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ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) 120–131
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Optimisation of polyherbal gels for vaginal drug delivery by Box-Behnken statistical design

Shruti Chopra *, Sanjay K. Motwani, Zeenat Iqbal, Sushma Talegaonkar, Farhan J. Ahmad, Roop K. Khar

Department of Pharmaceutics, Jamia Hamdard, New Delhi, India

Received 12 October 2006; accepted in revised form 13 December 2006
Available online 27 December 2006

Abstract

The present research work aimed at development and optimisation of mucoadhesive polyherbal gels (MPG) for vaginal drug delivery. As the rheological and mucoadhesive properties of the gels correlate well to each other the prepared MPGs were optimised for maximum mucoadhesion using a relationship between the storage modulus (G') and Gel Index (GI), by employing a 3-factor, 3-level Box-Behnken statistical design. Independent variables studied were the polymer concentration (X_1), honey concentration (X_2) and aerosil concentration (X_3). Aerosil has been investigated for the first time to improve the consistency of gels. The dependent variables studied were the elastic modulus, $G'(Y_1)$, gel index (Y_2), and maximum detachment force (Y_3) with applied constraints of $500 \leqslant Y_1 \leqslant 700$ and $4 \leqslant Y_2 \leqslant 5$. Response surface plots were drawn, statistical validity of the polynomials was established and optimised formulations was selected by feasibility and grid search. Three types of Carbopol studied were Carbopol 934P, Carbopol 974P and Polycarbophil. *In vitro* release studies were carried out for the optimised formulations and the data were fitted to release kinetics equations. Validation of the optimisation study with 8 confirmatory runs indicated high degree of prognostic ability of response surface methodology. Gels showed a gradual sustained release by a non-Fickian diffusion process. Incorporation of aerosil to gels was found to improve the rheological and mucoadhesion properties by about 50–54% and 7–11%, respectively. The Box-Behnken design facilitated the optimisation of polyherbal gel formulations for enhanced vaginal drug delivery by optimum mucoadhesion and longer retention.

Keywords: Optimisation; Box-Behnken design; Formulation; Polyherbal gels; Mucoadhesion

1. Introduction

For effective vaginal delivery of antimicrobial agents, the drug delivery system should reside at the site of infection for a prolonged period of time. From the various reported vaginal dosage forms, patients are known to better tolerate gels than inserts or ointments [1]. However, the direct application of gels onto the infected sites of the vagina might be difficult, inconvenient as well as have frequent dosing because the conventional gels do not remain for long time at the site of application.

In situ-gelling systems have been proposed as an alternative solution as they provide the more convenient liquid application in field of topical delivery. The liquid applied to topical areas turns into gels as a result of some physical and/or chemical change induced by physiological environments such as pH for cellulose acetate phthalate [1], the concentration of calcium ions for Gelrite [2,3], temperature for poloxamers [4–6], etc. Mucoadhesive drug delivery systems are reported to provide the advantage of intimate and prolonged contact of the formulation with diseased mucosa and high resistance to physiological removal mechanisms [7]. There are several ways to sustain the release of a drug from gels in order to take full advantage of the contact

* Corresponding author. Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110 062, India. Tel.: +91 11 2605 9688; fax: +91 11 2605 9663.
E-mail address: shrutichopra21@yahoo.com (S. Chopra).

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doi:10.1016/j.ejpb.2006.12.013

time. The drug can either be dispersed in the gel, giving a concentration that is higher than that corresponding to the solubility of the drug [8], formulated as particles [9,10], distributed in liposomes [11,12], or interacting with an oil phase that has been included in the gel [13].

Several materials capable of acting as adhesives have been investigated as bioadhesive materials [14–16], including sodium carboxy-methylcellulose, poly(acrylic acid), tragacanth, etc. The molecular characteristics required for bioadhesive materials have been recognised and can be listed as the presence of hydrogen bonding groups (OH, COOH), strong anionic charges, flexibility to allow penetration in the mucus network, surface tension properties appropriate for wetting the mucus or mucosal tissue surfaces, a high molecular weight and fast hydration capability, as this will allow the formation of the polymer interactions and entanglements necessary for adhesion [17].

Traditional medicines provide an interesting and still largely unexplored source for the creation and development of potentially new antimicrobial agents [18,19]. The primary and indispensable step towards this goal is screening of plants in popular medicine to provide rational means for many diseases that are obstinate and incurable with other systems of medicine. These are gaining popularity because of several merits such as often fewer side effects, better patient tolerance, relatively less expensive and better acceptance due to long history of use. Medicinal effects of plants tend to normalize the physiological function and correct the underlying cause of the disorder [20] and also they are often less prone to the emergence of drug resistance [21]. In our previous study, a novel antimicrobial combination (NAC) extracted from *Trigonella foenum-graecum* [22], *Azadirachta indica* [23], *Cichorium intybus* [24] and *Curcuma longa* [25] has shown a significant antibacterial activity in *in vitro* microbiological tests [26]. Development of this NAC into a suitable mucoadhesive drug delivery system in the form of a polyherbal gel was sought in order to provide the advantages of prolong retention and improved efficacy from vaginal route for local treatment of female-related conditions.

The evaluation of rheological properties for the gel type dosage forms is important for predicting their behavior in vivo. The rheological properties of eye gels were reported to affect the ocular residence time of the gels [1,10,27]. The flow properties of semi-solid vaginal dosage forms might be of use to predict the spreading and coating of the formulations over the vaginal epithelia. Especially for the mucoadhesive polyherbal gels (MPGs), the rheological characteristics need to be controlled and understood since the multi-component gels might exhibit complex flow behaviors due to the possible interaction among the components. The rheological properties of bioadhesive gels have been investigated by several authors [28–31], and it is reported that the prevalence of elastic modulus over viscous modulus (solid-like behavior) is highly desirable for mucoadhesive systems [32,33].

For prolonged retention on the mucous with sustained therapeutic effect, research efforts have been made on using hydrophilic polymers with bioadhesive characteristics to improve drug delivery by vaginal route [34]. The primary goal of bioadhesive controlled drug delivery systems is to localize the delivery device within the body to enhance the drug absorption process in a site-specific manner. Bioadhesion is affected by the synergistic action of the biological environment, the properties of the polymeric device, and the presence of the drug itself.

Aerosil 200 is chemically silicon di-oxide and is hydrophilic in nature. It provides a large surface area (200 m^2/g), which in contact with water immediately imbibes water and causes thickening of liquids. Although different aerosil grades have been used to regulate the rheological properties of marine paints [35], the present investigation is a first attempt to improve the rheological and mucoadhesion properties of hydrophilic Carbopol gels by using aerosil 200.

The purpose of the present study was to design and evaluate a new polyherbal NAC vaginal mucoadhesive drug delivery system for the local treatment of aerobic vaginitis using Carbopol® resins as they have well-proved excellent bioadhesive properties on the mucosal surfaces [36]. As the rheological and mucoadhesive properties of the gels correlate well to each other the prepared MPGs were optimised for maximum mucoadhesion using a relationship between the elastic modulus and gel index, by response surface methodology.

2. Materials and methods

2.1. Materials

Trigonella foenum-graecum, *Azadirachta indica*, *Cichorium intybus* and *Curcuma longa* were received as gift samples from Green Earth Products, New Delhi, India, and were assessed biologically by Department of Botany. Carbopol 934P, 974P, and Noveon AA-1 (polycarbophil) were provided ex-gratia by B.F. Goodrich (OH, USA). All three materials are structurally similar and are cross-linked acrylic acid polymers. The cross-linking agents for the whole Carbopol 900 series are allyl ethers of either sucrose or pentaerythritol [37]. Triethanolamine (S.D. Fine Chemicals, India) was used as neutralising agent. Mucin type III (partially purified from porcine stomach, Sigma, UK) was used as received and kept at 4 °C. Double distilled water was used for all studies. All other reagents and chemicals used were of analytical grade. Aerosil 200 (hydrophilic fumed silica) was kindly provided as free sample by Degussa AG, Germany.

Vaginal fluid simulant (VFS) was prepared from 3.51 gL^{-1} NaCl, 1.40 gL^{-1} KOH, 0.222 gL^{-1} $Ca(OH)_2$, 0.018 gL^{-1} bovine serum albumin, 2 gL^{-1} lactic acid, 1 gL^{-1} acetic acid, 0.16 gL^{-1} glycerol, 0.4 gL^{-1} urea, 5 gL^{-1} glucose and 15 gL^{-1} mucin. pH of the mixture was adjusted to 4.5 ± 0.02 using 0.1 M HCl [38].

2.2. Methods

2.2.1. Preparation of gels

Carbopol 974P (CP-974) gels were prepared by dispersing the polymer powder in small aliquots into stirred mixtures of neutralising agent and water. After continuous stirring at 1000 rpm for 5 min, the gel samples were left to hydrate completely and then centrifuged at 3000 rpm for 15 min to remove the air bubbles. The pH values were determined using digital pH meter (Mettler Instruments, Germany) with glass micro-electrode. The weight ratio between the polymer and the triethanolamine used was 1:1.6.

Different mucoadhesive polyherbal gel formulations of the 250 mg NAC were prepared using the following excipients: CP-974 (0.25–1.0 % w/w), honey (3–5% w/w), aerosil (0.5–2.0% w/w) and water (q.s. to 50 g).

The formulations were prepared using Box-Behnken experimental design and characterised for elastic modulus (G'), gel index (GI) and maximum detachment force (MDF) as defined later. The optimised formulation generated using statistical screening was prepared with three different Carbopol grades viz. Carbopol 934P (CP-934), Carbopol 974P (CP-974) and Noveon AA-1 (CP-AA1) for comparative evaluation of the rheological profile of MPGs with three polymers and for their comparative mucoadhesive potential.

2.2.2. Experimental design

Box-Behnken statistical screening design was used to statistically optimise the formulation parameters and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the MDF of mucoadhesive polyherbal gel formulations. Response surface methodologies, such as the Box-Behnken and Central Composite Design (CCD), model possible curvature in the response function [39,40]. A 3-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert® (Version 7.0.0, Stat-Ease Inc., Minneapolis, MN). The Box-Behnken design was specifically selected since it requires fewer runs than a CCD in cases of three or four variables. This cubic design is characterised by set of points lying at the midpoint of each edge of a multidimensional cube and center point replicates ($n = 3$) whereas the 'missing corners' help the experimenter to avoid the combined factor extremes. This property prevents a potential loss of data in those cases [41]. A design matrix comprising of 15 experimental runs was constructed. The non-linear computer-generated quadratic model is given as

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} 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 and X_i^2 ($i = 1$, 2 or 3) represent the interaction and quadratic terms, respectively [40,41]. The dependent and independent variables selected are shown in Table 1 along with their low, medium and high levels, which were selected based on the results from preliminary experimentation. The concentration range of CP-974 (X_1), honey (X_2) and aerosil (X_3) used to prepare the 15 formulations and the respective observed responses are given in Table 2.

2.2.3. pH Evaluation

The pH of the MPGs was recorded with a glass microelectrode (Mettler Instruments, Germany), by bringing it in contact with the MPGs and allowing it to equilibrate for 1 min. Experiments were performed in triplicate to check for the neutralisation of gels. The gels were also diluted with VFS in 1:1 ratio and the pH recorded. pH evaluation was carried out for all 15 experimental formulations and the optimised formulations prepared with three different Carbopol grades.

2.2.4. Rheological measurements

Two instruments were used to perform the rheological measurements, an AR 500 Advanced Rheometer (TA Instruments, England, UK) and a Brookfield Model DV-III+ Digital Rheometer (Brookfield Engineering Laboratories Inc., MA, USA).

For determination of G', thermorheological scans (from 30 to 90 °C at a rate of 10 °C/min) were performed on AR 500 Rheometer in the oscillatory mode, using a controlled-stress rheometer with the cone-and-plate geometry. The cone had a 20 mm diameter and an angle of 1° 58′ 58″. Truncation gap was 51 μ. The viscoelastic region was determined by torque sweep from 100 to 2000 μN m at a frequency of 1 Hz, and the torque of 1350 μN m was chosen for the frequency sweep analyses. Preliminary isothermal oscillatory runs were carried out at 25 °C, using a frequency range from 1 to 10 Hz. The equilibrium time before

Table 1
Variables in Box-Behnken design

Factor	Levels used, Actual (Coded)		
	Low (−1)	Medium (0)	High (+1)
Independent variables			
X_1 = polymer concentration (% w/w)	0.25	0.63	1.00
X_2 = honey concentration (% w/w)	3.00	4.00	5.00
X_3 = aerosil concentration (% w/w)	0.50	1.25	2.00
			Constraints
Dependant variables			
Y_1 = storage modulus, G' (Pa)			$500 \leqslant Y_1 \leqslant 700$
Y_2 = gel index, GI			$4 \leqslant Y_2 \leqslant 5$
Y_3 = maximum detachment force, MDF (g)			Maximize

Table 2
Observed responses in Box-Behnken design for polyherbal gel

Batch	Independent variables			Dependent variables (mean ± SD)			pH measurement	
	X_1	X_2	X_3	Y_1 (Pa)	Y_2	Y_3 (g)	Neutralised gels	Diluted gels (1:1)
1	−1	−1	0	547.7 ± 11.2	3.88 ± 0.35	20.4 ± 0.3	6.9	5.2
2	1	−1	0	695.4 ± 8.7	4.83 ± 0.48	27.8 ± 0.5	7.1	5.1
3	−1	1	0	588.7 ± 13.8	4.27 ± 0.23	22.5 ± 0.4	7.1	5.4
4	1	1	0	728.7 ± 4.9	5.31 ± 0.16	29.5 ± 0.3	7.0	5.0
5	−1	0	−1	565.2 ± 4.1	3.93 ± 0.09	21.3 ± 0.6	7.1	5.3
6	1	0	−1	703.3 ± 16.2	4.96 ± 0.19	28.1 ± 0.6	6.9	5.3
7	−1	0	1	568.7 ± 3.8	4.08 ± 0.07	21.9 ± 0.2	6.9	5.2
8	1	0	1	717.7 ± 13.7	5.11 ± 0.24	28.3 ± 0.4	7.1	5.5
9	0	−1	−1	519.4 ± 14.5	4.13 ± 0.17	26.7 ± 0.8	7.0	5.1
10	0	1	−1	586.2 ± 10.9	4.78 ± 0.29	30.1 ± 0.5	7.0	5.3
11	0	−1	1	528.3 ± 12.3	4.28 ± 0.13	27.1 ± 0.7	6.9	5.5
12	0	1	1	607.6 ± 8.8	4.69 ± 0.31	31.8 ± 0.3	7.0	5.2
13[a]	0	0	0	568.4 ± 16.2	4.28 ± 0.22	28.7 ± 0.8	6.9	5.2
14[a]	0	0	0	558.3 ± 11.4	4.38 ± 0.19	26.1 ± 0.5	6.9	5.5
15[a]	0	0	0	562.3 ± 5.6	4.32 ± 0.15	27.9 ± 0.2	7.1	5.0

[a] Indicates the center point of the design.

every measurement was 5 min and the sample volume used was approximately 1 mL. Calculation of G' was performed using rheology solution software (TA Instruments, USA). The tests were performed in triplicate, with a coefficient of variation of less than 10% being found.

For determination of GI, Brookfield Rheometer was used in which the test material is placed between two surfaces, one surface is rotated, and the torque resisting flow is measured. This allows the determination of relationship between applied shear rate and shear stress experienced by the test material. All the measurements were conducted using SC4-14 spindle using about 4 ml sample volume. The tests were performed in triplicate, with a coefficient of variation of less than 5% being found.

Since, *in vivo*, vaginal formulations will experience the dilution with vaginal fluids and it has been reported that the rheological behavior of gels could be affected by various factors such as copolymer compositions and solutes [6], the MPGs were also characterised for any change in G' after 1:5 dilution with VFS and after addition of the NAC to the blank neutralised gels.

2.2.5. Evaluation of mucoadhesive strength

The mucoadhesiveness of each formulation was determined by measuring the force required to detach the formulation (maximum detachment force, MDF) from Cellophane membrane treated with VFS [38] using a software-controlled penetrometer, TA-XT2 Texture Analyzer (Stable Micro Systems, UK) with a 5 kg load cell, a force measurement accuracy of 0.0025% and a distance resolution of 0.0025 mm. Gels were placed between two cellophane membranes hydrated with VFS and attached horizontally to upper and lower probes. The pre-test speed was set up at 1 mm/s, the test speed at 0.5 mm/s, and the penetration depth at 5 mm with an acquisition rate of 100 points/s. A downward force of 10 g was applied for 3 min to ensure intimate contact between the membrane and the sample. The probe was then moved upwards at a constant speed of 0.5 mm/s and the force required to detach the membrane (upper probe) from the surface of each formulation was determined as the peak value in the resultant force–time plot. The study was carried out at room temperature (25 °C) in five replicates for each sample with a coefficient of variation of less than 5% being found.

Since, *in vivo*, vaginal formulations will experience the dilution with vaginal fluids and it has been reported that the mucoadhesiveness of gels could be affected by various factors such as copolymer compositions and solutes [6], the MPGs were also characterised for any change in MDF after 1:5 dilution with VFS and after addition of the NAC to the blank neutralised gels.

2.2.6. In vitro release kinetics

Dissolution studies were performed using the USP XXVIII, paddle-rotating method (Electrolab dissolution tester, Electrolab, India) at 37 °C ± 0.5°C and 75 rpm using two dissolution media: phosphate-buffered saline pH 4.5 (PBS), and VFS. The gels were first packed into a dialysis bag and placed into the dissolution media. Dissolution studies were carried out in triplicate, maintaining the sink conditions for all the formulations. A 5 ml aliquot of sample was withdrawn at regular time intervals, filtered and assayed spectrophotometrically at 269 nm (Shimadzu 1601 UV–VIS Spectrophotometer, Japan). The cumulative % drug release was calculated for the formulations and the drug release data were curve fitted using *PCP Disso v2.08* software to study the possible mechanism of drug release from mucoadhesive polyherbal gels.

2.2.7. Optimisation data analysis and optimisation-model validation

Statistical validation of the polynomial equations generated by Design Expert® was established on the basis of ANOVA provision in the software. A total of 15 runs with

triplicate center points were generated. The models were evaluated in terms of statistically significant coefficients and R^2 values.

Various feasibility and grid searches were conducted to find the composition of optimised formulations. Various 3-D response surface graphs were provided by the Design Expert software. By intensive grid search performed over the whole experimental region, eight optimum checkpoint formulations were selected to validate the chosen experimental domain and polynomial equations. The optimised checkpoint formulations were prepared and evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with the predicted values to calculate the percentage prediction error. Also, linear regression plots between actual and predicted values of the responses were produced using MS-Excel.

2.2.8. Stability studies

Stability study of the optimised MPGs was carried out as per ICH guidelines at 25 °C ± 2 °C/60% ± 5% RH and 40°C ± 2°C/75% ± 5% RH. Physicochemical and rheological characterisation of the mucoadhesive gels was carried out over a period of 6 months at different time intervals of 1, 3 and 6 months.

3. Results and discussion

3.1. Characterisation of gels

The prepared gels were brown in color with slight aromatic odor. The pH of all the 15 gel formulations prepared as per Box-Behnken design and optimised formulations were found in the range of 6.8–7.2 after neutralisation with triethanolamine. After 1:1 dilution of gels with VFS the pH of the gels was found in the range of 5.0–5.5 (Table 2). The NAC content of the gel varied from 99.19% to 101.64%.

3.2. Rheological measurements

The principal parameter characterising the rheological behavior of mucoadhesive gels i.e. elastic modulus (G') for various formulations are presented in Table 2 along with the pH values of the different gels. Rheological results have been expressed in terms of the elastic modulus or storage modulus G', which is a measure of the energy stored and recovered per cycle of deformation and reflects the solid-like component of viscoelastic behavior of the material, while the viscous or loss modulus (G'') is a measure of the energy lost per cycle and reflects the liquid-like component [42]. The elastic modulus is closely related to the connectivity of the polymer network and is found to be directly proportional to the number of entities, which can support stress [43] including the physical entanglements and chemical bonds.

Unlike the Carbopols, polycarbophil (CP-AA1) is cross-linked with divinyl glycol and is insoluble in water after neutralisation. The lower G' for CP-AA1 as compared to CP-934 or CP-974 suggests the formation of a less elastic gel structure with polycarbophil. The content of –COOH groups is reported to be 56–68% and 62.5% for CP-934 and CP-974, respectively, whereas no information is available regarding the charge and cross-linking density of CP-AA1. Since the viscosity of mucoadhesive gels made with polyelectrolytes like Carbopol is primarily influenced by the number of charges present along the polymer chain [33,44], it is possible that CP-AA1 has a lower number of negative charges than CP-934 and CP-974. Less flexible network of CP-AA1 could also be caused by a higher cross-linking density of the polymer, which is known to affect the chain segment mobility of the polymer [45].

SC4-14 spindle was used for the viscometric characterisation of gels as the working range for this spindle as reported by manufacturers is 500–5000 cps (at 250 rpm spindle speed) or 1.25×10^7–1.25×10^8 cps (at 0.01 rpm). The decrease in viscosity of the gels observed with an increasing shear rates can be described well by an exponential function and hence the obtained data were analysed using the "Power Law" [46] and Ostwald-De Waele rheological model [47–50] as expressed by the following equations, respectively:

$$\sigma = K \cdot \gamma^n \tag{2}$$

$$\eta = K \cdot \gamma^{n-1} \tag{3}$$

where σ is shear stress; K is gel index (GI) or consistency index; γ is shear rate; η is viscosity; and n is pseudoplasticity index. 'Rheocalc 32' software was used to automatically apply the models to generated data and the value of GI was recorded.

The GI value for different formulations is presented in Table 2. When the GI of neutralised and VFS diluted gels (1:1) was compared, it was observed that the gel index is higher at neutral pH as compared to the acidic pH (4.0–5.5) of the vagina. This behavior can be explained by the fact that initially at acidic pH (about pH 3) the polymer chains exist in a spiral coiled form, exhibiting a relatively low viscosity. As the neutralisation progresses, the carboxyl groups of the Carbopol become ionized, causing an increased repulsion of negative charges. It leads the molecular structure to unwind and a gradual rise in viscosity.

Addition of aerosil not only improves the consistency of gels by increasing the elastic modulus of gels but also adds to smoothness and transparency of the gels. It could be related to the hydrophilic character of the aerosil 200 combined with a very large surface area, which is available for interaction with water and polymer chain molecules. Any increase in physical entanglement or formation of weak physical and/or chemical bonds like hydrogen bonds might be responsible for the increase in G' of mucoadhesive gels.

3.3. Evaluation of mucoadhesive strength

The results for MDF as obtained by mucoadhesion experiments are shown in Table 2. This has been done

in order to facilitate discussion of the relationship between the gel properties and bioadhesive performance and for optimisation of MPGs considering the three important response variables G', GI and MDF. In general, the mucoadhesive strength of CP-934 and CP-974 systems is higher than that of CP-AA1, which is in agreement with the finding of the studies by Tamburic and Craig [33]; and Dyvik and Graffner [31]. It is known that the expanded nature of the polymer network contributes significantly to the mucoadhesion strength, the prevalence of solid-like behavior (G') over liquid-like behavior (G'') is highly desirable in neutralised systems, where the repulsion between the charged group may expand the gel network to provide a higher mucoadhesion strength.

In vivo, vaginal formulations experience the dilution with vaginal fluids. The possibility exists that the rheological behavior and mucoadhesiveness of the formulations might be affected by dilution with environmental fluids [51]. The volume of vaginal fluids is reported to be about 0.75 ml [38] and the mucoadhesive gels would be applied in volumes of 1–3 ml, resulting in dilution when thoroughly mixed. To mimic the situation in the vagina, we diluted the gel formulations with VFS (1:5), and tested the influence of the dilution on G' and mucoadhesion in terms MDF. The dilution with simulated vaginal fluid reduced the elastic modulus, which can be ascribed to the breaking of gel structure on excessive dilution. MDF was almost unchanged or slightly higher with dilution and suggests that the dilution may infact help in the better wetting of the polymer and hence formation of strong mucoadhesive bonds either by physical entanglements and/or chemical bonds.

It has been reported that the rheological behavior and mucoadhesion could be affected by various factors such as copolymer compositions and solutes [6], the MPGs were also characterised for any change in G' and MDF after addition of the NAC to the blank neutralised gels. When the NAC incorporated gels were compared with blank neutralised gels, there was no statistically significant difference (t-test, $p > 0.05$), suggesting that the interaction of NAC with gel network is not significant at the dosage incorporated of the NAC into gels.

3.4. Fitting of data to the model

A three-factor, three-level Box-Behnken statistical experimental design as the RSM requires 15 experiments. The independent variables and the responses for all 15 experimental runs are given in Table 2. The 15 experimental formulations of MPGs were prepared using CP-974 polymer. Value of MDF was found to be significantly higher (27.8–30.1 g) only when the CP-974 is used at 0.50% or 1% concentration level. The ranges of other responses, Y_1 and Y_2 were 519.4–728.7 Pa and 3.88–5.31, respectively. All the responses observed for 15 formulations prepared were simultaneously fitted to first order-, second order- and quadratic models using Design Expert®. It was observed the best-fitted model was quadratic model and the comparative values of R^2, SD and % C.V. are given in Table 3 along with the regression equation generated for each response. Only statistically significant ($p < 0.05$) coefficients are included in the equations.

A positive value represents an effect that favors the optimisation, while a negative value indicates an inverse relationship between the factor and the response. It is evident that all the three independent variables viz. the concentration of CP-974 (X_1), honey (X_2) and aerosil 200 (X_3) have positive effects on the three responses viz. $G'(Y_1)$, GI (Y_2) and MDF (Y_3). The effect of aerosil on G′ and MDF was about 13- and 10-fold less, respectively, as compared to the polymer (CP-974) itself. However, the effect of aerosil on gel index or consistency was almost equivalent to that of polymer whereas it is about twofold as compared to effect of honey, which is in agreement with the hypothesis that the aerosil can be used to improve the consistency or gelling property of the mucoadhesive hydrophilic gels.

Coefficients with higher order terms or more than one factor term in the regression equation represent quadratic relationships or interaction terms, respectively. It also shows that the relationship between responses and factors is not always linear. Used at different levels in a formulation or when more than one factors are changed simultaneously, a factor can produce different degree of response. The interaction effect of X_1 and X_2 was favorable (positive) for response Y_2, whereas it was unfavorable (negative) for responses Y_1 and Y_3. Higher and positive

Table 3
Summary of results of regression analysis for responses Y_1, Y_2 and Y_3 for fitting to quadratic model

Quadratic model	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Response (Y_1)	0.9990	0.9972	0.9910	4.62	3.34
Response (Y_2)	0.9989	0.9968	0.9937	2.85	1.22
Response (Y_3)	0.9970	0.9923	0.9898	3.08	4.52

Regression equations of the fitted quadratic model[a]

$$Y_1 = 563.20 + 71.85X_1 + 27.58X_2 + 5.77X_3 - 2.1X_1X_2 + 3.0X_1X_3 + 3.0X_2X_3 + 77.45X_1^2 - 0.85X_2^2 - 1.8X_3^2$$
$$Y_2 = 4.33 + 0.51X_1 + 0.24X_2 + 0.45X_3 + 0.022X_1X_2 - 0.06X_2X_3 + 0.15X_1^2 + 0.098X_2^2 + 0.45X_3^2$$
$$Y_3 = 27.57 + 3.45X_1 + 1.49X_2 + 0.36X_3 - 0.1X_1X_2 - 0.1X_1X_3 + 0.32X_2X_3 - 3.27X_1^2 + 0.75X_2^2 + 0.6X_3^2$$

[a] Only the terms with statistical significance are included.

quadratic effects of X_1 and X_3 were observed for the responses Y_1 and Y_2, respectively. Quadratic effects of X_2 and X_3 were positive and almost similar for the response Y_3.

From these equations, it is quite clear that the aerosil plays an important role in the improvement of the gel index of MPGs and this can especially be employed in texture and rheological property enhancement of herbal gels.

3.5. Contour plots and response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are presented in Figs. 1–6, which are very useful to study the interaction effects of the factors on the responses. These types of plots are useful in study of the effects of two factors on the response at one time. In all the presented figures, the third factor was kept at a constant level. All the relationships among the three variables are non-linear, although Figs. 1 and 2 exhibit a nearly linear relationship of factor X_1 with factors X_2 and X_3, in the form of almost straight lines up to the medium

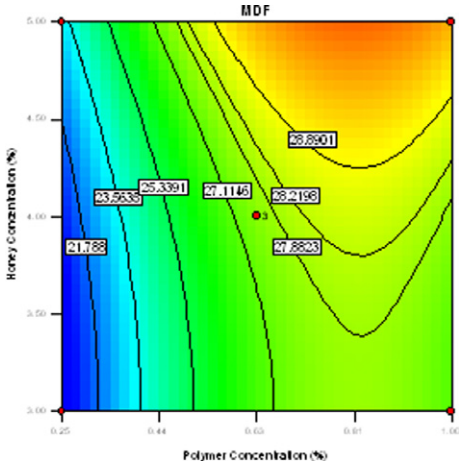

Fig. 1. Contour plot showing effect of polymer concentration (X_1) and honey concentration (X_2) on response Y_3.

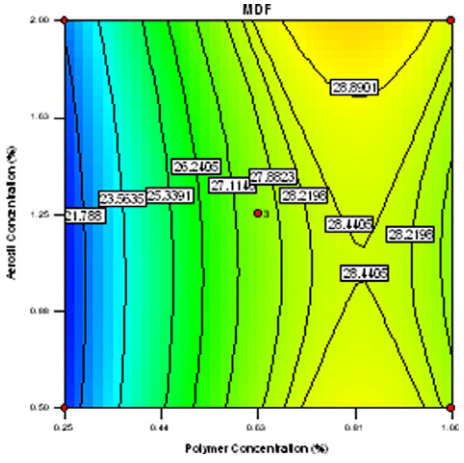

Fig. 2. Contour plot showing effect of polymer concentration (X_1) and aerosil concentration (X_3) on response Y_3.

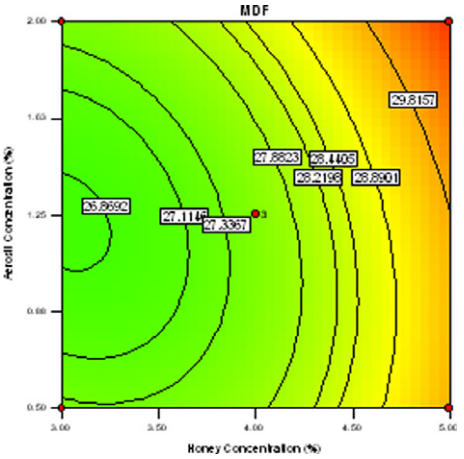

Fig. 3. Contour plot showing effect of honey concentration (X_2) and aerosil concentration (X_3) on response Y_3.

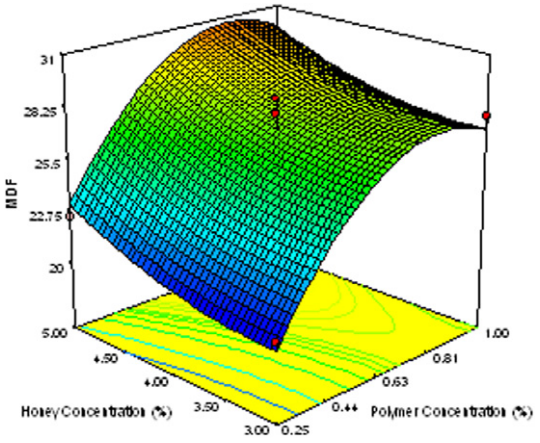

Fig. 4. Response surface plot showing effect of polymer concentration (X_1) and honey concentration (X_2) on response Y_3.

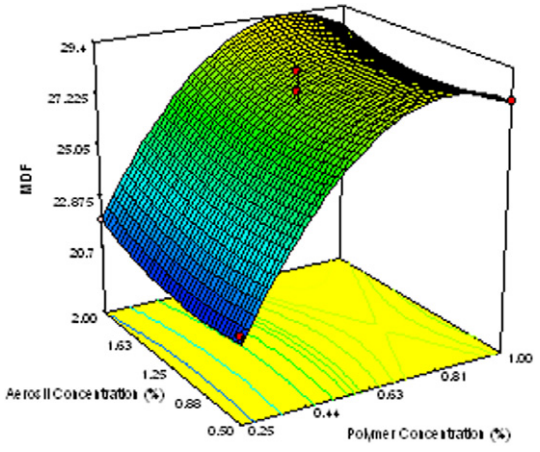

Fig. 5. Response surface plot showing effect of polymer concentration (X_1) and aerosil concentration (X_3) on response Y_3.

level of polymer concentration. At higher polymer concentrations these become curvilinear or non-linear. Factors X_2 and X_3 have non-linear relationship at all levels of the two variables (Fig. 3). Response surface plots show the

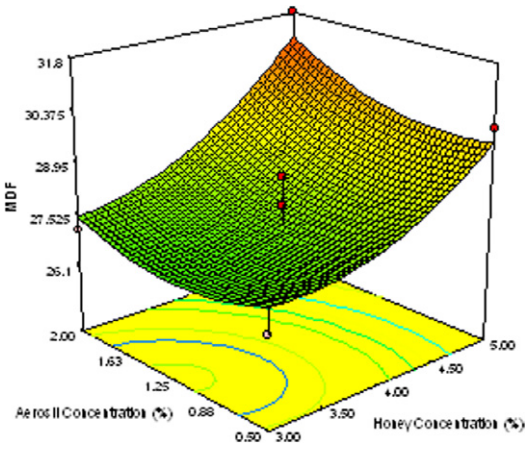

Fig. 6. Response surface plot showing effect of honey concentration (X_2) and aerosil concentration (X_3) on response Y_3.

relationship between these factors even more clearly. Figs. 4 and 5 show that the MDF increases with increasing concentrations of either honey or aerosil at constant concentration of polymer. Honey resulted in higher increase of MDF at 5% concentration as compared to increase with aerosil at 2% level. However, if used at equivalent concentrations of 2%, the increase in MDF is higher with aerosil, which supports the hypothesis that the aerosil being hydrophilic can be used to improve the rheological and mucoadhesive properties of Carbopol gels. Also the interaction effects of aerosil and honey were observed on MDF and the maximum MDF of 31.8 g was observed at 5% honey and 2% aerosil (Fig. 6).

3.6. Optimisation

The optimum formulation was selected based on the criteria of attaining the maximum value of mucoadhesion i.e. MDF by applying constraints on Y_1 ($500 \leqslant Y_1 \leqslant 700$) and Y_2 ($4 \leqslant Y_2 \leqslant 5$). Upon 'trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with polymer concentration of 0.76%, honey 4.50% and aerosil 1.32% was found to fulfill the maximum requisite of an optimum formulation because of optimum MDF considering the applied constraints on Y_1 and Y_2. The optimised formulation was prepared using three different grades of Carbopol viz. CP-934, CP-974 and CP-AA1; and the value of G', GI, MDF and pH observed are presented in Table 4.

The optimised formulation with three different grades of Carbopol was also prepared without aerosil to evaluate the % improvement by aerosil in G', GI, MDF and the observations are presented in Table 5. Aerosil was found to improve the rheological and mucoadhesion properties by about 50–54% and 7–11%, respectively.

3.7. In vitro release kinetics

In order to develop a mucoadhesive drug delivery system for localised and sustained vaginal delivery, it is necessary to check the release profile of MPGs in conditions simulating vaginal environment. Since, healthy human vaginal mucous is characterised by pH ranging between 4.0 and 5.0, [52] experiments were performed in the pH 4.5 phosphate buffer [53]. A variety of compounds from the vaginal fluid are known to affect the flow, retention, drug delivery kinetics, and bioactivity of therapeutic formulations applied vaginally. Therefore, *in vitro* release kinetics of MPGs was examined in the VFS, too. As can be seen in Fig. 7(a), at pH 4.5 a fast release of the NAC was observed within first hour at 37 °C ± 0.5 °C and sustained release in the following 23 h. Even after 24 h, about 17% of the drug still remained in the gels, regardless of the dissolution media used. When experiments were carried out in VFS, similar *in vitro* release profiles were observed as shown in Fig. 7(b), and there was no statistically significant difference (t-test, $p > 0.05$) between the release profiles of NAC from gels in PBS and VFS for the optimised formulation with three different Carbopol grades.

Table 4
Characterisation of optimised formulations with different Carbopol grades

Polymer used in MPG	Neutralised gels				Gels diluted with VFS (1:1)			
	G'	GI	MDF	pH	G'	GI	MDF	pH
Carbopol 934P	598.1	4.17	28.74	7.1	511.5	3.85	28.96	5.4
Carbopol 974P	648.3	4.89	31.26	7.1	637.9	4.26	31.87	5.1
Noveon-AA1	308.7	3.88	25.69	6.9	278.2	3.63	25.74	5.3

Table 5
Comparison of rheological and mucoadhesion properties of opimised formulations in presence and absence of aerosil

Polymer used in MPG	Neutralised gels with aerosil			Neutralised gels without aerosil			% Improvement with aerosil		
	G'	GI	MDF	G'	GI	MDF	G'	GI	MDF
Carbopol 934P	598.1	4.17	28.74	386.4	2.91	26.23	54.79	43.29	9.57
Carbopol 974P	648.3	4.89	31.26	429.1	3.46	29.03	51.08	41.33	7.68
Noveon-AA1	308.7	3.88	25.69	205.3	2.68	23.14	50.36	44.78	11.02

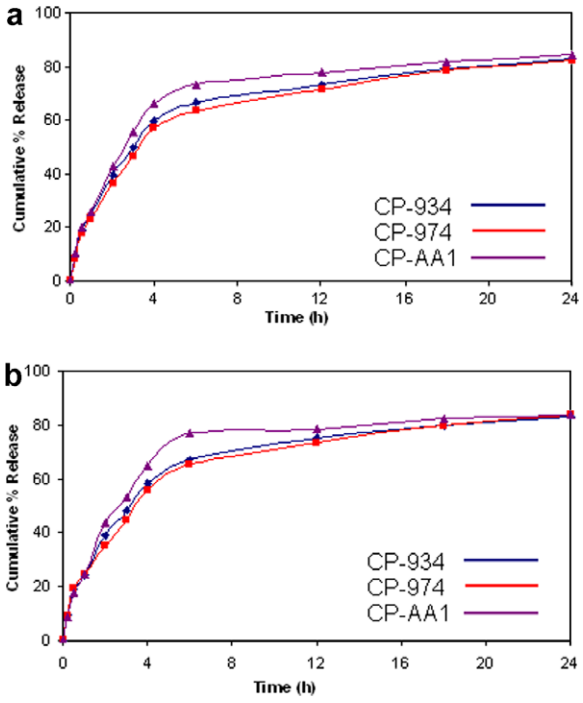

Fig. 7. In vitro release profiles of Carbopol mucoadhesive polyherbal gels in (a) PBS, (pH 4.5) and (b) VFS.

To study the release mechanism, various dissolution models including zero order-, first order-, Higuchi's-, Korsmeyer–Peppas- and Hixson–Crowell model were applied to the *in vitro* release profiles of the 15 different formulations. Table 6 shows the equations used to determine the appropriate models and presents the mean and standard deviation of R^2 values for all formulations.

Overall curve fitting showed that the drug release from mucoadhesive gels followed zero-order model ($R^2 = 0.9929$) for burst release during first hour and followed Korsmeyer–Peppas model ($R^2 = 0.9976$) for sustained release phase during later 23 h (the critical value of $n = 0.7266$–0.8593 suggesting non-Fickian diffusion). This is further supported by the fact that the combinations of polymer hydration, drug dissolution and polymer erosion determine the drug release from hydrophilic gels. Water-soluble drugs are released primarily by diffusion of dissolved drug molecules across the hydrated gel layer, while poorly soluble drugs are primarily released by polymer erosion mechanism [26,54]. Comparative evaluation of the release profile of gels with three polymers showed that the burst release was highest with CP-934 gels and was lowest with CP-974 gels, which can further be linked to the solid-like behavior G' of the gels and their comparative hydration rate. Burst release can be ascribed to dissolution of the drug present initially at the surface of non-hydrated gels but as the hydrophilic polymers imbibe water and the permeation of dissolution medium takes place in the hydrated gels, it initiates dissolution of drug from the inner layers.

3.8. Validation of response surface methodology

For all of the 8 checkpoint formulations, the results of the physicochemical evaluation were found to be within limits. Table 7 shows the composition of optimum check-

Table 7
Composition of checkpoint formulations, the predicted and experimental values of response variables and percentage prediction error

Optimised formulation composition (X_1:X_2:X_3)	Response variable	Experimental value	Predicted value	Percentage prediction error
0.76:5.00:1.98	Y_1 (Pa)	625.83	633.95	−1.28
	Y_2	4.99	4.91	+1.57
	Y_3 (g)	32.09	31.80	+0.93
0.80:4.97:2.00	Y_1 (Pa)	649.69	647.36	+0.36
	Y_2	4.86	4.96	−2.06
	Y_3 (g)	31.24	31.81	−1.79
0.82:5.00:1.85	Y_1 (Pa)	653.23	654.74	−0.23
	Y_2	4.87	4.99	−2.29
	Y_3 (g)	32.26	31.55	+2.26
0.76:5.00:1.55	Y_1 (Pa)	617.21	627.05	−1.57
	Y_2	4.99	4.87	+2.54
	Y_3 (g)	31.58	30.93	+2.11
0.82:5.00:0.79	Y_1 (Pa)	645.46	638.69	+1.06
	Y_2	4.89	4.99	−2.01
	Y_3 (g)	30.69	30.50	+0.62
0.90:4.53:2.00	Y_1 (Pa)	666.31	679.01	−1.87
	Y_2	5.06	5.00	+1.18
	Y_3 (g)	30.94	30.35	+1.93
0.92:4.36:2.00	Y_1 (Pa)	690.24	684.49	+0.84
	Y_2	4.93	4.99	−1.13
	Y_3 (g)	30.41	29.86	+1.84
0.83:3.00:0.50	Y_1 (Pa)	596.54	591.45	+0.86
	Y_2	4.36	4.43	−1.56
	Y_3 (g)	28.06	28.41	−1.23

Table 6
Dissolution model study by fitting in vitro release study[a]

Model	Equation	R^2 value for burst release phase (15 runs)	R^2 value for sustained release phase (15 runs)
Zero order	$m_0 - m = kt$	0.9929 ± 0.0716	0.8833 ± 0.0352
First order	$\ln m = kt$	0.9571 ± 0.0221	0.9481 ± 0.0115
Higuchi's model	$m_0 - m = kt^{1/2}$	0.9202 ± 0.0417	0.9641 ± 0.0216
Korsmeyer–Peppas	$\log(m_0 - m) = \log K + n \log t$	0.9106 ± 0.0318	0.9976 ± 0.0372
Hixson–Crowell	$m_0^{1/3} - m_{1/3} = kt$	0.9665 ± 0.0572	0.9314 ± 0.0311

[a] m_0 is the initial drug amount (100%, when represented as percentage); m the amount of drug remaining at a specific time (calculated as % of m_0); k the rate constant; t is the time.

point formulations, their predicted and experimental values for all the response variables, and the percentage error in prognosis. Percentage prediction error is helpful in establishing the validity of generated equations and to describe the domain of applicability of RSM model. Linear correlation plots between the actual and the predicted response variables were plotted and the residual plots, showing the scatter of the residuals versus actual values, are presented in Fig. 8.

For validation of RSM results, the experimental values of the responses were compared with the anticipated values and the prediction error was found to vary between −2.29% and +2.54%. The linear correlation plots drawn between the predicted and experimental values demonstrated high values of R^2 (ranging between 0.9839 and 0.9957) indicating excellent goodness of fit ($p < 0.001$). 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 RSM.

3.9. Stability studies

Stability studies on the optimised formulations were carried out as per ICH guidelines for 6 months. The stability samples were evaluated for pH, G', GI, MDF and *in vitro* release profiles after a period of 1, 3 and 6 months. The values obtained for G' and MDF of optimised formulations were found to be within ±5% of initial value whereas the pH and GI were within ±3% of initial value. Stability studies did not reveal any degradation of the NAC and also

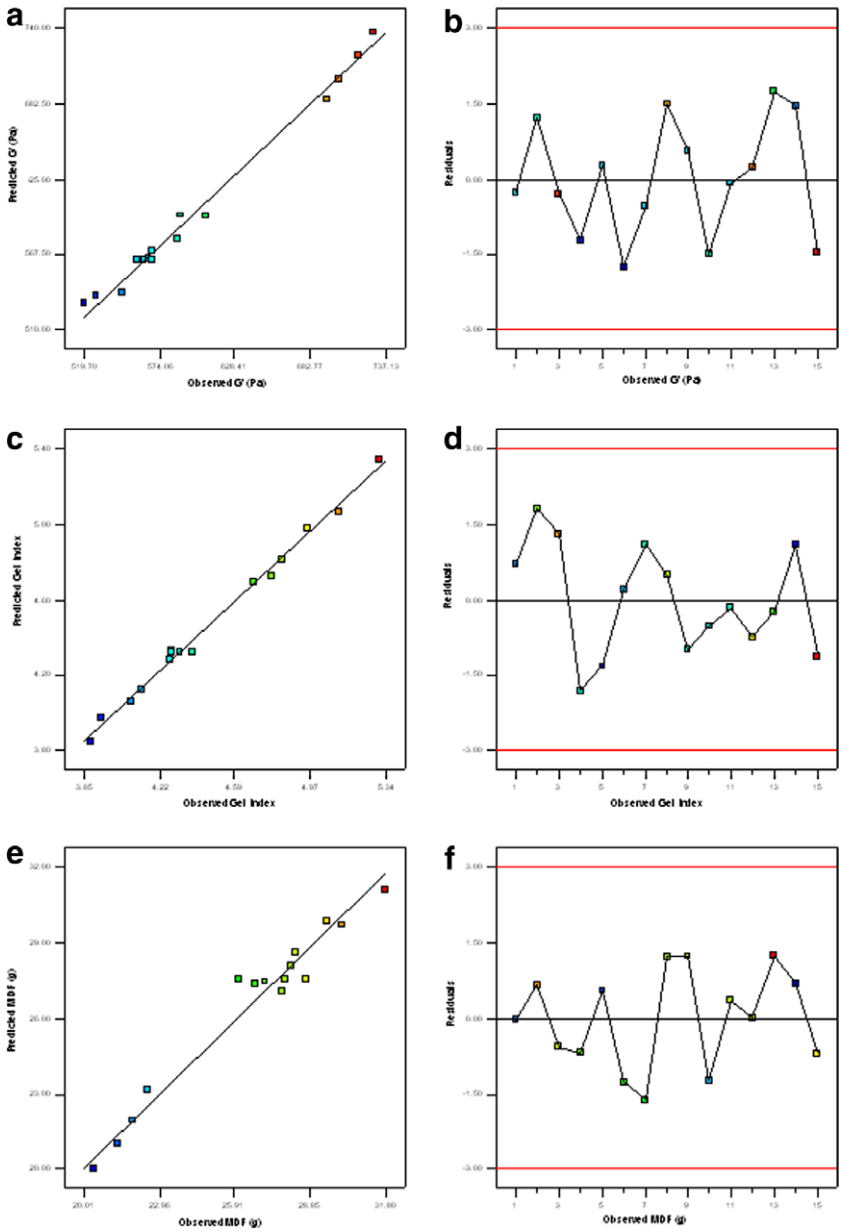

Fig. 8. Linear correlation plots (a, c, e) between actual and predicted values and the corresponding residual plots (b, d, f) for various responses.

changes in the *in vitro* release profiles of the optimised formulation after storage for 6 months were statistically insignificant as compared to the refrigeration control sample (ANOVA, $p > 0.05$).

4. Conclusion

Mucoadhesive polyherbal vaginal gels of a NAC using Carbopol 974P were prepared and optimised using a three-factor, three-level Box-Behnken design. The quantitative effect of these factors at different levels on the maximum detachment force could be predicted by using polynomial equations. Linearity observed between the actual and predicted values of the response variables suggested the prognostic ability of the RSM design. The optimised formulation was prepared with three different grades of Carbopol and the relationship between oscillatory rheology and mucoadhesive performance of the gels was studied. Stability study of the optimised formulation proved the integrity of the developed gels. Carbopol 974P gels were more consistent and provided better regulation of G' and MDF over a stability period of 6 months than Carbopol 934P and Noveon AA1 gels. Aerosil 200 was also found to contribute significantly to the improvement of consistency and rheological properties of gels. Thus, high degree of prediction obtained using RSM is quite efficient in optimising drug delivery systems that exhibit non-linearity in responses.

Acknowledgment

Authors wish to thank AYUSH, Ministry of Health and Family Welfare, Govt. of India, for providing financial assistance for this project.

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