The effect of plasticizers and crosslinking on the printability, structural properties and release behaviour of boric acid-loaded chitosan films prepared by 3D printing

Ayse Nur Buke¹,², Muge Kilicarslan¹*, Osama Ali Hindy³, Pinar Yilgor Huri³

¹Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06560, Ankara, Turkey
²Ankara University, Graduate School of Health Sciences, 06110, Ankara, Turkey
³Ankara University, Department of Biomedical Engineering, 06830, Ankara, Turkey

Introduction

The use of three-dimensional (3D) printing technology for tissue engineering and to produce various therapeutic systems has gained significant interest in recent years. Therapeutic systems with any desired shape and size can be easily manufactured through 3D printing, using various natural polymers including chitosan. By semi-solid extrusion (SSE), it becomes possible to prepare films and hydrogels with sufficient mechanical and biological properties that mimic natural tissue (Wei et al., 2023).

In this study, boric acid-loaded chitosan hydrogels were prepared as an antimicrobial film formulation by SSE printing. The effect of different plasticizers with various concentrations and drug concentrations were investigated. The effect of the plasticizer and the crosslinking onto the 3D film was investigated in terms of structural properties, printability, and drug release.

Materials and methods

Chitosan (75–85% DD, Sigma-Aldrich, USA), Boric acid (BA) (BOREN, Turkey), Propylene glycol (PG) (Aklar Chemicals, Turkey), Glycerol (Riedel-de Haen, Germany), and Polyethylene glycol 400 (PEG) (Aldrich, Germany) were used.

BA-loaded chitosan hydrogels were prepared by dissolving chitosan and BA in 1.5% (v/v) acetic acid at room temperature. PG (F1), PEG (F2) and glycerol (F3) were used as plasticizers with constant concentration of 10% (w/w), and 10mg of BA. Then, for F4-F6, the amount of glycerol was increased from 25% to 75%. Besides, for F7-F10, the amount of BA was increased from 10 mg to 90 mg gradually with the same amount of plasticizer (25%).

3D film model was designed as a cubic mesh scaffold using 3D Builder software (3D Builder, USA) exported as a STL file and sliced by AxoSuite (Turkey). The films were fabricated by an Axo A3 3D printer (Axolotl, Turkey) at room temperature using the following printing parameters: 25-gauge conic needle, layer height of 0.15 mm, speed of 9 mm/s, and pressure of 10.0 psi.

The viscosity of the hydrogel formulations (V) was analysed using a viscometer (Brookfield RVTDV-2, USA) with T spindle-No: 95 at 100 rpm (n=3). The weight of each film was determined, and then the thickness (T) of 3D films was measured by a micrometer (NSK, Japan) (n=6).

The swelling degree of the 3D films calculated through the equation (n=3): Swelling degree (%) =100× (weight of the swollen film at time t - initial weight of film) / initial weight of film.

The adhesiveness (AF) and tensile strength (TS) of the films were examined with a texture analyser (TA.XT plus, Stable Micro Systems, U.K.).

Drug content (DC) was determined using UV spectrophotometric method (430 nm) and in vitro drug release studies were performed by static method in 5 mL of pH 7.4 phosphate buffer at a temperature of 37°C and shaken at 75 rpm and analysed by UV spectrophotometric method (419 nm).

Results and discussion

*cmkilicarslan@gmail.com; muge.kilicarslan@pharmacy.ankara.edu.tr
Formulations ranging from F1 to F9 with different viscosities were successfully used in 3D film printing using the SSE method. Although films prepared with PG (F1) and PEG (F2) exhibited higher viscosity than film containing glycerol (F3), no significant difference was observed in the average film thickness. The use of PG resulted in a minor decrease in film adhesion. The highest swelling (2305% ± 192.5) was observed in the film containing the most hydrophilic plasticizer, PEG, while the lowest swelling rate (1267% ± 116.4) was observed in the films containing less hydrophilic PG. Films containing highly hydrophilic PEG revealed fragmentation during swelling. The effect of plasticizers on the tensile strength and adhesion of these films was found relatively higher in films containing glycerol (Table 1).

Table 1. Structural properties of the films (value ± SD)

<table>
<thead>
<tr>
<th></th>
<th>V (Pa.s)</th>
<th>T (μm)</th>
<th>DC (%)</th>
<th>TS (N)</th>
<th>AF (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>35.70 ± 81.67 ±</td>
<td>*</td>
<td>29.85</td>
<td>2.382 ±</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>35.33 ± 82.50 ±</td>
<td>*</td>
<td>32.51 ±</td>
<td>2.720 ±</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>26.67 ± 85.83 ±</td>
<td>90.83 ±</td>
<td>31.99 ±</td>
<td>2.796 ±</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>29.57 ± 103.3 ±</td>
<td>84.05 ±</td>
<td>14.75</td>
<td>2.092 ±</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>31.53 ± 117.5 ±</td>
<td>84.10 ±</td>
<td>7.862 ±</td>
<td>1.559 ±</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>36.53 ± 111.7 ±</td>
<td>83.88 ±</td>
<td>0.830 ±</td>
<td>0.478 ±</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>27.83 ± 134.2 ±</td>
<td>87.47 ±</td>
<td>18.29 ±</td>
<td>2.711 ±</td>
<td></td>
</tr>
<tr>
<td>F8</td>
<td>27.90 ± 126.7 ±</td>
<td>94.87 ±</td>
<td>17.32 ±</td>
<td>2.559 ±</td>
<td></td>
</tr>
<tr>
<td>F9</td>
<td>28.57 ± 142.5 ±</td>
<td>97.45 ±</td>
<td>8.140 ±</td>
<td>2.591 ±</td>
<td></td>
</tr>
</tbody>
</table>

*Drug content has not been determined for F1 and F2 due to their improper structure.

The viscosity of the hydrogel increased with the increase of the glycerol content from 10% w/w to 75% w/w (F3-F6) (Table 1). The thickness of the film also increased with the increase of the film content. However, the increase of the plasticizer content decreased water absorption capacity, strength, and adhesion properties of the films. This indicates that glycerol, through its classical plasticizer effect, penetrates into the chitosan matrix reducing the intermolecular interactions and polymer mobility (Thakhiew et al., 2010).

With a constant amount of glycerol, a nine-fold increase of the BA amount in the films (F10) resulted in a significant increase in viscosity (39.83 Pa.s ± 0.42), hence, it could not be printed. The increase of BA amount (from 10 mg to 60 mg) in films (F4, F7-F9) did not affect their adhesion, it led to a decrease in their mechanical strength. Furthermore, up to a three-fold increase of BA amount in films (F4, F7-F8) led to a decrease in the swelling ratios, which can be attributed to a crosslinking effect between BA and chitosan (Fig. 1a). Additionally, a six-fold increase of BA (F9) resulted to a high increase of swelling ratio, which could be attributed to a higher rate of crosslinking, resulting in a more brittle structure (Liang et al., 2019).

![Fig. 1. (a) Swelling degrees of films, (b) in vitro release of BA (→:F4, ←:F5, F6:→:F7, F8:←:F9)](image)

The release profiles of BA and the swelling properties of the films were similar, where the film with a higher swelling ratio showed a higher release rate (Fig. 1b). Besides, due to the BA hydrophilicity, a burst release followed by a decreasing release profile was observed in all profiles.

**Conclusion**

Hydrogels with different viscosities were successfully printed as 3D films using various types and concentrations of plasticizers that affect the printability, swelling degree, strength, and adhesion of the films. Furthermore, the release profiles and the swelling properties of the films were proportional. The changes observed in the structural properties of the films with the addition of varying concentrations of BA indicates the possibility of crosslinking between BA and chitosan.

This study was supported by TÜBİTAK 1001 project numbered 121R076.

**References**


Maced. pharm. bull., 69 (Suppl 1) 109 - 110 (2023)