Preparation and evaluation of an oral mucoadhesive gel containing nystatin-loaded alginate microparticles

Mohammadi Samani S.1, Karimaddini S.1, Sobhani Z.2, Ahmadi F.1

INTRODUCTION

Oral aphthous and oral lichen planus are two common oral mucosal diseases that are diagnosed by very painful and recurrent ulcers disturbing the normal activities of the oral mucosa and the patients' quality of life. These two chronic inflammations are mainly presented in immunocompromised patients and are often associated with oral mycosis. Therefore, drug therapy regimens of these inflammatory diseases mostly contain an antifungal agent (Conklin and Blasberg 1991, Ujević, Lugović-Mihić et al. 2013). Nystatin (Nys) belongs to the polyene antifungal antibiotics, which is produced by Streptomyces noursei strains and is effective against fungal infection unresponsive to azoles like Candida species. Nys interferes with the synthesis of fungal cell membrane and is widely used for treatment of susceptible fungal infections, especially oral mucosal infections (Lacy, Armstrong et al. 2004, Sean and Paul 2009, Sawant and Khan 2017). Conventional pharmaceutical dosage forms are not very effective for treatment of oral mucosal pathologies, because drugs are removed from the site of action by swallowing or salivary clearance (Roque, Castro et al. 2018). For Nys, there is an extra problem for systemic therapy. Nys is a large water-insoluble molecule that cannot penetrate the intestinal mucosa, and the drug therapy with Nys is limited to topical effects (Croy and Kwon 2004, Martín, Calpena et al. 2015). Therefore, designing a formulation that could keep the drug in the oral cavity for longer times seems a potentially successful approach for delivery of Nys. One of these formulations is mucoadhesive drug delivery systems, which utilize polymers that can be quickly and strongly bonded to the oral mucosa (Sudhakar, Kuotsu et al. 2006, Netsomboon and Bernkop-Schnürch 2016). Mucoadhesive dosage forms can be formulated in the form of multiparticulate systems to provide a more uniform and controlled mode of drug delivery (Madhav and Kala 2011). Alginate is a polysaccharide polymer with very interesting properties. Besides its biocompatibility and mucoadhesion properties, alginate forms gels and particles in the presence of cations such as Ca2+. This very simple and rapid
molecular weight (without use of any harsh solvents or pH) makes alginate a good choice for delivery of biological agents and cells (Takka 1999, Tafaghodi, Tabasi et al. 2006). Therefore, the aim of the present study was to develop and evaluate drug-loaded alginate microparticles to provide a multiunit drug delivery system. This part is the most challenging part as the drug molecule is very large and insoluble. To further enhance the mucoadhesion properties, the particles were introduced into the carbomer gel to provide a mucoadhesive oral dosage form that could be retained in the mouth. The formulation then was characterized in terms of particle size, drug loading, encapsulation efficiency, in vitro release profile, measurement of mucoadhesion, and physicochemical properties using DSC and FT-IR.

**MATERIALS AND METHODS**

**Materials**

Nys was purchased from Raha. Pharmaceutical Company (Isfahan, Iran). Sodium alginate medium viscosity and sodium citrate was obtained from Sigma (USA); **Carbopol 934** (cp934), calcium chloride, sodium lauryl sulfate (SLS), and triethylamine were purchased from Merck (Germany). All other chemicals and solvents were obtained from certified sources.

**Preparation of alginate microparticles**

The formulation of Nys-loaded alginate microparticles was based on the simple and well-known direct ionic gelation method. Alginate solution was prepared by dissolving sodium alginate in de-ionized water. Briefly, Nys powder was suspended thoroughly into aqueous solution of sodium alginate under magnetic stirrer, and then, the resulting solution containing Nys was extruded from a syringe into aqueous solution of CaCl₂. Alginate microparticles were filtered, collected, and washed using distilled water and dried at room temperature (Heng, Chan et al. 2003).

**Preparation of mucoadhesive gel of nystatin containing alginate microparticles**

The oral gel was prepared using cp934. Alginate microparticles containing Nys were incorporated into a 1% w/v cp934 gel under magnetic stirrer. For preparation of gel, 1 g cp934 was dissolved in water, and then, by adding triethylamine dropwise, the viscosity of the gel was modified, and the final pH was set to 5.5 ± 0.3.

**Experimental design**

To determine the effects of different parameters and choosing the most optimum formulation, experimental design was performed with four different factors at two levels. The corresponding runs and designed formulations are given in Table 1.

**Particle size analysis**

The particle size was evaluated by sieving method. A series of standard sieves were used to sieve the resulting particles, and the optimum alginate microparticles were considered the ones with particle size less than 250 µm. These microparticles were passed from sieve with mesh 60.

**Determination of drug loading and encapsulation efficiency**

4 mg of alginate microparticles containing Nys were added to sodium citrate solution 1% (w/v) under magnetic stirring until complete dissolution of microparticles. Nys extraction was done by centrifugation at 5,000 rpm for 30 min, and separation of the settled drug from the supernatant. Nys was dissolved by adding sodium lauryl sulfate (SLS) solution 1% (w/v), and then, the solution was analyzed by a UV spectrophotometer. The percentage of drug loading and encapsulation efficiency were determined based on the following equations:

**Equation 1.** Drug loading % = \( \frac{\text{weight of drug loaded in microparticles}}{\text{total weight of microparticles}} \times 100 \)

**Equation 2.** Encapsulation efficiency % = \( \frac{\text{weight of drug used in microparticles}}{\text{weight of drug used in microparticles}} \times 100 \)

**Fourier transform infrared spectroscopy measurement**

Fourier transform infrared (FT-IR) spectroscopy was performed using FT-IR spectrophotometer (Bruker FTIR-Vertex 70, USA). Spectra of pure drug, sodium alginate, and alginate microparticles containing Nys were obtained using KBr pellet method at a wave number region of 400–4,000 cm⁻¹.

**Differential scanning calorimetry**

Thermal properties of Nys, sodium alginate, blank alginate microparticles, and physical mixture of Nys and blank microparticles were analyzed by differential scanning calorimetry (DSC). A known amount of each was weighed and placed in sealed aluminum pans. Heating was performed under a flow of nitrogen gas at a rate of 5°C/min in the temperature range 25–300°C using DSC 302 apparatus (BAHR Thermoanalyse GmbH, Germany). A blank aluminum pan was used as the reference.

**Measurement of mucoadhesive force**

There are various methods for measuring the force of adhesion of mucoadhesive products; most of them are based on measuring the force required to detach the product from a smooth surface. This research employed a homely designed apparatus, which consisted of a fixed base, a jack, a digital scale fixed to a metal
frame, and two pieces of smooth glasses with the product placed between them. Once the product is placed between the two surfaces, by turning a knob and lowering the movable part a certain shear stress is created, which is shown by the digital scale (Mohammadi-Samani, Bahri-Najafi et al. 2005).

A biological substrate (sheep's oral mucosa) with a cross section of 4 cm² was used for examining mucoadhesion of the product. The biological mucosa was applied on one of the glass surfaces. Half a gram of cp934 gel containing alginate microparticles was also put between the two glass surfaces in contact with the mucosa. The jack was lifted to make the digital scale show a certain value. Consequently, the jack was lowered slowly, and the maximum value shown by the scale when the two glass surfaces detached was considered as the gel's adhesion strength. The force was calculated in g/cm² by dividing the value into the cross-section area. The adhesion determination process was repeated five times, and the average was reported in g/cm².

**Drug release studies**

USP dissolution apparatus II (Erweka, Germany) was used for studying drug release. 250 ml of buffer solution containing 1% w/v SLS solution in water was poured into the apparatus vessels, and the temperature and rotation speed were set to 37±0.5°C and 50 rpm, respectively. Consequently, three 3 cm-diameter petri dishes were filled equally (5.3 g) with cp934 gel containing alginate microparticles. Each petri dish was fixed individually at the bottom of the dissolution vessels, and samples were taken at 10, 20, 30, and 60 min. At each interval, 5 ml of the solution of each vessel was taken and replaced by 5 ml of fresh buffer. Absorption of the samples was measured by a UV spectrophotometer at 306 nm against buffer with 1% SLS as the blank solution. The amount of released drug was calculated using the standard curve equation. Average of the amount of drug released was calculated and converted into drug release percentage.

To validate the quantification method, the test was also carried out on drug loaded alginate microparticles without gel base and drug-free alginate microparticles, and absorption of each sample was recorded using a spectrophotometer at a wavelength of 306 nm.

**RESULTS AND DISCUSSION**

Mucoadhesive drug delivery systems have opened new horizons in drug delivery, as they can increase retention time of the dosage form in the place and hence improve the therapeutic efficacy. By merging multi-unit dosage forms and mucoadhesion, a controlled release drug delivery system with improved patient compliance could be achieved. These dosage forms can attach to mucosa, stay in the site, and release an acceptable amount of drug content at a given time. These are slow-releasing systems, and if properly designed, can release the drug regularly and controlled (Helliwell 1993, Lee and Chien 1996). Alginate microparticles are one of these multi-unit systems that can be readily prepared without need to harsh conditions or toxic solvents (Alipour, Montaseri et al. 2016).

**Preparation of microparticles**

Sodium alginate, a sodium salt of alginic acid, is soluble in water and is crosslinked with divalent or polyvalent cations such as calcium ion, to form an insoluble network (Østberg, Lund et al. 1994). Being biodegradable and biocompatible polymer, with low toxicity and immunogenicity and high availability and affordability, alginate microparticles could be an attractive choice for development of controlled release drug delivery systems. Indeed, because of mucoadhesive properties, alginate microparticles could stick to the mucosa for prolonged period of time and have been exploited for the site-specific drug delivery to mucosa (Tafaghodi, Tabasi et al. 2006, Laffleur, Shahnaz et al. 2013).
Mucoadhesive gel of nystatin for oral mucosal delivery

Table 2. Effect of different parameters on size of microparticles

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Alginic (w/v%)</th>
<th>CaCl₂ (w/v%)</th>
<th>Alginic to CaCl₂ ratio</th>
<th>Drug to polymer ratio</th>
<th>Particle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1:10</td>
<td>1:1</td>
<td>&lt;250</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>1</td>
<td>1:10</td>
<td>1:1</td>
<td>710</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1:10</td>
<td>1:1</td>
<td>296</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1:5</td>
<td>1:1</td>
<td>450</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1:10</td>
<td>1:5</td>
<td>710</td>
</tr>
</tbody>
</table>

Alginate microparticles were prepared using the ionic gelation method. To this end, the drug was added to an aqueous solution of sodium alginate under stirring with a magnetic stirrer. Then, the mixture of drug and sodium alginate was added to the aqueous solution of calcium chloride using an insulin syringe under stirring with the magnetic stirrer. Alginate microparticles were formed through ionic interaction between positive ions of calcium and negative carboxylic ions of alginate polymer. The impact of parameters effective on particle size is shown in Table 2. As presented in the table, using 0.5% (w/v) sodium alginate did not yield small size microparticles. 2% (w/v) calcium chloride resulted in a high content of calcium ions in the system, leading to binding of a high number of calcium ions to carboxyl groups of glucuronic acid in the structure of sodium alginate. Then, the system viscosity was increased, which resulted in large size microparticles. By reducing the volume of calcium chloride solution to 5 ml, more microparticles were accumulated and bound to each other, and hence, bigger microparticles were obtained. A drug to polymer ratio of 1:5 did not result in small size microparticles. The finest microparticles were obtained from 1% (w/v) alginate and 1% (w/v) calcium chloride, alginate to calcium chloride ratio of 1:10, and drug to polymer ratio of 1:1. They were smaller than 250 µm in size, and all passed through mesh no. 60. Previous studies on alginate microparticles also reported similar results. Tafaghodi et al. observed that increasing alginate concentration to more than 2% (w/v) results in increased particle size (Tafaghodi, Tabasi et al. 2006). There are many reports on the optimum concentration of calcium chloride solution to prepare fine particles. Heng et al. showed that high concentrations of calcium ions would raise the viscosity of the system and size of the particles (Heng, Chan et al. 2003).

Drug loading and encapsulation efficiency

The loaded drug was measured through a simple and direct method. After washing the microparticles deposited by centrifugation with 1% (w/v) solution of sodium citrate, the sample was diluted with an appropriate amount of SLS 1% (w/v). Sodium citrate can detach calcium ions from calcium alginate due to its ability to create complex between citrate and calcium ions and therefore increase the solubility of alginate. There are many controversies in choosing an optimum concentration for calcium chloride to achieve high drug loading efficiency (Østberg, Lund et al. 1994, El-Kamel, Al-Gohary et al. 2003). Calcium chloride solution 1% (w/v) was used in this study, which was suitable for loading the drug and drug loading efficiency. The results of the amount of drug loaded in alginate microparticles and encapsulation efficiency were 49.1 and 98.2%, respectively, which is quite high for loading such an insoluble large molecule in a hydrophilic carrier.

FT-IR results

The infrared spectra of Nys, alginate and Nys-loaded alginate microparticles are presented in Figure 1. IR spectrum of Nys showed a strong absorption frequency at the wavenumber of about 3,410 cm⁻¹, which is related to stretching vibrations of hydrogen bonds. While absorption frequency of 2,934 cm⁻¹ is due to asymmetric stretching vibration of methylene groups and absorption frequency of 1,706 cm⁻¹ to stretching vibration of ester carbonyl and carboxylic acid groups. Absorption frequencies of 1,556 cm⁻¹ and 1,844 cm⁻¹ are related to CH=CH double bonds, absorption frequency of 1,400 cm⁻¹ to flexural vibrations of C-H group, and 1,070 cm⁻¹ to hydroxyl groups. IR spectroscopy of alginate showed a strong absorption frequency in 3,075 cm⁻¹, which is related to the primary amino group, and 1,423 cm⁻¹ and 1,624 cm⁻¹ to asymmetric and symmetric stretching vibration of the carboxyl group, respectively. IR spectroscopy of Nys-loaded alginate microparticles showed a high frequency absorption at 3,275 cm⁻¹, which is related to stretching vibrations of hydrogen bonds in C=O, C-C-O, and O-C-C groups (Martín-Villena, Fernández-Campos et al. 2013). These spectra prove that Nys has been successfully loaded to alginate microparticles, and the main peaks of drugs could be seen in the spectrum of the particles.

DSC results

The results of DSC analysis for Nys, alginate, physical mixture of alginate and Nys, drug-free alginate microparticles, and Nys-loaded alginate microparticles are presented in Figure 2. Nys melting peak was seen at 160.3°C, above which the drug was degraded. Alginate melting peak was appeared at
227.1°C. Melting peaks of physical mixture of alginate and Nys were observed at 169.6°C and 228.4°C, which is the proof of compatibility. The polymer peak in the blank alginate microparticles was seen at 151.3°C; therefore, the polymer was not degraded. Two peaks were appeared at 145°C and 168.6°C for Nys-loaded alginate microparticles. The peak at 168.6°C can be related to free Nys on the microparticles surface. The DSC results are almost similar to the study of Martín-Villena et al. (Martín-Villena, Fernández-Campos et al. 2013).

**Measurement of mucoadhesive force**

Pharmaceutical mucoadhesive systems are used mainly to achieve two goals: first, slow and delayed release of active substance of formulation, which can lead to a uniform and appropriate plasma concentration of systemic products, and second, to locally focus the drug molecules of interest with a maximum absorption peak and hence a maximum effect. This property is applied in both topical and systemic products. The results of measurement of mucoadhesion capability of gel 1% (w/v) of cp934 containing microparticles and the gel of cp934 without alginate microparticles are shown in Table 3. In this study, the mucoadhesive polymer cp934 was used to prepare the mucoadhesive gel. This synthetic high molecular weight polymer is derived from acrylic acid and contains 56 to 68% carboxyl groups. The water absorption capacity of cp934 is low; thus, it has weak hygroscopicity, but a high tensile strength. The binding mechanism of poly-carboxylic acids such as cp934 to mucin is performed through swelling of the polymer in water, which results in interacting of polymer chains with superficial mucus (Llabot, Manzo et al. 2004). We used a cp934 solution 1% (w/v), because the system integrity was acceptable with this concentration and the system was low porous. According to other studies, use of high concentrations of carbopol934 creates a strong acidity in the mouth, resulting in significant adverse effects in the mucosa (Llabot, Palma et al. 2007, Llabot, Palma et al. 2007).

**Drug release study**

The profiles of drug release from gel-free and gel-based alginate microparticles are shown in Figure 3. This formulation showed an initial burst release, because the release environment contains 250 ml SLS solution 1% (w/v) in water, which can affect the structure of alginate, leading to rapid release of the drug. SLS 1% (w/v) solution in water was used in this study for the release medium to establish sink conditions, because the drug is easily dissolved in SLS, leading to a concentration gradient, while no concentration gradient is provided in case of low solubility, and hence, precipitation of the drug may happen. As depicted in the figure, drug release from alginate microparticles was completed in 20 min, but it...
was continued until 30 min when the particles were loaded in cp934 gel, which is not statistically different (P > 0.05). As alginate is a hydrophilic polymer and the drug is dissolved in SLS easily and quickly, it results in the maximum solubility of Nys in the release medium. It should be noted that the large molecule of the drug may be adsorbed on the microparticles surface, leading to the burst release.

Distilled water has been used for the release study of Nys-loaded alginate microparticles by Martín-Villena et al. As Nys is not dissolved in water, no burst release was reported (Martín-Villena, Fernández-Campos et al. 2013). Other studies on the release behavior of Nys have also used distilled water for the release environment and reported a slow drug release (Llabot and Manzo 2002).
CONCLUSION

A new and stable mucoadhesive gel of Nys-loaded alginate microparticles with desired size and high drug loading efficiency was developed by ionic gelation method. The size of the particles was dependent on different factors, including concentration of alginate and calcium solutions and their ratios, as well as to drug to polymer ratio. Carbopol base of the gel makes it very strong mucoadhesive system. Ability of this system to adhere to the oral mucosa has great appeal for the treatment of localized oral infections. In addition, the dosage form can mask the bitter taste of drug and retain it in the mouth for long periods.

ACKNOWLEDGMENT

This paper was based on a Pharm D. thesis written by Samaneh Karimaddini and was financially supported by Shiraz University of Medical Sciences.

References