

Formulation and Evaluation of Mucoadhesive Nasal Microspheres of Hydralazine Hydrochloride

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Abstract

Nasal delivery protects drugs like Hydralazine Hydrochloride from hepatic first-pass metabolism. The study aimed to formulate mucoadhesive sodium alginate and Carbopol 934P microspheres loaded with Hydralazine Hydrochloride. Methods: Microspheres were prepared by emulsion crosslinking method, with mucoadhesive polymers (Sodium alginate-carbopol-934P), for potential nasal delivery bypassing first-pass metabolism. Relevant physicochemical properties, in vitro release and Mucoadhesion, drug content and Particle size analysis were investigated. Microspheres were spherical in shape, distinct, with smooth properties favorable for intranasal absorption. Results: Kinetic modeling result suggested that the prepared formulation followed the korsmeyer-Peppas. Pronounced sustained drug release over 8 hours was exhibited upon incorporation of Carbopol 934P and Sodium alginate. An invitro mucoadhesive test showed that Hydralazine Hydrochloride mucoadhesive microspheres adhered more strongly to the mucous layer and retained in the gastrointestinal tract for an extended period of time.

Index terms—

1 Introduction

Microsphere systems which are made from the naturally stirring biodegradable polymers have attracted considerable attention for past few years in sustained release drug delivery. Recently, dosage forms that can accurately control the release rates and target drugs to a specific body site have made the massive impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems (1)(2)(3). They have varied applications and are prepared using various polymers (4). However, the success of these microspheres is limited owing to their short residence time at the site of absorption. Hence the efforts have been made for providing an intimate contact of the drug delivery system with the absorbing membranes (5)(6)(7)(8). This can be achieved by blend of mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site (9)(10)(11)(12). Carbopol-934P (acrylic acid homopolymer) is an anionic polymer that has been used in mucoadhesive systems by several researchers (13)(14)(15)(16)(17). Carbopol-934P has been selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive properties and is not absorbed by body tissues and being totally safe for human oral consumption. Whereas Sodium alginate Used for the aqueous microencapsulation of drugs in contrast with the more conventional microencapsulation techniques used in combination with an H₂ receptors antagonist in the management of gastro esophageal reflex. [18] Hydralazine Hydrochloride is Antihypertensive, Cardiovascular agent, having biological Half life 3-7 hrs necessitates the need for its administration 50 mg at Every 6 hrs. Thus, the development of controlled-release dosage forms would clearly be advantageous.

In context of the above principles, a strong need was felt to develop a dosage form that delivered Hydralazine Hydrochloride as mucoadhesive nasal microspheres and would increase the efficiency of the drug, providing a sustained action. Thus, an attempt was made in the present investigation to use Carbopol-934P as a mucoadhesive polymer and sodium alginate as carrier polymer, in order to prepare mucoadhesive nasal Hydralazine Hydrochloride microspheres. The microspheres were characterized by in-vitro and iv-vivo tests and factorial design was used to optimize the variables. [19] M II.

2 Experimental a) Materials

Hydralazine hydrochloride (powder) was obtained as a gift sample from Zydus Cadila (Ahmedabad, India). Carbopol-934P (CP, molecular weight of 3×10^6 Da,) was obtained as a gift sample from Noveon® (Mumbai, India). Sodium alginate and petroleum ether 80:20 were procured from Willson Lab (Mumbai, India) and S. D. Fine Chemicals Ltd. (Mumbai, India), respectively. Liquid paraffin and span 80 were purchased from Loba Chemie Pvt Ltd. (Mumbai, India). All other ingredients were of analytical grade.

3 b) Animals

Six-months-old mixed sex, specific pathogenfree healthy Indian white rabbits (lupas) (Body weight 2.3 to 2.65 kg), were gifted from Zydus Cadila (Ahmedabad, India) and maintained under standard laboratory conditions (room temperature, $23 \pm 2^\circ\text{C}$; relative humidity, $55 \pm 5\%$; 12/12 h light/dark cycle) with free access to a commercial rodent diet and tap water.

4 c) Preparation of mucoadhesive hydralazine hydrochloride microspheres

Microsphere were prepared by emulsification method. Sodium alginate and the mucoadhesive carbopol 934 polymer were dispersed in purified water to form a homogeneous polymer mixture. The Hydralazine hydrochloride was added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. Polymer solution were dispersed in by a syringe with a needle in 30 ml of DCM containing 2 %w/w span 80 using a using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm. Stirring was continued for 3 h. The prepared emulsion was added into a syringe and allow to fall as a droplet into a calcium chloride (10 %w/v) containing 1% tween 20 and allow to react with the polymer globules for the 30 min to induce cross linking and solidify the microspheres.

The amount of emulsifying agent and time for stirring were varied in preliminary trial batches from 1-3 % v/v and 1-3 h, respectively. In factorial design batches F1-F9, 2.0 % v/v Span 80 was used as an emulsifying agent and time for stirring was kept to 3 h. The drug-topolymer-to-polymer ratio and stirring speed were varied in batches F 1 -F 9 , as shown in Table 1. All other variables were similar to the preliminary trial batches. Microspheres thus obtained were filtered and washed several times with petroleum ether (80:20) to remove traces of oil. The microspheres were then dried at room temperature (25°C and 60 % RH) for 24 h. The effect of formulation variables on characteristics of the microspheres of factorial design batches has been summarized in Table 1.

Various batches of Hydralazine hydrochloride mucoadhesive microspheres, prepared using the 3 2 full factorial design layout. Translation of coded levels in actual unit Variable levels: Low (-1) Medium (0) High (+1) Drug-to-polymer-to polymer ratio (X 1):

1:3:0.5 1:3:1 1:3:1.5 Stirring speed (X 2) rpm: 800 1000 1200 All the batches were prepared using 2 % v/v Span 80 and a stirring time of 3 h.

5 d) Optimization of microspheres formulation using 3 2 full factorial design

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses. $Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$ (1)

Where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and b_i is the estimated coefficient for the factor X_i . The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms ($X_1 X_2$) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) show the non-linearity. On the basis of the preliminary trials, a 32 full factorial design was employed to study the effect of independent variables, i.e. drug-to-polymer-to-polymer (X_1) and the stirring speed (X_2) on dependent variables which were the percentage of mucoadhesion, drug entrapment efficiency, particle size and the time required for 80 % drug dissolution (t_{80}).

6 e) Determination of Hydralazine hydrochloride

The amount of Hydralazine hydrochloride was estimated, using a UV/Vis spectrophotometric method (Shimadzu UV-1700 UV/Vis double beam spectrophotometer, Kyoto, Japan). Aqueous solutions of Hydralazine hydrochloride were prepared in 7.2 phosphate buffer and absorbance was measured on a Shimadzu UV/Vis spectrophotometer at 272 nm. The method was validated for linearity, accuracy and precision. The method

obeyed from the Beer's Law in the concentration range of 5-40 µg/ml. When a standard drug solution was analyzed repeatedly (n = 5), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.86% and 1.1%, respectively.

f) Drug entrapment efficiency

Two hundred milligrams of accurately weighed microspheres were crushed in a glass mortar and the powdered microspheres were suspended in 10 mL of 0.1 N hydrochloric acid (pH=1.2). After 24 h, the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula: Practical drug content/Theoretical drug content × 100.

The drug entrapment efficiency for batches F1-F9 has been reported in Table 1.

g) Particle size of microspheres

The particle size of the microspheres was determined, using an optical microscopy method (20). Approximately 300 microspheres were counted for particle size, using a calibrated optical microscope (Labomed CX RIII, Ambala, India). The particle size of microspheres of batches F 1 -F 9 has been reported in Table 1.

h) In-vitro wash-off test for microspheres

The mucoadhesive properties of the microspheres were evaluated, using an in-vitro wash-off test, as reported by Lehr et al (21). A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch-by-1 inch), using thread. Microspheres were spread (approximately 50) onto the wet rinsed tissue specimen and the prepared slide was hung onto one of the grooves of a USP tablet disintegration test apparatus, with continuous oxygen supply. The disintegration test apparatus was operated, giving the tissue specimen was given regular up and down movements within the beaker of the disintegration apparatus, which contained the simulated gastric fluid (pH=1.2). At the end of 30 min, 1 h and at hourly intervals up to 12 h, the number of microspheres still adhering onto the tissue was counted. The results of in-vitro wash-off test of batches F 1 -F 9 have been shown in Table 1.

i) Scanning electron microscopy

Scanning electron photomicrograph of drugloaded Carbopol-934P mucoadhesive microspheres were taken. A small amount of microspheres was spread on a glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. A scanning electron photomicrograph was then taken at an acceleration voltage of 20 KV, and a chamber pressure of 0.6 mm Hg, at different magnifications. The photomicrograph of batch F 4 has been depicted in Figure 1. The drug release studies were carried out using a USP XXIV basket apparatus (Electrolab, TDT-06T, India) at 37°C ± 0.5°C and at 100 rpm, within 900 mL of 0.1 N hydrochloric acid (pH=1.2) as the dissolution medium, as per USP XXVI dissolution test described for Hydralazine hydrochloride tablets. Microspheres equivalent to 40 mg of Hydralazine hydrochloride were used for this purpose. Five milliliters of the sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted suitably and analysed spectrophotometrically at 289nm. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage of drug dissolved at different time intervals was calculated, using the Beer-Lambert equation. The t₈₀ was calculated, using the Weibull equation (22). The average values of t₈₀ for batches F 1 -F 9 have been shown in Table 1. The percentage of drug released from batch F 4 in pH 1.2 has been shown in Figure 4. The effects of independent variables on the response parameters were visualized from the contour plots. Numerical optimization, using the desirability approach, was employed to locate the optimal settings of the formulation variables so as to obtain the desired response (??3). An optimized formulation was developed by setting constraints on the dependent and independent variables. The formulation developed was evaluated for the responses, and the experimental values obtained were compared with those predicted by the mathematical models generated. Counter plots showing the effect of drug-to-polymer-to-polymer (X₁) and stirring speed (X₂) on the percentage of mucoadhesion, drug entrapment efficiency, particle size and t₈₀ have been shown in Figure 5.

l) In-vivo study

In-vivo studies on Hydralazine hydrochloride mucoadhesive microspheres were on normal healthy Indian white rabbits (lupas) of mixed sex, weighing 2.3 to 2.65 kg each. The approval of the Institutional Animal Ethics Committee was obtained, before starting the study. The study was conducted in accordance with the standard intuitional guidelines. Two groups of normal healthy rabbits (three in each group) that were fasted (with water) at least 12 h before studies, were used for this investigation. Before oral drug administration of respective dosage forms, normal heart rate was recorded for 15 min. After recording the normal heart rate, i.v. isoprenaline (0.25 µg/kg) was administrated for induction of heart rate at a fixed interval. A dose of 2.5 mg/kg of Hydralazine hydrochloride-containing mucoadhesive microspheres and Hydralazine hydrochloride powder were administrated orally using long food needles. After oral administration of dosage forms, heart rate was continuously recorded

14 B) FACTORIAL EQUATION FOR THE PERCENTAGE OF MUCOADHESION

for 12 h, using pulse transducer (MI) through a Powerlab-multichannel computerized data acquisition system (AD Instruments, Australia) from each rabbit.

12 Results and Discussion

13 a) Preliminary trials

The mucoadhesive Hydralazine hydrochloride microspheres prepared from Carbopol-934P and sodium alginate were made using the emulsion crosslinking technique. Carbopol-934P chosen for the preparation of mucoadhesive microspheres, owing to its good mucoadhesive properties. Sodium alginate was used as a carrier polymer. Different concentrations of span 80, from 1-3% v/v, were used as the emulsifying agent. Span 80 was found to have a significant influence on the percentage of mucoadhesion observed (i.e. percentage of microspheres adhered and remained on the mucous layer), particles size and drug entrapment efficiency. Results showed that increase in the concentration of span 80, increased the particle size of microspheres, as well as the percentage of mucoadhesion. However, the drug entrapment efficiency was decreased. At a 1% v/v span80 concentration, percentage of mucoadhesion, particle size and drug entrapment efficiency of microspheres were 62%, 100 μ m and 56 % respectively. However, formation of irregularly shaped microspheres was observed. On the other hand, at 3% v/v span 80 concentration, the percentage of mucoadhesion, particles size and drug entrapment efficiency of microspheres were 78%, 200 μ m and 49 % respectively. The shapes of microspheres were found to be spherical, particles were coalesced. However, a 2 % v/v of concentration of span 80 was used for further studies. Table ?? : Hydralazine hydrochloride mucoadhesive microspheres F4 batch using release models One of the important factors related to microspheres, as reported by Lee et al (24), is the viscosity of the polymer solution. Polymer concentrations of 0.5%, 1%, and 2% w/v were selected for preliminary trials. Flake formation was observed when Sodium alginate and Carbopol-934P concentration was used at a level of 0.5% w/v, whereas maximum Spherical particles were observed at the 1% w/v level. Non-spherical microspheres were formed, when 2% w/v using polymer concentrations. Therefore, a 1% w/v concentration of Sodium Alginate and Carbopol-934P, each in ethanol, was found to be the optimum concentration for the polymer solution. A 1:1 mixture of heavy and light liquid paraffin was found to be suitable as the dispersion medium.

Preliminary trial batches were prepared, in order to investigate the effect of stirring time and speed on the percentage of mucoadhesion, drug entrapment efficiency, and characteristics of the resulting microspheres. An increase in the stirring time 1 h to 3 h, showed an increase in the percentage of mucoadhesion, but a decrease in drug entrapment efficiency and particles size of microspheres. Thus, a stirring time 3h was selected for further studies. Since, the stirring speed had a significant effect on the percentage of mucoadhesion, drug entrapment efficiency and particles size of microspheres, it was selected as an important factor for further studies.

On the basis of the preliminary trials, a 3 2 full factorial design was employed to study the effect of independent variables (i.e. drug-to-polymer-to-polymer ratio [X1] and the stirring speed [X2]) on dependent variables, which were the percentage of mucoadhesion, drug entrapment efficiency, particle size and t80. The results depicted in Table 1 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables, as they show a wide variation among the nine batches (F1-F9). The fitted equations (full models), relating the responses (i.e. percentage of mucoadhesion, drug entrapment efficiency, particle size and t80) to the transformed factor are shown in Table 3. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). The high values of correlation coefficient (Table 3) for the dependent variables indicate a good fit. The equations may be used to obtain estimates of the response, since a small error of variance was noticed in the replicates.

14 b) Factorial equation for the percentage of mucoadhesion

The in-vitro mucoadhesiveness test represented the percentage of mucoadhesive microspheres remaining on the mucosal layer (Table 1). The mucoadhesive microspheres of all the factorial design batches were spherical (Figure 1, batch F 4) and freeflowing.

The linear model generated for the percentage of mucoadhesion was found to be significant, with an Fvalue of 20.64 ($p < 0.0001$) and R² value of 0.9809: % mucoadhesion = 77.42+ 14.47X₁ -8.19X₂ -2.81X₁ X₂ -9.6 X₂² (5)

The counter plot shows that the in-vitro wash-off test carried out for determining the percentage of mucoadhesion of microspheres, increased from 42 to 58 and 74 to 92, at lower and higher levels of drug-topolymer-to-polymer ratio, respectively, as the stirring speed decreased. Results obtained, indicated that the effect of X₁ (drug-to-polymer-to-polymer) is more significant than X₂ (stirring speed). Moreover, stirring speed had a negative effect on the percentage of mucoadhesion (i.e. as the stirring speed increased, the percentage of mucoadhesion decreased). This finding may be attributed to a change in particle size, which can consequently affect mucoadhesion. As the drug-topolymer-to-polymer ratio increases; the percentage of mucoadhesion also increases; since the greater amount of polymer results in a higher amount of free -COOH (carboxyl) groups (17), which are responsible for binding to the sialic acid groups within the mucus network. Hence, it results in an increase in the mucoadhesive properties of microspheres. In-vitro mucoadhesive tests showed that hydralazine hydrochloride mucoadhesive microspheres adhered more strongly to the gastric mucosa and could be retained in

the gastrointestinal tract for an extended period of time (Figures 2 and and3). Figure 3 also showed that even after 8 h some of the microspheres were remained on the gastric mucous layer.

15 c) Factorial equation for particle size

The linear model generated for particle size of microspheres was found to be significant, with an Fvalue of 88.76 ($p < 0.0001$) and R^2 value of 0.9955: Particle size = $103.77 + 7.3X_1 - 6.6X_2 - 1.0X_1X_2 - 1.0X_1^2 - 1.0X_2^2$ (6) The counter plot showed that the particle size of microspheres increased from 88 to 100 μ m and 102 to 115 μ m, at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as the stirring speed decreased. The results obtained indicate that the effect of X_1 (drug -to-polymer-to-polymer) is more significant than X_2 (stirring speed). This means that, as the stirring speed increases, the particle size decreases, and as a result the percentage of mucoadhesion could be directly affected.

Thus, it can be concluded that the amount of polymer (Carbopol-934P) and the stirring speed directly affect the percentage of mucoadhesion, as well as the particles size of microspheres.

16 d) Factorial equation for drug entrapment efficiency

The drug entrapment efficiency and t_{80} are important variables for assessing the drug loading capacity of microspheres and their drug release profile. These parameters are dependent on the process of preparation, physicochemical properties of drug, and formulation variables.

The linear model generated for drug entrapment efficiency was found to be significant, with an F-value of 4.39 ($p < 0.0001$) and R^2 value of 0.9165: Drug entrapment efficiency = $48.14 + 7.71X_1 - 3.28X_2 - 4.57X_1X_2 - 16.71X_1^2 + 0.28X_2^2$ (7) The results obtained, indicate that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Moreover, the stirring speed had negative effect on the percentage of drug entrapment efficiency (i.e. as the stirring speed increased, the particle size decreased and consequently the drug entrapment efficiency also decreased).

17 e) Factorial equation for t_{80}

The linear model generated for t_{80} was found to be significant, with an F-value of 115.91 ($p < 0.0001$) and R^2 value of 0.9997: $t_{80} = 533.47 - 167.16X_1 + 43.88X_2 - 25.57X_1X_2 - 58.71X_1^2 + 5.57X_2^2$ (8)

The results depicted in Table 3 indicate that the percentage of drug released in-vitro is highly dependent on the drug-to-polymer-to-polymer ratio and the stirring speed. The drug-to-polymer-to-polymer ratio has a negative effect on t_{80} , while stirring speed has a positive effect on t_{80} . Consequently, as the particle size decreases, the drug release also decreases.

A numerical optimization technique, using the desirability approach, was employed to develop a new formulation with the desired responses. Constraints like maximizing the percentage of mucoadhesion, drug entrapment efficiency, particle size and the amount of drug released after 12 h, in addition to minimizing the t_{80} , were set as goals to locate the optimum settings of independent variables in the new formulation.

The optimized microsphere formulation was developed, using a 1:3:1.25 drug-to-polymer-to-polymer ratio and a stirring speed of 950 rpm. The optimized formulation was evaluated for the percentage of mucoadhesion, drug entrapment efficiency, particle size and the amount of drug released. The results of experimentally observed responses and those predicted by mathematical models, along with the percentage prediction errors were compared. The prediction error, for the response parameters ranged between 0.52 and 2.18%, with an absolute error value of $1.26 \pm 0.72\%$. The low values of error indicate the high prognostic ability of factorial equation methodology. The amount of drug released from the optimized formulation was found to be low and it had a t_{80} value of 405 min. Thus, batch F 4 was selected for further studies, since it exhibited a high t_{80} value of 496 min and seems to be a promising candidate for achieving drug release up to 12 h. The drug release profile of batch F 4 is shown in Figure 4. This graph revealed that drug release rate slowed down after 2 h.

The results of curve fitting of the best batch into different mathematical models are given in Table ??.

The mechanism of drug release from the microspheres was found to be diffusion controlled, since the plots of the cumulative percentage of drug release versus the square root of time were found to be linear with the regression coefficient (R^2) values, ranging from 0.9784 to 0.9879 for the best batch. The release profile fitted to the Korsmeyer-Peppas equation, as the value of correlation coefficient was found to be 0.999. The values of slope and intercept were found to be 2.121 and -2.045, respectively.

18 f) In-vivo studies

A rapid reduction in heart rate was observed with pure hydralazine hydrochloride and the heart rate also recovered rapidly to the normal level within 5 h (Figure ??). In the case of hydralazine hydrochloride mucoadhesive microspheres, the reduction in heart rate was slow and reached a maximum reduction of 47 percent within 5 h after oral administration. This reduction in heart rate was sustained over a longer period of time (10 h). The 40 percent reduction in heart rate could be considered as a significant antihypertensive effect. In pure drug, the significant anti-hypertensive effect (40 percent) was maintained during the periods from 0.5 to 5 h following oral administration of hydralazine hydrochloride. Whereas, in case of mucoadhesive microspheres, the effect was maintained for a period of 0.5 to 10 h. The sustained antihypertensive effect observed over a longer period of

19 CONCLUSION

time in case of mucoadhesive microspheres was due to a slow release rate of drug, as well as the mucoadhesive properties of microspheres.

IV.

19 Conclusion

It could be said that the Hydralazine hydrochloride mucoadhesive microspheres developed, using a 3×2 full factorial design, showed a high percentage of mucoadhesion and drug entrapment efficiency. They also exhibited a sustained drug release property for peroral use in the form of capsule. Drug-to-polymer-to-polymer (Hydralazine hydrochloride-Sodium Alginate-Carbopol-934P) ratio, as well as the stirring speed had a significant influence on the percentage of mucoadhesion, drug entrapment efficiency, particle size and t_{80} . The optimized Hydralazine hydrochloride mucoadhesive microsphere formulation, developed using the desirability approach, showed a greater effect over a period of 8 h, compared to the Hydralazine powder. This would indicate the potential of mucoadhesive Hydralazine hydrochloride microspheres for use in the provision of a sustained therapeutic effect.

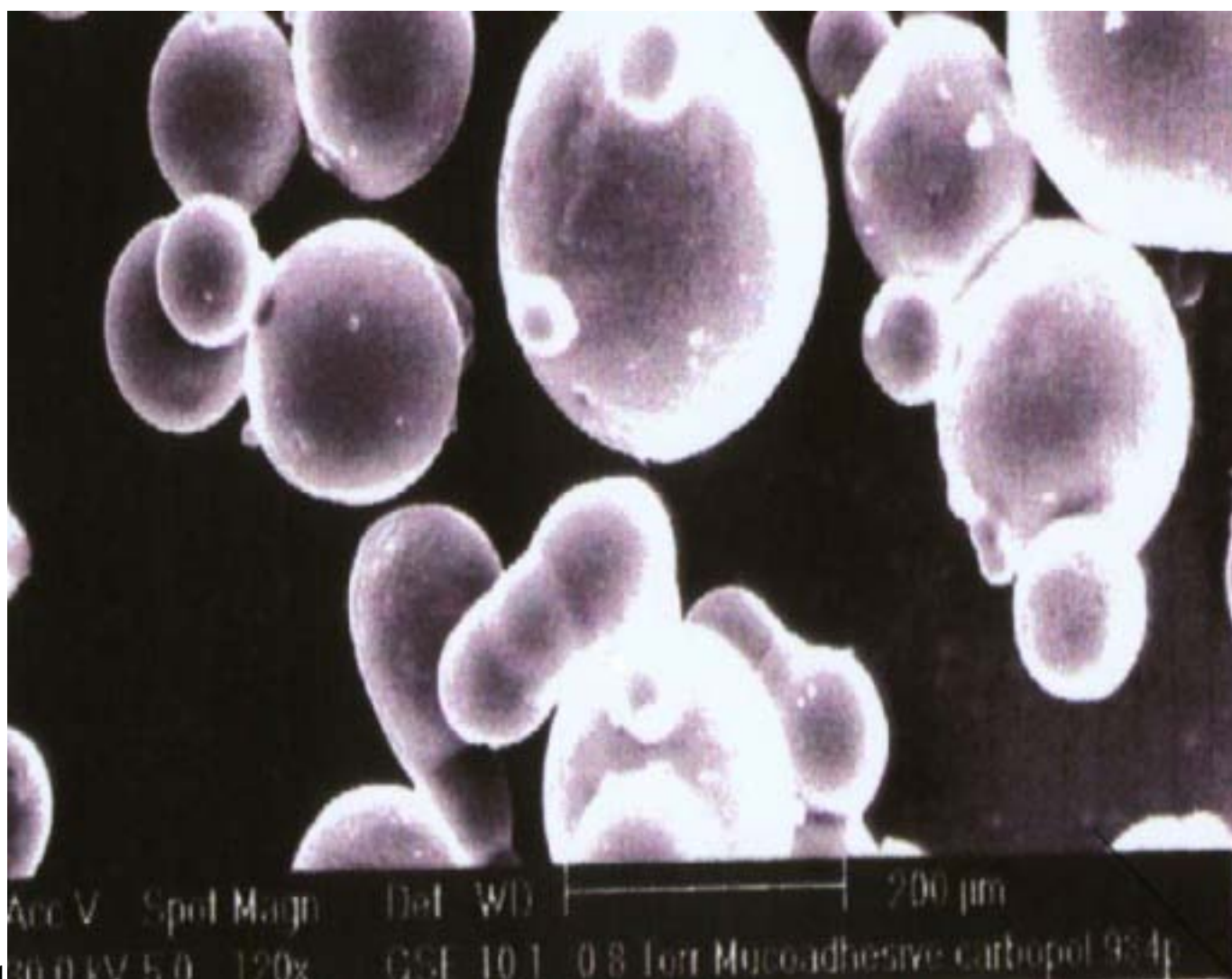
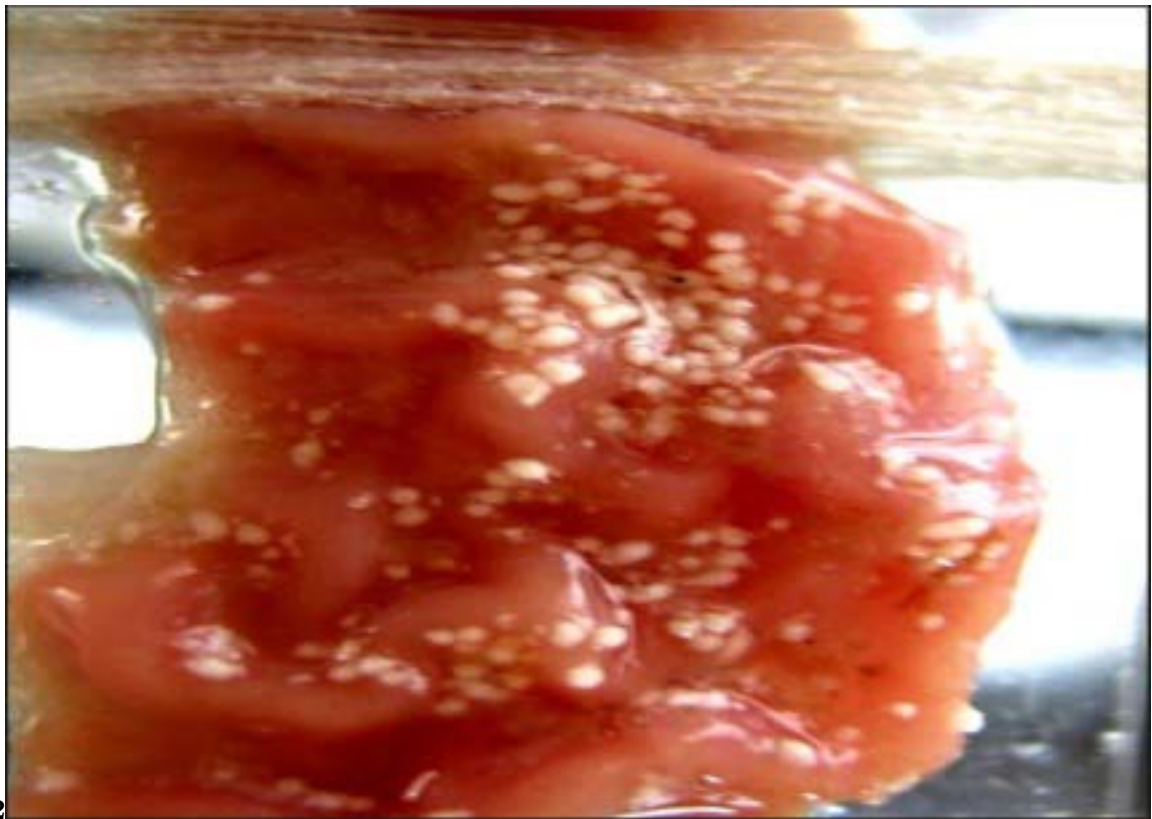


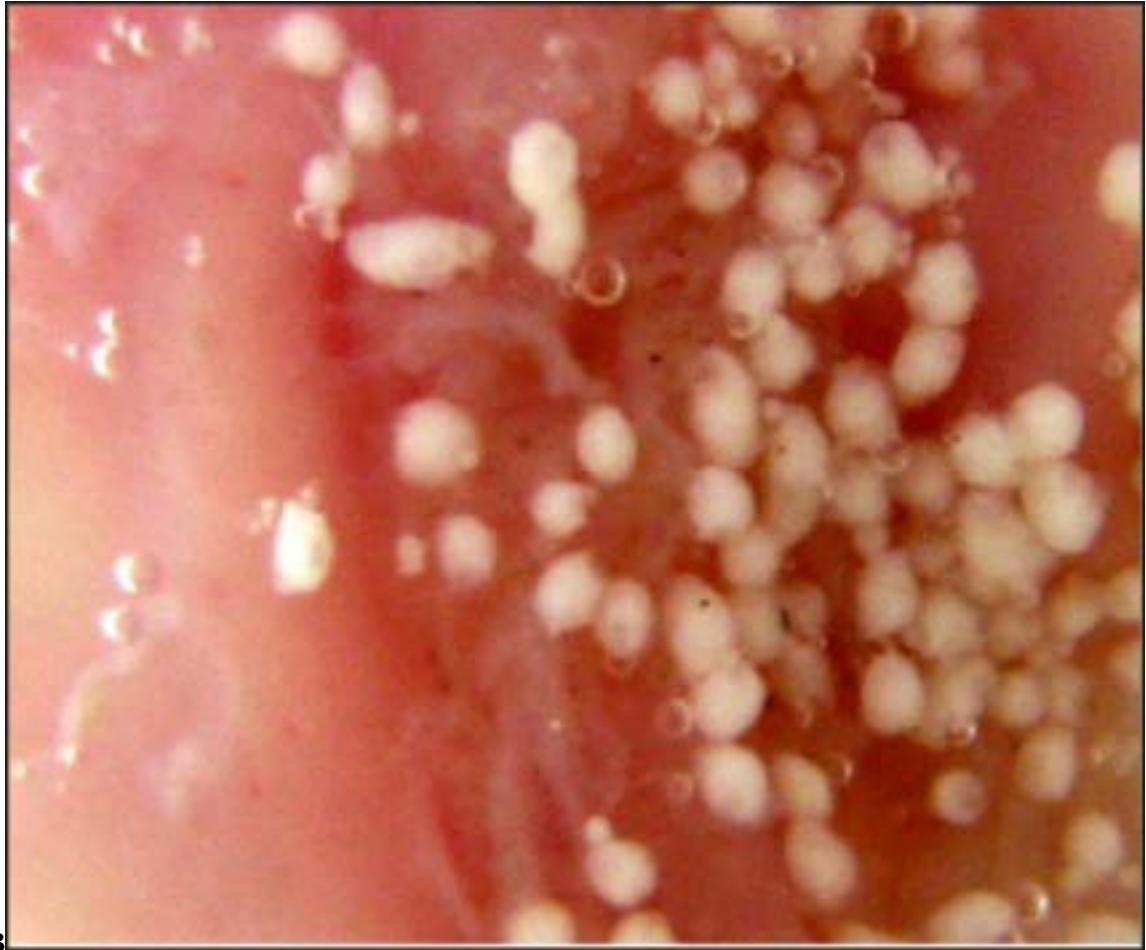
Figure 1: Figure 1 :

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Figure 2: Figure 2 :



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Figure 3: Figure 3 :



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Figure 4: Figure 4 :

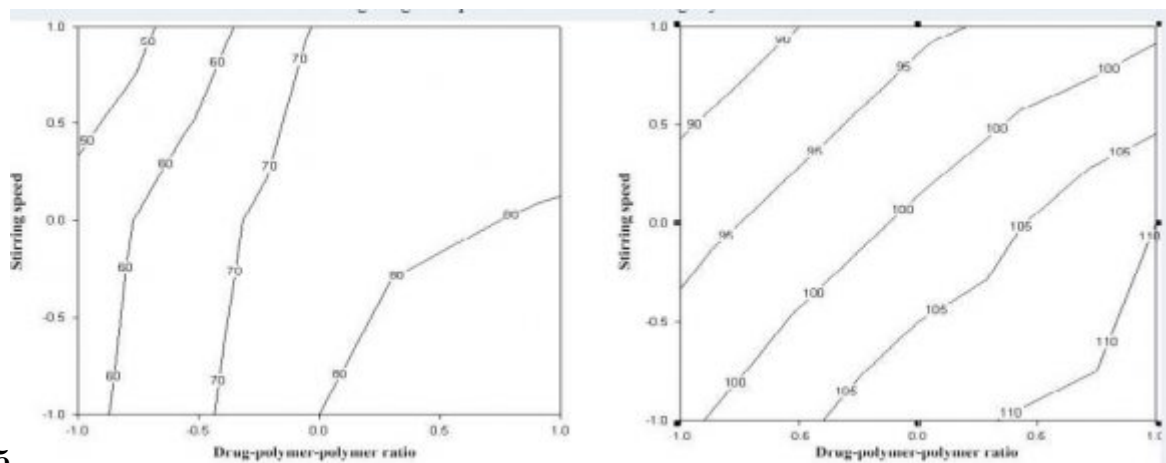


Figure 5: Figure 5 :

1

Batch code	Variable levels		In-vitro wash-off test mucoadhesion after 1 h)	wash- off (%)	Drug entrapment efficiency (%)	Particle size (?m)	t 80 (min)
	X 1	X 2					
F 1	-1	-1	58		25	100	589
F 2	-1	0	56		20	95	640
F 3	-1	1	42		29	88	720
F 4	0	-1	82		54	110	496
F 5	0	0	78		51	103	537
F 6	0	1	70		40	96	579
F 7	1	-1	92		45	115	294
F 8	1	0	80		40	111	306
F 9	1	1	74		33	102	333

Figure 6: Table 1 :

3

Coefficient	b 0	b 1	b 2	b 11	b 22	b 12	R 2
% Mucoadhesion	77.42	14.47	-8.19	-2.81	-9.60	-0.50	0.9809
Drug entrapment efficiency	48.14	7.71	-3.28	-4.57	-16.71	0.28	0.9165
Particle size	103.77	7.30	-6.60	-1.10	-1.01	-1.25	0.9955
t 80	533.41	167.50	43.88	-46.19	3.18	-23.49	0.9997

Figure 7: Table 3 :

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