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Research Article

# Almotriptan Loaded Sodium Alginate Microspheres for Nasal Delivery: Formulation Optimization Using Factorial Design, Characterization and *In Vitro* Evaluation

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**ABSTRACT**

Almotriptan malate, indicated for the treatment of migraine with or without aura in adults is not a drug candidate feasible to be administered via oral route during the attack due to its associated symptoms such as nausea and vomiting. This obviates an alternative dosage form. Nasal delivery of this drug is a good substitute to oral and parenteral administration, due to its numerous advantages. In the present study, sodium alginate microspheres of Almotriptan malate, for intranasal delivery, were prepared by water-in-oil (w/o) emulsification cross-linking technique. A $2^3$ factorial design was employed with drug to polymer ratio, calcium chloride concentration and cross-linking time as independent variables while particle size and *in vitro* mucoadhesion of the microspheres were the dependent variables. Regression analysis was performed to identify the best formulation conditions. The microspheres were evaluated for characteristics like particle size, incorporation efficiency, swellability, zeta potential, *in vitro* mucoadhesion, thermal analysis, X-ray diffraction study and *in vitro* drug release. The shape and surface characteristics were determined by scanning electron microscopy which revealed spherical nature and nearly smooth surfaces of the microspheres. The drug encapsulation efficiency was found to be in the range of 69.62 ± 1.15 – 89.11 ± 0.95%. *In vitro* mucoadhesion was performed by adhesion number using sheep nasal mucosa and was observed in a range from 77.58 ± 1.49 – 93.15 ± 1.25%. Differential scanning calorimetry and X-ray diffraction results indicated a molecular level dispersion of drug in the microspheres. *In vitro* drug diffusion studies in phosphate buffer, pH 6.4 indicated non-Fickian or anomalous type of transport for the release of Almotriptan from the microspheres.



## INTRODUCTION

Migraine is a recurrent incapacitating neurovascular disorder characterized by attacks of debilitating pain associated with photophobia, phonophobia, nausea and vomiting [1]. Almotriptan Malate (ALM), a triptan derivative is a novel selective 5-hydroxytryptamine$_{1B/1D}$ receptor agonist indicated for the acute treatment of migraine with or without aura in adults [2]. During an attack, the blood vessels in the brain dilate and then draw together with stimulation of nerve endings near the affected blood vessels. These changes in the blood vasculature may be responsible for the pain. However, the exact cause of migraine, whether it is a vascular or a neurological dysfunction still remains unclear. Therapeutic approaches for management of migraine has a strong rationale however, it is still a poorly understood phenomenon [3].

ALM is generally given by oral route and available commercially as conventional immediate release solid oral dosage form. ALM is well absorbed after oral administration [4], with absolute bioavailability of about 70%. The optimal dose for ALM is a 12.5 mg at the start of a migraine headache, which may be repeated once in 2 h to a maximum of 25 mg/24 h. Low oral bioavailability, frequent administration due to lower plasma half-life of 3 - 4 h and associated symptoms such as nausea and vomiting makes oral treatment unsatisfactory and justifies a need of an alternate route for drug delivery [5, 6].

Amongst the different routes for drug administration, nasal delivery offers an

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interesting alternative for achieving systemic drug effects to the parenteral route, which can be inconvenient or oral administration, which can result in unacceptably low plasma drug levels. Conventionally, the nasal cavity is used for the treatment of local diseases, such as rhinitis and nasal congestion [7]. However, in the past few decades, nasal drug delivery has been paid much more attention as a promising drug administration route for the systemic therapy. The nasal cavity as a site for the systemic absorption of drugs has some advantages such as relatively large surface area, porous endothelial basement membrane, highly vascularized epithelial layer, enhanced blood flow, avoiding the first-pass metabolism and ready accessibility. However, the major limitation in respect to the nasal route of drug administration is the poor contact time of the formulation with the nasal mucosa [8, 9].

The nasal mucociliary clearance system transports the mucus layer that covers the nasal epithelium towards the nasopharynx by ciliary beating [10]. Its function is to protect the respiratory system from damage by inhaled substances. Normal mucociliary transit time in humans has been reported to be 12 to 15 min. The average rate of nasal clearance is about 8 mm/min, ranging from less than 1 to more than 20 mm/min. Nasal mucociliary clearance is one of the most important limiting factor for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. Several approaches are discussed in the literature to increase the residence time of drug formulations in the nasal cavity, resulting in improved nasal drug absorption [11].

Amongst the various approaches available to enhance the transnasal delivery of drugs, the mucoadhesive microsphere drug delivery system is an attractive concept that has the ability to control the rate of drug clearance from the nasal cavity as well as to protect the drug from enzymatic degradation [12]. The microspheres swell in contact with nasal mucosa and form a gel-like layer, which controls the rate of clearance from the nasal cavity. In the presence of microspheres, the nasal mucosa is dehydrated due to moisture uptake by the microspheres. This results in reversible shrinkage of the cells, providing a temporary physical separation of the tight (intercellular) junction, which increase the absorption of the drug. Hence, a formulation that would increase residence time in the nasal cavity and at the same time increased absorption of drug would be highly beneficial in all respects [13].

Sodium alginate is a water-soluble, natural, linear polysaccharide which is most widely used as a polymer matrix due to its non-toxicity, biocompatibility and gel formation ability [14]. It has been reported that polyanion polymers are more effective bioadhesives than polycation polymers or non-ionic polymers [15]. Alginate, with its carboxyl end groups, is classified as anionic mucoadhesive polymer and studies have shown that alginate has the highest mucoadhesive strength compared with polymers such as polystyrene, chitosan, carboxymethylcellulose and poly (lactic acid). Sodium alginate develops a simple and rapid gelation with divalent metal ions such as $Ca^{2+}$, therefore many researchers used it as the matrix to prepare microparticles. It can be cross-linked with divalent or polyvalent cations to form an insoluble meshwork. $Ca^{2+}$ and $Zn^{2+}$ have been reported for cross-linking of acid groups of alginate [16]. However, $Ca^{2+}$ is preferred as it selectively binds to the guluronic acid units to form an 'egg-box' model. Preparation of alginate microparticles is reported by emulsification cross-linking with calcium salts [17], polylysine [18] or chitosan [19] by ionotropic gelation [20].

Optimization using factorial designs is a powerful, efficient and systematic tool that shortens the time required for the development of pharmaceutical dosage forms and improves research and development work. Factorial designs, where all the factors are studied in all possible combinations, are considered to be the most efficient in estimating the influence of individual variables and their interactions using minimum experiments [21]. The application of factorial design in pharmaceutical formulation development has played a key role in understanding the relationship between the independent variables and the responses to them. The independent variables are controllable, whereas responses are dependent. The contour plot gives a visual representation of the values of the response. This helps the process of optimization by providing an empirical model equation for the response as a function of the different variables [22, 23].

The current investigation was aimed at improving the therapeutic efficacy of ALM by preparing Sodium alginate (SA) microspheres for nasal administration. The microspheres were

prepared by utilizing a $2^3$ factorial design. The effect of some factors, such as drug: polymer ratio, concentration of cross-linking agent and cross-linking time on particle size and *in vitro* mucoadhesion was investigated.

## MATERIALS AND METHODS

Almotriptan Malate was obtained as gift sample from Apotex Research Private Limited, Bangalore. Sodium Alginate was purchased from Finar Chemicals Private Limited, Ahmedabad. n-octanol, calcium chloride and Span 80 were procured from S.D. Fine chemicals, Mumbai, India. All other reagents used were of analytical grade commercially available from Merck Pvt. Ltd., Mumbai, India.

### Preparation of sodium alginate microspheres

The formula for the various batches of microspheres is shown in Table 1. ALM loaded sodium alginate microspheres were prepared by water in oil emulsification method followed by cross-linking with calcium chloride [24]. ALM was dispersed in an aqueous solution containing 3%, w/v sodium alginate. The solution was dispersed in n-octanol containing 2% v/v Span 80 using a mechanical stirrer (Remi stirrer, Mumbai, India) at 1800 rpm. The ratio of the aqueous to n-octanol phase used was 1:20. The resultant w/o emulsion was stirred for 30 min. Calcium chloride solution was added drop-wise and the dispersion was stirred for another 5 min. The microspheres were collected by vacuum filtration, washed three times with isopropyl alcohol and dried in air at room temperature. Various variables like drug: polymer ratio, concentration of cross-linking agent and time of cross-linking were considered for optimization of the formulation.

### Experimental design

Various batches of alginate microspheres were prepared based on the $2^3$ factorial designs. The independent variables were drug to polymer ratio($X_1$), calcium chloride concentration ($X_2$) and cross-linking time ($X_3$). The independent variables and their levels are shown in Table 2. Particle size of the microspheres ($Y_1$) and in vitro mucoadhesion ($Y_2$) were taken as response parameters as the dependent variables. Table 1 shows the independent and dependent variables.

### Characterization of ALM loaded alginate microspheres

#### Percentage Yield:

The practical percentage yield was calculated from the weight of dried microspheres recovered from each batch in relation to the sum of the initial weight of starting materials. The percentage yield [25] was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{Practical mass (Microspheres)}}{\text{Theoretical mass (Polymer + Drug)}} \text{ x } 100$$

#### Shape and Surface Morphology:

The shape and surface characteristics of the microspheres [26] were evaluated by means of scanning electron microscopy (JEOL – JSM - 840A, Japan). The samples were prepared by gently sprinkling the microspheres on a double adhesive tape, which is stuck to an aluminium stub. The stubs were then coated with gold using a sputter coater (JEOL Fine coat JFC 1100E, ion sputtering device) under high vacuum and high voltage to achieve a film thickness of 30 nm. The samples were then imaged using a 20 KV electron beam.

#### Drug Encapsulation Efficiency:

Microspheres equivalent to 10 mg of ALM were crushed in a glass mortar and pestle and the powdered microspheres were suspended in 25 mL of phosphate buffer pH 6.4. After 24 h, the solution was filtered, 1 mL of the filtrate was pipetted out and diluted to 10 ml and analyzed for the drug content using Elico SL- 159 UV Visible spectrophotometer at 228 nm [27]. It was confirmed from preliminary UV studies that the presence of dissolved polymers did not interfere with the absorbance of the drug at 228 nm. The drug encapsulation efficiency [28] was calculated using the following formula:

$$\% \text{ Drug encapsulation efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

#### Particle Size Measurement:

Particle size of the microspheres [29] was determined by optical microscopy using an optical microscope Olympus BH2-UMA (Olympus, NWF 10x, India). The eye piece micrometer was calibrated with the help of a stage micrometer. The particle diameters of more than 300 microspheres were measured randomly. The average particle size [30] was determined by using Edmondson's equation.

$$D_{mean} = \frac{\sum nd}{\sum n}$$

Where, n = Number of microspheres checked; d = Mean size range

**Table 1:** Formulation of the microspheres employing a $2^3$ factorial design

| Formulation code | X1 | X2 | X3 | Y1* | Y2* |
|---|---|---|---|---|---|
| **ASM1** | 0.5:1 | 2 | 5 | 27.32 ± 1.22 | 93.15 ± 1.25 |
| **ASM2** | 1:1 | 2 | 5 | 45.65 ± 1.66 | 85.21 ± 1.32 |
| **ASM3** | 0.5:1 | 4 | 5 | 29.33 ± 1.36 | 88.24 ± 1.18 |
| **ASM4** | 1:1 | 4 | 5 | 51.64 ± 1.12 | 81.53 ± 1.62 |
| **ASM5** | 0.5:1 | 2 | 10 | 31.78 ± 1.27 | 87.65 ± 1.14 |
| **ASM6** | 1:1 | 2 | 10 | 55.67 ± 2.03 | 80.82 ± 1.01 |
| **ASM7** | 0.5:1 | 4 | 10 | 34.33 ± 2.51 | 82.91 ± 1.19 |
| **ASM8** | 1:1 | 4 | 10 | 53.48 ± 1.05 | 77.58 ± 1.49 |

* Values are expressed as mean ± SD. Y1 and Y2 are particle size and *in vitro* mucoadhesion, respectively.

**Table 2:** Factorial design parameters and experimental conditions

| Factors | Levels used, Actual (coded) | |
|---|---|---|
| | **Low (-1)** | **High (+1)** |
| **X1 = Drug to polymer weight ratio** | 0.5:1 | 1:1 |
| **X2 = Concentration of $CaCl_2$ (%)** | 2 | 4 |
| **X3 = Cross-linking time (min)** | 5 | 10 |

### Zeta potential study

Microspheres ASM1 to ASM8 were subjected to zeta potential measurements [31] using zeta sizer (Nano ZS, Malvern Instruments, UK). The microparticles were dispersed in distilled water and placed into the electrophoretic cells of the instrument and a potential of 100mV was applied. Zeta potential was determined for 25 distinct particles.

### *In Vitro* Mucoadhesion Studies

The *in vitro* mucoadhesion study of microspheres was assessed using Falling liquid film technique [32]. A strip of sheep nasal mucosa was mounted on a glass slide and 50 mg of accurately weighed microspheres were sprinkled on the nasal mucosa. This glass slide was incubated for 15 min in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed on the stand at an angle of $45^0$. Phosphate buffered saline of pH 6.4; previously warmed to 37 ± $0.5^0$C was allowed to flow over the microspheres and membrane at the rate of 1 mL/min for 5 min with the help of a peristaltic pump [33]. At the end of this process, the detached particles were collected and weighed. The % mucoadhesion was determined by using following equation.

$$\% \text{ Mucoadhesion} = \frac{\text{Weight of sample} - \text{weight of detached particles}}{\text{Weight of sample}} \text{ X } 100$$

### Degree of Swelling:

The Swellability [34] of microspheres in physiological media was determined by allowing the microspheres to swell in the phosphate buffer saline pH 6.4. 100 mg of accurately weighed microspheres were immersed in little excess of phosphate buffer saline of pH 6.4 for 24 h and washed thoroughly with deionised water. The degree of swelling was arrived at using the following formula:

$$\propto = \frac{W_S - W_O}{W_O}$$

Where, α is the degree of swelling; Wo is the weight of microspheres before swelling and Ws is the weight of microspheres after swelling

### Thermal analysis

Differential scanning calorimetry (DSC) was performed on ALM, blank microspheres and ALM loaded microspheres. DSC measurements [35] were performed on a differential scanning calorimeter (DSC 823, Mettler Toledo, Switzerland). The thermograms were obtained at a scanning rate of 10°C/min over a temperature range of 25 - $250^0$C under an inert atmosphere flushed with nitrogen at a rate of 20 mL/min.

### X-Ray diffraction (XRD) studies

The qualitative X-ray diffraction studies [36] were performed using an X-ray diffractometer (PAnalytical, X Pert Pro). ALM, blank microspheres and ALM loaded microspheres were scanned from 0-40° diffraction angle (2θ) range under the following measurement conditions: source, nickel filtered Cu-Kα

radiation; voltage 40 Kv; current 30mA; scan speed 0.05/min. Microspheres were triturated to get fine powder before taking the scan. X-ray diffractometry was carried out to investigate the effect of microencapsulation process on crystallinity of the drug.

### *In Vitro* Drug Diffusion Studies

***Preparation of nasal mucosa:*** Fresh sheep nasal mucosa was collected from a nearby slaughter house. The nasal mucosa of sheep was separated from sub layer bony tissues and stored in distilled water containing few drops of Gentamycin injection. After complete removal of blood from mucosal surface, it was attached to the donor chamber tube [37].

*In vitro* nasal diffusion study was done using nasal diffusion cell, having three openings each for sampling, thermometer and donor tube chamber. The receptor compartment has a capacity of 60 mL in which Phosphate buffer, pH 6.4 was taken [38]. Within 80 min of removal, the nasal mucosa measuring an area of 3 $cm^2$ was carefully cut with a scalpel and tied to the donor tube chamber and it was placed establishing contact with the diffusion medium in the recipient chamber. Microspheres equivalent to 10 mg of ALM were spread on the sheep nasal mucosa. At hourly intervals, 1 mL of the diffusion sample was withdrawn with the help of a hypodermic syringe, diluted to 10 mL and absorbance was read at 228 nm. Each time, the sample withdrawn was replaced with 1 mL of pre-warmed buffer solution (pH 6.4) to maintain a constant volume of the receptor compartment vehicle.

### Optimization data analysis and model-validation

ANOVA was used to establish the statistical validation of the polynomial equations generated by Design Expert® software (version 9.0, Stat-Ease Inc, Minneapolis, MN). Fitting a multiple linear regression model to a $2^3$ factorial design gave a predictor equation which was a first-order polynomial, having the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

where Y is the measured response associated with each factor level combination; $b_0$ is an intercept representing the arithmetic average of all quantitative outcomes of eight runs; $b_1$ to $b_{123}$ are regression coefficients computed from the observed experimental values of Y and $X_1$, $X_2$ and $X_3$ are the coded levels of independent variables. The terms $X_1X_2$, $X_2X_3$ and $X_1X_3$ represent the interaction terms. The main effects ($X_1$, $X_2$ and $X_3$) represent the average result of changing one factor at a time from its low to high value. The interaction terms show how the response changes when two factors are changed simultaneously. The polynomial equation was used to draw conclusions after considering the magnitude of coefficients and the mathematical sign it carries, i.e. positive or negative. A positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonistic effect [22].

In the model analysis, the responses: the particle size of the microspheres and *in vitro* mucoadhesion of all model formulations were treated by Design Expert® software. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient ($R^2$), adjusted multiple correlation coefficient (adjusted $R^2$) and the predicted residual sum of square (PRESS), provided by Design Expert® software. Among them, PRESS indicates how well the model fits the data and for the chosen model it should be small relative to the other models under consideration. Level of significance was considered at $p < 0.05$. Three dimensional response surface plots and two dimensional contour plots resulting from equations were obtained by the Design Expert® software. Subsequently, the desirability approach was used to generate the optimum settings for the formulations [39, 40].

$$Linear\ model: Y = b_1X_1 + b_2X_2 + b_3X_3$$

$$2FI(interaction)model: Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

## RESULTS AND DISCUSSION

ALM loaded microspheres of Sodium alginate were successfully fabricated by water in oil emulsification cross-linking method employing calcium chloride as cross linking agent. During the process of microsphere preparation, the drug may partition out into the aqueous phase due to its hydrophilic nature, hence in the present investigation n-Octanol was used as the harvesting medium. In this condition, ALM would find it non-favourable to diffuse out of the microspheres before they harden thus resulting in sufficiently high encapsulation efficiency.

**Table 3:** Characteristics of the prepared ALM-loaded alginate microspheres

| Formulation code | % Yield | % encapsulation efficiency* | Degree of swelling* |
|---|---|---|---|
| **ASM1** | 85.62 | 89.11 ± 0.95 | 1.104 ± 0.151 |
| **ASM2** | 89.24 | 74.19 ± 1.21 | 1.098 ± 0.069 |
| **ASM3** | 76.55 | 84.59 ± 1.62 | 0.843 ± 0.210 |
| **ASM4** | 84.98 | 71.23 ± 1.94 | 0.895 ± 0.168 |
| **ASM5** | 88.95 | 85.29 ± 2.16 | 0.914 ± 0.054 |
| **ASM6** | 92.84 | 72.44 ± 1.47 | 0.902 ± 0.176 |
| **ASM7** | 89.62 | 83.39 ± 0.62 | 0.780 ± 0.235 |
| **ASM8** | 94.73 | 69.62 ± 1.15 | 0.766 ± 0.319 |

* Values are expressed as mean ± SD.

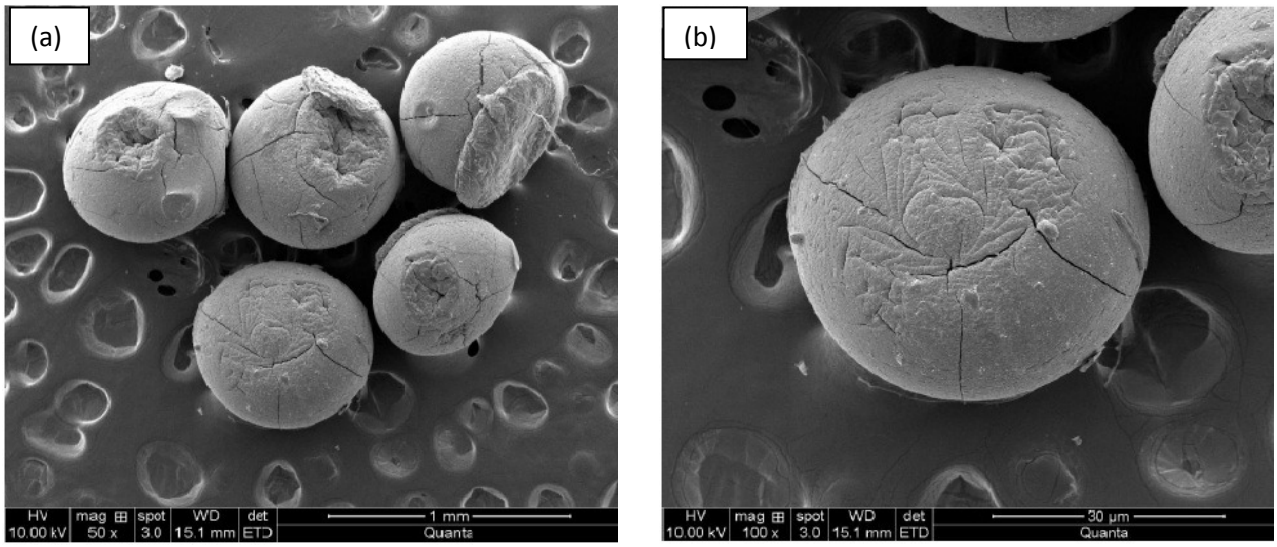


**Figure 1:** SEM Photograph of ALM Loaded alginate microspheres at low (a) and high (b) magnification

It was observed that as the drug to polymer ratio increased from 0.5:1 to 1:1, the product yield also increased. The low percentage yield in some of the formulations may be due to loss of microspheres during the washing process. The percentage yield was found to be in the range of 76.55 to 94.73%. The photographs of the optimized formulation (ASM1) taken by scanning electron microscope are depicted in the Figure 1. The SEM photographs revealed that the microspheres were discrete and spherical in shape with nearly smooth surface morphology. These microspheres had no pores on the surface; such morphology would result in slow clearance and good deposition pattern in nasal cavity.

As the drug to polymer ratio was varied from 0.5:1 to 1:1, it was observed that the particle size increased, whereas, encapsulation efficiency decreased. The drug encapsulation efficiency was found to be in the range between 69.62 ± 1.15% - 89.11 ± 0.95% and revealed its dependency on drug loading, amount of cross-linking agent and time of cross-linking. The formulations loaded with higher amount of drug (ASM2, ASM4, ASM6, ASM8) exhibited decrease encapsulation efficiencies which could be related to the increased extent of drug diffusion to the external phase due to greater flux at higher drug content during the emulsification and microsphere formation process. The decrease in encapsulation efficiency with increase in concentration of calcium chloride and cross-linking time could be attributed either to an increase in cross-link density, which will reduce the free volume spaces within the polymer matrix or incomplete emulsification as a result of higher viscosity of the internal phase. The % yield and drug encapsulation efficiency of the prepared microspheres is compiled in Table 3.

The prepared microspheres were in the mean particle size range of 27.32 ± 1.22 µm to 55.67 ± 2.03 µm, ideal for intranasal absorption. Preliminary studies showed that as the concentration of polymer was increased, the particle size also proportionally increased. Lower sodium alginate concentrations (1% w/v and 2% w/v) resulted in clumping of

microspheres, whereas high sodium alginate concentration (4% w/v) resulted in formation of discrete microspheres with a mean particle size greater than 80 µm which could be attributed to an increase in the relative viscosity at higher concentration of polymer and formation of larger particles during emulsification. Hence an optimum sodium alginate concentration of 3% w/v was selected for preparing the different batches of the microspheres. The mean particle size of the microspheres increased with an increase in drug loading. This can be attributed to the corresponding increase in viscosity of drug–polymer dispersion comprising the internal phase of the emulsion. The increase in viscosity within the internal phase results in the generation of a coarser emulsion with larger droplets, leading eventually to the formation of larger microspheres. A similar increase in the size of microspheres was also observed with increase in calcium chloride concentration as well as cross-linking time. The addition of higher amount of $Ca^{2+}$ will result in relatively more crosslinking of the guluronic acid units of sodium alginate, thereby leading to formation of larger microspheres. Similarly, increasing the cross-linking time will increase the extent of cross-linking and thereby increase the particle size. The mean particle size ($Y_1$) of the prepared microspheres is presented in Table 1.

Zeta potential analysis was performed to get the information about the surface properties of the microspheres. All microspheres prepared were negatively charged, indicating the presence of SA at the surface of all microspheres formed. Studies have cited that polymers with charged density can serve as good mucoadhesive agents. It has also been reported that anion polymers are more effective bioadhesive than polycations or non-ionic polymers. Zeta potential distribution curve of the optimum formulation (ASM1) is depicted in Fig. 2.

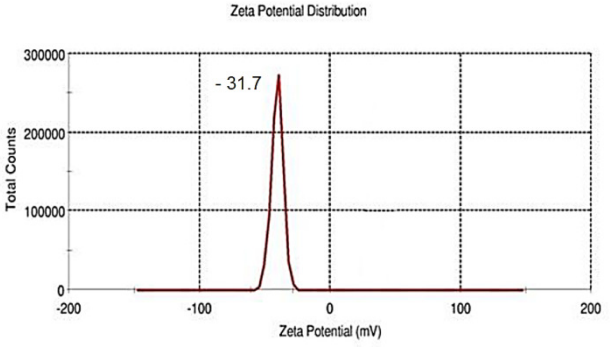


**Figure 2:** Zeta potential distribution curve of ALM-Loaded alginate microspheres (ASM1)

The results of *in vitro* mucoadhesion test ($Y_2$) are displayed in Table 1. The prepared microspheres had satisfactory mucoadhesive properties ranging from 77.58 ± 1.49% to 93.15 ± 1.25% and could adequately adhere on nasal mucosa. The results also showed that, with increasing polymer ratio, higher mucoadhesion percentages were obtained. This could be attributed to the availability of a higher amount of polymer for interaction with mucus. Increase in calcium chloride concentration and cross-linking time decreased the mucoadhesive property of the microspheres. Most of the studies showed that the pre-requisite for a good mucoadhesion is the high flexibility of polymer backbone structure and its polar functional groups. Such a flexibility of the polymer chains, however, is reduced if the polymer molecules are cross-linked either with each other or with coagulation agents like calcium ions. Although the cross-linked microspheres will absorb water, they are insoluble and will not form a liquid gel on the nasal epithelium but rather a more solid gel-like structure. This decrease in flexibility imposed upon polymer chains by the cross-linking makes it more difficult for cross-linked polymers to penetrate the mucin network [41]. Thus, cross-linking effectively limits the length of polymer chains that can penetrate the mucus layer and could possibly decrease the mucoadhesion strength of the microspheres. The formulation, ASM1, with highest mucoadhesion (93.15 ± 1.25%) was considered to be the best formulation.

Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Swellability data revealed that the amount of polymer plays an important role in solvent transfer. It can be concluded from the data shown in Table 3 that, with an increase in calcium chloride concentration and cross-linking time, the degree of swelling decreased in the range from 1.104 ± 0.151 to 0.766 ± 0.319. This tendency could be attributed to greater crosslinking degree of the polymer resulting in rigid microspheres which lowers the solvent transfer rate, reduced swelling and thus reduced mucoadhesiveness.

In an effort to assess the physical state of the drug in the SA microspheres, we attempted to analyze ALM, blank microspheres and drug-loaded microspheres (ASM1) using DSC. The results are displayed in Figure 3. The DSC thermogram showed a sharp endothermic peak at 169.9°C due to the melting of the ALM but, in

the case of ALM loaded microspheres, no characteristic peak was observed at 169.9°C, suggesting that ALM is molecularly dispersed in the matrix.

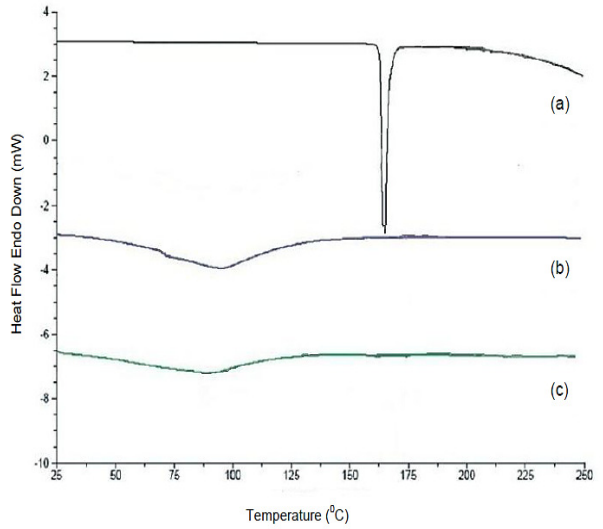


**Figure 3:** DSC thermograms of (a) pure ALM (b) blank microspheres and (c) drug -loaded microspheres

XRD studies are useful to investigate the crystallinity of drug in the polymeric microspheres. The X-ray diffractogram recorded for pure ALM, blank microspheres and drug-loaded microspheres (ASM1) are presented in Figure 4. ALM peaks observed at 2θ of $16^0$, $17^0$ and $22^0$ are due to crystalline nature of ALM. But in case of blank microspheres and drug-loaded microsphere no intense peaks were observed between 2θ of $16^0$, $17^0$ and $22^0$. This indicates that drug particles are dispersed at molecular level in the polymer matrices since no indication about the crystalline nature of the drug was observed in the drug loaded microspheres.

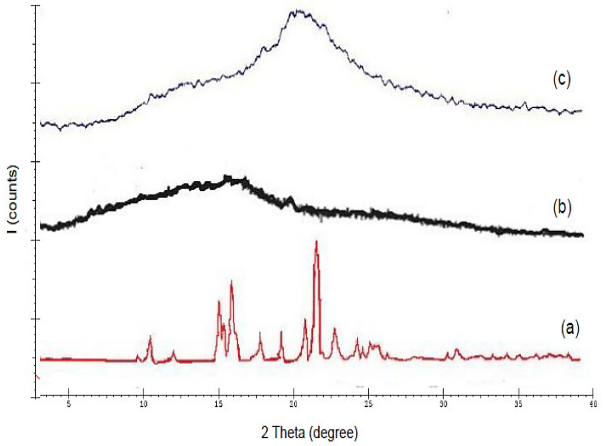


**Figure 4:** Powder X-ray diffractograms of (a) pure ALM (b) blank microspheres and (c) drug -loaded microspheres

In order to understand the mechanism and kinetics of drug release, the data was analysed by Peppas equation:

$$Mt/M^{\infty} = kt^n$$

Where M*t* is the amount of drug released at time t, $M^{\infty}$ is the amount released at time$^{\infty}$, $Mt/M^{\infty}$ is the fraction of drug released at time t, *k* is a constant characteristic of the drug-polymer system and n is the diffusional exponent, a measure of the primary mechanism of drug release. Using the least squares procedure, the values of n, k and correlation coefficient (r) were estimated and presented in the Table 4. In spherical matrices, if n≤0.43, a Fickian diffusion (case-I), 0.43≤n≤0.85, anomalous or non-Fickian transport and n≥0.85, a case-II transport (zero order) drug release mechanism dominates. The values of n for all the batches ranged from 0.610 – 0.742, with correlation coefficient close to 0.9951, indicating a non-Fickian or anomalous type of transport. The drug release behaviour of optimized batch is shown in Figure 5, which indicates initial burst release followed by near zero order release.

**Table 4:** Release kinetics parameters of ALM loaded-alginate microspheres

| Formulation Code | n | k | Correlation coefficient, r |
|---|---|---|---|
| **ASM1** | 0.610 | 0.246 | 0.9895 |
| **ASM2** | 0.642 | 0.239 | 0.9811 |
| **ASM3** | 0.668 | 0.230 | 0.9854 |
| **ASM4** | 0.613 | 0.221 | 0.9516 |
| **ASM5** | 0.624 | 0.236 | 0.9789 |
| **ASM6** | 0.701 | 0.202 | 0.9951 |
| **ASM7** | 0.698 | 0.211 | 0.9782 |
| **ASM8** | 0.742 | 0.189 | 0.9823 |

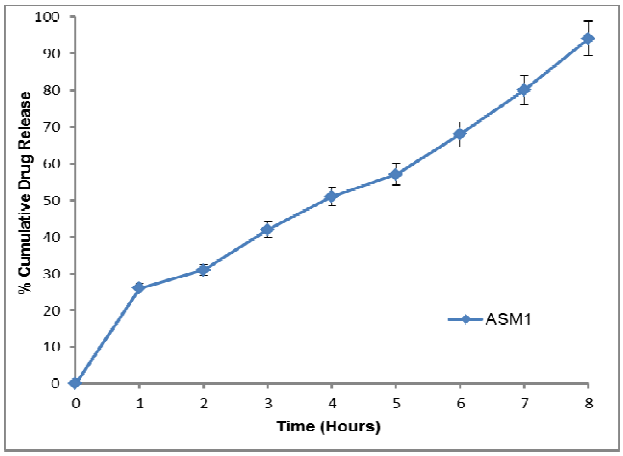


**Figure 5:** *In vitro* drug diffusion profile of optimized batch (ASM1) formulation

**Table 5:** Summary of results of regression analysis for responses Y1 and Y2

| Models | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | SD | % CV | *P* |
|---|---|---|---|---|---|---|
| **Response $Y_1$, Linear model** | 0.9816 | 0.9679 | 0.9266 | 2.10 | 5.10 | 0.0006 |
| **Response $Y_2$, Interactive model** | 0.9897 | 0.9821 | 0.9590 | 0.66 | 0.78 | 0.0002 |

SD: Standard Deviation, CV: Coefficient of Variation; *P:* Probability value
Regression equations of the fitted linear and interactive model:
$Y_1 = 41.15 + 10.46X_1 + 1.04\,X_2 + \ 2.66X_3$
$Y_2 = 84.64 - 3.35X_1 - 2.07X_2 - 2.40X_3 + \ 0.34X_1X_2 + \ 0.31X_1X_3 + \ 0.076X_2X_3$

**Table 6:** Results of analysis of variance for measured responses

| Parameters | DF | SS | MS | F | Significance *F* |
|---|---|---|---|---|---|
| ***Particle Size*** | | | | | 0.0006 |
| **Model** | 3 | 940.85 | 313.62 | 71.30 | Significant |
| **Residual** | 4 | 17.59 | 4.40 | - | |
| **Total** | 7 | 958.44 | - | - | |
| ***In Vitro* Mucoadhesion** | | | | | 0.0002 |
| **Model** | 3 | 170.10 | 56.70 | 128.70 | Significant |
| **Residual** | 4 | 1.76 | 0.44 | - | |
| **Total** | 7 | 171.87 | - | - | |

DF: Degrees of Freedom, SS: Sum of Square, MS: Mean Sum of Square, F: Fischer's ratio

## Optimization data analysis and model-validation

### Fitting of data to the model:

The three factors with lower and upper design points in coded and uncoded values are shown in Table 2. The ranges of responses $Y_1$ and $Y_2$were 27.32 ± 1.22 to 55.67 ± 2.03 µm and 77.58 ± 1.49% to 93.15 ± 1.25%, respectively. All the responses observed for eight formulations prepared, were fitted to various models using Design- Expert® software. It was observed that the best-fitted models were linear and interactive. The values of $R^2$, adjusted $R^2$, predicted $R^2$, SD and %CV are given in Table 5, along with the regression equation generated for each response. The results of ANOVA in Table 6 for the dependent variables demonstrate that the model was significant for both the response variables.

It was observed that all the three independent variables viz $X_1$ (drug:polymerratio), $X_2$ (concentration of $CaCl_2$) and $X_3$ (cross-linking time) had a positive effect on particle size ($Y_1$), but, a negative effect on *in vitro* mucoadhesion ($Y_2$). The coefficients with more than one factor term in the regression equation represent interaction terms. When more than one factor is changed simultaneously and used at different levels in a formulation, a factor can produce different degrees of response. The interaction effects of $X_1$ and $X_2$; $X_1$ and $X_3$; $X_2$ and $X_3$ were favourable (positive), for response $Y_2$.

### Contour plots and response surface analysis:

Three dimensional response surface plots and two dimensional contour plots generated by the Design Expert® software are presented in Figures 6 and 7 for the studied responses, i.e. particle size and *in vitro* mucoadhesion respectively. Figures 6(a) depicts response surface and contour plots of the effects of drug: polymer ratio ($X_1$) and $CaCl_2$concentration ($X_2$) on particle size, which indicate a linear effect on particle size of the microspheres. The combined effects of $CaCl_2$ concentration ($X_2$) and cross-linking time ($X_3$) and drug: polymer ratio ($X_1$) and cross-linking time ($X_3$) on particle size, as shown in Figure 6(b) and 6(c) are also linear. This explains that the higher the amount of $CaCl_2$ or higher the time of cross-linking, the more will be the cross-linking of the guluronic acid units of sodium alginate leading to formation of larger microspheres.

The combined effect of $X_1$ and $X_2$ on *in vitro* mucoadhesion of the microspheres was observed to be non-linear, as in Figures 7(a). At low value of drug: polymer ratio and $CaCl_2$ concentration, a higher value for *in vitro* mucoadhesion was observed. Similar effects were observed for factors $X_2$, $X_3$ and $X_1$, $X_3$, as shown in Figures 7(b) and 7(c) respectively. As the $CaCl_2$ concentration and cross-linking time increased from low to high, the value for *in vitro*mucoadhesion of the microspheres was decreased.

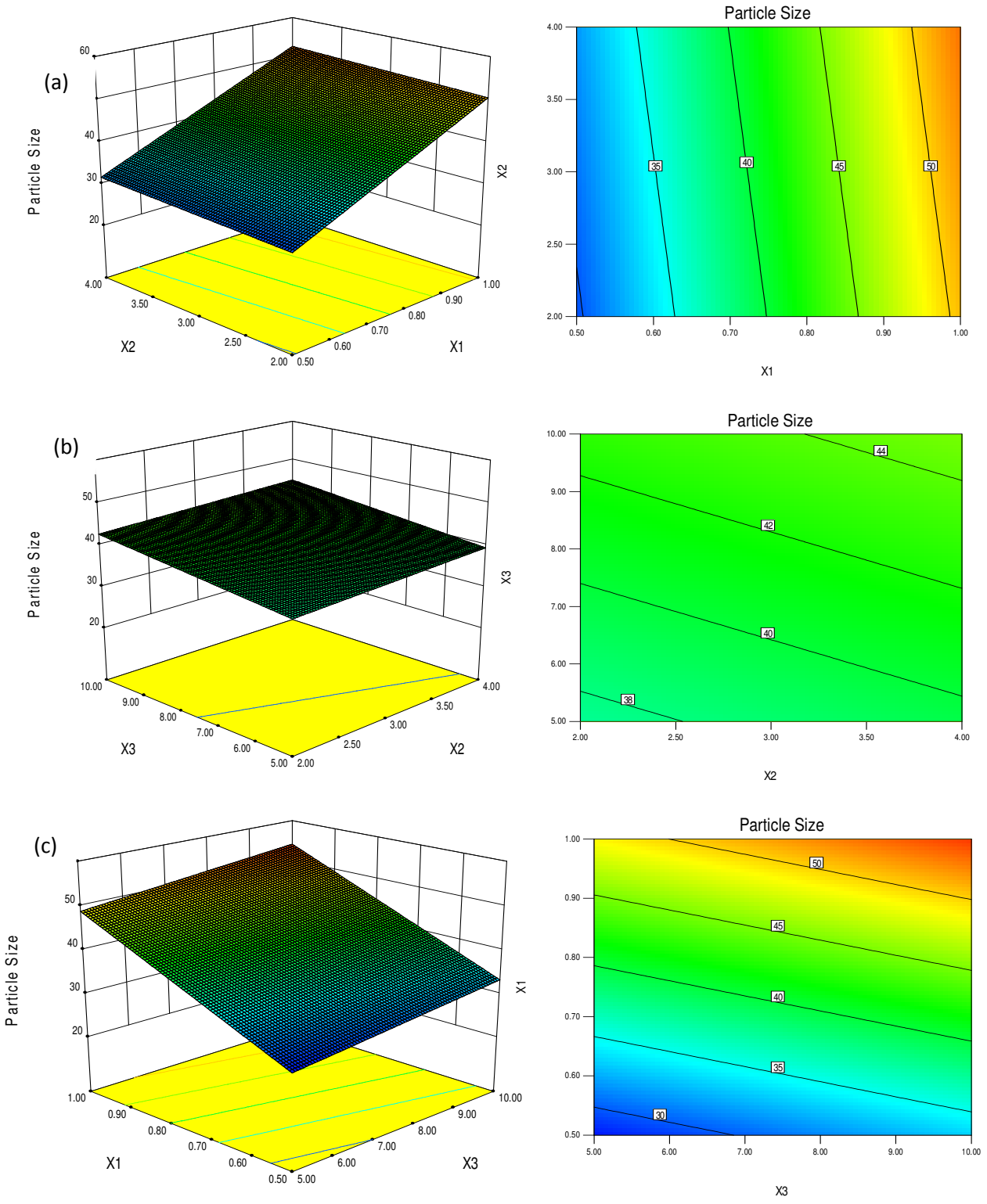


**Figure 6:** Response surface and contour plots for the (a) effect of drug:polymer ratio ($X_1$) and $CaCl_2$ concentration($X_2$), (b) effects of $CaCl_2$ concentration($X_2$) and cross-linking time ($X_3$) and (c) effect of drug:polymer ratio ($X_1$) and cross-linking time ($X_3$) on particle size

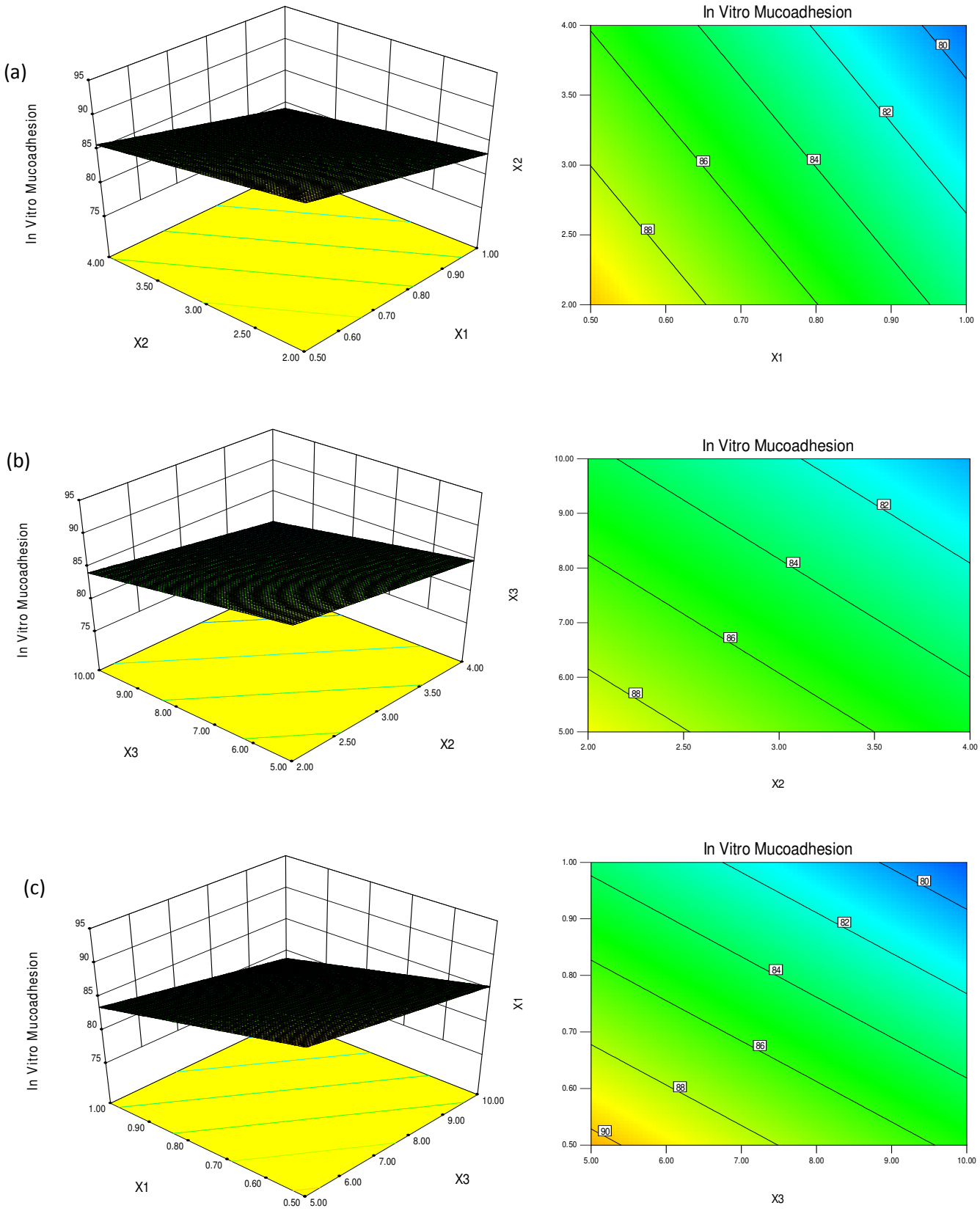


**Figure 7:** Response surface and contour plots for the (a) effect of drug:polymer ratio ($X_1$) and $CaCl_2$ concentration($X_2$), (b) effects of $CaCl_2$ concentration($X_2$) and cross-linking time ($X_3$) and (c) effect of drug:polymer ratio ($X_1$) and cross-linking time ($X_3$) on *In vitro* mucoadhesion

**Table 7:** The predicted and observed response variables of the microspheres

| Responses | Formulation | Predicted Value | Actual Value | Prediction error * (%) |
|---|---|---|---|---|
| **$Y_1$** | ASM1 | 26.98 | 27.32 | 1.260 |
| | ASM2 | 47.90 | 45.65 | -4.697 |
| | ASM3 | 29.07 | 29.33 | 0.894 |
| | ASM4 | 49.99 | 51.64 | 3.301 |
| | ASM5 | 32.31 | 31.78 | -1.640 |
| | ASM6 | 53.23 | 55.67 | 4.584 |
| | ASM7 | 34.40 | 34.33 | -0.203 |
| | ASM8 | 55.32 | 53.48 | -3.326 |
| **$Y_2$** | ASM1 | 92.46 | 93.15 | 0.746 |
| | ASM2 | 85.75 | 85.21 | -0.630 |
| | ASM3 | 88.31 | 88.24 | -0.079 |
| | ASM4 | 81.61 | 81.53 | -0.098 |
| | ASM5 | 87.66 | 87.65 | -0.011 |
| | ASM6 | 80.96 | 80.82 | -0.173 |
| | ASM7 | 83.52 | 82.91 | -0.730 |
| | ASM8 | 76.82 | 77.58 | 0.989 |

*Prediction error (%) = (Actual value – Predicted Value) / Predicted Value x 100
$Y_1$ and $Y_2$ are Particle size and *in vitro* mucoadhesion respectively

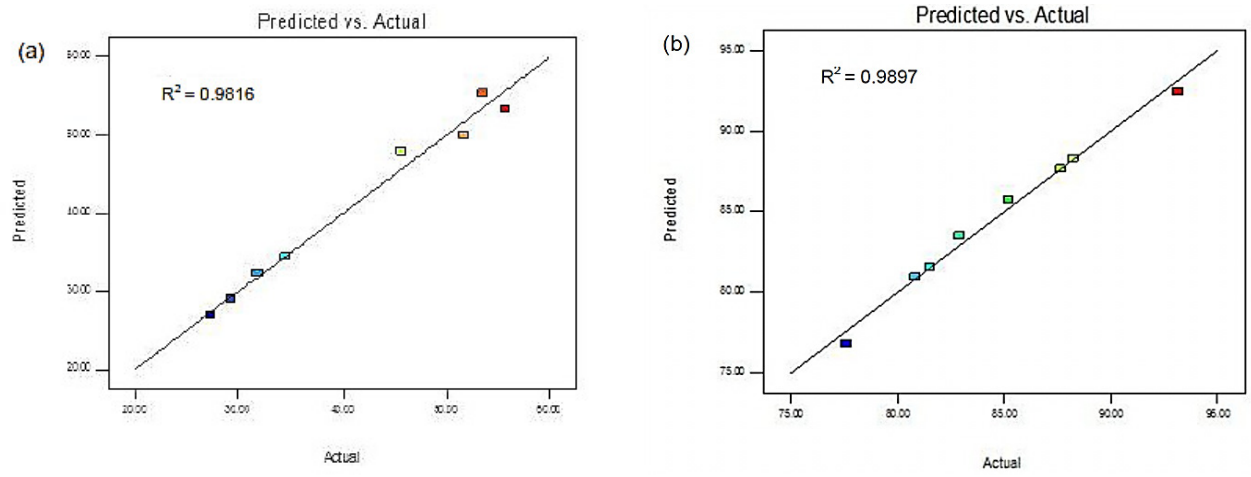


**Figure 8:** Correlation between actual and predicted values for (a) particle size and (b) *in vitro* mucoadhesion

**Optimization and validation:**

A numerical optimization technique by the desirability approach was used to generate the optimum settings for the formulation. The process was optimized for the dependent (response) variables $Y_1$ and $Y_2$. The optimum formulation was selected based on the criteria of attaining the minimum value of particle size and maximum value of *in vitro* mucoadhesion. Formulation ASM1 having drug: polymer ratio (0.5:1), $CaCl_2$ concentration (2%) and cross-linking time (5 min) fulfilled all the criteria set from desirability search. To gainsay the reliability of the response surface model, a new optimized formulation (as per formula ASM1) was prepared according to the predicted model and evaluated for the responses. The result in Table 7 illustrates the comparison between the observed and predicted values of both the responses $Y_1$ and $Y_2$ for all the formulations presented. It can be seen that in all cases there was a reasonable agreement between the predicted and the experimental values, as prediction error was found to vary between -0.011% and +4.584%. For this reason it can be concluded that the equations describe adequately the influence of the selected independent variables on the responses under study. This indicates that the optimization technique was appropriate for optimizing the

alginate microsphere formulation. The linear correlation plots drawn between the predicted and experimental values for all the batches of the microspheres are shown in Figure 8, which demonstrated high values of $R^2$ (0.9816 and 0.9897). Thus, the low magnitudes of error as well as the significant values of $R^2$ in the present investigation prove the high prognostic ability of the optimization technique by factorial design.

## CONCLUSION

In the present investigation, Almotriptan malate loaded sodium alginate microspheres were prepared by water-in-oil (w/o) emulsification cross-linking technique. Various process variables such as the drug: polymer ratio, calcium chloride concentration and the cross-linking time were optimized by the factorial design. A $2^3$ experimental design was employed to identify optimal formulation parameters for a microsphere preparation with the minimum value of particle size and maximum value of *in vitro* mucoadhesion. From the mathematical models generated, an optimal formulation comprising of drug: polymer ratio (0.5 : 1), $CaCl_2$ concentration (2%) and cross-linking time (5 min) was identified to provide desired values for particle size (27.32 ± 1.22 µm) and *in vitro* mucoadhesion (93.15 ± 1.25%). SEM confirmed the spherical nature and nearly smooth surfaces of the microspheres. Particle size was in the range of 27.32 – 55.67 µm, which is considered to be favourable for intranasal absorption. All batches showed good *in vitro* mucoadhesion (77.58 – 93.15%). Results of DSC and XRPD study indicated drug–polymer compatibility and a molecular level dispersion of Almotriptan in the microspheres. *In vitro* release studies indicated non-Fickian or anomalous type of transport for the release of Almotriptan from the microspheres. Hence, the results of the present study clearly indicated promising potentials of sodium alginate microspheres for delivering Almotriptan intranasally and could be viewed as a potential alternative to conventional dosage forms.

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