ORIGINAL ARTICLE

Mucoadhesive Film for Local Delivery to Oral Cancer: Formulation Development, Box–Behnken Experimental Design, and *In vitro* Characterization

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Abstract

Background: Oral cancer affects millions of people worldwide, which is more common in those 35 and older. Low-targeted therapy options for oral malignancies and poor drug uptake by lesions in the oral cavity followed by systemic injection contribute to high mortality and poor patient quality of life. **Aim:** The present study was undertaken for optimization and assessment of the designed cisplatin mucoadhesive film made of hydroxyl propyl methyl cellulose and chitosan that can be used locally to treat oral cancer. **Materials and Methods:** The films were prepared by solvent casting method, employing a Box–Behnken design, and subsequently confirmed by ANOVA analysis. The formulations were optimized by swelling index and residence time. Physiochemical characteristics of oral films, including pH, weight, thickness, tensile strength, folding endurance, and *in vitro* drug release, were also assessed. **Results and Discussion:** According to the factors selected for the optimization, the formulation (F3) was selected as the optimized formulation with highest desirability of 0.997. *In vitro* release profile indicated initial burst release and subsequently a sustained release of cisplatin from the film for 24 h. The release kinetics data displayed a Korsmeyer–Peppas release model with the best-fit match R^2 value (0.9257). *In vitro* exposing the KB-3-1 cell line to the optimized film resulted in dose-dependent cancer cell death. The IC_{50} of free cisplatin and cisplatin mucoadhesive film (F3) on KB-3-1 was found to be 94.25 µg/ml and 23.64 µg/ml, respectively. This study demonstrates that local bioadhesive therapies are effective in treating cancer of the oral cavity. **Conclusion:** On the basis of data, it could be concluded that the number of polymers used was the critical factor for the production of cisplatin mucoadhesive film that had a substantial influence on their physical attributes. This factorial design study has served as a valuable tool for optimizing mucoadhesive film for the delivery of cisplatin.

Key words: Cisplatin, mucoadhesive film, optimization, quality by design

INTRODUCTION

Oral cancer is malignant neoplasia that impacts human health worldwide and ranks fifth among the top 10 etiologies of cancer-related deaths.[1,2] Tumors located in the tongue and floor of the mouth highly correlate with poor prognosis, affected by 5–10 years survival rate of 60% and 48%, respectively.[3] Tobacco and local consumption are linked to ~72% of oral cavity and pharynx cancers, while human papillomavirus is the second cause of the disease.[4] Treatment usually involves tumor resection and radiation, resulting in poor quality of life and long reconstructive efforts.[5] Since there are not any efficient commercially accessible drug delivery devices that completely solve these issues, this continues a significant unmet therapeutic need.[6,7] In recent decades, numerous scientific studies have shown that the most desirable form of treatment for oral cancer is targeted therapy which aims to deliver the drug to the specific site, thereby lowering the side effects and levels of systemic toxicity.[8] This study has driven by the hypothesis that local drug delivery to the tumor will reduce tumor size and improve treatment outcomes.

Numerous variables impede drug delivery across the oral mucosa, including rapid clearance of the drug or the

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delivery system due to bulk salivary flow and limited mucus permeability.[9,10] Mucoadhesive polymers can overcome these hurdles by adhering the drug delivery system to the oral mucosa for extended periods, through the formation of physical and weak chemical bonds between the delivery system and mucin.[11-13] Important aspect to consider for the development of mucoadhesive film is the contact time of the drug and cancer cells.[14-16] The residence time of mucoadhesive oral films on the oral mucosa has been enhanced to improve the drug partitioning to target tissue to overcome the oral mucosa penetration barrier.[17] As a result, these strategies may significantly advance the development of a sustained and targeted drug delivery system for oral cavity tissues.

Chitosan (CH) prepared buccal patches have outstanding mucoadhesive characteristics and a high capacity for drug absorption through the buccal mucosa.[18,19] Sivasankarapillai *et al*. prepared CH and hydroxypropyl methylcellulose (HPMC) films to deliver propranolol hydrochloride by solvent casting method using glycerol as the plasticizer.[20] The results showed that the CH-HPMC blend has acceptable mechanical properties and the optical examination revealed that the drug is evenly dispersed throughout the polymeric network. Diffusion study indicates that single polymeric formulations of CH and HPMC release drugs more quickly than CH and HPMC blend, demonstrating the importance of the polymeric molar ratio for controlled drug delivery from films.[20]

In patients with oral cancer, cisplatin is the first line of choice for post-operative chemotherapy.[21] Cisplatin is a well-known chemotherapeutic drug.[22-24] As a potential endpoint for a successful therapeutic result, Rui *et al*. did the Phase III trial of docetaxel, cisplatin, and 5-fluorouracil induction chemotherapy for resectable oral cancer predicts a favorable pathological response.[25] Quality by design is tried and tested systemic method that can be applied during the preliminary stages of formulation development, designing, and optimization.[26-29] In the present study, Box–Behnken design was implemented to optimize cisplatin mucoadhesive film with a high swelling index and residence time. The optimized film was evaluated for appearance, pH, thickness, weight uniformity, folding endurance, percentage moisture absorption (PMA), percentage moisture loss (PML), content uniformity, *in vitro* drug release, and *in vitro* cytotoxicity study.

MATERIALS AND METHODS

Materials

Cisplatin was procured from Biotechno Labs, New Delhi, India. o-phenylenediamine obtained from Merck (Germany). Cholesterol, HPMC, CH, polyvinyl alcohol (PVA), and polyethylene glycol 400 (PEG 400) were all purchased from Sigma-Aldrich. All other reagents, solvents, and chemicals used in the experiments were of analytical reagent grade.

Experimental design

For maximizing the experimental efficiency, a 3-level, 3-factor Box–Behnken design was used in the study. Using this method, minimal members of experiments were required to optimize the preparation of mucoadhesive films. The mucoadhesive films were formulated by solvent casting method and the influence of independent variables on the dependent variables was studied using Design Expert software (Version 10.0.1 Stat-Ease Inc., MN). The independent variables were the amount of HPMC: CH (ratio) (A), amount of PVA (B), and amount of PEG400 (C), as shown in Table 1. Other constraints, that is, the concentration of the drug, acetic acid, and method of preparation, were kept constant to minimize fluctuation. The above three factors were selected at three different levels low (-1), medium (0), and higher (+1), based on the literature survey. Seventeen runs of the experiment were evaluated for responses: Swelling index (Y_1) and residence time (Y_2). The data analysis was performed by ANOVA.

The polynomial quadratic equation generated from the Box–Behnken experimental design in Equation 1.

$$Y = \beta_0+\beta_1 A+\beta_2 B+\beta_3 C+\beta_4 AB+\beta_5 AC+\beta_6 BC+\beta_7 A^2+\beta_8 B^2+\beta_9 C^2 \quad (1)$$

where, Y is the dependent variables, β_0 is the intercept, β_1–β_9 the regression coefficients computed through the experimental values observed for the response, and A, B, and C are the coded levels of various independent variables. The terms AB, A^2, B^2, and C^2 indicate interaction and quadratic terms, respectively. The statistical validation of the polynomial equation was conducted with ANOVA, the statistical significance of coefficients, and R^2 values. $P \leq 0.005$ was considered statistically significant.

Preparation of mucoadhesive film

The oral mucoadhesive films were prepared by solvent casting method. Based on the literature, polymers such as HPMC: CH, PVA, and PEG 400 were used for the preparation

Table 1: Independent variables and the coded levels of the Box–Behnken design

Factors	Coded levels		
Independent variables	**Lower (-1)**	**Middle (0)**	**Higher (+1)**
A=Amount of HPMC: chitosan ratio	1:1	1.5:1	2:1
B=Amount of PVA (%w/v)	1	1.5	2
C=Amount of PEG (%w/v)	1	2	3
Dependent variables	Constraints		
Y_1=Swelling index (%)	Maximum		
Y_2=Residence time (min)	Maximum		

of the mucoadhesive film. The casting solution was a mixture of different ratios of these polymers to form a viscous mixture. The required percentage of the polymer solution was prepared by dispensing HPMC and CH in distilled water with continuous stirring. Films made with PEG and glycerol showed hygroscopic nature so PEG 400 was selected as the plasticizer. After complete hydration of the polymer with water, a drug complex solution containing other excipients was added and stirred. The solution was cast into a glass Petri dish and allowed to dry at room temperature (25°C) and 40–45% humidity for 48–72 h until a flexible film was formed. A weighed amount of ethyl cellulose was dispersed in 15 ml of chloroform containing a fixed concentration of the plasticizer (2% w/v diethyl phthalate) with continuous stirring using a magnetic stirrer. This solution was casted onto glass Petri dishes and kept in vacuum desiccators for drying for up to 24 h for the total removal of chloroform. The final oral mucoadhesive film is a composite of the above-mentioned two layers. The two layers: The mucoadhesive films layer containing the drug and the backing membrane layer were joined by spraying chloroform. Films were cut into 2 × 2 cm^2 size, wrapped in aluminum foil, and stored in the desiccator to avoid moisture loss and thus to maintain integrity and elasticity.

Optimization and validation

Design Expert software was used for graphical and numerical analysis to determine the best values [Table 1] for the variables based on the desirability criteria. For the evaluation of the selected experimental domain and polynomial equations, the optimum variables were utilized to build a preliminary mucoadhesive formulation and the predicted error was compared to the predicted values.

Characterization of mucoadhesive film

Swelling index

The swelling index procedure was used to determine the general swelling characteristics of the film. A drug-loaded film was cut into 2 cm^2 and weighed (W1) and allowed to swell in Petri dishes containing 10 ml of isotonic phosphate buffer solution (pH 6.8). Since, the pH of cancer cells is 6.8, the phosphate buffer solution of pH 6.8 has been chosen. The films were incubated at 30°C in distilled water for 30 min and stored at room temperature. After definite time intervals (30–60 min), the films were removed, and excess moisture was absorbed using tissue paper, until 4 h. Following this, the films were reweighed (W2), and the percentage of swelling was calculated using Equation 2.

$$Swelling\ index = \frac{(w2 - w1)}{w1} \times 100 \quad (2)$$

Residence time

The residence time ($n = 3$) of the oral film was determined after the application of the mucoadhesive film on freshly cut porcine buccal mucosa. The fresh porcine buccal mucosa obtained from a slaughterhouse was stored in Krebs' buffer pH 7.4 at 4°C after collection. A piece of cleaned porcine buccal mucosa was fixed on the internal side of a beaker using suitable glue. Each film was divided into portions of 2 cm^2 and cut, a side of each film was wetted with 50 ml of simulated saliva fluid (SSF) and was pasted to the porcine buccal mucosa by applying a light force with the fingertip for 20 s. The beaker was filled with 800 ml of the SSF (pH 6.8) and was kept at 37°C. After 2 min, a 100 rpm stirring rate was applied to simulate the oral environment, and residence was monitored for 8 h.

Physical appearance, weight, and thickness of the film

The film was observed visually for their physical appearance such as color, transparency, and texture. Weight uniformity was determined by weighing three films of each set individually using a digital weighing balance, and the average weight was determined with standard deviation. Each combined layer formulation was taken and the thickness was measured using a screw gauge at different points of the plane surface. The average film thickness was calculated.

Surface pH

The surface pH of the film was determined by allowing the film to swell by keeping it in contact with 1 ml of phosphate buffer (pH 6.8) for 1 h in a glass Petri dish. The surface pH was noted by bringing a combined glass electrode near the surface of the film for 1 min using a pH meter. The pH was recorded and the average of three determinations was calculated.

Folding endurance

Folding endurance determines the flexibility and mechanical strength of the mucoadhesive films. A non-flexible film without sufficient folding endurance leads to mechanical irritation, drug loss, and discomfort. The folding endurance was measured manually. A small strip of film measuring 2 cm^2 of each formulation was taken and folded at the same place till it broke or folded up to 250 times without breaking. The number of times a film could be folded at the same place gave the value of folding endurance.

Percent moisture absorption (PMA)

The PMA test was carried out to check the physical stability of the buccal films at high humid conditions. Three 2 × 2 cm^2 areas of films were cut out and weighed accurately then the films were placed in desiccators containing a saturated solution of aluminum chloride, keeping the humidity inside the desiccators at 60 ± 5% RH (relative humidity). After 3 days, the films were removed, weighed, and the average PMA of the three films was calculated using Equation 3.

$$\text{Percentage moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad (3)$$

The PML was also carried by taking three 2 × 2 cm^2 area films, weighed accurately, and kept in desiccators containing anhydrous calcium chloride. After 3 days, the film was removed, weighed and the average PML of three films was calculated using Equation 4.

$$\text{Percentage moisture loss} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad (4)$$

Morphology examination (scanning electron microscope [SEM])

The morphological examination of optimized cisplatin mucoadhesive film was performed by SEM with an accelerating voltage of 10 keV. The samples were mounted on metal stubs with double-sided adhesive tape and coated with a fine gold layer under a vacuum before obtaining the micrographs.

Drug content uniformity

The casted film was cut from three different places to evaluate drug content uniformity. Then, drug content measurement was carried out by completely dissolving each sample in 100 mL of distilled water (pH = 6.8). After filtration of solutions through 0.45 µm filter paper, drug content was then determined at 706 nm using the aforementioned derivatization method of UV–visible spectrophotometric for cisplatin. Equation 5 was used for the calculation of drug content uniformity.

$$\text{Content Uniformity} = \frac{\text{Actual amount of drug in film}}{\text{Theoritical amount of drug present in film}} \times 100 \quad (5)$$

In vitro drug release study

In vitro release of cisplatin from the optimized mucoadhesive film was performed by paddle over disk method using USP XXIV dissolution apparatus with 900 ml of simulated saliva pH 6.75 ± 0.05 as diffusion medium (simulated saliva was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, and 8.0 g NaCl in 1000 ml distilled water). The film selected was cut into specified sizes (2 cm × 1 cm) which were cut and adhered to the central shaft using a cyanoacrylate adhesive. During the release study, the temperature and rotation speed of the apparatus were maintained at 37 ± 0.5°C and 50 rpm, respectively. After designated time intervals (1, 2, 3, 4, 5, 6, 7, and 24 h), a 1 ml sample was taken, and a similar volume of fresh medium was added immediately following this to maintain a constant medium volume. Samples were filtered through a Millipore filter (0.45 mm) and were analyzed for the amount of cisplatin released using the UV–visible spectrophotometric method. To study the release mechanism of the optimized cisplatin mucoadhesive film, the data obtained from *in vitro* release study were fitted to various kinetic models including zero-order model, first-order, Higuchi, and Korsmeyer–Peppas models which were applied respectively. The correlation coefficient (R^2) for each model was calculated. Model giving near to 1 was selected as the best-fit model for the drug release.

Cell toxicity determination

The cytotoxicity potential of the fabricated oral film was evaluated by (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl-tetrazolium bromide (MTT) test. First, the oral film was exposed to UV irradiation for sterilization and immersed into the serum-containing medium at 37°C in a 5% CO_2 environment for 24 h. For the initial screening, confluent KB-3-1 cells were seeded into 96-well plates at 1 × 10^5 cells/ml and incubated for 24 h in a humified atmosphere of 5% CO_2 at 37°C. Then, the cells were treated with sample supernatants for 24 h. The supernatant was removed and 100 µl of MTT solution (5 µg/ml in the cellular medium) was added to the 96-well plates and incubated for 4 h at 37°C to allow the formation of blue formazan crystals. Finally, 100 µl of DMSO was added to each well and incubated for 20 min at 37°C. The optical density (OD) of the formazan solution was measured at 570 nm using the multiplate reader. Untreated cells were taken as control with 100% viability. The calculation of cell viability was calculated from the average OD values based on Equation 6. The IC_{50} was determined using GraphPad Prism (GraphPad Software Inc., USA). All the data are shown as the mean value ±SD of three independent experiments.

$$\text{Cell viability} = \frac{\text{OD values of sample}}{\text{OD values of control}} \times 100 \quad (6)$$

Statistical analysis

The experimental results of the study were expressed as mean ± standard deviation. The statistical significance of the data graphing and statistical analysis was performed using GraphPad Prism 9 (GraphPad Software Inc., USA) and Microsoft Excel (Microsoft Corporation, USA). $P < 0.05$ was considered statistically significant in the present study.

RESULTS AND DISCUSSION

The preparation of cisplatin-loaded mucoadhesive film was successful. In the current research, a novel mucoadhesive oral film of HPMC: CH was fabricated using the solvent casting method and was optimized to develop soft, flexible, and malleable films. As mentioned earlier, HPMC: CH polymer

is a suitable candidate for the preparation of a mucoadhesive oral film by virtue of its high mucoadhesive performance, biocompatibility, and non-toxicity. In addition, using solvent casting as a simple and cost-effective method for embedding the drug into the matrix can create oral films with the ability of controlled drug delivery while maintaining the above characteristics.

Data analysis and formulation optimization

The three polymers, HPMC, CH, and PVA, with PEG400 as a plasticizer, were used for the development of mucoadhesive oral films. The casting solution was a blend of these in various concentrations [Table 2].

Optimization was done by the response surface Box–Behnken experimental design. The formulations were prepared by independent variables, that is, HPMC and CH ratio (A), the concentration of PVA (B), and concentration of PEG400 (C), and two dependent/responses variables, that is, swelling index (Y1) and residence time (Y2). Using multiple linear regression analysis, different polynomial equations (β1–β9) with β0 as intercept were evaluated for best fitting to the experimental data by determining the values of coefficients in the polynomial equations. Considerable effects of each independent variable with their respective levels toward the dependent variable were predicted through analysis of variance (ANOVA).

The generated model for both the response variables obtained a high statistically significant *P*-value ($P < 0.0001$). The R^2 values ranging from 0.9949 to 0.9972 for all generated models indicate an excellent fit for the polynomial equation created for the response data. A "lack of fit" was found, to be insignificant for both models as $P = 0.3163$ and $P = 0.3802$, assuming that the proposed model was appropriate. The proximity of magnitudes in adjusted R^2 and predicted R^2 (ranges 0.9883–0.9935 and 0.9549–0.9731, respectively) also confirms the excellent fit of the data to the model generated. The summary of ANOVA results for the selected quadratic mixture model, that is, statistical *P*-values, R^2, adjusted R^2, and predicted R^2 for both responses, is shown in Table 3. Based on the criteria, the quadratic model was found to be best fitted to the observed responses.

Effect on swelling index

The hydration and swelling behavior of the polymer were reported to be crucial for its bioadhesive character because the former is necessary to initiate intimate contact of the film with the mucosal surface and consequently the drug release from the film.[30] All the prepared films showed swelling which initiated immediately after coming in contact with the aqueous medium. The percentage swelling of the mucoadhesive film containing different ratios of polymers is summarized in Table 2. The formulations with a higher concentration of HPMC and plasticizer showed a higher swelling. The higher swelling percentage of the formulation containing higher HPMC was due to the presence of more hydroxyl groups.

Table 2: Generated table of formulation composition and the effect on different formulation variables

Run	Formulation code	Factor 1 A: HPMC: CHITOSAN (ratio)	Factor 2 B: PVA (%w/v)	Factor 2 C: PEG (%w/v)	Response 1 R1: Swelling Index (%)	Response 2 R2: Residence time (min)
1	F1	1:1	1	2	18.2	281
2	F2	2:1	1.5	1	47.6	503
3	F3	2:1	2	2	53.1	519
4	F4	1.5:1	1	1	29.2	425
5	F5	2:1	1	2	46.7	504
6	F6	1.5:1	2	3	34.8	474
7	F7	1.5:1	1.5	2	32.9	468
8	F8	1:1	2	2	21.6	262
9	F9	1:1	1.5	3	19.3	276
10	F10	1.5:1	1.5	2	32.4	463
11	F11	2:1	1.5	3	49.1	521
12	F12	1:1	1.5	1	16.5	268
13	F13	1.5:1	2	1	31.4	473
14	F14	1.5:1	1.5	2	33.2	486
15	F15	1.5:1	1.5	2	34.1	482
16	F16	1.5:1	1	3	31.7	482
17	F17	1.5:1	1.5	2	34.2	472

Table 3: ANOVA summary table for the quadratic mixture model for responses

Source	R1: Swelling index	R2: Residence time
Unit	%	min
Standard deviation	0.88	10.27
Statistical *P*-value	0.0097	<0.0001
Lack of fit *P*-value	0.3163	0.3802
R-squared	0.9972	0.9949
Adjusted R-squared	0.9935	0.9883
Predicted R-squared	0.9731	0.9549
Model	Quadratic	Quadratic

According to the results, non-cross-linked HPMC: CH and PVA have the highest swelling ratio due to the presence of hydrophilic groups. The hydrophilic groups such as –OH, -COOH, and $–NH_2$ provide the possibility of hydrogen bonding formation and good water absorption properties. In addition, the development of the hydrogel network is caused by the presence of physical entanglements between the chains of PVA and CH. On applying the factorial, a quadratic model was suggested by the Design-Expert® software (Version 10.0.1). The final equation in terms of actual factors was found to be

$$\text{Swelling index } (Y_1) = 33.36 + 15.11 \times A + 1.89 \times B + 1.28 \times C + 0.75 \times AB - 0.33 \times AC + 0.22 \times BC + 1.44 \times A^2 + 0.0095 \times B^2 - 1.68 \times C^2 \quad (7)$$

The equation given above indicates that the concentration of HPMC in the film has a positive effect on the swelling index. This signifies that the swelling index of the film increases with an increase in the concentration of HPMC in the film. This observation is also consistent with the experimental data reported in the literature.[31] The response surface plot for the swelling index is shown in Figure 1. The lack of fit value was found to be not significant in the ANOVA model for swelling index indicating that the regression equation was well fitted.

Effect on residence time

Adequate residence is a prerequisite for the effective delivery of drug molecules from the film matrix into the oral mucosa. The values of residence time are presented in Table 2. The relationship between the swelling index and mucoadhesion time has been well established.[32] The residence time of the tested films ranged between 262 and 521 min. It could be implied that the increased swelling capacity of the film contributes to enhanced mucoadhesion. The residence time property was polymer dependent because as the concentration of the polymer increases, the residence time also increases. The presence of hydrophilic groups such as –OH, -COOH, and –NH2 and the formation of a physical hydrogel network between HPMC: CH and PVA chains increases the water absorption properties, leading to excessive swelling.

Application of factorial design suggested a quadratic model for the response variable which was found to be significant. The response surface plot for particle size is shown in Figure 2. The lack of fit value was found to be not significant in the ANOVA model for vesicle size, indicating that the regression equation was well fitted. In this case, A, C, BC, and A2 are significant model terms.

$$\text{Residence time } (Y_2) = 474.2 + 120.00 \times A + 4.50 \times B + 10.5 \times C + 8.50 \times AB + 2.50 \times AC - 14 \times BC - 77.10 \times A^2 - 5.60 \times B^2 - 5.10 \times C^2 \quad (8)$$

This equation indicates that the residence enhanced with an increase in the amount of HPMC: CH (A). Based on the literature, the mucoadhesion of the HPMC is attributable to the formation of physical (including hydrogen) bonds with the mucus components. It possesses a large number of hydroxyl groups that are responsible for adhesion. The effect of factors A, B, and C when put together can be understood by making use of 3D response surface plots [Figure 2].

Optimization and validation

The optimized mucoadhesive film was selected using the desirability function to get the most robust formulation with desired quality and characteristics satisfying the maximum target of all responses within the given constraints [Table 4]. The batch of the formulation was sorted with the highest desirability of 0.997. ANOVA was used to identify the significant effect of factors on response regression coefficients. Design space plots confirmed a suitable option of the possible region for optimized liposome formulations. The swelling index and residence time of the optimized batch were found to be 53.1% and 519 min, respectively. According to the criteria selected and calculations of desirability using Design-Expert®, formulation (F3) achieved the highest desirability of 0.997. Therefore, it was chosen for further investigations.

Characterization of mucoadhesive film

Mucoadhesive polymers, including HPMC, CH, and PVA, have potential in the development of film formulation as they form a swellable polymeric matrix to control drug release. The physical attributes of the films were characterized. As the CH polymeric solution was viscous, the developed films were pale yellow colored, opaque, and slightly hard, but varying the ratio of HPMC along with PVA and PEG400 improved the texture of the film and malleability. The prepared films were soft, flexible, and uniform in appearance. The developed films were suitable in terms of mechanical properties and malleability, with good esthetic and formulation performance. In the developed film formulations, a high concentration of HPMC causes the film most fragile and easily erodible due to the highest swelling of the HPMC polymer, whereas CH strengthened the polymer network and reduced the erosion capacity of the films, alongside a controlled release of the

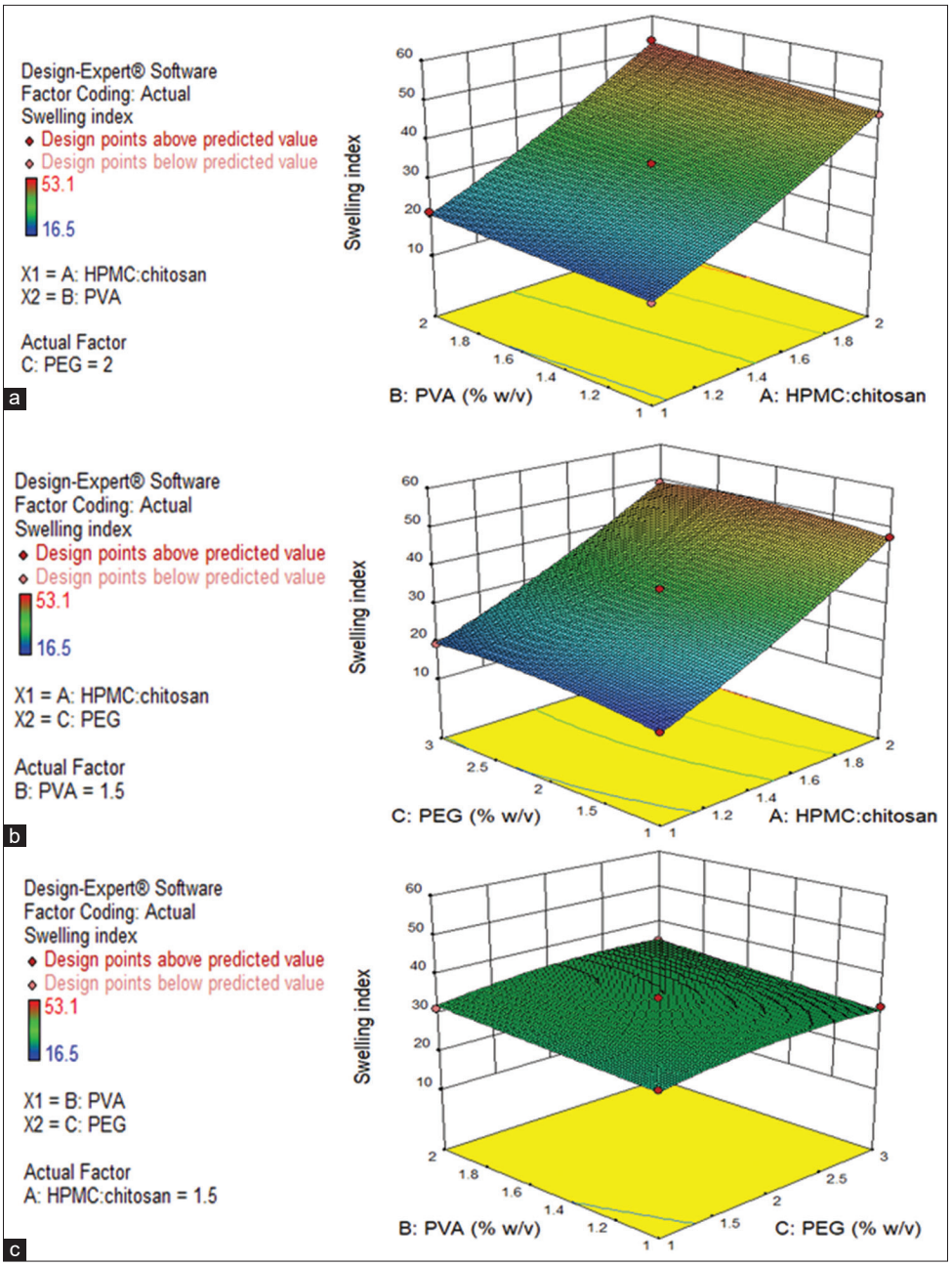

Figure 1: 3D response surface plot of (a) HPMC: chitosan-PVA, (b) HPMC: chitosan-PEG, and (c) PVA-PEG on concentration interaction on swelling index

drug. Films with different polymer concentrations were prepared (F1 to F17) and were characterized based on physiochemical properties. Table 5 shows the optimized film with its different properties with respect to average thickness, weight, pH, folding endurance, PMA, and PML.

The surface pH of the film was found to be 7.02 ± 0.08. This indicates the suitability of the film for application in oral drug delivery systems without any irritation. Regardless of the folding endurance, it appears mucoadhesive film possesses good flexibility. The folding endurance was found to have good folding endurance greater than 250 revealing satisfactory flexibility of the films. Checking the physical stability of the film at high humid conditions and integrity of the film at dry conditions, the films were evaluated for PMA and PML. The observed results of PMA and PML are shown in Table 5. SEM image was employed to investigate the surface morphology of the film, as shown in Figure 3. The cisplatin film exhibited smooth, porous, and homogeneous surfaces indicating good properties of film forming.

Drug content uniformity

The percentage of drug content from different places of the film was found to be 98.24 ± 12%. The observed result of content uniformity indicated that the drug was uniformly distributed throughout the film.

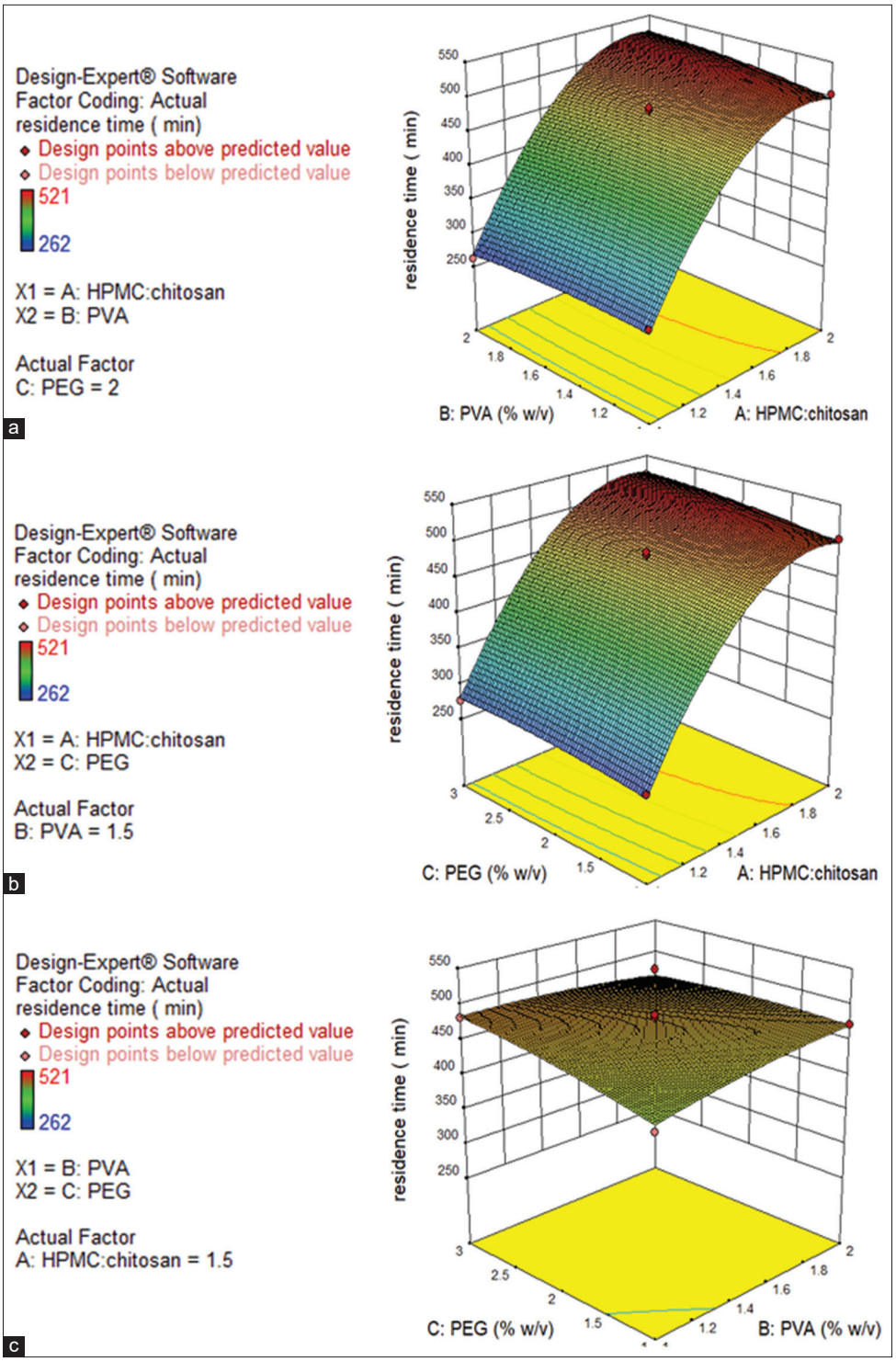

Figure 2: 3D response surface plot of (a) HPMC: chitosan-PVA, (b) HPMC: chitosan-PEG, and (c) PVA-PEG on concentration interaction on residence time

In vitro drug release

The release profile of cisplatin Figure 4 shows the release profile of cisplatin from HPMC: CH mucoadhesive film (F3). As can be observed in Figure 5, the drug release behavior of the film depicts a biphasic pattern, an initial rapid (burst) release phase followed by a slow (sustained) release phase. Within the first 2 h, the percentage of cumulative cisplatin released from the film reached 34.6%, indicating the burst release of cisplatin from the drug entrapped near the surface. The drug release after 24 h was found to be 88.4%. According to the obtained kinetics parameters, it is concluded that the Korsmeyer–Peppas release equation is the best model for the oral

Table 4: Constrains for optimization and predicted solutions of mucoadhesive film

A: (HPMC:Chitosan) (ratio)	B: PVA (% w/v)	C: PEG (% w/v)	R1: Swelling Index (%)	R2: Residence Time (min)	Desirability
2	2.0	2.0	53.1	519	0.997
2.000	2.000	2.352	52.855	523.515	0.997
2.000	2.000	2.335	52.855	523.591	0.997
2.000	2.000	2.391	52.852	523.332	0.997
2.000	2.000	2.406	52.850	523.251	0.997
2.000	2.000	2.442	52.841	523.062	0.996

Table 5: Characterization of optimized mucoadhesive oral film (*n*=3)

Physical appearance	Thickness (mm)	Weight (mg)	pH	Folding endurance	PMA (%)	PML (%)
Pale yellow, opaque, and flexible	0.54±0.04	34.2±0.14	7.02±0.08	>250	2.19±0.32	1.89±0.99

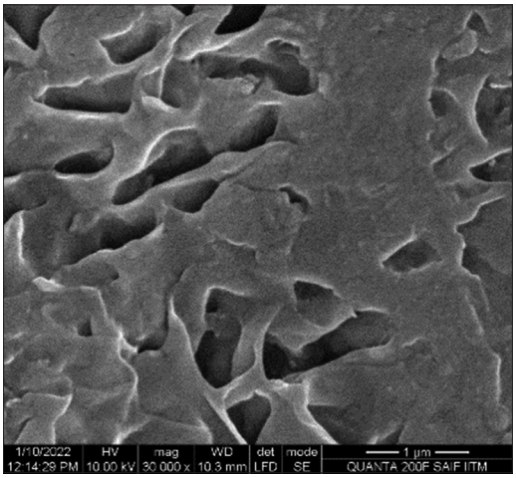

Figure 3: Scanning electron microscope micrographs of mucoadhesive film

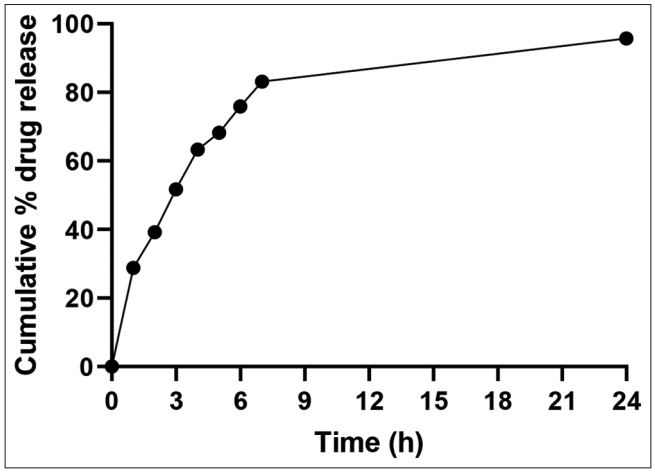

Figure 4: Drug release profile of optimized mucoadhesive film (F3) indicating 88.4% drug release after 24 h

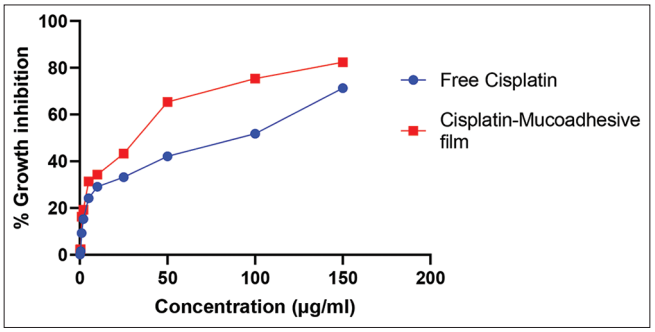

Figure 5: % cell inhibition of the free drug and cisplatin-mucoadhesive film on KB-3-1 cell line

mucoadhesive film with a higher R^2 (0.9257) value than those of other models.

Cytotoxicity evaluation

An *in vitro* cytotoxicity screening was performed by MTT assay on the KB-3-1 cancer cell line to establish the cytotoxic effect of the formulation. The formulation was found to be more cytotoxic at all concentrations. Determination of half-maximal inhibitory concentration (IC_{50}) values was done using GraphPad Prism 9. The IC_{50} of free cisplatin and cisplatin mucoadhesive film (F3) on KB-3-1 was found to be 94.25 µg/ml and 23.64 µg/ml, respectively. This result showed a good cytotoxic effect on the cancer cells.

CONCLUSION

In this study, cisplatin mucoadhesive film with the potential for oral cancer targeting was successfully developed by solvent casting method and optimized by Box–Behnken design. With 17 runs generated through factorial design, the resultant

polynomial equations, and response surface plots, the optimum formulation with the desired properties could be prepared. On the basis of the data, it could be concluded that the number of polymers used was the critical factor for the production of cisplatin mucoadhesive film that had a substantial influence on their physical attributes. This factorial design study has served as a valuable tool for optimizing mucoadhesive film for the delivery of cisplatin. The initial choice of composition was to strike a balance between swelling, residence, and drug release. HPMC: CH and PVA have a significant effect in the water absorption and swelling behavior of the film because of their hydrophilic nature and the formation of hydrogen bonding with mucosa. On the basis of experimental results, it can be deduced that the selling index, residence time, and pH of the mucoadhesive film are suitable for oral cancer. Results of the kinetic analysis of the dual drug delivery system for both drugs well fitted to the Korsmeyer–Peppas model. Furthermore, the oral film indicated excellent anticancer activity on KB-3-1 cancer cells. In addition, oral films showed excellent cell biocompatibility without any irritation to the oral mucosa. In conclusion, the fabricated mucoadhesive film can be used for clinical applications in oral cancer.

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