 10 min at 1000 rpm with the help of a magnetic stirring; the required quantity of ELM was then mixed to polymeric compositions and agitated for 1 h, assisted by a mechanical stirrer at about 1000 rpm. A gauze size #18 needle gently dropped the liquid into a 10 % w/v calcium chloride. To obtain appropriately rounded microspheres, the extra microspheres were allowed to soak in 10 % w/v calcium chloride for around 3h. The microspheres were then collected using the decantation process, rinsed with purified water, and evenly dried at 45 °C for about 12h.

Critical quality characteristics (CQAs) and the quality target product profiles (QTPPs)

In a broader sense, QTPPs refers to a drug's predetermined anticipated characteristics, which are necessary to establish the product's intended performance with respect to safety, efficacy further to enable the recognition of product CQAs. The QTPP was determined based on regulatory, scientific requirements as listed in table 1. QTPPs, which regulate the development of goods and processes, create CQAs. They are also coupled to in-process materials like critical material attributes (CMAs) and process parameters like critical process parameters (CPPs) in the manufacturing of microspheres [11].

Table 1: Quality target product profile (QTPP) and critical quality attributes (CQAs) for developing mucoadhesive microspheres of enalapril maleate (ELM)

QTTPs	Target	CQAs	Pre-determined target	Justification
Dosage type	Controlled-release dosage forms	Cumulative drug release	≥ 90-95%	Controlled release of drug is the objective of the study and is important for better absorption.
Dosage form	Mucoadhesive microspheres	% mucoadhesion	≥ 80-95%	Highly critical factor for developing optimized dosage form.
Drug release and absorption	C_{max} and AUC higher compared to pure drug	Mean particle size (µm)	300 µm-600 µm	Particle size in these ranges is highly critical and important for better absorption of drug.

Optimization of formulation by central composite design

The ELM-loaded microsphere was optimized using Design Expert 12.1.1. (State-Ease Inc., Minneapolis, MN). Three independent factors were considered: sodium alginate concentration (A), HPMC K4M concentration (B), cross-linking duration in hours (C), the additional impact of these individual variables on observed responses (drug release, mucoadhesion, entrapment efficiency, and particle size). Table 2 depicts the optimization design with the three components and 3 levels. Twenty different runs have been undertaken, and the responses for each run were documented. The composition with the optimal outcomes was chosen for future research [12].

Table 2: Composition of different runs of mucoadhesive microspheres of ELM as per CCD along with the obtained CQAs responses as per the coded values for the central composite design

Runs	Factor (X1)	Factor (X2)	Factor (X3)	Response (Y1)	Response (Y2)	Response (Y3)	Response (Y4)
	A: Na Alginate conc. (mg)	B: HPMC K4M conc. (mg)	C: Cross-linking time (h)	Drug release (%)	Mucoadhesion (%)	Entrapment efficiency (%)	Particle size (µm)
1	0	0	0	59.112	65.442	68.659	553.21
2	-1	1	1	16.741	19.457	22.365	835.63
3	0	0	-1	18.149	20.112	25.369	790.25
4	-1	-1	1	20.146	25.102	36.998	750.36
5	-1	0	0	26.179	30.116	42.154	693.11
6	-1	1	-1	29.569	36.232	44.697	689.17
7	0	0	0	60.897	69.448	69.785	520.23
8	0	0	0	62.214	70.399	72.449	487.75
9	1	-1	-1	32.147	42.189	56.215	675.23
10	1	1	-1	22.214	29.471	39.445	703.69
11	0	0	0	69.365	75.344	78.214	420.45
12	1	0	0	38.147	49.576	59.336	638.98
13	1	-1	1	49.235	56.216	59.766	620.14
14	1	1	1	15.362	12.321	10.563	850.25
15	0	-1	0	54.213	60.752	65.337	565.47
16	0	0	0	72.621	79.336	80.364	446
17	0	0	1	82.796	92.167	83.697	410.54
18	0	1	0	89.263	96.149	85.221	389.43
19	0	0	0	79.548	88.976	82.365	440.08
20	-1	-1	-1	19.789	22.03	30.264	735.32
Independent variables					Levels		
					Low (-1)	Medium (0)	High (1)
X1: Sodium Alginate conc. (mg)					250	500	750
X2: HPMC K4M conc. (mg)					50	100	150
X3: Cross-linking time (h)					3	4.5	6

Characterization of mucoadhesive microspheres of ELM

FT-IR analysis

Following the fabrication of microspheres, an FT-IR investigation was conducted to assess the interactions of enalapril with polymers, and it is one of the most successful strategies for anticipating functional groups. Sequential, FT-IR analysis of enalapril maleate microspheres and pure drug along with polymer (HPMC K4M) were done. To validate major functional groups, the FT-IR spectra of microspheres was compared to the FT-IR spectrum of the pure medication. All the sample preparation involves mixing the sample with ethanol and dichloromethane, triturating in a glass motor, and placing it in the sample holder. The enalapril maleate and HPMC K4M polymer spectrum and the optimised formulation were determined [13].

Particle size analysis

Optical microscopy was applied to estimate the size of the particles of the produced microspheres. In this operation, an optical microscope, namely the Olympus BX53 utilised. Every batch with over 300 microspheres was placed on the surface slide, and the particle dimensions were determined randomly. Then, the eyepiece micrometre was calibrated using a stage micrometer [14].

Percentage yield

It can be calculated by measuring the total weight of the dried mucoadhesive microspheres to that of the weight of the drugs and polymer using equation-1[15].

$$\% \text{ Yeild} = \frac{\text{Weight of dried mucoadhesive microspheres}}{\text{Weight of pure drug and polymers}} \text{X } 100 \ldots\ldots (1)$$

Drug entrapment efficiency

By weighing about 25 mg of microspheres then dissolving them in 100 ml of pH 7.4 phosphate buffer, the solution was set aside for 24h and filtrated via What man filter paper. The drug content of enalapril was assessed employing UV spectroscopy adjusted to λ_{max} 221 nm, and the entrapment efficiency was estimated using equation-2 [16].

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{X } 100 \ldots\ldots (2)$$

Loose surface crystal study

The additional proportion of drug located on the surface of the drug-loaded microspheres were determined utilising loose surface crystal analysis. 100 mg of microspheres are been agitated for 5 min in 20 ml of pH 7.4 phosphate buffer prior filtering through a 0.45 m membrane filter. The overall drug content was estimated by spectrophotometric analysis, which shows the quantity of drug contained within formulations [17].

Micrometric studies of mucoadhesive microspheres

Angle of repose

The resistivity of the particle flow was estimated using the angle of repose (α). The fixed funnel method was used to measure the angle of repose. The funnel's height was carefully controlled such that there was just a little space between the funnel's tip and the pile of microspheres. Weighed microspheres passed through the funnel and onto the surface without difficulty [18]. The height and radius of the powder were measured, and the angle of repose was calculated using equation-3 [19].

$$\alpha = \tan^{-1}\left(\frac{h}{r}\right) \ldots\ldots (3)$$

Where α = angle of repose, h = height, and r = radius of a heap of microspheres

Bulk density

Accurately weighed microspheres being introduced in a graduated cylinder to determine the bulk density (100 ml). The volume was measured by extrapolating the bulk quantity, which comprises both the true powder amount and even the microspheres void area. Prior to actually tapping to ascertain bulk density using equation-4 [20].

$$\text{Bulk density} = \frac{\text{Mass of mucoadhesive microspheres}}{\text{Bulk volume of mucoadheshive microspheres}} \ldots\ldots (4)$$

Tapped density

An appropriate volume of microspheres was inserted in a graduated cylinder and tapped 100 times utilising bulk density apparatus. The maximum tapped volume of the microspheres was examined. The tapped density was computed using equation-5 [21].

$$\text{Tapped density} = \frac{\text{Mass of mucoadhesive microspheres}}{\text{Tapped volume of mucoadheshive microspheres}} \ldots (5)$$

Compressibility) index

The compressibility index of the prepared microspheres was determined by using the equation-6,

$$\% \text{ Compressibility} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \ldots\ldots\ldots (6)$$

For the powders with good flow characteristics, Carr's index value usually lies below 15%, whereas Carr's index above 25% indicates the poor flowability [22].

Hausner's ratio

The Hausner's ratio for microspheres was calculated as the ratio of tapped density to bulk density and the value was calculated by using the equation-7 [23],

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \ldots\ldots\ldots\ldots\ldots (7)$$

Scanning electron microscopy (SEM)

Scanning electron microscopy was used to examine the physical features of microspheres. On one side of a double sticky stub, the samples were dispersed on the microspheres. The gold on the stub was coated using a Jeol JFC 1100 sputter coater (Jeol Ltd., Tokyo, Japan). The formed microspheres were analysed using a model Jeol JSM 5300 (Jeol Ltd.) and examined at a 15-20 kV accelerating voltage [24].

Swelling index

The swelling index was measured by soaking 25 mg of mucoadhesive microspheres in a pH 7.4 phosphate buffer for 12h, filtering and weighing the results, and then using equation-8 to determine the swelling index [25].

$$\text{Swelling index} = \frac{\text{Wt} - \text{Wo}}{\text{Wo}} \times 100 \ldots\ldots\ldots\ldots\ldots (8)$$

Where, W_t= final weight of the microspheres, W_o= initial weight of the microspheres

Percent moisture loss

The microspheres were weighed and is noted as (W1). The weighed microspheres were kept in a desiccator contains $CaCl_2$ at 37 °C for 24h. After the time period again weighed the microspheres and is noted as (W2). The percent moisture loss was calculated by using equation-9 [26].

$$\% \text{ Moisture loss} = \frac{\text{W1} - \text{W2}}{\text{W1}} \times 100 \ldots\ldots\ldots\ldots\ldots (9)$$

***In vitro* mucoadhesion test**

According to the monograph, the mucoadhesive property of the microspheres is assessed on the goat's intestinal mucosa by utilizing phosphate buffer pH 7.4 (PBS) support. Gauged microspheres are spread onto wet flushed tissue example, and quickly from that point, the slides are clung to the arm of a USP disintegration test machine with reasonable uphold at 37 °C. The heaviness of microspheres drained out at various spans is estimated. The % mucoadhesion is determined by the following equation-10 [27].

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres adhered on to the mucosa}}{\text{No. of microspheres applied}} \times 100 \ldots\ldots (10)$$

In vitro drug release study

A USP type II dissolution unit was employed to evaluate drug release *in vitro*. Mucoadhesive microspheres (equal to 10 mg ELM) were weighed and placed in a dissolving media of 900 ml containing 0.1N HCl. The tween 80 (0.02%), the surface-active agent, was incorporated to aid in the solubilization of medicines in the dissolving media. The paddle revolving at 100 rpm was used to agitate the medium. 5 ml samples were taken at 30 min, 1, 2, 4, 6, and 12 h. 5 ml of new dissolving media was added after each sample to keep the sink condition. A Shimadzu 1700-E double-beam spectrophotometer was used to measure the absorbance of these solutions at λ_{max} 221 nm. The cumulative percentage of drug release was ascertained using an equation emanating from a standard curve. The pure drug (10 mg) was used as a reference in a comparable dissolving trial. Both the pure medication and formulations fared well in the comparative solubility testing. Finally, the data from the *in vitro* drug release studies were fitted using multiple kinetics models (Zero-order, First-order, Higuchi, and Korsmeyer–Peppa's) to determine the release kinetics and grasp the whole process of drug release from the microspheres. Finally, each kinetic release model evaluated the coefficient of correlation values (R^2), comparing the prepared formulations [28].

In vivo pharmacodynamic study

For *in vivo* studies, the improved formulation 18 of ELM-loaded mucoadhesive microspheres produced optimal results in terms of % mucoadhesion. Albino rabbits weighing 1.5 to 2.5 kg was chosen and were subjected to suitable housed state in a temperature and relative humidity were implemented for the research according to the Institutional Animal Ethical Committee (IAEC) recommendations with Approval No: CPCSEA/IAEC/JLS/06/05/17/006. The animals (Saha Enterprises Kolkata 700051) were divided into six groups, with Group I receiving an optimum formulation of ELM-microspheres corresponding to (40 mg) administered as an oral solution. Enalapril maleate pure drug suspension (10 mg/kg body weight) was administered to Group II. At 0, 2, 4, 6, 8, and 12 h after these formulations were administered, blood samples were obtained from the marginal ear vein.

Pharmacokinetic analysis

The area under the curve (AUC), peak plasma concentration (C_{max}), and time to reach peak concentration were indeed estimated by using plasma concentration versus time plot (t_{max}). A semi-logarithmic plot of plasma concentration vs time was used to compute the elimination rate constant (Kel) and the elimination half-life ($t_{1/2}$). The area under the curve was statistically investigated using one-way ANOVA at 0.05 levels in Graphpad Software version 5.01 software [29].

Stability studies

The optimized microspheres' stability studies were done in compliance with ICH recommendations. The microspheres were properly packaged in high-density plastic bottles and kept in the stability chamber at 40°/75% RH for three months. To determine their stability, the microspheres were tested for physicochemical parameters, drug content, and *in vitro* drug release at different time intervals (0, 1, 2, and 3 mo) [30].

RESULTS AND DISCUSSION

Results

Enalapril maleate calibration curve in pH 7.4 phosphate buffer

A UV spectrometer was used to test the solution's absorbance at λ_{max} 221 nm at pH 7.4 phosphate. As shown in fig. 1(a-b) a graph of absorbance vs. concentration was generated, indicating that Beer's law was followed in the concentration range of 10 to 80 µg/ml [31].

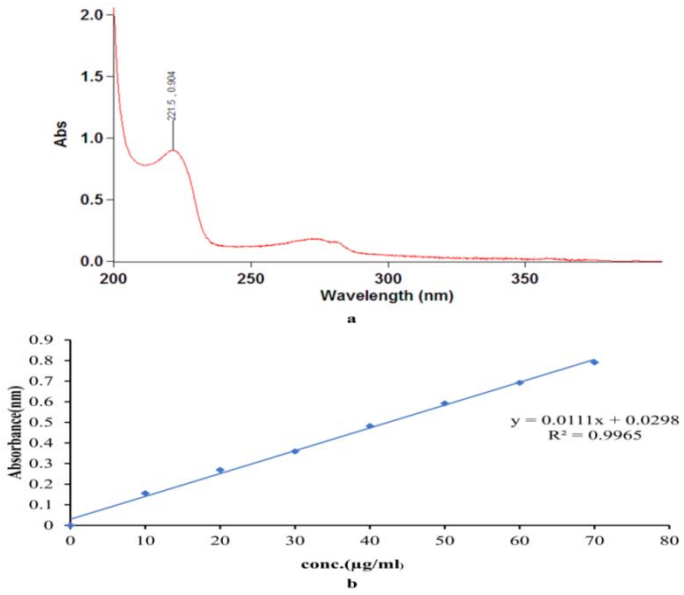

Fig. 1: UV spectrum of enalapril maleate (ELM) using pH 7.4 phosphate buffer (a), Calibration curve of enalapril maleate at λ_{max} 221 nm using phosphate buffer pH 7.4 (b)

FT-IR spectra analysis

There was no significant interaction between various rational combinations combining a physical mixture of drug with polymers (i.e., sodium alginate and HPMC K4M) and optimised microspheres, as shown in fig. 2 (a-c) [30, 31].

Particle size analysis

Microspheres of particle sizes ranged from 389.43 to 835.63 µm. Factors including polymer concentration, cross-linking agent amount, and stirring duration all influenced size distribution variability between batches [31].

Response surface approach for statistical optimization

The direct effect of chosen independent factors such as sodium alginate concentration (A), HPMC K4M concentration (B), and cross-linking time (h) (C) on dependent variables such as % drug release, % mucoadhesion, % entrapment efficiency, and particle size was observed, with responses for 20 generated trials examined. The

outlines of the model were determined by fitting it into an appropriate mathematical model, particularly a quadratic model, demonstrating interaction effects among the variables. ANOVA and lack of fit were also tested statistically on the model. A substantial p-value was found in the model terms (0.05), and the model was best suited due to an insignificant lack of fit. Furthermore, among the three parameters, the concentration of HPMC K4M had a substantially more significant impact on mucoadhesion. The concentration of sodium alginate, on the other hand, had a substantial effect on drug entrapment efficiency. It was observed that elevating the concentration of cross-linking substances and HPMC K4M had a greater impact on the size of microsphere particles. Finally, a numerical optimization method and a desirability function were used to identify the best formulation. The optimal formulation comprised 500 mg sodium alginate and 150 mg HPMC K4M, with a 4.5-h cross-linking period, as shown in 2D and 3D plots in fig. 3 (a-h) [32].

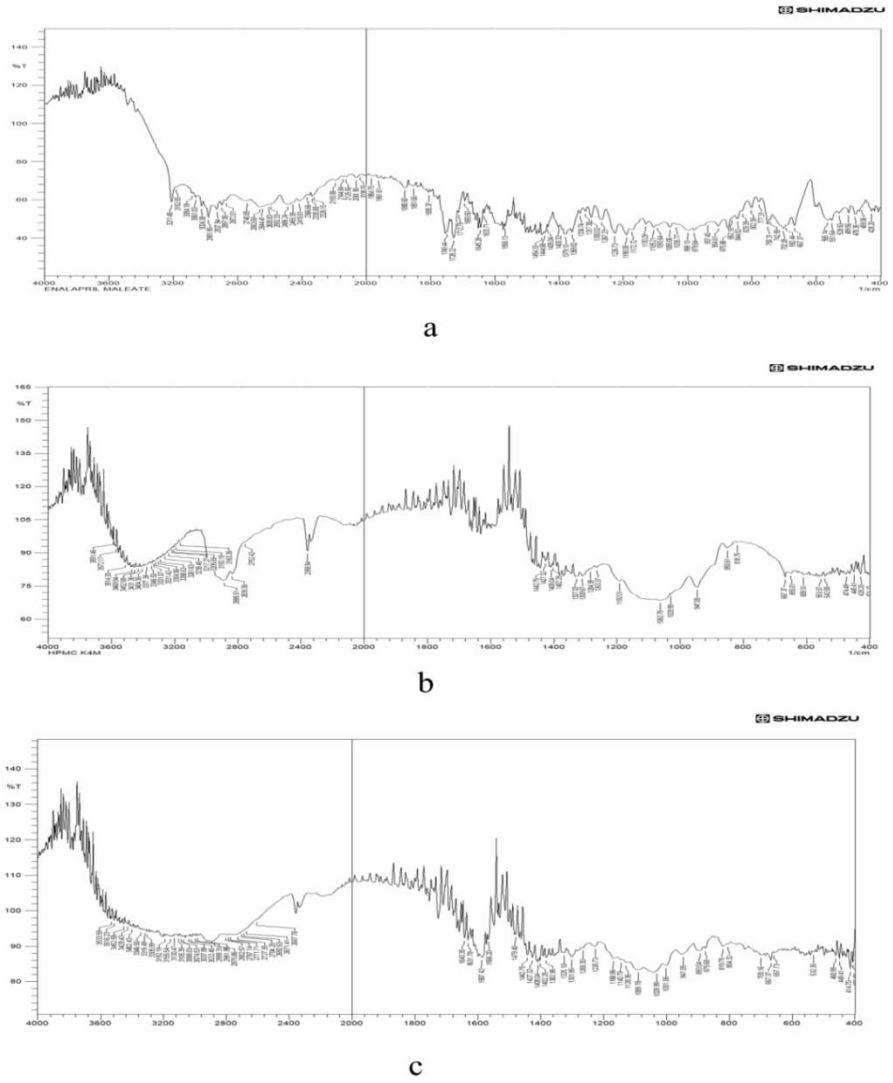

Fig. 2: The FT-IR spectrum of pure drug ELM (a), FT-IR spectrum of HPMC K4M (b), FT-IR spectrum of ELM-loaded mucoadhesive microspheres of F18 formulation (c)

Percentage yield

Table 3 shows that the percentage yield of several batches of microspheres ranged from 64.39 to 80.25%. The yield was excellent, implying that the crosslinking agent binds all polymers to generate microspheres. The proportion of drug content in the prepared microspheres ranged from 10.26 % to 48.32 % [33].

Drug entrapment efficiency

Entrapment efficiency, on the other side, was estimated to vary from 10.56 to 85.22 %, as seen from table 3. The efficiency of entrapment was assessed by the sodium alginate concentration [33].

Loose surface crystal study

The microsphere formulations featuring a smaller quantity of the matrix-forming polymer, HPMC K4M, had the highest fraction of free drug on the surface, according to the loose surface crystal analyses. The largest quantity of loose surface crystals was found in formulation F18 (8.22±0.01), whereas the least amount was found in formulation F14 (2.14±0.02) [34].

Micrometric studies

The reported tapped density ranged from 0.4520±03 to 0.610±0.23 g/cm^3, with bulk density ranging from 0.2890±0.02 to 0.524±0.06 g/cm^3. As shown in table 4, the produced microspheres had acceptable flow characteristics, with Carr's index ranging from 6.30±0.03 to 15.20±0.10 and the angle of repose ranging from 10.03° to 28.42°, indicating favourable flow behaviour [35].

Scanning electron microscopy

A scanning electron microscopy (SEM) was used to look at the surface morphology of the formed mucoadhesive microspheres. The

formulation-18 is presented in scanning electron photomicrographs in fig. 4. These images were taken with a magnification of 100. According to SEM examination, all of the formulated mucoadhesive microspheres were well-formed and spherical [36].

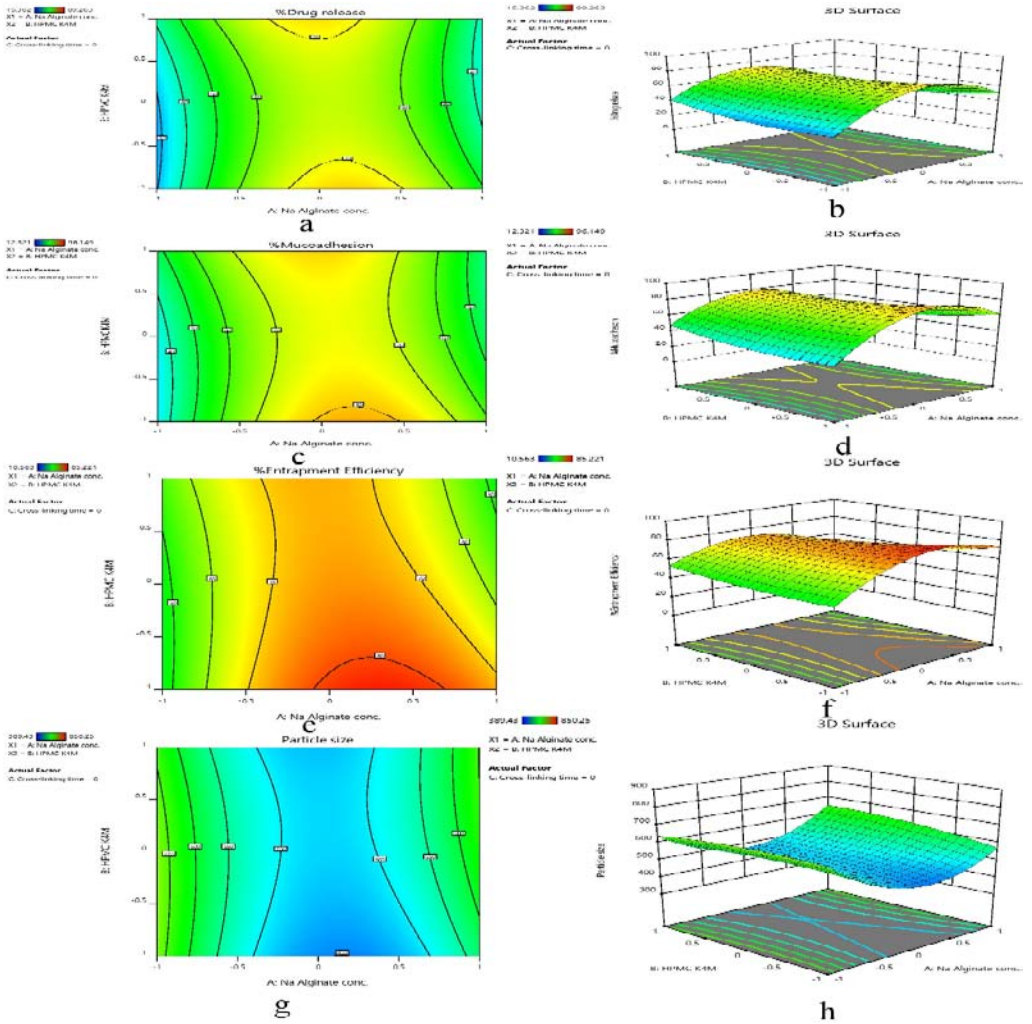

Fig. 3: 2D-plot depicting % drug release of F18 (a), 3D-plot depicting % drug release of F18 (b), 2D-plot depicting % mucoadhesion of F18 (c), 3D-plot depicting % mucoadhesion of F18 (d), 2D-plot depicting % EE of F18 (e), 3D-plot depicting % EE of F18 (f), 2D-plot depicting particle size of microspheres of F18 (g): 3D-plot depicting particle size of microspheres of F18 (h)

Table 3: Particle size (µm), % yield, drug entrapment efficiency (%), swelling index (%) and moisture loss (%) of formulations F1 to F20

Formulation code	Particle size (µm) (mean±SD)	% Yield (mean±SD)	Drug entrapment efficiency (%) (mean±SD)	Swelling index (%) (mean±SD)	Moisture loss (%) (Mean)
F1	553.21±0.043	33.8±0.03	68.659±0.01	27.2±0.20	1.65
F2	835.63±0.09	46.6±0.02	22.365±0.02	33.9±0.12	1.74
F3	790.25±0.04	38.1±0.01	25.369±0.22	44.2±0.18	1.13
F4	750.36±0.05	42.6±0.021	36.998±0.31	20.2±0.15	1.76
F5	693.11±0.061	23.8±0.023	42.154±0.12	42.60±0.11	1.04
F6	689.17±0.006	35.2±0.028	44.697±0.11	13.06±0.15	2.10
F7	520.23±0.01	46.1±0.029	69.785±0.24	20.54±0.02	2.55
F8	487.75±0.03	39.7±0.032	72.449±0.31	34.25±0.14	2.34
F9	675.23±0.001	45.6±0.012	56.215±0.01	45.02±0.04	1.99
F10	703.69±0.04	26.2±0.024	39.445±0.002	50.78±0.05	2.26
F11	420.45±0.06	29.3±0.032	78.214±0.02	60.66±0.04	1.45
F12	638.98±0.002	42.6±0.028	59.336±0.04	43.66±0.14	1.54
F13	620.14±0.06	49.2±0.034	59.766±0.06	24.36±0.13	1.29
F14	850.25±0.015	64.39±0.045	10.563±0.05	40.20±0.008	1.26
F15	565.47±0.01	49.5±0.027	65.337±0.03	52.58±0.07	1.15
F16	446±0.001	25.6±0.032	80.364±0.003	43.65±0.06	1.14
F17	410.54±0.033	39.6±0.034	83.697±0.01	30.54±0.04	1.21
F18	389.43±0.017	80.25±0.029	85.221±0.09	79.2±0.06	0.86
F19	440.08±0.011	50.23±0.002	82.365±0.04	20.32±0.15	1.46
F20	735.32±0.012	29.3±0.023	30.264±0.03	21.32±0.13	1.34

n = 6, n is the number of observations

Table 4: Angle of repose, bulk density (g/cm³), tapped density (g/cm³), carr's index and Hausner's ratio of formulations (F1 to F20)

Formulation	Angle of repose (Degree)*	Bulk density (g/cm³)*	Tapped density (g/cm³)*	Carr's index*	Hausner's ratio
F1	25.23°±0.01	0.338±0.03	0.326±0.02	12.72±0.20	0.645
F2	29.32°±0.02	0.466±0.02	0.425±0.031	13.39±0.12	0.794
F3	23.12°±0.03	0.381±0.01	0.568±0.002	11.44±0.18	1.133
F4	22.02°±0.001	0.466±0.021	0.654±0.03	11.20±0.15	0.706
F5	25.66°±0.005	0.438±0.023	0.495±0.014	12.60±0.11	0.604
F6	27.45°±0.031	0.352±0.028	0.472±0.024	13.06±0.15	0.120
F7	27.36°±0.014	0.361±0.029	0.528±0.014	12.54±0.02	0.545
F8	25.34°±0.012	0.397±0.032	0.543±0.021	14.25±0.14	0.354
F9	22.13°±0.022	0.456±0.012	0.539±0.034	15.02±0.04	0.929
F10	16.36°±0.031	0.362±0.024	0.503±0.026	9.78±0.05	0.726
F11	19.78°±0.003	0.293±0.032	0.512±0.031	10.66±0.04	0.445
F12	23.93°±0.013	0.426±0.028	0.468±0.022	13.66±0.14	0.954
F13	21.89°±0.004	0.492±0.034	0.493±0.011	14.36±0.13	0.629
F14	10.03°±0.015	0.289±0.045	0.452±0.041	6.30±0.008	0.726
F15	23.02°±0.019	0.295±0.027	0.492±0.022	12.58±0.07	0.145
F16	23.54°±0.023	0.256±0.032	0.469±0.025	9.65±0.06	0.154
F17	19.11°±0.011	0.396±0.034	0.553±0.015	10.54±0.04	0.291
F18	28.42°±0.017	0.524±0.029	0.610±0.09	15.20±0.06	1.214
F19	19.25°±0.019	0.510±0.002	0.529±0.06	10.32±0.15	0.456
F20	20.36°±0.020	0.493±0.023	0.496±0.05	12.32±0.13	1.354

*mean±SD, n = 6, n is the number of observations

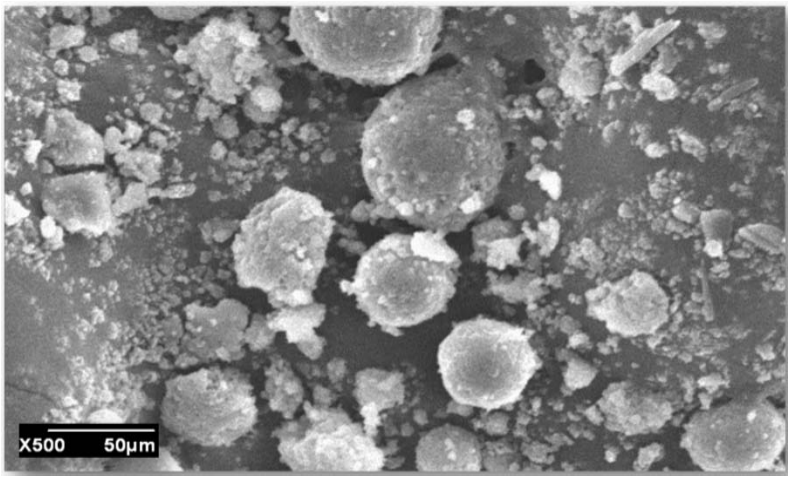

Fig. 4: SEM analysis of ELM-loaded microspheres of F18

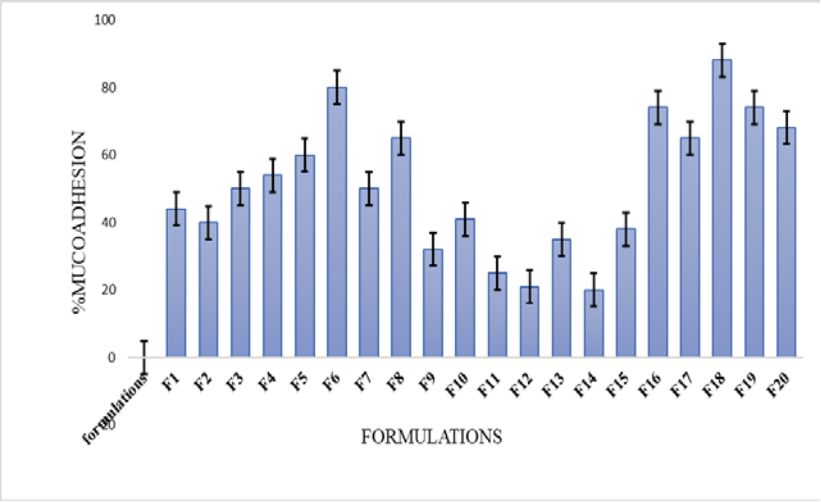

Fig. 5: The percentage of mucoadhesion of all the formulations of ELM-loaded microspheres where: mean±SD, n = 6, n is the number of observations

Swelling index

The mucoadhesive microspheres' swelling index was calculated according to the experimental design. Table 3, shows that formulation F18 has the most extensive swelling index, which might be attributed to more excellent water absorption and concentration of the mucoadhesive polymer, HPMC K4M [37].

Percent moisture loss

As shown in table 3, formulation F18 had the most negligible moisture loss, while formulation F14 had the most moisture loss. This confirmed that the availability of simulating water content was related to the use of water as a manufacturing vehicle during the synthesis of microspheres, as well as the water-absorbing characteristics of the drug or mucoadhesive polymers. Furthermore, the reduced water content suggested that the microspheres had appropriately dried [38].

***In vitro* mucoadhesion test**

The microsphere formulations demonstrated good mucoadhesion properties in both 0.1N HCl and pH 7.4 phosphate buffer wash-off tests. After 3h, microspheres exposed to 0.1N HCl have washed away. Microspheres in pH 7.4 phosphate buffer, on the other hand, remained on the tissue for 6h. As shown in fig. 5, the formulation F18 showed significantly higher mucoadhesive strength in a pH 7.4 phosphate buffer than the other formulations [39].

***In vitro* drug release study**

The *in vitro*, drug release studies revealed that all mucoadhesive microsphere batches exhibited a consistent release profile. The drug release % from the microcapsule compositions generated ranges from 15.36 to 89.26%. In compositions with a greater proportion of the mucoadhesive polymer, the releasing action was shown to be higher, and vice versa. When the sodium alginate concentration in the microspheres was elevated, the ELM delivery from the polymer matrix was decreased. Furthermore, as shown in fig. 6, the F18 formulation had the maximum degree of controlled release mechanism for roughly 12 h, i.e., 89.26% drug release [40].

***In vivo* pharmacodynamic study**

The serum % drug concentration of dug loaded microspheres v/s pure drug suspension and the results for the pharmacokinetics parameters as the graph shown in fig. 7. The pure drug solution and optimized formulation (F18) serum drug concentration versus time profiles revealed that C_{max} after the oral dosage was 50.22 ng/ml and 75.39 ng/ml, t_{max} was 6 h and 8 h, AUC was 50.25 ng/h/ml and 53.55 ng/h/ml, and Kel was 0.0365 and 0.0392, respectively. The pure drug had a $t_{1/2}$ of 10h, whereas formulation F18 had a $t_{1/2}$ of 12 h. After the drug's C_{max} was reached, the drug's concentration in the plasma dropped rapidly. Following C_{max}, the formulation demonstrated regulated drug release for up to 8 h, with pure drug concentrations declining after 6 h. The pharmacodynamic data of drug-loaded mucoadhesive microspheres in control group I (a); DOCA salt+group II (b); DOCA salt+optimized formulation group III (c); DOCA salt+pure drug group IV (d) are shown in fig. 8. As a result, when enalapril maleate was formulated as mucoadhesive microspheres instead of pure medication alone, the bioavailability of the drug was shown to be significantly enhanced. Because of its ideal particle size, high entrapment efficiency, and efficient *in vitro* drug release, optimized formulation (F18) was chosen for *in vivo* research. The primary basis of the study was to assess the antihypertensive consequences of formulation (F18) and the pure drug (Enalapril maleate) *in vivo* and the results are listed in table 5 [41].

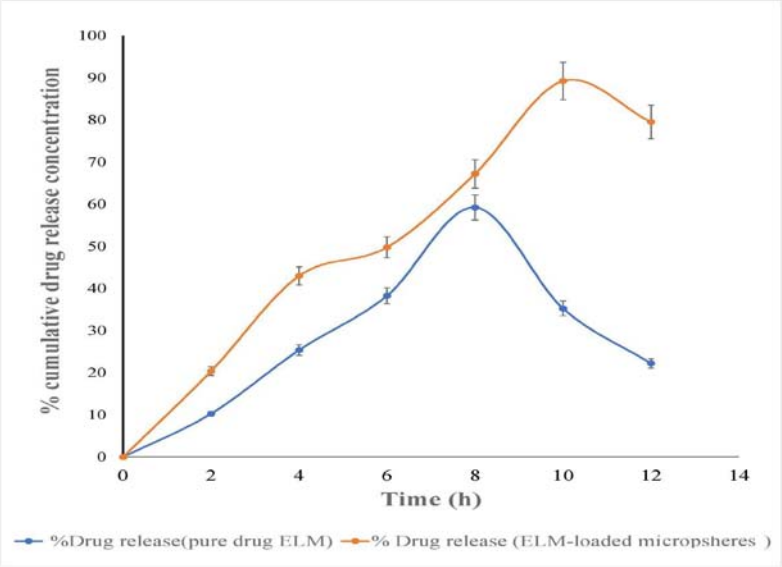

Fig. 6: *In vitro* cumulative drug release concentration of ELM-loaded microspheres for F18

Table 5: *In vivo* studies of formulation (F18) for anti-hypertensive effect in rabbit model

Time (h)	Control (Group I)		DOCA salt (Group II)		DOCA salt+formulation (Group III)		DOCA salt+pure drug (Group IV)	
	Systolic BP	Diastolic BP	Systolic BP	Diastolic BP	Systolic BP	Diastolic BP	Systolic BP	Diastolic BP
0	120±0.2	90±0.25	158±1.3	118±2.0	156±1.0	116±2.5	160±1.5	118±0.60
1	120±0.4	90±0.35	158±0.5	117±0.35	145±2.5	115±2.0	152±2.2	105±2.5
2	118±0.5	91±0.40	157±2.0	118±1.2	132±3.0	105±3.0	141±3.0	98±2.1
6	119±0.35	91±0.54	159±1.2	117±2.5	130±0.36	95±1.2	138±0.5	95±2.4
12	122±0.22	89±0.36	156±2.5	116±0.9	123±0.23	93±1.0	135±0.5	93±3.2
24	121±0.29	90±0.32	157±0.56	118±0.6	122±0.21	91±1.5	130±0.9	91±2.5

Mean±SD, n = 6, n is the number of observations

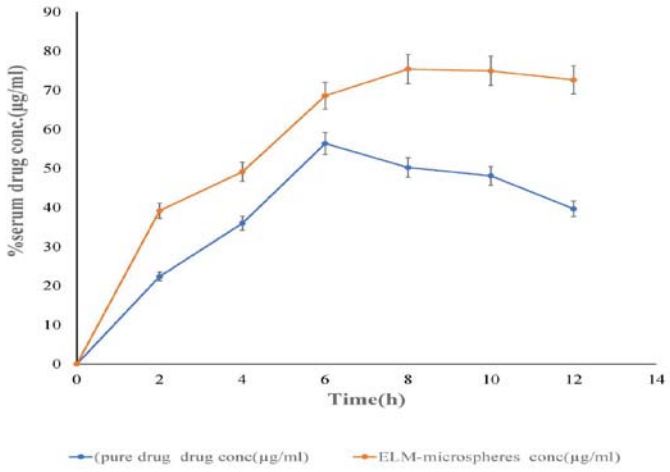

Fig. 7: Serum % drug concentration of ELM-loaded microspheres v/s pure drug suspension

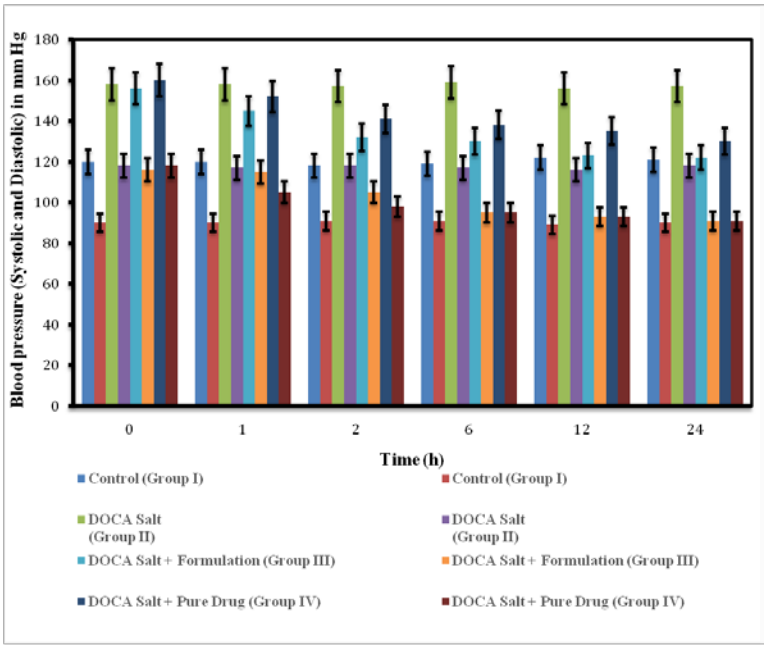

Fig. 8: Pharmacodynamic data of drug-loaded mucoadhesive microspheres in control group I (a); DOCA salt+group II (b); DOCA salt+optimized formulation group III (c); DOCA salt+pure drug group IV (d)

Table 6: Short-term stability studies data for the formulation (F18) of drug-loaded mucoadhesive microspheres

Stability studies as per ICH temperature conditions	**4±1 °C**			**25±2 °C (60±5% RH)**			**37±2 °C (65±5% RH)**		
No. of months of studies(mo)									
Parameters	**1M**	**2M**	**3M**	**1M**	**2M**	**3M**	**1M**	**2M**	**3M**
% Drug content	65.32	61.29	52.98	55.74	50.85	45.232	60.25	59.20	43.65
% Entrapment efficiency	80.22	79.69	70.22	79.48	73.22	70.47	71.25	69.27	62.54
% Drug release	85.58	79.21	71.25	80.25	79.58	76.24	79.22	68.23	65.25
% Mucoadhesion	92.25	89.25	79.36	90.89	82.47	79.15	89.14	75.14	72.96
Particle size (µm)	395.22	405.27	435.12	401.25	452.12	502.25	404.53	462.22	495.35
p-value	0.115	0.065	0.062	0.089	0.135	0.061	0.075	0.069	0.125

p-value ≤0.05 indicates significant difference, *p-value* ≥0.05 indicates no significant difference

Stability studies

The generated enalapril maleate microspheres were tested for stability by storing the formulation F18 at 4±1 °C, 25±2 °C; 60±5 %RH and 37±2 °C; 65±5 %RH for 3 mo as shown in table 6 [42].

DISCUSSION

The FT-IR data for enalapril shows C=O stretching (ester), 1648 cm^{-1} for N-H bending, and 1454 cm^{-1} for C-H (alkanes) bending bands at 1751 cm^{-1}, 1648 cm^{-1}, and 1454 cm^{-1}, respectively. The peak visible at

1053 cm^{-1} revealed the presence of a glucose ring. C-H absorptions are seen at 1452 cm^{-1}. In ketone, stretching was seen at 3436 cm^{-1} N-H, 2931 cm^{-1} C-H, 1745.33 cm^{-1} C=O, 1352 cm^{-1} C-N stretching, and 1245 cm^{-1} C=O, according to FT-IR results. All the defined drug bands, including polymers, occurred, with no significant difference in peak heights, indicating that the drug and excipients were compatible. The drug-polymer mixture's FT-IR spectra revealed no change in peak wave numbers or intensity, implying a lack of interaction. In formulations containing a higher concentration of mucoadhesive polymer, greater mucoadhesion strength and more extended wash-off periods were observed. This might be because the polymer chains and mucin have an electrical attraction.

The mucoadhesion strength is also affected by the polymer's swelling index. The zero-order, first-order, Higuchi, and Korsmeyer-Peppa's model was used to fit the drug release from the microspheres. In addition, the Korsmeyer-Peppa's model found that the F18 formulation had the greatest R^2 value of (0.988) in the simulated gastric fluid (pH 1.2).

On the other hand, the R^2 value of 0.988 is similar to the Higuchi model. For the first-order model and zero-order model, respectively, R^2 values of 0.986 and 0.932 were reported.

In the control group (Group-I), the systolic and diastolic arterial pressures were 120±0.3 and 90±0.03 mm of Hg, respectively. The administration of DOCA (deoxycorticosterone acetate) for 30 d to other groups II, III, and IV resulted in systolic and diastolic arterial pressures of 159±1.2 and 117±2.5 mm Hg, respectively. At 24 h, blood pressure in the DOCA salt group was 157±0.56 and 116±0.9 mmHg; in the DOCA salt+formulation group, 125±0.36 and 95±1.2 mm of Hg; and in the DOCA salt+pure drug group, 130±0.9 and 91±2.5 mm of Hg. Group-III maintains systolic and diastolic arterial pressures of 122±0.21 and 91±1.5 mm of Hg, respectively, after 24 h of investigation, which is close to group-baseline I's arterial pressure as reported by Nanjwade B. K. *et al.*, 2014 [43], Group-III with optimized formulation (F18) maintains lower systolic and diastolic arterial pressure for 24 h. Still, group-III and IV with formulation and pure drug show a rise in pressure before 6 h.

After 3 mo of storage, the percent drug content, percent entrapment efficiency, and percent drug release, percent mucoadhesion, and particle size (µm) of the chosen formulation (F18) were determined. The studies revealed that there no noticable change in drug content, entrapment efficiency, mucoadhesion, drug release and particle size after storage for 3 mo at 4±1 °C, 25±2 °C; 60±5% RH and 37±2 °C; 65±5% RH as reported by Bahadur K. V. *et al.*, 2020 [12]. It was also revealed that formulation F18 stored at 4±1 °C showed maximum drug content of (65.32 %) followed by the storage at 25±2 °C; 60±5% RH and 37±2 °C; 65±5% RH conditions. Hence, the results indicated that there was no significant change was observed from the respective *p-values* for the determined parameters.

CONCLUSION

The research and its results indicate that using an ionic gelation process with sodium alginate and HPMC K4M as suitable polymers, it is possible to make enalapril maleate (ELM) mucoadhesive microspheres. The adoption of the CCD design enabled the emergence of an effective formulation with an intended controlled drug release profile of drug which boosted mucoadhesion and anti-hypertensive properties. The drug's release profile was successfully found; it was diffusion regulated and marked Higuchi kinetics. As per FT-IR analysis, there seems to be no physiochemical interference between the drug and the polymers. SEM investigation showed a smooth texture with particles which seem to be spherical. Further, *in vivo* pharmacodynamic studies reported significantly better anti-hypertensive function of mucoadhesive microspheres than the pure drug's suspension. Accelerated stability studies indicated that the optimised compositions were stable and might be used as an alternate dose form for hypertension patients being treated orally.

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Nil

ABBREVIATIONS

AUC: Area under the curve, CCD: Central composite design, C_{max}: Maximum plasma drug concentration, CQAs: Critical quality attributes, DOCA: Deoxycorticosterone acetate, EE: Entrapment efficiency, ELM: Enalapril maleate, FT-IR: Fourier-transform infrared spectroscopy, HPMC: Hydroxy propyl methyl cellulose, IAEC: Institutional animal ethical committee, ICH: The international council for harmonisation, RH: Relative humidity, SD: Standard deviation, SEM: Scanning electron microscopy, t_{max}: Time to reach maximum concentration, TPPs: Target product profiles, USP: United states of pharmacopeia, UV: Ultraviolet.

AUTHORS CONTRIBUTIONS

MEBR was involved in planning and supervised of the work, SK drafted the manuscript and designed the figures, aimed in data analysis, collect the sample and performed the measurement, formulated the drug, performed the calculation, SKS performed the characterization, aided in interpreting the results and worked on the manuscript, DG performed the statistical analysis. All authors discussed the results and commented on the manuscript.

CONFLICT OF INTERESTS

All authors declare that they have no conflicts of interest

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