Formulation and optimization of gellan gum-poloxamer based dexamethasone mucoadhesive *in situ* gel

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ABSTRACT: The main objective of the present study was to formulate and evaluate mucoadhesive *in situ* buccal gels of dexamethasone based on gellan gum-poloxamer 407. Formulations were characterized for gelling capacity, drug content, pH, viscosity, rheological studies, mechanical studies and *in vitro* drug release. The drug content, clarity and pH of the formulations were found to be satisfactory. Mucoadhesive *in situ* gels showed thermoresponsive behavior, existing as a liquid at room temperature and gel at 30–37ºC. Formulations exhibited pseudoplastic flow and typical gel-type mechanical spectra (G' > G'') at different frequecy values and 37ºC. Prepared gels resulted in preparations with desirable rheological features as well as texture (appropriate hardness, compressibility, adhesiveness, cohesiveness and elasticity) properties, which could benefit the therapeutic efficacy, by increasing the residence time and easiness for local application on the buccal mucosa. Additionally, the developed preparations exhibited sustained drug release up to 72 h as intended for these systems. Optimized formulation containing 14% w/v poloxamer 407 and 0.4% w/v gellan gum exhibited desired characteristics (mechanical and rheological properties) for developing buccal drug delivery systems. Thus, buccal dexamethasone loaded mucoadhesive *in situ* gel was found to be a promising formulation.

KEYWORDS: Dexamethasone; gellan gum; poloxamer; *in situ* gel; buccal drug delivery.

1. INTRODUCTION

Use of the oral mucosa for drug delivery has many positive factors: it is very accessible and responsive, as well as being diverse and dynamic. The positive features for drug delivery include that it is very vascular and therefore encourages a systemic effect. It also avoids first-pass metabolism and has fairly low enzymatic activity, which may result in increased bioavailability. Patient compliance may also be greater in orally delivered drugs [1-3]. Furthermore, local treatment to the oral mucosa is commonly used for treating fungal and bacterial infections, in addition to dental, periodontal and gum diseases and so forth [4]. However, one disadvantage of buccal drug delivery is that the drug remains at the site of action for only a brief period of time and therefore multiple dosage regimens are often needed to be effective [5].

Mucoadhesive *in situ* gels are drug carrier systems which are liquid prior to application and are converted into gel form after application. The most important advantages of mucoadhesive *in situ* gels is that they provide easiness of application, enhance the bioavailability, reduce the dose concentration and frequency and improve the patient compliance and comfort and sustained and prolonged action. Also, these systems are not too complex and the manufacturing processes is simple and cheap. The formulations provide covering substantially all oral cavity when they are sprayed into the mouth. Then the mucoadhesive polymers gelling at body temperature and adhering to oral mucosa and finally providing the active agent in an improved and effective way for treatment of the diseases [6,7].

In this work, mucoadhesive *in situ* gels were formulated using poloxamer (Plx) 407 and gellan gum (GG). Dexamethasone (DEX), a poor-water soluble glucocorticoid, was used as a model drug, since it is used clinically as an anti-inflammatory and immunosuppressive agent [8]. Relevant data have shown that dexamethasone has a certain effect in the treatment of oral ulcers [9]. Furthermore, DEX ointment has been used as a topical glucocorticoid agent to treat oral ulcers for many years [10]. For this reason, mucoadhesive *in situ* gels containing DEX were prepared for patient comfort.

Poloxamer (Plx) 407 is composed of 70% polyethyleneoxide and 30% polypropyleneoxide. It is frequently used, due to its low toxicity, high solubilizing capacity and excellent drug-release properties. This also means that it could be used in controlled drug delivery systems [11-13].

GG is a water-soluble, mucoadhesive, linear, anionic deacetylated exocellular bacterial polysaccharide produced by *pseudomonas elodea* [7,14]. It was primarily used as a stabilizer and thickening or gelling agent, and has been added to many different foods due to these properties. More recently, GG has been investigated as a polymer in pharmaceutical technology due to its biocompatibility, biodegradability and low cytotoxicity. It is able to form soft gels at low concentrations [15,16].

The objective of this study was to assess the potential use of these polymers in controlled drug delivery of DEX in the oral mucosa, using a straightforward method and through assessing the properties of mucoadhesive *in situ* gels.

2. RESULTS AND DISCUSSION

In our previous study, DEX loaded poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) were successfully prepared using the emulsification/solvent evaporation method for the local treatment of oral precancerous lesions [17]. NPs with different rates of PLGA showed adequate properties in relation to pH, particle size, polydispersite index, zeta potential, encapsulation efficiency, *in vitro* drug release, stability, *ex vivo* drug diffusion and cell culture cytotoxicity. In the present study, *in situ* gels were successfully prepared using Plx 407 and GG improve for the patient compliance and comfort. *In situ* gels offer several advantages such as easy to administer into the desired body cavities, high spreadability at certain temperatures, a reduction in the frequency of administration, improve patient compliance and comfortable in comparison to conventional dosage forms [6,7].

2.1. Measurement of gelation temperature

The gelling temperature of *in situ* gelling systems is one of the prime goal. For drug delivery it is ideal for the *in situ* gel to be sol state at room temperature, thereby facilitating administration of the drug, and then for the formulation to rapidly form a gel when in the oral cavity. Moreover, these gels should not dissolve, but rather remain in the gel form for a length of time [18].

The gelation temperatures of *in situ* gel formulations have been considered to be suitable if they are in the range of body temperature. Gelation temperature range suitable for mucosal formulations is 30–36°C [19]. In order to create these desired properties, the optimum concentration of the gel base and complexing agents will need to be known. Changes in micellar number due to temperature changes is known to cause gelation of Plx. Due to the negative solubility co-efficient of these micelles, an increase in temperature leads to an increase in the number of micelles formed. At high temperatures there are sufficient micelles in a small space so that the micelles can no longer move and it therefore forms a gel. Gelation occurs due to changes which take place in the centre of the micelle, where it undergoes changes in the methyl groups of the polymer side chains and water is expelled. At the different concentrations of Plx 407 gelation temperatures of the formulations were obtained. It was found that the gelation temperature of formulations decreased with an increase in the concentration of the thermosensitive polymer Plx 407 [20]. However, gelation temperature of G7 and G8 formulations was not influenced by Plx 407 concentration. The gellation temperatures of prepared mucoadhesive *in situ* gels were shown in Figure 1. The gelling temperature of the formulations ranged from 28.613±0.026 to 39.191±0.025°C. As seen in Table 2, G2, G3, G4, G6, G7 and G8 coded formulations had 30-37°C gelation temperature. However, it was decided that the most suitable formulations were G6, G7 and G8 coded *in situ* gel formulations by looking at their organoleptic (clarity) and rheological properties. The gelation temperature of G6, G7 and G8 coded formulations were found 35.151±0.038°C, 30.653±0.032°C and 31.184±0.016°C, respectively. Formulation G6 containing Plx 407 (10%) showed excellent gelation as compared to the G7 containing Plx 407 (12%) and G8 containing Plx 407 (14%) due to increasing the concentration of Plx 407 for the same GG concentration. The GG and Plx 407 concentration in the formulations was determined as 0.4% and 10-12-14% based on the gelation temperature studies, respectively. Therefore, studies continued with these formulations. Baloglu *et al.* [19] prepared *in situ* gel formulations using Plx 407 and Plx 188 for use as drug delivery platform via mucosal route. In this study, Plx 407 and Plx 188 were used alone and together. The results showed that with the increase in the concentration of Plx 407, the gelation capacity was decreased. In another study, Garala *et al.* [20] was developed *in situ* gels containing Chlorhexidine hydrochloride using Plx 188, Plx 407, GG, and Carbopol 934P for the treatment of periodontal disease. Gelation temperatures for Plx 188 and Plx 407 gels were

observed for the different concentration range of polymer, and it was found that the gelation temperature of formulation decreased with increasing concentration of polymer.
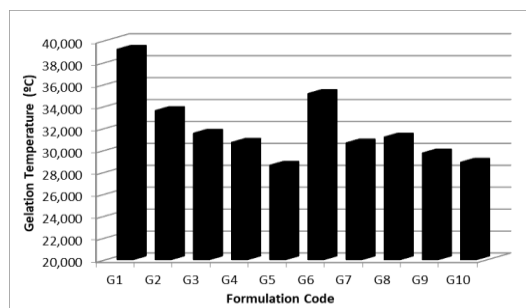

Figure 1. The gelation temperature of the formulations.

2.2. Drug content

The method for analysis was designed and DEX underwent validation studies. The acceptance criteria of 2% standard deviation was used, and the analysis method for the determination of the assay was verified if the standard deviation was lower than the acceptance criteria. The limit of detection (LOD) of DEX was 0.182 µg/mL and the limit of quantification (LOQ) of DEX was 0.487 µg/mL. The used method for DEX analysis was found to be linear (r^2=1).

The drug content of all mucoadhesive *in situ* gels containing DEX was found to be within the acceptable range of 95.307-97.026%, which indicates content uniformity (Table 2).

2.3. Determination of pH

pH is a very important factor for buccal formulations. The normal physiological pH of the buccal mucosa ranges from 5.5–7.5 [17,18]. The pH of all *in situ* gel formulations containing DEX was observed in the range of 6.72-6.82 (Table 1). There was no need for pH adjustment by any alkalinizing agent. Besides, an acidic or alkaline formulation may irritate buccal mucosa, and hence, this parameter assumes importance in the formulation of mucoadhesive dosage forms [4,21]. The obtained pH values of formulations are considered acceptable to avoid the risk of irritation upon application to the buccal mucosa.

Table 1. Drug content and pH of gel formulations.

| Formulation Code	Drug content (%)±S.D.	pH±S.D.
G6	97.026±1.378	6.82±0.01
G7	95.426±2.938	6.74±0.02
G8	95.307±1.391	6.72±0.02

2.4. Rheological studies

The rheological properties for the gel formulations would be important for predicting their behavior/spreadability *in vivo* conditions. Rheological properties have fundamental in retention on the buccal surface for gels efficacy. The better elastic characteristic and enhancement the rheologic properties of the mucoadhesive gel extend the residence time at desired area [22,23]. The sufficient retention and distribution capacity in the buccal mocosa is provided the selection of correct viscosity of the formulation. For buccal delivery of a drug the viscosity of the *in situ* gel will need to be low at the time of application to the oral mucosa, but a high viscosity is required thereafter in order for the drug to remain in the area. Representative viscosity curves of prepared mucoadhesive *in situ* gel formulations were graphically presented in Figure 2. As anticipated due to its thermoresponsiveness, at 37±0.1°C all of the preparations showed non-Newtonian pseudoplastic flow, as is expected for Plx above the sol-gel transition temperature. It is precisely the non-Newtonian pseudoplasticity flow that is beneficial in buccal application, as it causes the gel to break down and disperse over the oral mucosa and then after being applied it regains its initial structure [18,23-25]. In addition, at 25±0.1°C the formulations demonstrated Newtonian flow. With an increase in shear strain the viscosity decreases, as seen in shear thinning. Our findings demonstrated sheer thinning at 37±0.1°C, in line with the literatures [26-29]. In our study the G8 formulations gave rise to the highest viscosity value and had the most consistent gel structure.

G6   G7   G8
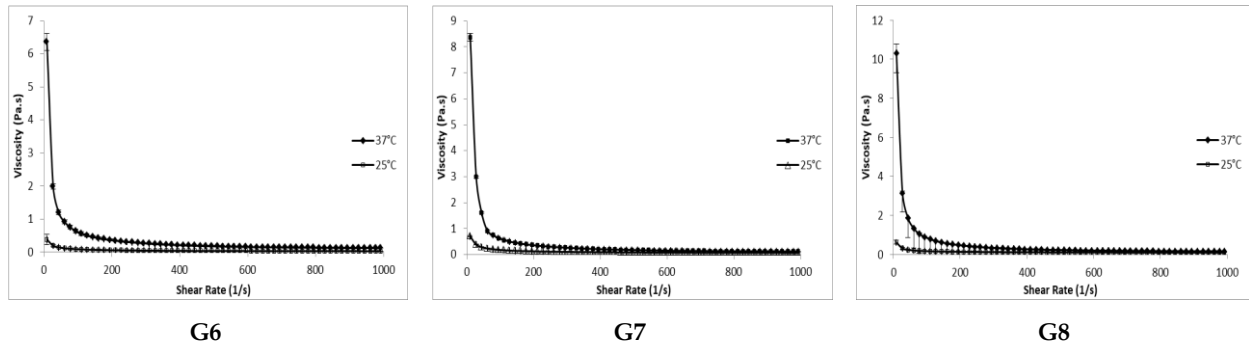
Figure 2. Viscosity curves of the formulations at 25°C and 37°C

The oscillatory properties of semisolid systems are influencing the application and retention on the buccal mucosa. The good elastic character and the beneficial rheologic properties prolong the residence time of the drug delivery system in the buccal mucosa. The storage modulus, G′, represents the elastic component of a viscoelastic material and the loss modulus, G′′, refers to the viscous portion. These values are obtained when an oscillatory force is applied to the formulations and the strain is measured. When a single oscillatory force is applied semi-solid formulations do not have time to separate out and therefore G′ will be higher than G′′ [22,30]. All optimum formulations were frequency independent solid-like spectra (G'> G'') at 37°C (Figure 3).

G6   G7   G8
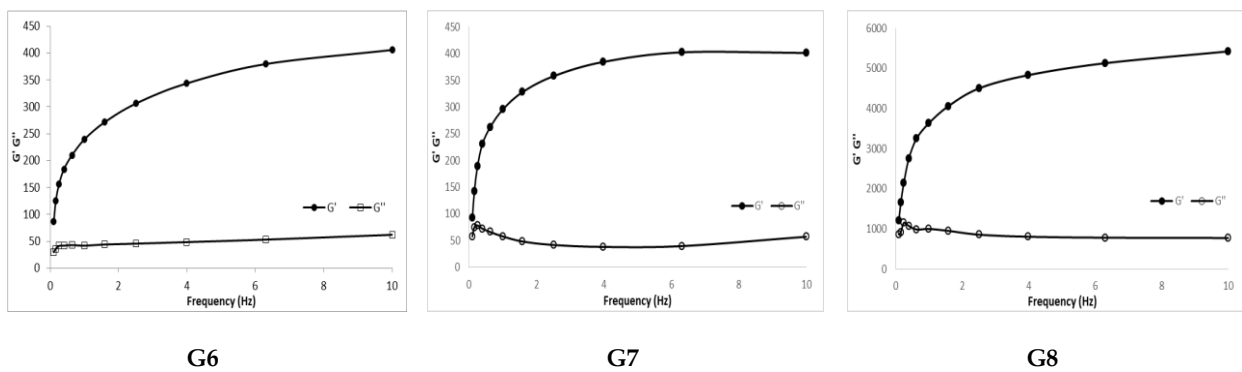
Figure 3. Frequency-dependent changes of viscoelastic properties of the formulations at 37°C

The value of phase angle (tan δ=G″/G′) is representing the relative contribution of viscous components to the mechanical properties of the formulations with values lower than 1, representing a solid gel response (Figure 4) [30].

G6   G7   G8
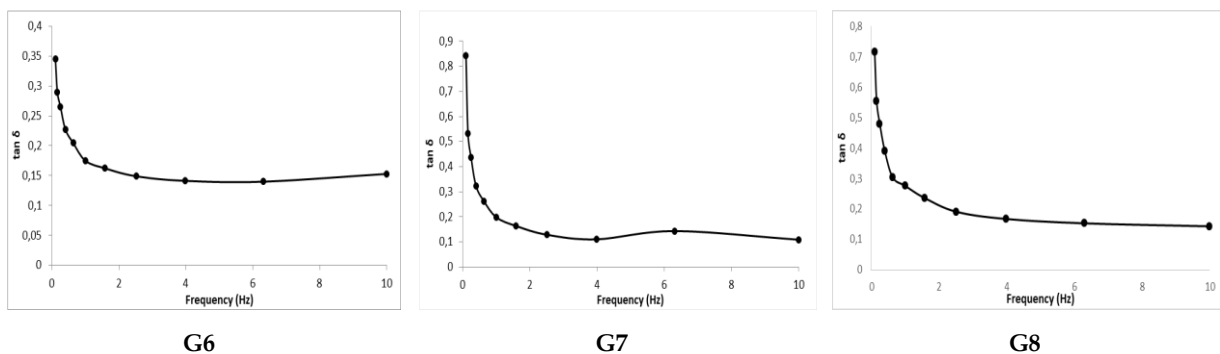
Figure 4. Frequency-dependent changes of tan δ values of the formulations at 37°C.

2.5. Mechanical properties of formulations

To better understand the physical and mechanical properties of gel formulations a Texture Profile Analysis (TPA) can be carried out. The TPA can be used to predict the behavior of the gels in different

conditions and can also be used to understand and identify the physical and chemical interactions between the different gel components. A successful buccal gel formulation must have the right mechanical properties to provide the patient with the most effective and efficient treatment. TPA provides information on the following parameters: compressibility, hardness, adhesiveness, elasticity and cohesiveness [31-34]. Table 2 represented the obtained results of mechanical properties from textural analysis.

The maximum force needed to achieve a specific degree of deformation of a gel is known as the hardness, thus it provides information about the buccal application of the gel. As the hardness value increases, the *in situ* gel formation will be thicker and have higher consistency. The effort needed to deform the gel in the first pass of the probe is termed compressibility. In terms of ease of use, a gel will need to have low hardness and compressibility values to facilitate application and allow its spread on the site of action. The disadvantage of very low hardness and compressibility is that low values mean the gel does not remain in the oral cavity for the desired time (retention) [31,35,36].

Adhesion is the attraction between the surface formulation and the probe and therefore the effort needed to overcome this reaction is the adhesiveness. In order to satisfy the need for retention of the formulations at the site of action, the gel must have high adhesiveness.

The ratio of the positive force area during the second compression cycle to that of the first compression cycle is known as cohesiveness. This parameter assesses the ability of the gel to recuperate after it is applied [37]. The higher the cohesiveness value, the more complete the reconstruction of the gel after application. A high value will translate in increased efficacy of the application at the site of action [38, 39].

The ratio of time needed to obtain maximum structural deformation on the second compression cycle to the first compression cycle is the elasticity. A high value of elasticity in TPA refers to a low elasticity of the formulation. Retention of the gel at the desired site is increased with higher values of elasticity as this means the formulation tends to reconstruct at the site (34,35,38].

The G8 coded formulation demonstrated higher values of hardness, adhesiveness, and cohesiveness than G6 and G7 coded formulations, probably because its polymeric concentration is also greater. However, the compressibility and elasticity values of G8 coded formulation decreased than G6 and G7 coded formulations due to the increase in the polymer concentration. The results from our study were in accordance with the literatures [31,38]. Swain *et al.* [31] developed Moxifloxacin Hydrochloride loaded *in situ* gels with Plx, GG and carbopol for the treatment of periodontitis. The hardness values of these formulations were increased as the concentration of polymer increases.

Table 2. Mechanical properties of gel formulations.

Code	Hardness (mN)±S.D.	Compressibility (mN.s)±S.D.	Adhesiveness (mN.s)±S.D.	Cohesiveness ±S.D.	Elasticity ±S.D.
G6	45.653±0.635	183.521±1.533	273.521±0.851	0.898± 0.043	0.985±0.025
G7	53.232±0.187	182.308±0.549	305.895±0.292	1.021± 0.061	1.003±0.011
G8	60.760±3.736	180.452±1.415	341.699±2.613	1.210± 0.288	1.039±0.110

2.6. *In vitro* drug release studies

The *in vitro* release of drug from the dosage form plays an important role in the drug delivery systems and in determining the therapeutic effect of the drug. In order to plan and prepare for the use of the gel formulation *in vivo*, an *in vitro* study needs to be carried out [40].

The *in vitro* release profiles of DEX were evaluated in pH 6.8 phosphate buffer at 37°C and the results were displayed in Figure 5. As can be seen, the release of DEX was in range of 42-46% over 8 hours. In 24 hours, the release rates of DEX from G6, G7 and G8 reached 76.026, 73.128 and 72.618%, respectively. In addition, at the end of 72 hours, the release rates from G6, G7 and G8 were 100, 100 and 93.222%, respectively. These results indicated that combination of mucoadhesive agents with Plx 407 could prolong drug action time in buccal mucosa due to the increase of viscosity with addition of GG. In our previous DEX loaded NP study, after 8 h, the drug release in formulations was found to be approximately 60% [17]. Mucoadhesive *in situ* gel formulations showed a slower drug release profile than nanoparticles.

The literatures have suggested that gel viscosity affects drug release, showing that gels with greater viscosity demonstrate slower drug release [38,41-43]. Cavallari *et al.* [44] showed that a gel barrier forming on the surface of the formulation causes a high ratio of hydroxypropyl methyl cellulose and slower drug release. Topical application of Moxifloxacin Hydrochloride in a thermosensitive *in situ* gel was designed by

Swain *et al.* [31] and the findings showed that the concentration of GG and Plx had an effect on the *in vitro* release of the drug.
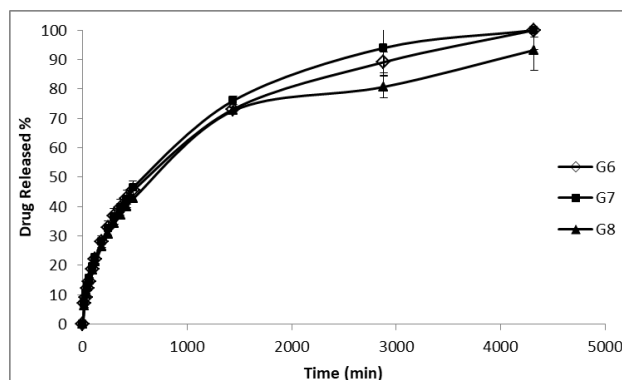

Figure 5. *In vitro* drug release of formulations.

3. CONCLUSION

In present research work mucoadhesive *in situ* buccal gel formulations containing DEX was developed with combination of gellan gum and Plx 407. It was seen that as the concentration of Plx 407 was increased, the gelation temperature of formulations decreased. GG tends to decrease the gelation temperature of Plx 407. A modulation of the gelation temperature to reach the desired range (30°C-37°C) was achieved through the use of a combination of the Plx 407 and GG. Moreover, mucoadhesive *in situ* gels resulted in formulations with desirable rheological and texture properties that can benefit the therapeutic efficacy of a drug administered by buccal route, increasing the retention time and the easiness for local application in the buccal mucosa. Considering the viscosity and mechanical properties of gels, these results suggest that G8 coded gel formulation may promote greater residence time on the mucosa, being more advantageous for buccal administration. Furthermore, the release profile studies showed that the DEX could be released from the formulation over a prolonged period of time (72 h). Thus from the above results it can be concluded that a mucoadhesive *in situ* gel of DEX can be formulated using optimum quantity of Plx 407 and GG combination (14 and 0.4%, respectively) of both to have a increase in buccal residence time and patient comfort.

4. MATERIALS AND METHODS

DEX was generously donated by Pharmacia&Upjohn Company LLC (A Subsidiary of Pfizer Inc, USA). GG was obtained from Sigma-Aldrich (St. Louis, MO). Plx 407 was donated by BASF Chemical Company (Germany). All the other chemicals were of analytical grade.

4.1. Preparation of mucoadhesive *in situ* gel formulations

In situ gels were prepared by dispersing the polymer in distilled water. GG powder was dispersed in distilled water maintained at 50°C with vigorous stirring using magnetic stirrer. The dispersion was stirred at 50°C for 20 minutes to facilitate the complete hydration of GG. The solution was cooled down to 25°C. The required amount of DEX was added to GG solution with continuous stirring until the entire drug was dispersed. Then required amount of Plx 407 was added with continuous mild stirring for 5 minutes. The formulation containing partially dissolved Plx 407 were stored in the refrigerator until entire polymer gets completely dissolved. The prepared formulation store at cool place [26,31,45]. The composition of the formulations was presented in Table 3.

4.2. Measurement of gelation temperature

Gelation temperature and gelation time were determined with rheometer (TA Discovery HR-1 hybrid rheometer, Newcastle, Britain). The geometry was a stainless steel plate/plate (diameter 40 mm) and it provided a homogeneous shear of the gel. The sol-gel transition temperatures of the gels were determined from oscillation measurements with a fixed frequency of 0.01 Hz. The samples were heated with a rate of 2°C/min, within a range of 20–50°C during the measurement [22,46].

Table 3. The composition of *in situ* gel formulations.

Formulation Code	DEX (%)	GG (%)	Plx 407 (%)
G1	0.1	0.2	10
G2	0.1	0.2	12
G3	0.1	0.2	14
G4	0.1	0.2	15
G5	0.1	0.2	16
G6	0.1	0.4	10
G7	0.1	0.4	12
G8	0.1	0.4	14
G9	0.1	0.4	15
G10	0.1	0.4	16

4.3. Determination of pH

The calibrated pH meter (Hana Instruments HI 221) was used to determine the pH values of formulation at room temperature to examine the compatibility of gels for buccal mucosa.

4.4. Drug content

To determine the drug content, 0.25 g of mucoadhesive *in situ* gel sample was taken from top, middle, and bottom of the gel and extracted by addition of 10 mL of phosphate buffer (pH 6.8) followed by mixing for 48 hours. The drug content was analyzed per validated HPLC method (n=5).

A validated HPLC method was used for determining the DEX amount (Hewlett-Packard Agilent 1100, Agilent Technologies, Santa Clara, CA, USA-equipped with UV-Visible detector). A 250 mm × 4.6 mm (5 μm particle size) reversed-phase C18 column was used for separation and quantitation. As mobile phase methanol: water: triethylamine (70:30:0.6, v/v/v) was used and pH of the mixture was adjusted to 3.0±0.05 with orthophosphoric acid. The flow rate was set as 0.9 mL/min at 25°C. UV detection was made at 240 nm [47,48]. The validation was performed following the ICH guidelines including determination of linearity, specificity, accuracy, precision (repeatability and reproducibility), stability, limit of detection, and limit of quantification [49].

4.5. Rheological studies

Rheological studies were performed using a rheometer device (TA Discovery HR-1 Hybrid Rheometer). The analysis was performed, in flow mode using parallel plate geometry (gap: 1 mm) at room temperature. Upward and downward flow curves were measured ranging from 10 s^{-1} to 1000 s^{-1} [22,23,50].

The stress sweep studies were used to determine the yield stress of gel formulations to predict the stress required to initiate flow. The stress was gradually conducted over the range of 0.1-1000 Pa and at a frequency of 1 Hz. The resulting viscoelastic parameters were monitored and determined its linear viscoelastic region, where the stress was directly proportional to the strain and the storage modulus remained constant. The yield stress value was detected.

The oscillatory analysis was performed after determination of its linear viscoelastic region. Frequency sweep analysis was performed over the frequency range of 0.1-10.0 Hz following application of constant stress. Elastic (storage) modulus (G'), viscous (loss) modulus (G'') and the loss tangent (tan δ) were examined [22,51,52].

4.6. Mechanical properties of formulations

The mechanical properties (hardness, compressibility, adhesiveness, cohesiveness and elasticity) were performed using a software-controlled penetrometer, TA-XT Plus texture analyzer (Stable Micro Systems, UK), with a 500 g load cell at 37±0.5°C. Each formulation was transferred into universal bottle and kept in the ultrasonic water bath to remove air bubbles for 20 min. The Perspex probe was twice compressed into each formulation at a defined rate of 2 mm.s^{-1} to a depth of 15 mm. A delay period of 15 sec was allowed between the two compressions. Mechanical properties of the gel formulations were derived from the resultant force-time curve [34]. (n=5).

4.7. *In vitro* drug release studies

Dialysis bag method was used for the *in vitro* drug release study of *in situ* gels. *In situ* gel containing DEX was put into the Spectra/Por Regenerated Cellulose Dialysis Membrane Tubes (12.000–14.000 MWCO). The release medium was 100 mL of phosphate buffer (pH 6.8) under sink conditions. In order to simulate the oral mucosa temperature, the temperature was set at 37°C±0.5°C and the stirring speed was kept at 300 rpm. At defined time intervals, samples were withdrawn, and the DEX content of each sample was analyzed using a validated HPLC method (n=5).

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