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Optimization of metronidazole SR buccal tablet for gingivitis using genetic algorithm

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Abstract: Gingivitis is a condition that needs sustained concentration of antibiotic locally over extended period of time. The current study aimed to formulate and evaluate the sustained and localized release of metronidazole (MTZ) as mucoadhesive buccal tablet containing hydroxypropylmethyl cellulose (HPMC), Carbopol 940® (CP), carboxymethylcellulose (CMC) and ethyl cellulose (EC) as mucoadhesive polymers. Tablets were directly compressed with proportions of polymeric blends (F1-F16). The results indicated that weight variation (249±2.10mg) and friability (0.21%) were within USP compendial limits. Maximum mucoadhesive strength and time were depicted by F1 and F14 which were 28.47g and 12hr respectively. Formulations, except F4, were within physiological pH limit. Maximum swellability index (261.9%) was exhibited by F16, at 8 hr, containing highest concentration of CP, HPMC and additional CMC. For *in vitro* release, the pre-set 8 hr complete release were shown by formulations, F15 and F16 which were 100% and 97%, respectively. Genetic algorithm was applied on the attributes to optimize polymeric response in accordance with desirability. The software predicted composition (F17) was tested which revealed that physical characteristics were in accordance with the compendial standards. The release kinetics, evaluated through DDsolver®, suggested that release of MTZ followed non-Fickian diffusion type in Korsmeyer-Peppas model. Therefore, MTZ, if delivered as mucoadhesive buccal formulation (F17) containing amounts (mg) of CP (16.4), HPMC (78.7), CMC (8.3) and EC (10.5) will simulate satisfactory release i.e. 96% at 8 hr in simulated buccal fluid.

Keywords: Metronidazole, mucoadhesive buccal tablet, optimization, ethylcellulose tablet, genetic algorithm.

INTRODUCTION

The oral cavity is a dynamic environment containing normal microbial flora, primarily bacteria (500 species approximately) and cavity infections occur when conditions are in favor of microbial residents (Xu *et al.,* 2015). Oral cavity ailments including dental caries and periodontal disease prevailed in approximately 90% of the world's population during any time point of their life. The therapeutic goal is to abate and control disease progression through mechanical cleaning of plaque and added antimicrobial agents. Treatments like mouth washes, fluoride pastes may be used but these chemicals alter taste buds (Bansal *et al.,* 2015). Antibiotic therapy is a cornerstone in periodontal infections. Metronidazole (MTZ) is a broad-spectrum antibacterial and antiprotozoal agent and is considered effective due to its selective efficacy against obligate anaerobes in periodontal infections (Razzaq *et al.,* 2018) where the dose of 20 mg has been used for local mucoadhesive drug delivery. Buccal mucoadhesive buccal route is preferred over systemic delivery, to avoid conventional high doses of drug (normally 400 mg t.i.d) compared with local dose and undesirable ranging from vision problems and gastrointestinal ulcer to seizures and neck stiffening. Unlike sublingual route, the buccal mucosa is less permeable and does not allow drugs to reach systemically rapidly; thus preferable for local action, if required (Montenegro and Morales, 2017). Additionally, buccal mucoadhesive delivery also possesses advantages like: i) bypass from intestinal first pass effect ii) direct removal of dosage form in case of any over dose and iii) apart from systemic delivery, it can also be designed to target the local release of drug(Graziani *et al.,* 2017). Aided by mucoadhesion, buccal delivery persists in close contact with oral cavity thereby enhancing the contact of the antibiotic to the affected places in buccal region (Sawant and Khan, 2017).

The gel matrix provides sustainability and mucoadhesion to the dosage form which provides retention of dosage form in the buccal cavity (Hussain *et al.,* 2016). For mucoadhesive polymers, hydroxypropylmethyl cellulose,

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carbopol and ethyl cellulose have been widely evaluated for mucoadhesion. Carbopol is strongly bonded with mucus and become localized to target site. Carbopol has gelling effect as similarly, like HPMC, upon hydration uncoils the chain and imbibe water molecule among polymeric chains forms gel and provide sustainability to dosage form and control release. Its important features are high swellability, sustained effect and satisfactory mucoadhesion (Caccavo *et al.,* 2015). Ethyl cellulose is also a sustained agent with defined mucoadhesive behaviour; however it is hydrophobic in nature (Yang *et al.,* 2016). Similarly, carboxy methylcellulose also posseses hydrophilic mucoadhesive nature . Among other cellulose derivatives HPMC has been widely used by researchers as it possesses great strength to retard the release and give prolong effect (Russo *et al.,* 2016).

The current research was designed to formulate and optimize oral mucoadhesive sustained release buccal tablet to deliver MTZ locally against gingivitis. The research was carried out in two phases i.e. the optimization study and the confirmation study. In the optimization study, different formulations were set using different amounts of mucoadhesive polymers to attain the physicochemical characteristics of the formulations. Such results were then used as input for the software to optimize the response of the polymers on physicochemical characteristics using historical design with genetic algorithm technique, in a lab generated software NeuronPower®. The prediction was then retested in the second phase i.e. confirmation formulation.

MATERIALS AND METHODS

Materials

Metronidazole (MTZ) was attained form Remington Pharmaceuticals (Pvt.) Ltd (Pakistan) as a gift sample. Hydroxypropylmethyl cellulose k4M (HPMC) was obtained from Servier Research Pharmaceuticals (Lahore, Pakistan) on kind basis. Similarly, carbopol 940® (CP), ethylcellulose (EC), magnesium stearate, carboxymethylcellulose (CMC) and sucrose were gifted by Harmann Pharmaceutical Pvt. Ltd. (Lahore, Pakistan). All agents, wherever used in the study, were of Merck analytical grade.

Fabrication of buccal tablets

All formulations with variable composition of mucoadhesive polymer were fabricated by direct compression method. All Ingredients were blended geometrically and shaken vigorously for 5 minutes before compression. Mixed ingredients compressed using single punch manual tablet machine at a pressure of 2 tons for 30 sec, having flat faced punch. 20 tablets were prepared for each formulation batch. After formulation, tablets were subjected to quality evaluation. The final weight of each tablet was 250 mg.

Physical characterization of mucoadhesive formulations (F1-F16)

Various tests were applied on the prepared formulation i.e. weight variation, diameter and thickness.

Weight Variation

The average of 20 tablets was calculated according to the procedures as prescribed and deviation was checked in accordance with the United States Pharmacopeia 28.

Thickness, hardness and diameter

The thickness and diameter for each formulation was calculated with Vernier Caliper and results were expressed in terms of standard deviation. Hardness of the tablet from each formulation was performed on using semi-automated hardness tester MGT 2020 (Hanif *et al.,* 2014).

Friability

Roche Friabilator® was used to estimate the percentage loss. Initially, preweighed tablets were run at 25 rpm for 4 minutes. After it, tablets were dusted and reweighed. The loss of ingredients was expressed as percentage (%) loss as depicted in Equation 1 (Husain *et al.,* 2016).

$$\text{Percentage loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \qquad \text{Eq. 1}$$

Physicochemical characterization of mucoadhesive formulations (F1-F16)

Surface pH

The surface pH for each tablet from individual batches was assessed by placing in phosphate buffer solution (PBS) pH 6.8. The pH value was recorded after 2 hr by touching electrodes of pH meter to the surface of tablet and noted the reading as meter was stable (Mylangam *et al.,* 2016).

Swelling index

Each initially preweighed dry tablet were placed in separate petri dishes containing 10 ml PBS pH 6.8. The extent of medium imbibition was analyzed by estimating the weight gain of tablet at defined interval i.e. 0.5hr, 1hr, 2hr…till 8 hr. This weight gain was considered as swelling index in accordance with Equation 2.

$$\text{Swelling index} = \frac{\text{Final swelling - Initial swelling}}{\text{Initial swelling}} \times 100 \qquad \text{Eq.2}$$

In vitro mucoadhesive time

To estimate *in vitro* mucoadhesive time, a method devised in the literature was used (Hussain *et al.,* 2016). One face of each tablet formulation was slightly wetted with distilled water adjusted to 6.8and pressed gently for about 20 sec against the surface of glass slide upon which freshly prepared cut rabbit buccal mucosa was fixed. Placed the slide in a beaker containing 900 ml distilled

(adjusted pH 6.8 at 37 °) water at an approximate angle of 45°. The speed of magnetic stirrer was set at 100rpm at which system was rotated. . The moment after which the tablet is detached from the surface was measured as the mucoadhesion time.

In vitro mucoadhesive strength

The evaluation of *in vitro* mucoadhesive force of each tablet was through a modified physical balance (Hanif *et al.* 2017). One pan of the balance was removed and a slide was fixed on the static base stage through a thread. The other arm was used to measure the force of detachment through added weights. To start with, both surfaces of the tablet were wetted with drop of PBS pH 6.8, in such a way that the tablet was sandwiched between both glass slides. One glass slide was kept static and fixed to the base, while other slide was attached with thread. The mucoadhesive strength was considered as the minimum weight required detaching the tablet from the mucosa.

In vitro MTZ release

The USP type II dissolution paddle apparatus (Erweka DT-700) was used to study the dissolution and MTZ release from mucoadhesive buccal formulations. Dissolution media containing 900 ml of buffer solution of pH 6.8 was filled in the apparatus and maintained at 37± 1°C throughout the experiment. The rotation of paddles were set at 50 rpm (USP). Samples of drug were collected at an interval of 0.5 hr, 1 hr...8 hr or complete dissolution and analyzed using UV spectrometry (Razzaq *et al.,* 2018).

In vitro release kinetics

Kinetic modeling was evaluated by DDsolver®. The mode of MTZ release from tablet was decided on the basis of best fit model either zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models.

Optimization of polymeric response using Artificial Intelligence

Computer aided optimization have been employed to investigate the effect of polymers on mucoadhesive strength, time, swelling index and *in vitro* release of the drug. The major challenge in designing the quantitative approach is to find out actual relationship among various factors (polymers) and responses. Another important quest for designing formulation is selection of desirable formulation that shows true relationship among all variables in that drug delivery system. To resolve these issues artificial intelligence which is a computer aided optimization technique and was used to select and find out trends of ingredients with respect to concentration (Hanif *et al.,* 2017). All the formulations were tested for physical and physicochemical evaluation through genetic algorithm (Quick Propagation) with multilayer perceptron (MLP) structure. Total input, output and hidden layers were 4, 9 and 1 respectively. Results were tabulated and software supported data were further analyzed to test the output.

STATISTICAL ANALYSIS

The statistical analysis for the estimation of standard deviation in physicochemical characterization was performed using SPSS v.20.0 and results were tabulated.

RESULTS

The current study was aimed with suitable mucoadhesion and swellability. In order to meet the objectives, polymers HPMC, CP, CMC and EC were added in different formulated tablets by direct compression method. Formulations (F1-F16) were designed with decreasing quantities of HPMC (F1-F10), compared with increasing concentration of CP in such. However, for formulation F11-F16, higher quantities of HPMC were used compared with rest of the formulations (F1-F10). The concentrations of HPMC and CP were manipulated from 4-40% and 0.5-10% respectively used in current study.

Table 1: Composition of different mucoadhesive buccal formulations, F1-F17 used in the study. The values are expressed as mg.

Code	MTZ	CP	HPMC	CMC	EC
F1	20	5.25	50	-	-
F2	20	7	40	-	-
F3	20	10	25	-	-
F4	20	12.3	20	-	-
F5	20	15	15	-	-
F6	20	17.5	13	-	-
F7	20	1.25	25	-	-
F8	20	2.5	20	-	-
F9	20	3.75	15	-	-
F10	20	4.5	10	-	-
F11	20	12.5	75	12.5	12.5
F12	20	15	75	7.5	-
F13	20	25	62.5	12.5	12.5
F14	20	15	75	15	10
F15	20	17	87.5	10	7.5
F16	20	20	100	7.5	12.5
F17*	20	16.4	78.7	8.3	10.5

*Software predicted optimized formulation tested in the Confirmation study.

Physical characterization of mucoadhesive tablets

All formulations were evaluated for physical parameters like weight variation, thickness, diameter, hardness and friability (table 2). As, weight per tablet was designed to be 250 mg, so 5% deviation was allowed according to USP28 specifications. The standard deviations of tested formulations complied with USP standards. Maximum

Table 2: Physical characterization of mucoadhesive buccal formulations, F1-F16 in terms of weight variation, thickness, hardness, friability and diameter

Code	*Weight Variation (mg)	*Thickness (mm)	*Diameter (mm)	Friability (%)	Hardness (kg/cm^2)
F1	243±2.48	4.74±0.08	8.77±0.04	0.62	6.57
F2	242±4.11	4.77±0.04	8.78±0.06	0.57	6.07
F3	249±2.11	4.67±0.04	8.74±0.09	0.61	6.23
F4	249±2.11	4.68±0.042	8.78±0.04	0.45	6.26
F5	246±3.94	4.57±0.04	8.79±0.05	0.67	6.74
F6	242±2.41	4.64±0.05	8.18±0.27	0.64	6.47
F7	249±3.16	4.78±0.04	8.75±0.05	0.43	6.02
F8	248±4.21	4.78±0.04	8.74±0.05	0.44	6.45
F9	242±2.41	4.77±0.04	8.76±0.05	0.64	6.09
F10	249±2.10	4.48±0.04	8.77±0.04	0.56	6.06
F11	240±2.35	4.86±0.042	8.07±0.04	0.34	6.72
F12	249±2.10	4.84±0.051	8.11±0.05	0.25	6.91
F13	249±2.10	4.75±0.08	8.87±0.04	0.21	6.59
F14	245±4.08	4.46±0.07	8.08±0.04	0.38	6.46
F15	249±2.11	4.57±0.05	8.77±0.04	0.45	6.31
F16	249±2.16	4.76±0.05	8.77±0.04	0.35	6.48

*Values are represented as mean ± S.D, n=10 except for weight variation where n = 20.

Table 3: Values of swelling indices of different mucoadhesive formulations, F1-F16 from time intervals 0.5-8 hr calculated according to Eq. 3.

Code	0.5 hr	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr
F1	23.52	35.29	17.39	17.39	56.52	60.86	99.43
F2	41.67	41.67	13.33	13.33	26.66	26.66	40.01
F3	26.08	43.47	65.21	65.21	52.17	65.21	73.91
F4	27.27	31.81	63.63	63.63	50.0	50.0	77.27
F5	16.67	25.0	25.0	27.0	13.33	13.33	16.67
F6	12.5	25.0	25.0	25.0	29.16	29.16	29.166
F7	30.43	43.47	43.47	43.47	56.52	56.521	60.86
F8	25.0	29.16	29.16	29.16	37.51	37.5	37.5
F9	23.81	38.09	38.09	38.09	38.09	38.09	42.85
F10	27.27	36.36	36.36	36.36	45.45	45.45	45.45
F11	75.0	83.33	108.33	133.33	145.833	150	179.17
F12	15.38	23.07	46.15	69.23	80.76	84.61	99.99
F13	45.83	54.16	66.66	70.83	83.33	104.16	116.67
F14	20.83	33.33	45.83	54.16	66.66	74.99	120.83
F15	20.83	33.33	54.16	58.33	83.33	100	125.01
F16	47.61	80.95	100.0	119.04	119.04	119.04	261.91

deviation was observed with formulations F2, F8 and F14, whereas, least was observed with F10, F12 and F13, which were 2.10. Least friability was found in F13, which was 0.21%. Maximum friability count was reported for F5 (0.67%) which might be due to external compaction problems. Likewise, the thickness and diameter of the formulations were found in the range of 4.46-4.86 mm and 8.07-8.79 mm, respectively.

Similarly, minimum values of standard deviation for thickness and diameter were found to be 0.04, whereas maximum deviation for thickness and diameter was found to be 0.08 and 0.27. As formulations were punched by direct compression method, the hardness was set in such a manner that it should neither be too high to retard the release of drug, nor it should be too soft to break up during handling. Hardness of the tablets was within range of 6.02-6.72 kgcm^{-2}.

Physicochemical characterization of mucoadhesive tablets

Swellability index

Quantitative measurement of water absorption at specific time duration was evaluated by swelling index. It inferences the degree of swellability *in vitro*. Generally, an increasing trend in swelling index was observed throughout the formulations (fig. 1) as organized in table 3.

The extent of swellability for formulations F1, F11-F16 was more than the initial dry weight. Swellability of formulations F2-F10 was not prominent might be due to higher concentrations of CP. Because CP shows its gelling effect only, up to 2.0% (Rowe *et al.,* 2009). Here addition of the carbopol is to reinforce the mucoadhesion strength of HPMC. Carbopol provide sustain effect and increase the adhesion along with HPMC. In formulation F12, slow sustained hydration was evident till 8 hr with

Table 4: Response of mucoadhesive strength, mucoadhesive time and surface pH of different mucoadhesive formulations, F1-F16 studied.

Code	Mucoadhesive Time (hr)	Mucoadhesive strength (gm)	pH
F1	9.6	21.47	5.88
F2	9.81	12.93	5.82
F3	7.73	15.67	5.71
F4	10.22	14.81	5.29
F5	9.71	9.03	5.58
F6	9.06	6.06	6.13
F7	9.5	16.39	6.39
F8	8.66	9.73	6.2
F9	9.46	9.06	6.01
F10	10.1	10.74	6.02
F11	6.21	18.07	5.8
F12	9.5	16.86	5.67
F13	9.62	8.59	5.68
F14	12.41	24.69	5.68
F15	10.61	18.76	5.5
F16	10.97	20.7	5.64

values of 99.99%. Among formulations F11-F16, F16 exhibited maximum swelling. Due to different swelling trends was observed, categories all formulations into three different pattern as shown in fig. (1). fig.1 (a) shows random pattern due to increasing and decreasing concentration of HPMC, and CP respectively.

Formulations F2-F5, exhibited the same trend initially steep was observed. The formulation F1 showed these tablets have maximum swelling after 1hr and then decreased due to erosion of the tablet. The swelling trend of F6-F10 indicated the gradual increase in to swelling and show smooth swelling behavior, increasing trend was observed gradually and showed sustain smooth effect. In formulations F11-F16 gradual increment of tablet weight occurred and this behavior was sustained at the end of the time. Formulations F1-F15 followed same pattern while F16 adopted different swelling trend i.e. it exhibited gradual increment of swelling till 6 hr, sharp peak was observed at the end of the time.

Surface pH

The rationale to investigate the surface pH of tablet was to recognize the local irritation might by occur on the mucosa. It should be in the range of 5.5-7.0. Surface pH of buccal formulations were in the range of 5.5- 6.39 except F4 which was 5.29 as depicted in Table 4. The lesser value of pH might be due to higher amount of CP. HPMC act neutral so decrease amount of HPMC dominate the effect of CP.

Thus, majority of formulations have pH which do not cause irritation and damage to mucosal membrane. Maximum pH was found for F7 which was 6.39 due to presence of highest quantity of neutral polymer HPMC comparatively to acidic CP that increases the pH and move towards the neutral (Hussain *et al.,* 2016).

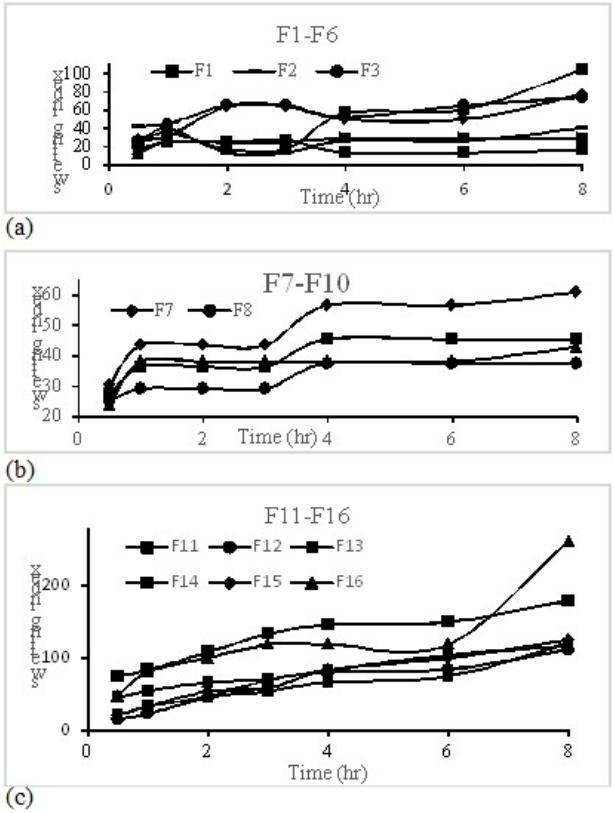

Fig. 1: Graphical depiction of trends swell ability indices of different mucoadhesive formulations, F1-F16 from time intervals 0.5-8 hr (a) F1-F6, (b) F7-F10, (c) F11-F16.

Mucoadhesive strength

Mucoadhesive strength test was carried out to access the mucoadhesion power of the tablet and it was calculated *in vitro* as force required to detach the tablet from mucosa. The strength is dependent on the nature, concentration and swelling behavior of polymer (Mansuri *et al.,* 2016).

A notable variation in the mucoadhesive was observed in the formulations. Higher forces of mucoadhesion were observed with F1 and F14, the values of which were 21.47 g and 24.69 g respectively. It might be due to fact that moderate to higher amounts of HPMC, CMC, EC and CP were present in F14 as higher amounts of polymers were associated with greater mucoadhesion. It is evident that, variability of polymers impact greatly over the properties of each other and final fabricated product. In formulations F1-F6 the concentration of CP was increasing downward from F1-F6 while HPMC has lesser concentration into these formulations.

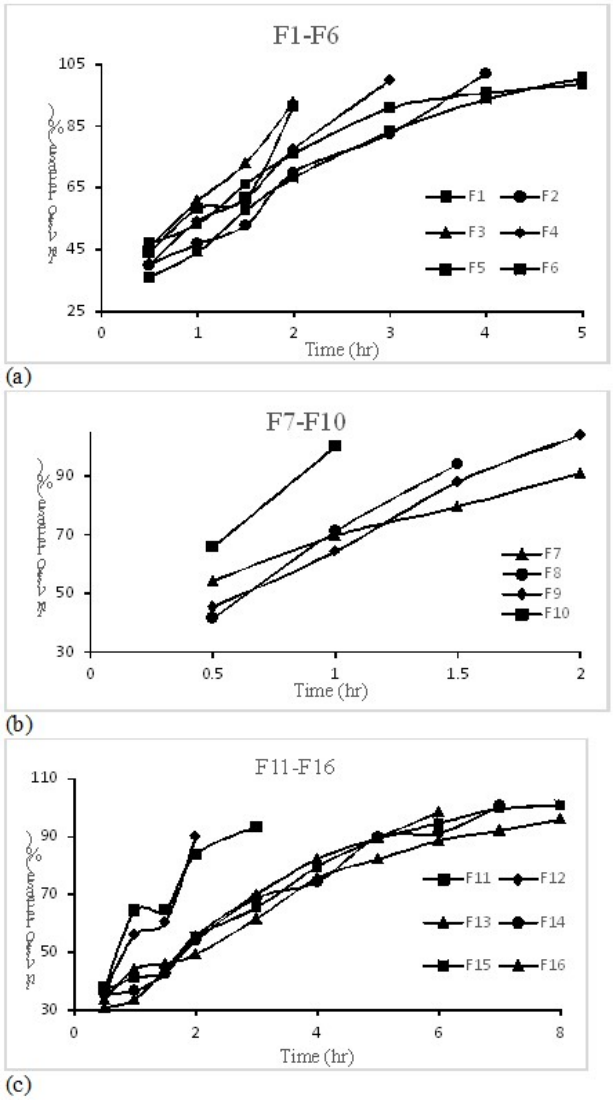

Fig. 2: Graphical depiction showing different patterns of *in vitro* release of MTZ from mucoadhesive buccal formulations, (a) F1-F6, (b) F7-F10, (c) F11-F16.

Mucoadhesive time

Highest mucoadhesive time was noted for F14 which was 12 hr, as it contained highest amount of mucoadhesive polymers as CP, EC increases its time to detach from mucosa, while F3 and F11 exhibited least time values i.e.7.71 hr and 6.21 hr respectively. It was found combination of 5-20% CP with HPMC was characterized with prolonged mucoadhesive time i.e. 10 hr as compared with HPMC (8 hr) alone (Hassan *et al.,* 2018).

In vitro MTZ release

For quantitative determination of MTZ in mucoadhesive buccal formulations, standard calibration curve was constructed in the dissolution media i.e. distilled water adjusted to pH 6.8 at a wavelength of 277 nm in UV visible spectroscopy. The curve showed linearity over a range of 2µg/ml to 20µg/ml with correlation value of 0.9994. Results showed that all mucoadhesive formulations released drug before 8 hr except F15 and F16 contain appropriate amount of EC and CMC. Most of the formulations were released at 5 hr or earlier showing retards the release of the drug (Table 5). In formulation F12, complete drug was release within 2 hr due to absence of EC and CMC was observed. As seen in the fig. 2, slow and smooth sustain behavior was observed in formulations F7, F8, where 50% of the drug was released within 2 hr as shown in fig. 2 (a), (b) and (c). In formulations F13-F16, slow and sustain trend was observed initially that released complete drug release till 8 hr. undesired release. Different release behaviors were observed in formulations F13-F16. F10 showed fastest release where the 50% of the drug was release within half an hour and complete release was attained at first hour. This behavior might be due to presence of low concentrations of HPMC. The presence of CP and HPMC enhance the formation of gel layer due to penetration of aqueous media into its polymeric chain that controls the release of drug from this barrier via diffusion process (Razzaq *et al.,* 2018).

Confirmation study (F17)

Artificial intelligence exhibits the property to develop formulation expert models by predicting formulation composition and conditions for manufacturing process (Table 6). From such computation, it reduces the time, error and cost of the process. As shown in fig. 3, the neural network generated outputs as 3D surface responses for by reading the trend of the polymers in physicochemical tests. Contouring in the plot indicated the maximum (pink to red) and minimum (yellow to orange) response of ingredients relative to parameter.

According to fig. 3, higher concentrations of HPMC and EC showed desirable effect on mucoadhesive strength while highest concentration of HPMC and CP imparted stronger effect on mucoadhesive time, pH and swellability index. The *in vitro* release of drug showed results that, at the initial stage, HPMC and CP control the release of the drug up till 50% concentration while EC was important in providing sustain effect up to 8hr and control the release of drug from the matrix.

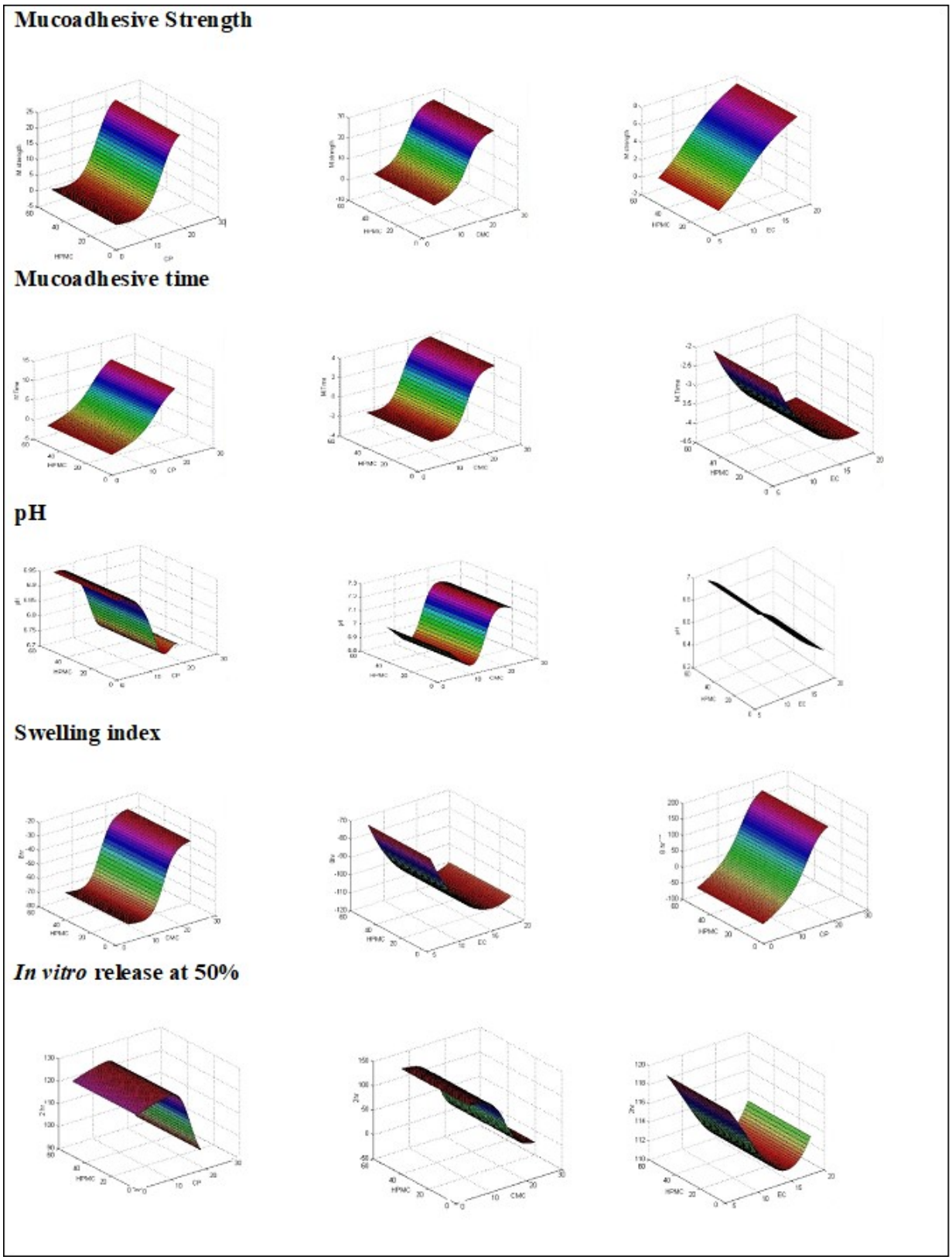

Fig. 3: Artificial Neural Network projected relative importance of HPMC, CP, CMC and EC on mucoadhesive strength, mucoadhesive time, pH, swellability index and *in vitro* drug release. Note that red area indicates maximum effect of relative polymers (input) against the output with relative desirability.

The release behavior of formulation F7-F10 shown in figure 3b, the 50% of drug release within one hr that indicated that less amount of HPMC along with CP cannot formed sufficient gel barrier that retard the release of drug in such concentrations (Hussain *et al.,* 2016). Also that, greater concentrations of HPMC relatively to CP

Table 8: Release kinetics of MTZ from optimized mucoadhesive buccal formulations (F17) depicting coefficient values for different models.

Kinetic Models	r^2 value
Zero order	0.3826
First order	0.9224
Higuchi	0.9808
Korsmeyer-Peppas	0.9938
Hixon Crowell	0.8564

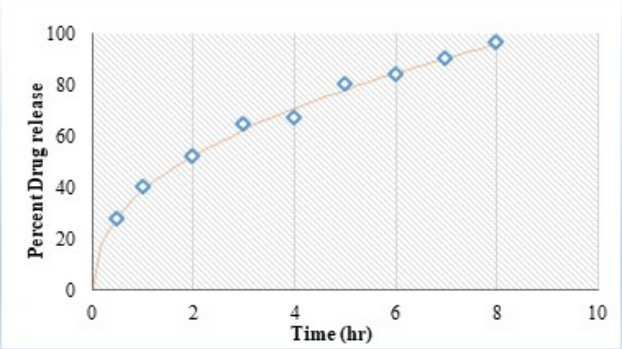

Fig. 4: Graphical depiction of MTZ release from optimized formulation (F17) showing best fit model i.e. Korsmeyer-Peppas using DDsolver®.

Confirmation formulation F17, (composition represented in Table 1 while outcomes in Table 7) was formulated and the response of different physicochemical parameters are presented in table7. The results of weight variation, hardness, friability, mucoadhesive strength, time, and pH coincided with the predicted results. The *in vitro* drug release was similar to forecasted value and 50% drug release within 2 hr, while complete MTZ was released at 8 hr. The swelling index results showed significant deviation at 8 hr; however this deviation might also occur due to experimental error or any other such factor cannot be denied.

In vitro release kinetics of Confirmation Formulation (F17)

DDsolver® was employed on all formulations which showed complete release and significant mucoadhesive results. For this F17 confirmation formulation was selected. All best fit models described above were applied on the release profile of such formulations. The highest R^2 value is indication of best fit release for that formulation (Hanif *et al.,* 2017). The Korsmeyer-Peppas model was found to have maximum r^2 value as shown in Table 8. The value of "n" for this model was 0.436 which depicts that the mode of MTZ release from HPMC matrix was diffusion only (fig. 4). It suggests that interlocking of HPMC matrix was only hindrance for the water soluble release of drug from mucoadhesive formulation.

DISCUSSION

Variable Concentration of HPMC and CP along with addition of a superdisintegrant (SSG) and binding agent (PVP k30) have been reported in previously reported preliminary work (Razzaq *et al.,* 2018) where the behavior of SSG was not significant due to declining mucoadhesive strength, time and swelling as well as undesirable faster drug release. There was no predefined direct increasing or decreasing trend of quantities of HPMC, CP, EC and CMC in a uniform manner. Because, it was easier for the artificial intelligence to observe or predict the impact of polymers on the behavior of formulation. The software would ultimately predict the ingredients based on desirability. Friability of the tablet formulations were compared with standard USP limit i.e. less than 1%. As depicted in Table 2, all the formulations were within USP standard limit.

Appropriate swelling behaviors are prerequisite for sustain release of drug so that water can diffuse inside the gel matrix and may allow controlled amount of MTZ to move out of the dosage form. Gradual swelling has been found initially, with plateau achieved after 8 hr with all formulation. Direct relationship was observed between the rate of swelling and concentration of polymer (HPMC, CP). The mechanistic behind swelling study depends on the imbibition of water into polymeric matrix, so, when hydrogen bond breaks as integrity of matrices losses that leads to hydration of polymer up to its extent. In fig. 1 (c) a gradual increasing steep was indicated the hydration of polymer and achieved highest plateau after 6 hr and maximum swelling achieved after 8 hr. This gradual increase in weight was due to presence of EC that reduces the hydration and delayed the swelling of polymer because EC is hydrophobic so showed hindrance in the hydration of water. HPMC hydrophilic polymer is time dependent polymer. When it exposed to water quickly swells and strongly adhere and then dissolve. With respect to mucoadhesion, HPMC has ability to form physical bonding due to presence of hydroxyl group in its chain that forms strong hydrogen bond with mucus, ultimately lead to mucoadhesion (Rao *et al.,* 2017). As CP is responsible for the formation of secondary bond with mucus and interpenetrate between polymer chains that allows it more adhesive strength. In F8-F10 less concentration of both polymers decreases the mucoadhesion strength, when concentration of both polymer increases in F11-F16 mucoadhesion increases from 18g to 20g due to incremental effect of these two mucoadhesive polymers and presence of CMC and EC as

Table 5: *In vitro* metronidazole release mucoadhesive buccal formulations (F1-F16) in distilled water adjusted to pH 6.8.

Code	0.5 hr	1 hr	1.5 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr
F1	46.8	53.1	66	75.9	90.6	95.7	98.4			
F2	39.9	46.8	52.8	69.9	82.5	102				
F3	45.9	60.6	72.9	92.7						
F4	40.2	54	61.5	77.4	99.7					
F5	44.1	58.2	61.5	91.2						
F6	35.7	44.1	57.6	68.1	83.1	93.6	100.5			
F7	54.3	69.6	79.5	90.9						
F8	41.4	71.1	93.9							
F9	45.3	64.2	87.9	101.91						
F10	65.7	100.2								
F11	37.8	64.2	64.5	83.7	93.3					
F12	36.3	56.1	60.3	90	99.41					
F13	30.6	33.6	44.1	55.5	69.9	82.2	89.7	98.4		
F14	35.7	36.6	42.6	54	68.7	74.4	89.7	91.2	100	
F15	36.9	41.1	43.2	55.2	65.4	79.5	89.1	94.5	99.6	100
F16	33.6	44.1	45.9	49.2	61.5	75.6	82.2	88.5	92.1	96

Table 6: Parameters set for performing Genetic Algorithm the optimization of mucoadhesive buccal tablet.

ANN Parameters	Settings
Learning Algorithms	Genetic and Natural Selection
Connection Type	Multilayer Normal Feed Forward
Total Layer Numbers	3
Node Number of Input Layer	4
Output Layer	1
Node Number of output layer	9
Transfer Function	Tanh
Hidden Layer	1
Node Number of hidden layer	8

Table 7: Comparison of the software predicted and experimental outcomes of different formulation parameters tested in the study for Confirmation formulation (F17).

Parameters	ANN predicted	Experimental	S.D
Weight variation (mg)	245	248	2.10
Hardness (kg/cm^2)	6.65	6.71	0.04
Friability (%)	0.63	0.32	0.21
Swelling index at 8 hr (%)	221	175.8	3.96
Mucoadhesive strength (g)	18.31	20.3	1.40
Mucoadhesive time (hr)	6.98	7.21	0.10
Surface pH	6.91	6.9	0.007
In vitro release $t_{50\%}$ (hr)	2	2	1.98
In vitro release $t_{90\%}$ (hr)	8	8	1.56

well. The mucoadhesion of F14 was 24.69g due to presence of highest concentration of CMC along with these two polymers provide additional effect on mucoadhesion.

It is manifested that escalating *in vitro* mucoadhesive time was noticed with higher polymer quantity. Here in such formulations main contributing polymer for controlling mucoadhesion times was CP and EC. The erosion front of HPMC and CMC did not allow tablet to reside for longer period of time but it contribute along with CP and EC to maintain its mucoadhesion up to required time.

CONCLUSION

The sustained release mucoadhesive buccal tablet to release MTZ locally for the treatment of orodental infections was formulated with the polymeric blend of

Hydroxypropyl methylcellulose (HPMC), Carbopol 940P® (CP), ethylcellulose (EC) and carboxymethylcellulose (CMC). The release of active ingredients was also found to be dependent on the relative concentration of HPMC, CP and EC. The formulation containing a higher concentration of the polymers HPMC and EC showed sustain release profile. ANN analysis revealed that the highest concentration of HPMC imparted a stronger impact on the mucoadhesive strength, while highest concentration of HPMC and CP provide best results of mucoadhesive time, pH, swelling index. Results of *in vitro* drug release indicated that HPMC and CP control the release of the drug below 50% while EC imparted sustain effect up to 8 hr.

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