



(This article was presented to the 28th National Chemistry Congress and submitted to JOTCSA as a full manuscript)

## **Production and Investigation of Controlled Drug Release Properties of Tamoxifen Loaded Alginate-Gum Arabic Microbeads**

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**Abstract:** The entrapment of tamoxifen onto alginate-gum arabic beads and the production of controlled drug release was investigated in this study. The polymeric system that would provide the controlled release of tamoxifen was formed using alginate and gum arabic. In the first phase of the study, the optimization of the alginate-gum arabic beads production was conducted; then the study continued with drug entrapment experiments. Tamoxifen entrapment yield was found to be approximately 90% of initial tamoxifen concentration. *In vitro* drug release experiments were performed in simulated gastric juice and intestinal fluid where the tamoxifen release was 20% and 53% of the initial drug present, respectively. As a result of this study, it is expected that a valuable contribution to the field of controlled drug release system production is realized.

**Keywords:** Tamoxifen, alginate, gum arabic, bead, controlled drug release.

**Submitted:** July 4, 2016, **Revised:** August 4, 2016, **Accepted:** August 11, 2016.

**Cite this:** Korkmaz O, Girgin B, Sunna Ç, Yavaşer R, Karagözler A. Production and Investigation of Controlled Drug Release Properties of Tamoxifen Loaded Alginate-Gum Arabic Microbeads. Journal of the Turkish Chemical Society, Section A: Chemistry. 2016;3(3):47-58.

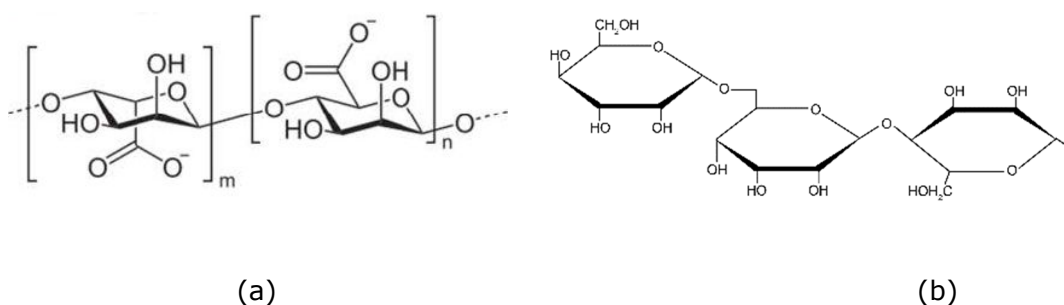
**DOI:** 10.18596/jotcsa.77290.

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## INTRODUCTION

Controlled drug release systems have a significant role in medicine. They are considered as useful systems that enable the drug to be more efficient. These systems have advantages such as decreasing the risk of harmful effects that may come from the drug and increasing the efficiency and availability of the drug compared to conventional drug applications (1). In controlled drug release systems, the drug is entrapped by the carrier. The carrier is required to release the drug in a desired manner during its voyage to the defined part of the body or when it reaches there.

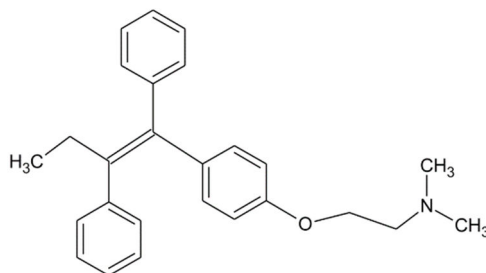
Natural polymers have gained attention as suitable materials to produce controlled drug release systems (2). Alginate (Figure 1a) is a natural polysaccharide composed of  $\alpha$ -1,4-guluronic acid and  $\beta$ -D-mannuronic acid blocks. Guluronic acid residues are responsible for the gelation properties of alginate. Alginate is a water-soluble, linear, and polyanionic biopolymer obtained from brown algae and it is employed in numerous applications such as food, textile, pharmaceutical, cosmetic industries as stabilizers, thickening and gelling agents, and adhesives. Alginate is reported to be safe when it is taken by the oral route since it is nontoxic and biodegradable (3). Alginate can be gelled with  $\text{Ca}^{2+}$  anions easily. Gel beads, microparticles and nanoparticles can be synthesized from alginate alone or together with other polymers like chitosan, gelatin, and albumin. Gum arabic (Figure 1b) is a polysaccharide obtained from acacia tree. Similar to alginate, gum arabic finds usage in food and other industries.



**Figure 1.** Molecular structures of alginate (a) (4) and gum arabic (b) (5).

Tamoxifen [(Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethyl-ethanamine, TMX] (Figure 2) is a synthetic drug used for cancer therapy. It is an antagonist for estrogen receptors in mammary tissue via its active metabolite hydroxytamoxifen (6). Antagonists attach to the cell receptor and act as a natural stimulator and prevent the natural response (7). TMX is being administered to patients with different stages of breast cancer and although it is a very effective drug, in high doses it may lead some harmful developments such as endometrial cancer and tumor progression (8-9). Entrapment materials were synthesized for minimizing these disadvantageous effects to capture tamoxifen and deliver

it to the tumor site. Polymeric micelles (10), nanoparticles (11-12), alginate/chitosan microparticles (13), and hydrogels (14) are examples of tamoxifen carriers.



**Figure 2.** Chemical structure of tamoxifen molecule (15).

In this study, the entrapment of tamoxifen into alginate/gum arabic beads and *in vitro* controlled tamoxifen release was investigated. The aim of the study was to develop a new carrier for tamoxifen with a high entrapment yield and good carrier properties.

## MATERIALS AND METHODS

### Materials

Tamoxifen citrate, alginic acid sodium salt from brown algae (medium viscosity), CaCl<sub>2</sub>, gum arabic, ethanol, and HCl were purchased from Sigma (Steinheim, Germany). Tween 80 was obtained from Merck (Darmstadt, Germany) and KCl was obtained from Carlo Erba (Ronado, Italy). Phosphate buffer tablets (PBS) were purchased from Oxoid (Hampshire, England). All chemicals used were of analytical grade.

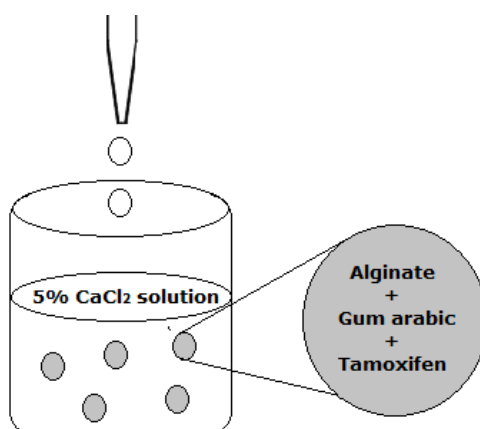
### Production of plain alginate-gum arabic beads

Plain beads were prepared by ionic crosslinking method (16). Two hundred milligrams of alginic acid sodium salt was dissolved by stirring the solution at 200 rpm at room temperature for 30 min in 20 mL distilled water. Gum arabic (100 mg) was added slowly and mixture was stirred for 30 min at 200 rpm and sonicated for 5 min. Alginate-gum arabic solution was added dropwise into continuously stirred (100 rpm) 5% CaCl<sub>2</sub> solution. The resulting beads were matured for 1 h, filtered, and washed with distilled water.

### Preparation of TMX entrapped alginate-gum arabic beads

TMX entrapped beads were produced by adding 1.0 mL of TMX solution (3 mg/mL in ethanol) into 20 mL of alginate solution (1%, w/v) containing 100 mg gum arabic. The

solution was mixed at 200 rpm for 30 min. The resulting solution was added dropwise into the crosslinker solution (5% CaCl<sub>2</sub>) and the drug-entrapped beads were obtained (Figure 3). The beads were matured for 1 hour, then filtered. All experiments were employed triplicate.



**Figure 3.** Production of TMX entrapped alginate-gum arabic beads.

### Entrapment efficiency (EE%)

Entrapment efficiency was calculated evaluating the drug content in the filtrate that indicates the untrapped TMX. Two mL of the filtrate was centrifuged at 18000 rpm at 4 °C for 30 min in order to remove any suspended molecules and the absorbance of the supernatant was recorded at 276 nm. The EE% of beads was calculated from the formula given below:

$$EE = \frac{\text{Total TMX added (mg)} - \text{Untrapped TMX (mg)}}{\text{Total TMX added (mg)}} \times 100$$

### *In vitro* release studies

*In vitro* release of TMX from alginate-gum arabic beads was carried out at 37±1 °C in 50 mL of simulated gastric fluid (SGF) (mixture of 100 mL 0.2 M HCl and 82.8 mL 0.2 M KCl, pH 1.5) and 103 mL of simulated intestinal fluid (SIF) (100 mL PBS solution containing 3.0 mL of 1% Tween 80, pH 7.4) separately. At 30 min intervals, 2.0 mL aliquots were sampled out. Prior to absorbance measurements at 276 nm, the samples were centrifuged at 18000 rpm at 4°C for 30 min to remove any suspended particles. The percent of TMX released was calculated and plotted versus time.

The release profile of TMX was also evaluated in SGF and SIF consecutively. Beads were transferred into 100.0 mL of SGF and allowed to stir at 150 rpm for 2 h. At each 30 min intervals, 2.0 mL of the medium was sampled and centrifuged at 18000 rpm at 4 °C for 30 min and the absorbance was recorded at 276 nm. Then, beads were removed from SGF and transferred into 103 mL of SIF and stirred for another 3 h. The release procedure was followed in intestinal medium for 22 hours. Release experiments were conducted at 37 °C in triplicate. The amount of released tamoxifen was calculated from TMX standard curve.

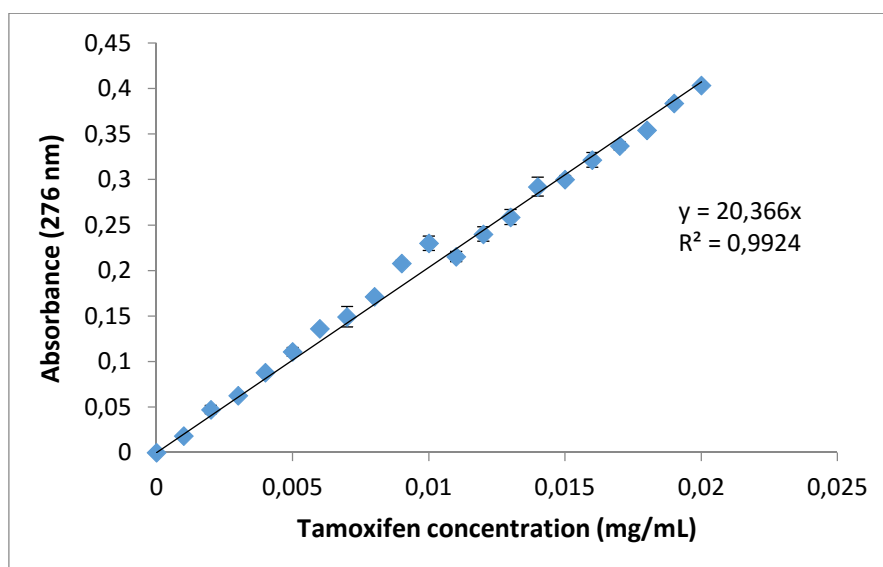
## RESULTS AND DISCUSSION

In this study, an anticancer agent, Tamoxifen, was selected as the model drug which was further entrapped by ionic crosslinking method in alginate and gum arabic natural polymers. Alginate-gum arabic beads were produced and their abilities to release the anticancer drug Tamoxifen in a controlled manner were investigated. A polysaccharide blend was prepared composed of alginate and gum arabic. Spherical beads were obtained about 3 mm size (Figure 4).



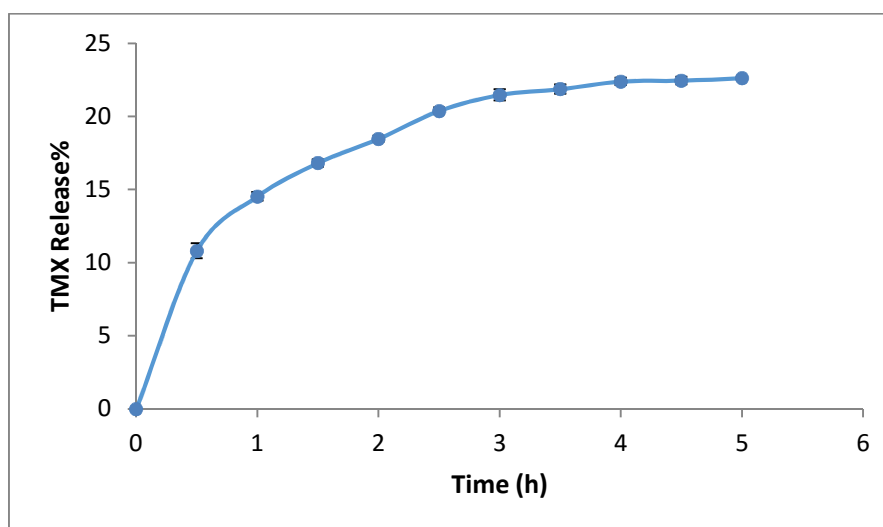
**Figure 4.** TMX entrapped alginate-gum arabic beads.

TMX entrapment efficiency was calculated using the TMX standard curve which is demonstrated in Figure 5. According to the results from entrapment studies, approximately 90% of TMX was entrapped in the beads.



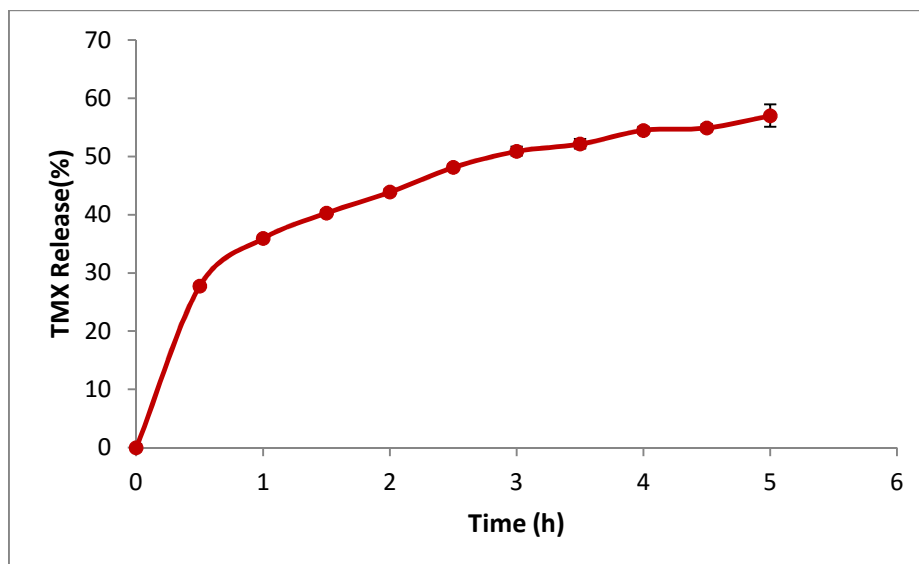
**Figure 5.** TMX standard curve.

The extend of drug release in *in vitro* stomach (gastric) and small intestine (intestinal) media were measured using pH 1.5 and pH 7.4 for 24 hours. The results revealed that the release of the initial TMX present in beads was 20% at pH 1.5 whereas it was 53% at pH 7.4. The release ratio did not change significantly after five hours. The release profiles in SGF and SIF are shown in Figures 6 and 7.



**Figure 6.** The release profile of TMX entrapped alginate-gum arabic beads in SGF.

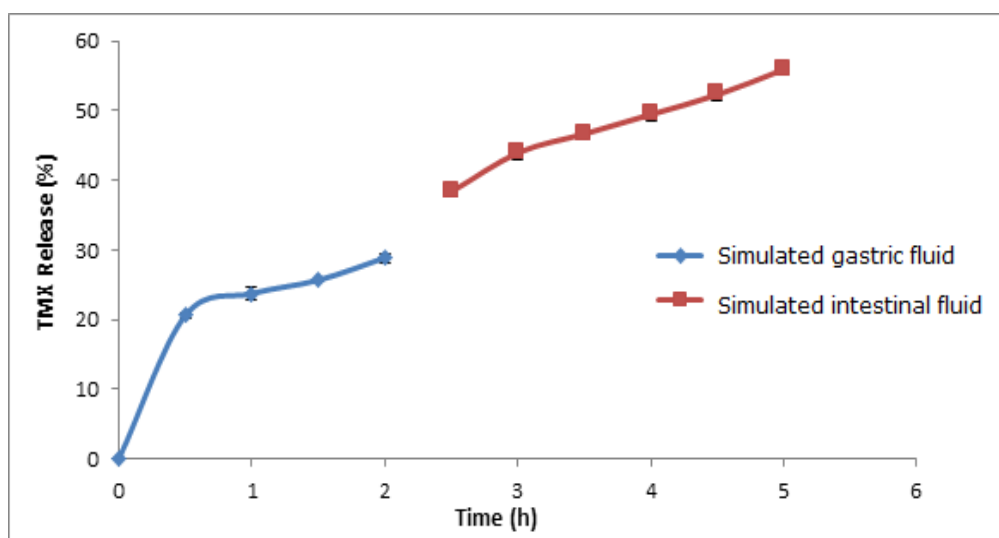
For *in vitro* drug release studies, the physiological conditions such as pH and temperature are important. Simulated gastric fluid (pH 1.5) and simulated intestinal fluid (pH 7.4) are usually preferred as drug release media. The temperature of the release medium is generally chosen as 37 °C since it is the temperature of the human body. By this way, the results reflect the behavior of drug carrier particle in the body.



**Figure 7.** The release profile of TMX entrapped alginate-gum arabic beads in SIF.

The release percentage in SGF was lower compared to SIF. This may be explained by the swelling behaviors of beads in these media. Alginate-gum arabic beads might have the capability to swell and release the drug entrapped slightly in gastric medium. However, they might swell more in intestinal media and also disintegrate and allow the drug to become free (17). Disintegration may be the major reason of higher release profile since calcium favors to form calcium phosphate (18), as it is observable that the release medium was turbid during the studies in SIF.

The consecutive TMX release in SGF and SIF was also studied and was illustrated in Figure 8. The results were similar to individual procedures applied in SGF and SIF alone. However, this treatment was assumed to be more convenient to mimic the stages that a drug passes in the human body. As seen in Figure 8, 30% of TMX was released in SGF and when the beads were transferred into SIF, with a shift of 10% in 30 min, 60% of cumulative TMX release was observed after 5 h.



**Figure 8.** Consecutive TMX release from alginate-gum arabic beads in SGF and SIF.

## CONCLUSION

The polymeric system that provided the controlled release of Tamoxifen was built using alginate and gum arabic natural polymers. *In vitro* drug release experiments were conducted in simulated gastric juice and intestinal fluid using the tamoxifen entrapped beads. It may be concluded that results presented here indicates that it is possible to manufacture a working drug carrier and release system for Tamoxifen using alginate and gum arabic natural polymers. The study needs further investigation for production of the drug carrier in real time manufacturing plants.

## ACKNOWLEDGEMENTS

The authors thank to Adnan Menderes University Research Fund for the financial support of this study under a project (Project Number: FEF 16006).



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**Türkçe Öz ve Anahtar Kelimeler**

**Tamoksifen Yüklü Aljinat-Gam Arabik Mikroboncukların Üretimi ve Kontrollü İlaç Salımı Özelliklerinin İncelenmesi**

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**Öz:** Bu çalışmada aljinat-gam arabik boncuklarına tamoksifenin tutturulması ve kontrollü ilaç salımının üretilmesi araştırılmıştır. Tamoksifenin kontrollü salımını sağlayacak polimerik sistem aljinat ve gam arabik kullanılarak oluşturulmuştur. Çalışmanın ilk kısmında, aljinat-gam arabik boncuklarının en uygun şartlarda elde edilmesi sağlanmıştır; ardından çalışma ilaç tutuklama deneyleriyle devam etmiştir. Tamoksifen tutuklaması ilk tamoksifen derişiminin yaklaşık %90'ı kadar tespit edilmiştir. *In vitro* ilaç salım deneyleri yapay mide ve bağırsak ortamına benzer ortamlarda gerçekleştirilmiştir ve tamoksifen salımı baştaki ilaç derişiminin sırayla %20'si ve %53'ü olarak bulunmuştur. Bu çalışmanın sonucu olarak, kontrollü ilaç salımı sistem üretimine değerli bir katkıda bulunulması beklenmektedir.

**Anahtar kelimeler:** Tamoksifen, aljinat, gam arabik, boncuk, kontrollü ilaç salımı.

**Gönderilme:** 4 Temmuz 2016, **Düzeltilme:** 4 Ağustos 2016, **Kabul:** 11 Ağustos 2016.

