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# DESIGN, DEVELOPMENT, AND CHARACTERIZATION OF *OCIMUM BASILICUM* MUCILAGE-BASED, MODIFIED RELEASE MUCOADHESIVE GASTROSPHERES OF CARVEDILOL

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**ABSTRACT**

**Objective:** The present investigations aim to develop mucoadhesive gastrospheres of carvedilol using sodium alginate and *Ocimum basilicum* seed mucilage combination blend oral use.

**Methods:** The gastrospheres were prepared by ionotropic gelation method using $3^2$ factorial designs, the concentration of sodium alginate and *O. basilicum* mucilage was independent variables while % drug content (DC), % entrapment efficiency, and % drug release at 12 h were dependent variables. The gastrospheres were evaluated for other parameters such as Fourier-transform infrared (FTIR), powder X-ray diffraction, differential scanning calorimetric, and *in vitro* mucoadhesion studies.

**Results:** In optimization studies from statistical second-order complete model equation among the polymers used *O. basilicum* mucilage had a more profound effect on DC and % encapsulation efficiency as compared to sodium alginate. The mean particle size of gastrospheres when measured by optical microscopy technique ranged from 774 to 882 µm. The percentage of gastrosphere adhering to goat intestinal mucosal tissue varied from 10% to 65% over 8 h in 0.1N HCl, whereas this was varied from 40% to 60% in phosphate buffer pH 6.8 with provided 12 h of controlled release following zero-order release pattern.

**Conclusion:** Studies conclude that mucilage of *O. basilicum* can be used as controlled release mucoadhesive material in the formulation of gastrospheres.

**Keywords:** Gastrospheres, Mucoadhesion, *Ocimum basilicum.*

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## INTRODUCTION

Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have an enormous impact on the health-care system. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microsphere and nanoparticles, which modulates the release and absorption characteristics of the drug. Microsphere/microparticles constitute an important part of this particulate DDS by virtue of their small size and efficient carrier characteristics. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity, improved patient compliance, and convenience [1,2].

A controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimum amount in the right period of time thereby causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microparticles as carriers for drugs. Microparticles can be described as small particles (in 1–1000 µm size range) for use as carriers of drugs and other therapeutic agents consisting of proteins or synthetic polymers which are biodegradable in nature [3,4].

Gastrospheres are the particulate DDSs which achieve the target of gastric retention by mainly two mechanisms, i.e., buoyancy and bio-adhesion. Due to their small size and efficient carrier characteristics, gastrospheres constitute an important part through the particulate novel DDS. The limitation of gastrospheres is due to their short residence time on the site of absorption, and it can be overcome by providing an intimate contact of the drug-delivery system with the absorbing membrane. This can be accomplished by coupling bio-adhesion characteristics to gastrospheres and developing mucoadhesive gastrospheres [5,6]. Extending the residence time with a dosage form at a particular site and controlling the release of drug from the dosage form are useful, especially for achieving controlled plasma level of the drug as well as improving bioavailability.

Mucoadhesive DDSs are the systems which utilize the property of mucoadhesion of certain polymers, which become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for an extended period of time. Bioadhesion is an integral phenomenon in which two materials, at least one of which is biological are held together by means of interfacial forces. In the case of polymer attached to mucin layer of a mucosal tissue, the term mucoadhesion is used. The mucosal layer lines a number of regions of the body including the nose, Gastrointestinal tract (GIT), urogenital tract, the airways, the ear and eye. Residence time for most mucosal routes is less than an hour, and typically in minutes, it can be increased by the addition of an adhesive agent in the delivery system which is useful to localize the delivery system and increases the contact time at the site of absorption. The exact mechanism of mucoadhesion is not known between the mucoadhesive polymer and mucin occurs, which is followed by the interpenetration of polymer and mucin [7].

Mucilages and gums obtained from natural sources are most widely used as pharmaceutical excipients for their different properties such as its binding, diluents, and disintegrant properties in tablets, suspending, gelling properties in gel, and thickening properties in oral liquids [8-10].

Mucilages are also used for their binding, thickening, stabilizing, humidifying, disintegrating, and release controlling properties in medicines [10,11].

Herbal products appear to be more preferred over synthetic ones due to their accessibility, biocompatibility, low cost and low toxicity potential. Mucilage's and gums obtained from natural sources are most widely used as pharmaceutical excipients due to their binding, diluents and disintegrant properties in tablets, due to suspending and gelling properties in gels and due to thickening properties in oral liquids[8-10]. *Ocimum basilicum* plant has been used in traditional medicine due to its medicinal properties such as antibacterial, antifungal, antispasmodic, carminative, diaphoretic, digestive, emmenagogue, expectorant, stimulant, and stomachic. The plant is generally used in treatments of problems concerning digestion and nervous system. *O. basilicum* gum has been used as an emulsifier [12]. Furthermore, it has been reported that *O. basilicum* seed gum is used as a raw material for the preparation of edible films and coatings [13].

Carvedilol (CRV) is a non-selective ß-adrenergic blocking agent with α1-blocking activity. It is well absorbed from the GIT but subjected to first-pass metabolism, and its human oral bioavailability is only 20%. For enhancement of CRVs absorption it has been formulated with a series of polymers from point of view of preventing its oral degradation. Since the drug has less solubility and dissolution rate, several methods are used to improve its dissolution rate such as solid dispersions, cyclodextrin complexes, and microcapsules [14]. CRV has short biological half-life of 2 h. Its main absorption site is proximal small intestine [15]. Therefore, it would be lucrative to develop mucoadhesive system of CRV using sodium alginate – *O. basilicum* seed mucilage (OBM) polymer blend for oral use, which might facilitate an intimate contact with the absorbing surfaces of mucous membrane, mucoadhesion and thus the gastric residence could be prolonged to release the drug at target site at controlled rate to maximize the therapeutic effect.

A $3^2$ factorial design based computer-aided optimization was employed to investigate the effects of two independent process variables, i.e., amount of OBM and sodium alginate on measured responses such as % drug content (DC), % drug entrapment efficiency (DEE), and % drug released. Therefore, the objectives of the present investigation were to prepare OBM-alginate mucoadhesive gastrospheres containing CRV and to optimize drug delivery using $3^2$ factorial designs.

## MATERIALS AND METHODS

### Materials

CRV was provided ex gratis by Alembic Pvt. Ltd., (Vadodara), sodium alginate (Research Lab. Pvt. Ltd. Mumbai), and calcium chloride ($CaCl_2$) (Research Lab. Pvt. Ltd. Mumbai). The *O. basilicum* seeds were procured from Green Pharmacy, Pune. All other chemicals employed were of analytical grade.

### Isolation and purification of *O. basilicum* mucilage

*O. basilicum* seeds were procured and cleaned by washing with distilled water and then soaked in 500 ml of distilled water with intermittent stirring for 12–14 h until soft. The soft seed material was subjected to slow stirring using overhead stirrer (Remi India), and seedless white-colored mucilaginous material was collected by filtering through muslin cloth. The mucilage was collected and purified by precipitation method by using 250 ml of 95% ethanol. Collected mucilage was dried in the oven at 50–55° for 4–5 h. Dried mucilage was scraped with spatula and powdered using mortar and pestle. Mucilage was sieved using mesh no.60 and stored in desiccators for further studies [16].

### Preparation of OBM-alginate gastrospheres containing CRV

The OBM-alginate gastrospheres containing CRV were prepared u1ing $CaCl_2$ as a cross-linking agent by ionotropic gelation method. Briefly, sodium alginate and OBM aqueous dispersions were prepared separately using distilled water. These dispersions were well mixed at 1000 rpm using magnetic stirrer (Remi Motors, India). Afterward, CRV was added to the mixture solutions of sodium alginate and *O. basilicum* mucilage for each formulation and mixed thoroughly using a magnetic stirrer. The final sodium alginate – *O. basilicum* mucilage mixture solutions containing CRV was ultra-sonicated for 5 min for debubbling. The resulting dispersion was then added through a 21-gauge needle dropwise into 40 ml of 10% (w/v) $CaCl_2$ solutions. Added droplets were retained in the $CaCl_2$ solutions for 15 min to complete the curing reaction and to produce spherical rigid gastrospheres. The gastrospheres were collected by decantation and washed repeatedly with distilled water and dried at 37°C for overnight. The dried gastrospheres were stored in a desiccator until used [6].

### Experimental design for optimization

To obtain "optimized product," nine different formulations were generated using $3^2$ factorial designs. The amount of sodium alginate ($X_1$) and amount of *O. basilicum* mucilage ($X_2$) were taken as independent formulation variables while % DC (Y1), % entrapment efficiency ($Y_2$), and % drug released at 12 h ($Y_3$) were considered as dependent or response variables. A statistical model incorporating interactive and polynomial terms were used to evaluate the responses. Design expert (Version 7.0.0) software was used during the generation and evaluation of the statistical experimental design [17,18]. The effects of independent variables were modeled using a quadratic mathematical equation generated by a $3^2$ factorial design such as:

$$Y = b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_{12}+b_{22}X_{22}$$

Where Y is the response; $b_0$ is the intercept, and $b_1$, $b_2$, $b_{12}$, and $b_{11}$; $b_{22}$ is regression coefficients. $X_1$ and $X_2$ are individual effects; $X_{12}$ and $X_{22}$ are quadratic effects; $X_1$ and $X_2$ are the interaction effect. One-way ANOVA was applied to estimate the significance of models ($p<0.05$). Individual response parameters were evaluated using the F test. The surface response plots were analyzed to reveal the effect of independent factors (amount of OBM mucilage and sodium alginate) on the measured responses (% DC, % DEE, and % DR). The details in the design are shown in Tables 1 and 2.

### Determination of DC

Drug loaded gastrospheres (100 mg) were powdered and suspended in 100 ml 0.1N HCl solution and kept for 24 h. Similar procedure was repeated in phosphate buffer pH 6.8 It was stirred for 5 min and filtered

**Table 1: Selection of levels of independent variables**

| Coded value | Sodium Alginate (mg) $X_1$ | *Ocimum basilicum* mucilage (mg) $X_2$ |
|---|---|---|
| −1 | 300 | 50 |
| 0 | 400 | 75 |
| 1 | 500 | 100 |

*$X_1$: Amount of sodium alginate, $X_2$: Amount of *Ocimum basilicum* mucilage, mg: Milligram, $p<0.05$

**Table 2: Design layout of $3^2$ factorial batches**

| Batch code | Coded value | | Actual value | | Drug |
|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | SA | *Ocimum basilicum* seed mucilage | |
| S1 | −1 | −1 | 300 | 50 | 400 |
| S2 | −1 | 0 | 300 | 75 | 400 |
| S3 | −1 | +1 | 300 | 100 | 400 |
| S4 | 0 | −1 | 400 | 50 | 400 |
| S5 | 0 | 0 | 400 | 75 | 400 |
| S6 | 0 | +1 | 400 | 100 | 400 |
| S7 | +1 | −1 | 500 | 50 | 400 |
| S8 | +1 | 0 | 500 | 75 | 400 |
| S9 | +1 | +1 | 500 | 100 | 400 |

*SA: Sodium alginate, $p<0.05$

by Whatman filter paper. CRV content in the filtrate was determined using spectrophotometer at 280 nm [6].

Drug content = Actual drug content/total wt. of gastrosphere taken×100%

**Determination of DEE**

Gastrospheres equivalent to 5 mg of CRV was taken and was crushed using pestle and mortar. The crushed powders of drug-containing gastrospheres were placed in 25 ml of 0.1N HCl and kept for 24 h with occasional shaking at 37±0.5°C. After the stipulated time; the mixture was stirred at 500 rpm for 20 min using a magnetic stirrer (Remi Motors, India). The polymer debris formed after disintegration of gastrospheres was removed filtering through Whatman® filter paper (No.40). The DC in the filtrate was determined using an ultraviolet (UV)-Vis spectrophotometer (Lab India) at 280 nm against appropriate blank. The DEE was calculated using the following formula [6].

$$DEE(\%)=\left(\frac{\text{Actual}}{\text{Theoretical}}\text{ Drug Content}\right)\times\text{Drug}$$

**Particle size analysis**

The particle size of 100 dried gastrospheres from each batch was measured by optical microscopic method for average particle size using an optical microscope. The ocular micrometer was previously calibrated by stage micrometer. OBM-alginate beads containing CRV were gold-coated using ion-sputter, and at 20 kv, their morphology was examined by Scanning electron microscope (JSM 6330 JEOL Japan).

***In vitro* mucoadhesive test**

The mucoadhesive property of optimized CRV gastrospheres was evaluated by *ex vivo* wash-off method. Freshly excised pieces of goat intestinal mucosa (2 cm×2 cm) (collected from the slaughterhouse) were mounted in the glass slide (7.5 cm×2.5 cm) using thread. About 20 gastrospheres were spread onto the wet tissue specimen, and the prepared slide was hung onto a groove of the disintegration test apparatus. The tissue specimen was given a regular up and down movement in a vessel containing 900 ml of 0.1N HCl at 37±0.5°C. After regular time intervals, the machine was stopped and the number of gastrospheres still adhering to the tissue was counted [9].

***In vitro* drug release (DR) study**

The release of CRV from various gastrosphere was tested using a dissolution apparatus USP Type II. The dissolution rate was measured at 37±0.5°C under 50 rpm speed. Accurately weighed quantities of gastrospheres containing CRV equivalent to 50 mg were added to 900 ml of 0.1 N HCl. 5 ml sample was collected at regular time intervals, and the same amount of a fresh dissolution medium was replaced into the dissolution vessel to maintain the sink condition throughout the experiment. The collected aliquots were filtered and suitably diluted to determine the absorbance using a UV–Vis spectrophotometer (Lab India) at 280 nm against an appropriate blank.

**FTIR spectroscopy**

Samples were reduced to powder and analyzed as KBr pellets using a FTIR spectroscopy. The pellet was placed in the sample holder spectral scanning was taken in the wavelength region between 4000 and 400 $cm^{-1}$ with a scan speed of km/s.

**Powder X-ray diffraction (PXRD)**

PXRD patterns were recorded using BRUCKER D2 PHASER A26-X1 ABOB2A, fitted with a copper target, a voltage of 40 kV, and a current of 30 mA. The scanning rate was 1°/min over a 2θ range of 1–50°. PXRD patterns were traced for CRV, physical mixture, and formulation. The samples were slightly ground and packed into the aluminum sample container.

**Differential scanning calorimetric (DSC)**

DSC analysis of the samples was carried out on a PerkinElmer DSC7, USA. Samples (6.5–10 mg) were heated under nitrogen atmosphere on an aluminum pan at a heating rate of 10 °C/min over the temperature range of 5 and 300°C. DSC analysis was carried out under nitrogen gas flow of 20 $lb/in^2$.

## RESULTS AND DISCUSSION

**Isolation of *O. basilicum* mucilage and preparation of OBM-alginate gastrospheres containing CRV**

OBM was isolated from raw seeds of *O. basilicum*, and the average yield of dried OBM was found 20.16% w/w. The OBM-alginate gastrospheres containing CRV was prepared through ionotropically gelation using $CaCl_2$ as cross linker. When dispersion mixture of sodium alginate, OBM and CRV were dropped into the solutions containing $Ca^{2+}$ ions, ionotropically gelled OBM-alginate gastrospheres containing CRV were formed instantaneously due to an electrostatic ionic interaction between negatively charged $COO^-$ groups of sodium alginate and positively charged $Ca^{2+}$ ions. Actually, $Ca^{2+}$ ions are accommodated in the interstices of two polycarbonate chains having a close ion-pair interaction with $COO^-$ anions of the sodium alginate and sufficient coordination by other electronegative oxygen atoms [19].

**Optimization studies**

The influences of factors on investigating responses were elucidated by response surface methodology (Table 3).

Response surface methodology is a widely used approach for the development and optimization of drug delivery formulations, which has been utilized by the formulators to investigate the combined effect of investigating factors on the desired responses. The three-dimensional response surface graphs are very useful in learning about the main effects and interaction effects of the independent variables. The three-dimensional response surface plots (Figs. 1 and 2) were presented to estimate the effects of the independent variables (factors) on each response investigated. The values of investigated responses measured for all trial formulations were fitted in the $3^2$ factorial designs to get model equations for responses analyzed in this investigation. These models were evaluated statistically by applying one-way ANOVA ($p<0.05$). The fitted model for DC is a quadratic model and expressed as the final equation in terms of actual factors as follows:

a. Drug content = +11.91500+0.043183 SA+0.011133OBM
[$R^2$=0.8450; adjusted $R^2$=0.7933; predicted $R^2$=0.6353; F=16.35; p<0.05]

From the RSM plot for DC (Fig. 1), it is observed that the change in concentration of sodium alginate and *O. basilicum* mucilage affects DC. It is observed that with increase in concentration of sodium alginate DC increases; however, increase in concentration of *O. basilicum* mucilage decreased DC. From the statistical second-order complete model equation, it can be concluded that among the polymers used

**Table 3: Result of ANOVA for factorial design batches**

| Source | Sum of square | Df | Mean square | F value | p>F |
|---|---|---|---|---|---|
| Drug content | | | | | |
| Model | 112.35 | 2 | 56.18 | 16.35 | 0.0037(S) |
| $X_1$ | 111.89 | 1 | 111.89 | 32.57 | 0.0013(S) |
| $X_2$ | 0.46 | 1 | 0.46 | 0.14 | 0.7256(NS) |
| Drug entrapment efficiency | | | | | |
| Model | 108.44 | 3 | 36.15 | 375.40 | <0.0001(S) |
| $X_1$ | 104.08 | 1 | 104.08 | 1080.96 | <0.0001(S) |
| $X_2$ | 3.38 | 1 | 3.38 | 35.05 | 0.0020(S) |
| $X_1X_2$ | 0.98 | 1 | 0.98 | 10.18 | 0.0243(S) |
| Drug release | | | | | |
| Model | 133.75 | 2 | 66.87 | 7.10 | 0.0262(S) |
| $X_1$ | 12.88 | 1 | 12.88 | 1.37 | 0.2867(NS) |
| $X_2$ | 120.87 | 1 | 120.87 | 12.83 | 0.0116(S) |

S and NS indicate significant and nonsignificant, respectively. d.f. indicate degree of freedom

*O. basilicum* mucilage had a more profound effect on DC as compared to sodium alginate.

b. Entrapment efficiency = +15.81111+0.056500SA+0.10920 OBM−1.98000E-004×SA×OBM
[$R^2$=0.9956; adjusted $R^2$=0.9929; predicted $R^2$=0.9905; F=375.40; p<0.05]

From the RSM plot for DEE (Fig. 2), it is observed that the change in concentration of sodium alginate and *O. basilicum* mucilage affects DEE. It was observed that with increase in concentration of sodium alginate DEE increases; however, increase in concentration of *O. basilicum* mucilage decreased DEE. From the statistical second-order complete model equation, it can be concluded that among the polymers used *O. basilicum* mucilage had a more profound effect on DEE as compared to sodium alginate.

c. Drug release = +55.29722+0.014650 SA+0.17953OBM.
[$R^2$=0.7029; adjusted $R^2$=0.6039; predicted $R^2$=0.3418; F=7.10; p<0.05]

From the RSM plot for DR (Fig. 3), it is observed that the change in concentration of sodium alginate and *O. basilicum* mucilage affects DR. It was observed that increase in concentration of sodium alginate increased the DR up to certain extent further increase in concentration of sodium alginate retarded the DR values. However, a decrease in DR values with the increasing *O. basilicum* mucilage ($X_2$) is indicated by the three-dimensional response surface graph relating DR (Fig. 3). Surface response plot indicates that sodium alginate concentration at optimum level 400 mg yielded microspheres with highest DR with *O. basilicum* mucilage concentration at 75 mg. Hence, the optimized batch was found to be S4 with optimum level of sodium alginate and *O. basilicum* mucilage.

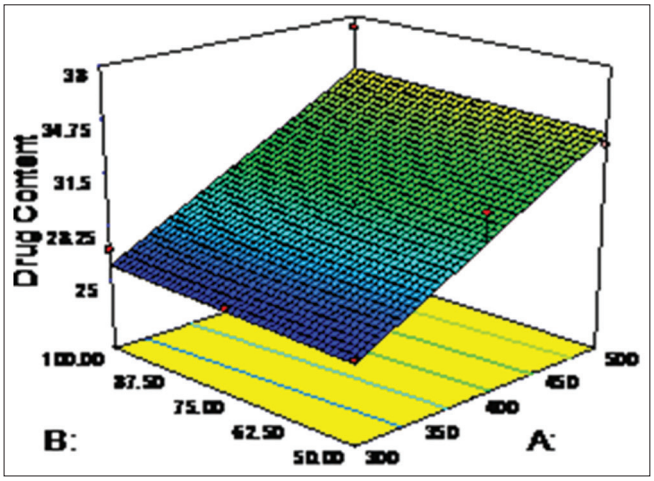


**Fig. 1: Response surface plot of drug content (A: Sodium alginate, B: *Ocimum basilicum* mucilage)**

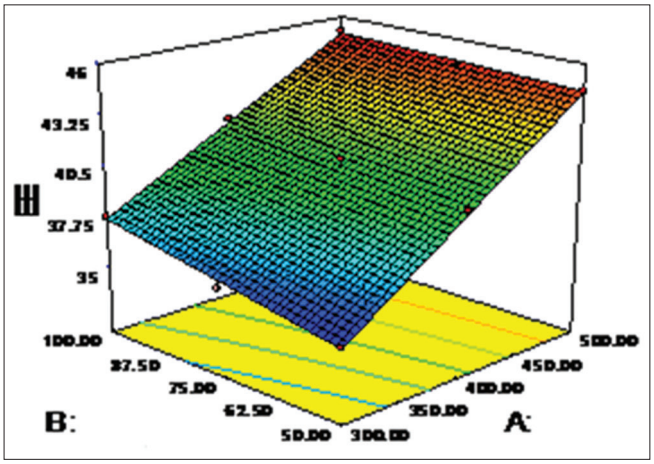


**Fig. 2: Response surface plot of daily energy expenditure (A: Sodium alginate, B: Ocimum basilicum mucilage)**

**Results of DC and entrapment efficiency**

The formulated CRV mucoadhesive gastrospheres were characterized for different parameters by varying the solvent pH. The results of DC and DEE were obtained in different solvent pH, namely, 0.1N HCl and phosphate buffer pH 6.8 for determining the effect of solvent pH on the above parameters.

*DC*

In these methods, drug is uniformly distributed in the polymer solution, so drug can be loaded easily in the polymer. DC analysis for the nine batches based on sodium alginate and *O. basilicum* mucilage concentration in both medium (0.1N HCl and phosphate buffer pH 6.8) is shown in Table 4, which concludes that DC values were ranging from 25.58% to 37.26% in 0.1N HCl and 46.05% to 57.45% DC values for phosphate buffer pH 6.8. It was found that DC increases with an increase in polymer concentration.

*DEE*

The entrapment efficiency determines the percentage of entrapped drug with respect to the total drug introduced into a polymer solution. Effect of *O. basilicum* mucilage content on entrapment efficiency was studied.

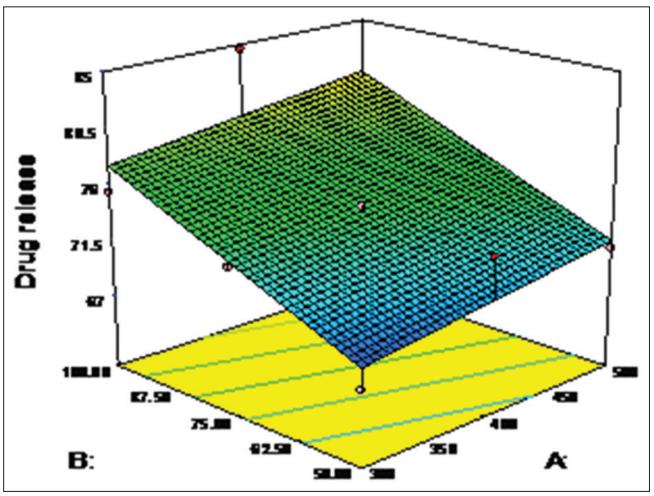


**Fig. 3: Response surface plot of drug release (A: Sodium alginate, B: *Ocimum basilicum* mucilage)**

**Table 4: Results of drug content of CRV gastrospheres**

| Formulation | Drug content % (0.1N HCl) | Drug content (%) (phosphate buffer pH 6.8) |
|---|---|---|
| S1 | 25.58±0.164 | 48.10±0.327 |
| S2 | 25.82±0.124 | 46.05±0.396 |
| S3 | 26.91±0.063 | 47.64±0.196 |
| S4 | 31.48±0.185 | 57.45±0.184 |
| S5 | 28.26±0.218 | 55.50±0.225 |
| S6 | 27.94±0.100 | 55.35±0.122 |
| S7 | 33.38±0.253 | 53.70±0.073 |
| S8 | 33.58±0.220 | 53.01±0.012 |
| S9 | 37.26±0.214 | 53.62±0.367 |

SD: Standard deviation for n=3. CRV: Carvedilol

Entrapment efficiency in 0.1N HCl and phosphate buffer pH6.8 was found to be in the range from 35.32 to 45.13% and 56.39 to 70.49%, respectively (Table 5); it is evident that with increase in *O. basilicum* mucilage concentration entrapment efficiency also increased. This may be due to increase in viscosity with increased *O. basilicum* mucilage concentration.

**Particle size analysis**

All the formulations were spherical in particle shape with a smooth surface. The mean particle size of gastrospheres ranged from 774 to 882 µm, (Table 6) which indicate the large particle size distribution. It was also noted that increase in drug to mucilage ratio increases the particle size due to increased viscosity of feed solution which influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle.

***In vitro* mucoadhesive test**

The *in vitro* wash off test using goat intestinal mucosa for assessing mucoadhesive of gastrosphere containing CRV was performed at both gastric pH (0.1N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 6.8) for 8 h. In 0.1N HCl, the percentage of gastrosphere adhering to the goat intestinal mucosal tissue varied from 10% to 65% over 8 h, whereas this was varied from 40% to 60% in phosphate buffer pH 6.8 (Tables 7 and 8).

The mucoadhesion test results reflect a decrease in mucoadhesion of CRV gastrospheres in both gastric pH (0.1N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 6.8) the reason may be due to the reason that at lower concentration the *O. basilicum* mucilage structure is more loose and the polymer chain have more space to extend within the mucus, as the number of *O. basilicum* mucilage chains penetrating in mucus is increased a strong bond, either chemical, mechanical or the both is formed between the mucus and the *O. basilicum* mucilage [20]. Among all nine batches S4 batch gave highest mucoadhesive property.

***In vitro* DR**

Gastrospheres were subjected to *in vitro* release using basket type dissolution apparatus in 900 ml of 0.1N HCl medium. The results of the *in vitro* DR studies were given in Table 9.

**Table 5: Results of entrapment efficiency of carvedilol gastrospheres**

| Formulation | E.E (0.1N HCl) | E.E (phosphate buffer pH 6.8) |
|---|---|---|
| S1 | 35.32±0.231 | 56.39±0.424 |
| S2 | 36.03±0.348 | 59.05±0.425 |
| S3 | 37.85±0.195 | 60.66±0.232 |
| S4 | 40.10±0.46 | 70.49±0.214 |
| S5 | 40.94±0.28 | 66.22±0.472 |
| S6 | 41.52±0.37 | 65.51±0.397 |
| S7 | 44.58±0.30 | 65.48±0.506 |
| S8 | 44.48±0.46 | 60.33±0.235 |
| S9 | 45.13±0.517 | 64.08±0.422 |

SD: Standard deviation for n=3

**Table 6: Carvedilol loaded mucoadhesive gastrospheres particle size analysis**

| Formulation | Particle size |
|---|---|
| S1 | 830±5.77 |
| S2 | 774±5.93 |
| S3 | 798±6.43 |
| S4 | 810±4.37 |
| S5 | 838±7.51 |
| S6 | 854±11.6 |
| S7 | 862±8.33 |
| S8 | 874±5.93 |
| S9 | 882±2.96 |

SD: Standard deviation for n=3

The release seems to be somewhat sustained with increase in the amount of *O. basilicum* mucilage. The release rate was found to be decreased in accordance with the increase in the ratio of *O. basilicum* mucilage used. CRV release from the gastrospheres was studied in gastric buffer 0.1N HCl for 12 h. In case of gastrosphere containing higher *O. basilicum* mucilage contents, the more hydrophilic property of the mucilage could probably bind better with water to form viscous gel structure, which might blockade the pores on the surface of gastrosphere and could delay the DR from these formulated gastrospheres [20]. The another reasonable explanation of the delayed DR can be attributed to increasing coating efficiency of the drug particles with the increasing *O. basilicum* mucilage content employed in the formulation. Among all the fabricated formulation, S4 was chosen as an ideal formulation showing an extended DR over a period of 12 h (84.58%) with acceptable mucoadhesive property.

**Analysis of DR kinetics and mechanism**

The *in vitro* DR data from various mucoadhesive gastrosphere containing CRV were evaluated kinetically using various mathematical models such as zero-order, first-order, and Higuchi, and Korsmeyer–Peppas model [20,21]. The results of the curve fitting into these above-mentioned mathematical models are given in Table 10.

When respective correlation coefficients of this gastrosphere were compared, the CRV release from this gastrosphere was found to follow zero-order model ($R^2$=0.969–0.998) over a period of 12 h. The best fit of zero-order model indicated that the drug released from this gastrosphere followed sustained release pattern. The values of release exponent (n) determined from *in vitro* DR data of various CRV gastrosphere S3, S4, S6, S7, S8, and S9 follow the anomalous non-Fickian DR (0.61–0.822), i.e., the rate of solvent penetration and DR is in the same range, and S1, S2, and S5 indicating the DR from this gastrosphere followed the Case-II transport mechanism controlled by swelling and relaxation of polymeric blend in gastrosphere matrix. This could be attributed due to *O. basilicum* mucilage polymer dissolution and enlargement or relaxation of polymeric chain [14,22].

**Table 7: Mucoadhesive test in 0.1N HCl**

| Formulation | % mucoadhesion test (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| S1 | 90 | 90 | 85 | 75 | 75 | 65 | 65 | 60 |
| S2 | 70 | 60 | 50 | 40 | 35 | 25 | 15 | 10 |
| S3 | 95 | 90 | 90 | 80 | 70 | 70 | 60 | 60 |
| S4 | 95 | 95 | 90 | 90 | 70 | 70 | 65 | 65 |
| S5 | 100 | 75 | 75 | 65 | 60 | 60 | 50 | 40 |
| S6 | 95 | 80 | 80 | 75 | 70 | 70 | 65 | 60 |
| S7 | 95 | 90 | 85 | 80 | 70 | 70 | 60 | 60 |
| S8 | 90 | 80 | 75 | 70 | 60 | 60 | 50 | 45 |
| S9 | 100 | 90 | 80 | 75 | 70 | 70 | 60 | 60 |

**Table 8: Mucoadhesion test in phosphate buffer pH 6.8**

| Formulation | % Mucoadhesion test (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| S1 | 90 | 75 | 50 | 25 | 10 | - | - | - |
| S2 | 90 | 65 | 55 | 30 | 20 | 10 | - | - |
| S3 | 100 | 75 | 70 | 70 | 60 | 60 | 50 | 50 |
| S4 | 100 | 95 | 80 | 75 | 65 | 65 | 60 | 60 |
| S5 | 95 | 95 | 90 | 80 | 70 | 60 | 50 | 50 |
| S6 | 100 | 100 | 95 | 90 | 75 | 65 | 55 | 50 |
| S7 | 90 | 80 | 75 | 75 | 65 | 60 | 60 | 50 |
| S8 | 95 | 85 | 80 | 80 | 75 | 70 | 60 | 55 |
| S9 | 100 | 95 | 85 | 80 | 80 | 65 | 50 | 40 |

**Table 9: Summary of results of dependent variables**

| Formulation | Variable levels in coded form | | Drug content (0.1N HCl) (%) | Entrapment efficiency (0.1N HCl) (%) | Drug release (0.1N HCl) (%) |
|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | | | |
| S1 | −1 | −1 | 25.58 | 35.32 | 67.00 |
| S2 | −1 | 0 | 25.82 | 36.03 | 72.67 |
| S3 | −1 | +1 | 26.91 | 37.85 | 75.50 |
| S4 | 0 | −1 | 31.48 | 40.10 | 84.58 |
| S5 | 0 | 0 | 28.26 | 40.94 | 74.37 |
| S6 | 0 | +1 | 27.94 | 41.52 | 73.52 |
| S7 | +1 | −1 | 33.38 | 44.58 | 74.65 |
| S8 | +1 | 0 | 33.58 | 44.48 | 70.97 |
| S9 | +1 | +1 | 37.26 | 45.13 | 78.34 |

**Table 10: Release kinetics data**

| Release kinetics | | | | | | |
|---|---|---|---|---|---|---|
| Formulation | Zero-order $R^2$ | First-order $R^2$ | Higuchi $R^2$ | Hixson-Crowell $R^2$ | Korsmeyer–Peppas $R^2$ | n (slope) |
| S1 | 0.991 | 0.966 | 0.942 | 0.978 | 0.993 | 1.004 |
| S2 | 0.992 | 0.962 | 0.953 | 0.978 | 0.969 | 1.061 |
| S3 | 0.993 | 0.978 | 0.972 | 0.989 | 0.983 | 0.702 |
| S4 | 0.994 | 0.938 | 0.959 | 0.97 | 0.976 | 0.756 |
| S5 | 0.996 | 0.953 | 0.96 | 0.975 | 0.987 | 0.873 |
| S6 | 0.998 | 0.944 | 0.943 | 0.965 | 0.966 | 0.677 |
| S7 | 0.969 | 0.878 | 0.896 | 0.916 | 0.976 | 0.822 |
| S8 | 0.987 | 0.94 | 0.928 | 0.961 | 0.947 | 0.61 |
| S9 | 0.986 | 0.918 | 0.925 | 0.948 | 0.947 | 0.701 |
| Best fit model | Zero-order | | | | | |

**FTIR spectroscopy analysis**

IR spectra of CRV and its combination with excipients are shown in Fig. 4. An IR spectrum of pure CRV showed characteristic peaks at 3352.05 $cm^{-1}$ (O-H and N-H stretching vibration peaks merged together), 2938.40 $cm^{-1}$ (C-H stretching vibrations), 1596.88 $cm^{-1}$ (N-H bending vibrations), and 1241.16 $cm^{-1}$ (O-H bending and C-O stretching vibrations). These peaks can be considered as characteristic peaks of CRV and were not affected and prominently observed in IR spectra of CRV along with excipients as shown in Fig. 4 indicated no interaction between CRV and excipients.

The IR spectrum of the formulation showed that there was no significant evidence for interaction between drug and the *O. basilicum* mucilage. Peaks of both drug and formulation were observed and interpreted. Hence, this clearly suggests that drug, polymers, and excipients used for the current study have not found any interaction.

**XRD analysis**

Substances in solid state can present crystalline or amorphous characteristics and in some cases both. A crystal has an ordered arrangement of molecules and atoms, maintained in contact through non-covalent interactions. On the other hand, amorphous solids are characterized by a random state. These characteristics are important in the absorption process. Amorphous solids are, in general, more soluble than the crystalline form, due to free energies involved in the dissolution process. Solids in amorphous state have randomly arranged molecules, and thus low energy is required to separate them and, consequently, their dissolution is faster than when in the crystalline form. The CRV has crystalline characteristics which are represented by peaks in X-ray diffractograms (Fig. 5), and the most evident peaks appear at 2θ=12.67, 13.18, 19.60, and 22.14. These peaks were not observed in the CRV loaded gastrospheres. This indicates that drug particles are dispersed at molecular level in the polymer matrices since no indication about the crystalline nature of the drugs was observed in the drug-loaded gastrospheres [23-26].

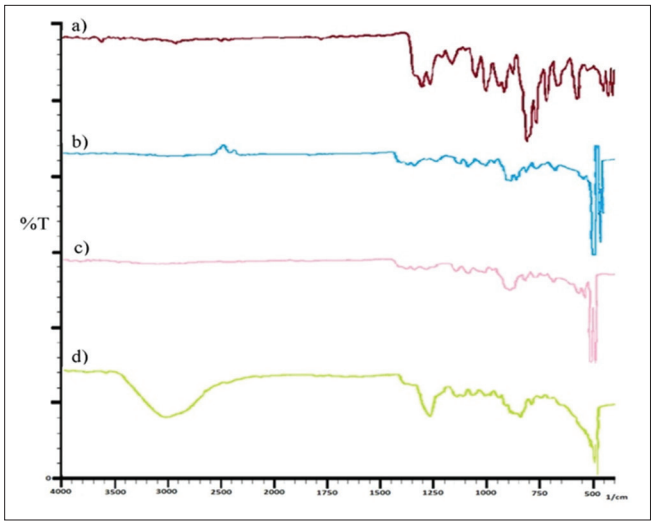


**Fig. 4: Infrared spectra of a) carvedilol (CRV) b) CRV + *Ocimum basilicum* mucilage c) CRV + *O. basilicum* mucilage + Sod. alginate d) formulation S4**

**DSC analysis**

DSC is a fast and reliable method for understanding the polymorphic transition when screening drugs and excipients for compatibility, obtaining information about possible interactions. The results of DSC studies are shown in Fig. 6. DSC thermogram showed an endothermic peak of CRV at 87°C, which corresponds to its melting point. The presence of detectable peaks of CRV in a physical mixture is an indication of uniform mixing of excipients. The absence of peaks of CRV loaded gastrospheres in formulation S4 clearly indicates that CRV was dispersed completely in the formulation, thus modifying the gastrospheres to an amorphous, disordered crystalline phase [25].

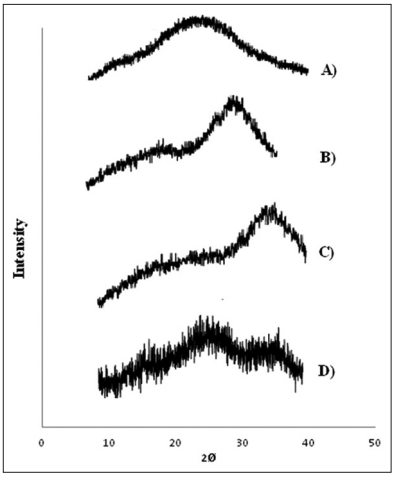


**Fig. 5: A) Carvedilol (CRV) B) CRV + *Ocimum basilicum* mucilage C) CRV+*O. basilicum* mucilage + NA. alginate D) formulation S4**

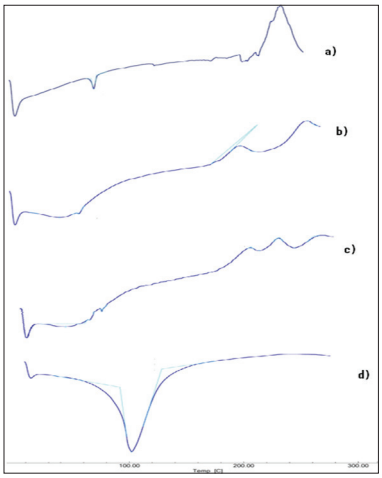


**Fig. 6: a) Carvedilol (CRV) b) CRV + *Ocimum basilicum* mucilage c) CRV + *O. basilicum* mucilage + NA. alginate d) formulation S4**

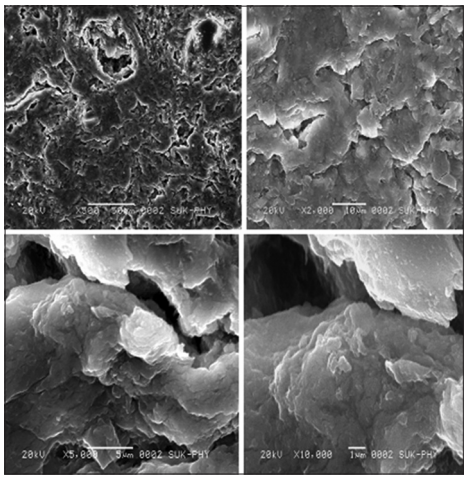

**Fig. 7: Scanning electron photograph of drug-loaded gastrosphere (magnification ×500, ×2000, ×5000, ×10,000)**

**Scanning electron microscopy (SEM)**

The morphological analysis of the mucoadhesive gastrospheres was studied by SEM. SEM photomicrographs of optimize formulation S4 gastrospheres were rough spherical and showed few pores on surface, due to uniform distribution of the drug in formulation, as shown in Fig. 7. Particulate matter of the drug and *O. basilicum* mucilage was seen on the surface of the gastrospheres, indicating uniform distribution of the drug in the polymeric network [23]. The SEM photographs indicated that the gastrospheres were completely covered with polymer the rough texture which may be due to removal of water from gastrospheres during drying. Thus the rate of removal of water from gastrospheres exerts an influence on the morphology of final product [23-28].

## CONCLUSION

The present study concludes that mucoadhesive gastrosphere of CRV can be prepared using the ionotropic gelation method. $3^2$ full factorial designs are suitable to study the effect of process variables on formulation characteristics by applying statistical analysis. From the study, we successfully developed microparticulate DDS of CRV using as mucilage/mucoadhesive polymer isolated from *O. basilicum* seeds and rate retardant sodium alginate polymer.

## AUTHORS' CONTRIBUTIONS

The first author carried out literature review, critical analysis, idea generation, and experimental work. The second author supervised the methodology implemented and helped in drafting of manuscript.

## CONFLICTS OF INTEREST

There are no conflicts of interest among the authors.

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