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## Production and Drug Release Assessment of Melatonin-Loaded Alginate/Gum Arabic Beads

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**Abstract:** Melatonin containing alginate-gum arabic beads were produced by ionotropic gelation. The most suitable alginate-gum arabic ratio for bead production was optimized. To the optimized beads the loading of changing amounts of melatonin was conducted to produce the beads with the best melatonin entrapment and release. Entrapment efficiency of the beads as melatonin carrier was calculated to be approximately 70%. Release profiles of melatonin from the beads were generated using *in vitro* gastric fluid (pH 1.5) and intestinal fluid (pH 7.4). According to results obtained, following a burst in the first half hour, melatonin release from the beads in pH 1.5 medium was approximately 45% in the first 5 hours whereas the release was approximately 50% in pH 7.4 medium. The results indicate that the beads manufactured may be used as carriers for controlled release of melatonin.

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

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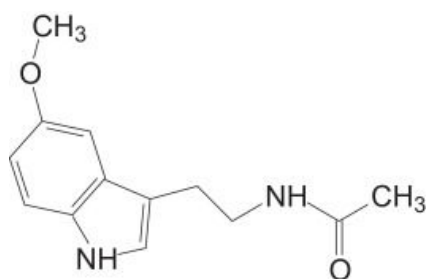
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## INTRODUCTION

Conventional application of drug doses for treatment of diseases involves repetitive introduction of the medicine with equal intervals (1). However, this method may cause fluctuation of the drug concentration and this may lead to low non-treating or high toxic levels. Controlled drug release systems aim to balance this fluctuation and provide a constant level of drug for a certain period of time (2). Controlled drug release systems are accepted as more reliable and high therapeutic carriers compared to conventional drug pills. Polymeric beads are regarded as good candidates for controlled drug release systems since they serve high bioavailability and stability. They also offer advantages such as limited fluctuation in the therapeutic range, reduced side effects, decreased dosing frequency and improved patient compliance (3).

Natural polymers have been widely used in controlled drug release formulations in view of their abundance and biocompatibility (3). Biopolymers are natural, biodegradable, and generally nontoxic materials. Due to these properties, they are often used in applications of pharmacology and food industry. Alginate is a natural polysaccharide which is mainly obtained from brown algae and is easily gelled when contacts with multivalent cations, especially  $\text{Ca}^{2+}$ , forming small pores which enable to entrap and release the desired molecule. The gelling and release profile of alginate beads differ depending on the conditions like the type and concentration of gelling agents, curing time, release medium, and the drug itself (4). Gums are naturally occurring polysaccharides which increase the viscosity of the solutions in which they are added even at very low concentrations. Gum arabic is the most frequently used one among them. It is also known as acacia gum since it is the leakage of acacia tree. Alginate and gum arabic are used in drug release formulations in several studies (5).

Melatonin (N-acetyl-5-methoxy tryptamine) (Figure 1) is a tryptophan-derived hormone which is synthesized and secreted from pinealocytes of pineal gland during night in human body. The most important task of melatonin molecule is to regulate circadian rhythm and sleep-wake cycle in humans. Melatonin also provides positive effects on immune system, reduction of tumor growth, antioxidative protection, and homeostasis (6). Low melatonin release may cause sleep disorder, tiredness, and memory impairment. In order to restore these disorders melatonin is available as over the counter supplement drug. Melatonin is excreted in urine as 6-sulphatoxymelatonin after metabolized by the liver (7).



**Figure 1.** Molecular structure of melatonin (8).

In this work, entrapment of melatonin molecule in alginate-gum arabic beads (AG) and their melatonin release properties were investigated. In order to achieve this, first, the most suitable alginate/gum arabic ratio was optimized. Then, using the optimal combination loading of changing amounts of melatonin was conducted to produce the beads with the best melatonin entrapment and release. Release profiles of the melatonin loaded beads (AGM) in simulated gastric (SGF) and intestinal (SIF) fluids were obtained.

## MATERIALS AND METHODS

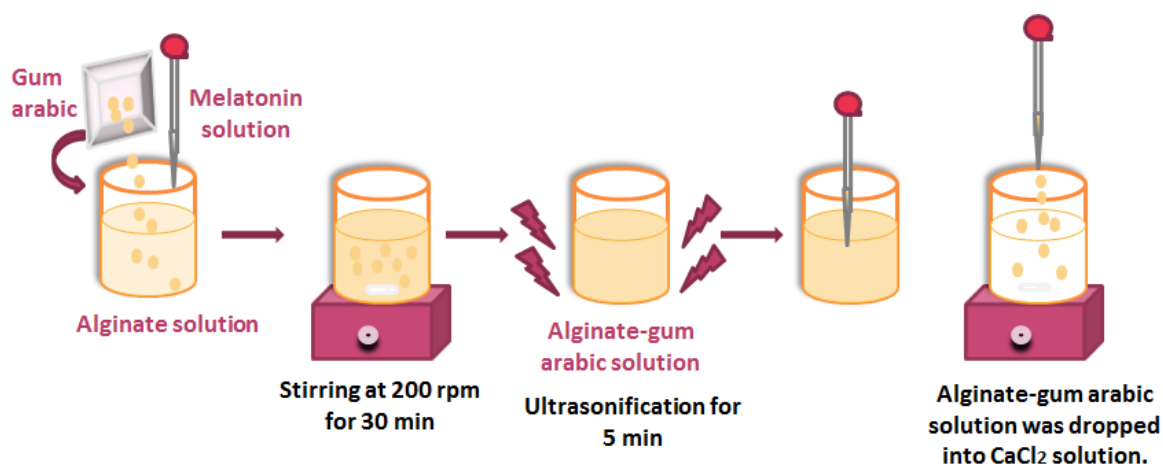
### Materials

Melatonin, alginic acid sodium salt from brown algae (low viscosity),  $\text{CaCl}_2$ , 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 were of analytical grade.

### Production of alginate-gum arabic beads and melatonin-loaded beads

AG beads were prepared by ionic crosslinking method reported by Malakar *et al.* (9). Briefly, 10.0 mL of aqueous alginate solution (1.5%, w/v) was prepared by stirring the solution gently at 200 rpm at room temperature for 1 h. Fifty milligrams of gum arabic 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%  $\text{CaCl}_2$  solution. The resulting matured beads were filtered and washed with distilled water. To produce AGM beads, 1.0 mL of melatonin solution (3 mg/mL in ethanol) was added slowly into alginate solution in order to achieve a homogenous mixture. Other steps

were employed as the same for production of AG beads (Figure 2). Optimization studies to determine the best bead formation were conducted using different alginate/gum arabic ratios. Also, the effect of initial melatonin concentration on entrapment efficiency was investigated at various drug quantities (1-10 mg). All experiments were employed in triplicate.



**Figure 2.** Preparation of melatonin loaded alginate-gum arabic beads.

### Determination of melatonin entrapment efficiency of AG beads

Entrapment efficiency (EE) of AG beads was calculated by measuring the absorbance of the supernatant obtained from the entrapment medium after centrifugation (Sigma 3-30KS, Germany) at 18000 rpm for 30 min employing a UV spectrophotometer (Shimadzu UV-1601, Japan) at 278 nm and calculating the % EE from the equation below.

Melatonin concentration was determined from the melatonin standard plot.

$$EE = \frac{\text{Total amount of melatonin added} - \text{Amount of free melatonin after entrapment}}{\text{Total amount of melatonin added}} \times 100 \quad (\text{Eq. 1})$$

### *In vitro* release of melatonin

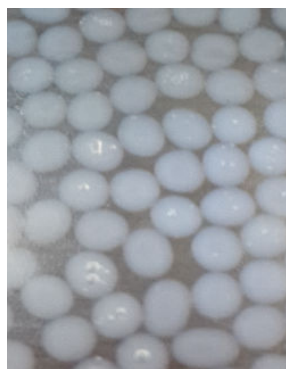
In order to determine the release profile of melatonin from AGM, beads were transferred into 50.0 mL of SGF (mixture of 100 mL 0.2 M HCL and 82.8 mL 0.2 M KCl, pH 1.5) and allowed to stir at 150 rpm for 2 h. At each 30 min intervals, 2.0 mL of the medium was collected and centrifuged at 18000 rpm at 4°C for 30 min and the absorbance was recorded at 278 nm. Then, beads were removed and transferred into 51.5 mL of SIF (50 mL PBS solution containing 1.5 mL of 1% Tween 80, pH 7.4) and stirred for 22 h more. The release

procedure was employed in intestinal medium for 22 hours. Release experiments were conducted at 37°C in triplicate. The amount of released melatonin was calculated from melatonin concentration curve.

## RESULTS AND DISCUSSION

### Production of alginate-gum arabic beads and melatonin loaded beads

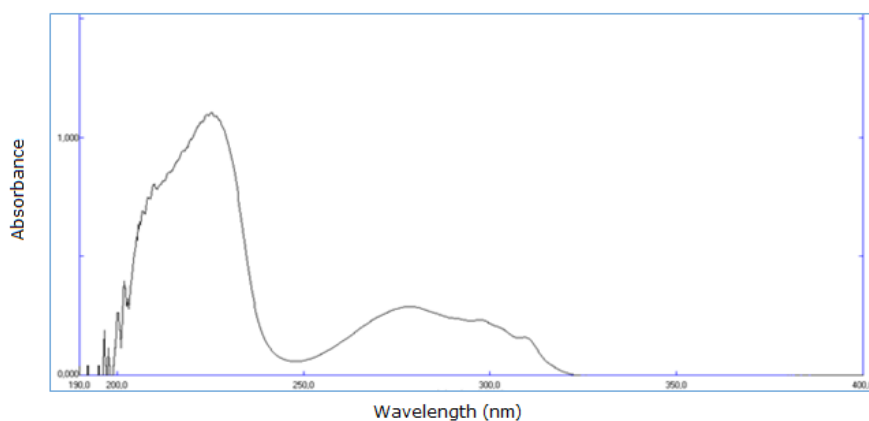
There are few studies concerning on synthesis of alginate-gum arabic beads and their usage in controlled drug release studies. Alginate and gum arabic are anionic polysaccharides. When they are exposed to calcium ions, carboxylate groups of both polymers interact with positively charged calcium ions by electrostatic interactions (10). AG and AGM beads produced in this study were spherical, semi-opaque, and their sizes were approximately 3 mm (Figure 3).



**Figure 3.** Melatonin loaded alginate-gum arabic beads.

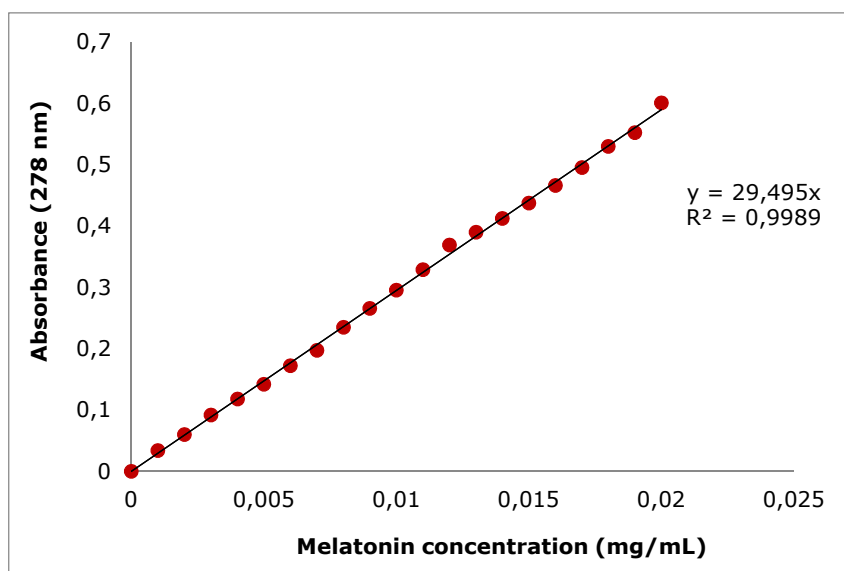
In order to study the best bead formation, ionically crosslinked alginate-gum arabic beads were produced using different amounts of alginate (0.5-2.0 w/v %) and gum arabic (0-100 mg). The most suitable combination was chosen as 1.5 % alginate solution containing 50 mg of gum arabic regarding to the stability, shape, and intensity of the beads.

Melatonin concentrations in entrapment and release media were measured spectrophotometrically. The UV spectrum (Figure 4) of melatonin solution (dissolved in ethanol) was obtained in order to determine the wavelength at which the measurements would be performed. All spectrophotometric measurements were recorded at 278 nm.



**Figure 4.** UV spectrum of melatonin solution.

Melatonin concentrations in the entrapment and release media were calculated using the plot (Figure 5) constructed with concentration dependent absorbance values at 278 nm.



**Figure 5.** Melatonin standard curve.

### Melatonin entrapment efficiency of AG beads

The effect of melatonin concentration on entrapment efficiency was evaluated and maximum EE% was calculated to be  $77.82 \pm 4.47\%$  with the optimized bead formulation (1.5 mg alginate-50 mg gum arabic) at the drug concentration of 3.0 mg in the entrapment medium (Table 1). Entrapment efficiency increased in the range of 1-3 mg melatonin and decreased beyond probably due to the saturation of the beads. Findings of Dubey *et al.* (2006) were consistent with our results where they loaded different amounts of melatonin into elastic liposomes (11).

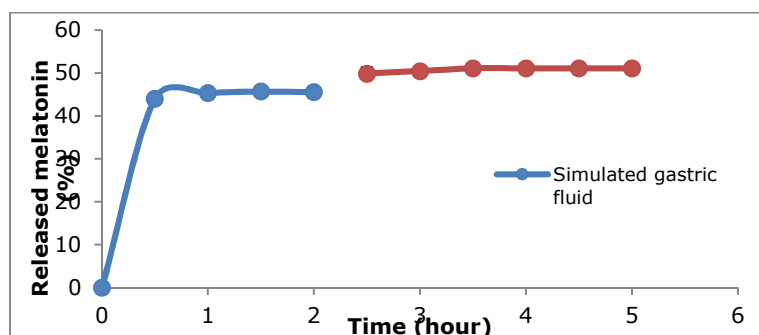
**Table 1.** Effect of melatonin concentration on EE%.

Alginate-Gum Arabic (% / mg)	Melatonin concentration (mg)	Entrapment (%)
1.5 - 50	1.0	58.45 ± 2.56
1.5 - 50	2.0	70.70 ± 5.41
1.5 - 50	3.0	77.82 ± 4.47
1.5 - 50	4.0	60.41 ± 4.60
1.5 - 50	5.0	60.73 ± 6.36
1.5 - 50	6.0	59.44 ± 3.62
1.5 - 50	7.0	62.58 ± 6.34
1.5 - 50	8.0	64.52 ± 3.63

***In vitro* release of melatonin from alginate-gum arabic beads**

The solubility and stability of drugs as well as increased pharmacologic efficiency can be enhanced by entrapping them in drug carriers (6). Melatonin has a short half-life, therefore, developing a controlled release system may be useful (12). Controlled release systems should release melatonin over 8-10 h to mimic the endogenous release of melatonin. Lee and Min (1995) have reported alginate beads for sustained release of melatonin for oral administration (13).

In this work, release experiments were carried out exposing the AGM beads to SGF for 2 h and SIF for 3 h consecutively to simulate the way that drugs pass in the human body. In SGF, totally 45.6% of the entrapped melatonin was release in 2 hours with a burst in the first 30 min. The cumulative percentage shifted to 51% when the beads were transferred to SIF (Figure 6). This increase may be explained by the tendency of alginate to erode and disintegrate in SIF. A burst release was also reported for melatonin loaded into stearyl alcohol microspheres. Drug release was retarded by coating the spheres with chitosan and alginate (14).



**Figure 6.** Consecutive melatonin release in SGF and SIF, respectively. (37°C, stirring rate: 100 rpm).

It was stated that melatonin had a solubility of  $3.11 \pm 0.90 \text{ mgmL}^{-1}$  and  $2.76 \text{ mgmL}^{-1}$  in gastric and intestinal fluids, respectively. Entrapment efficiency was  $60.6 \pm 4.5\%$ . Approximately 80% of melatonin was released in gastric medium whereas 90% in intestinal medium (4).

Generally, relatively low release is observed in gastric fluid compared to intestinal fluid due to the stability and low swelling of alginate beads in acidic conditions. Solubility, passive diffusion, and hydrogen bonding properties of the drug were suggested as the major factors that influence the drug release (4, 15, 16).

## CONCLUSION

Entrapment efficiency of melatonin in beads was calculated to be approximately 70%. Release of melatonin from the beads was measured using *in vitro* gastric fluid and intestinal fluid (pH 1.5 and pH 7.4; respectively) and release profiles were plotted. According to results obtained, melatonin release from the beads in pH 1.5 medium was approximately 45% in the first 5 hours whereas the release was approximately 40% in pH 7.4 medium. Melatonin release from alginate-gum arabic beads demonstrated an initial burst profile since the drug on the outer surface of the beads were able to be released easily. The results indicate that the beads manufactured may be used as carriers for melatonin.

## ACKNOWLEDGEMENTS

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**Türkçe Öz ve Anahtar Kelimeler**

**Melatonin Yüklü Aljinat/Gam Arabik Boncukların Üretimi ve İlaç Salım Özelliklerinin Değerlendirilmesi**

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**Öz:** Melatonin içeren aljinat-gam arabik boncuklar iyonotropik jelleşme ile üretilmiştir. Boncuk üretimi için en uygun aljinat - gam arabik oranı tespit edilmiştir. En iyi melatonin tutuklama ve salım özelliğine sahip boncukları üretebilmek için, değişen miktarlarda melatonin yüklenmiştir. Melatonin taşıyıcısı olarak boncukların tutuklama etkinliği yaklaşık %70 olarak hesaplanmıştır. Melatoninin boncuklardan salım profilleri *in vitro* mide özütü (pH 1,5) ve bağırsak özütü (pH 7,4) kullanılarak oluşturulmuştur. Elde edilen sonuçlara göre, ilk yarım saatte ani bir yükselmeyi takiben, boncuklardan melatonin salımı pH 1,5 ortamında ilk 5 saat için yaklaşık %45 olmuştur ve pH 7,4 ortamında ise salım yaklaşık %50 olarak tespit edilmiştir. Sonuçlara göre melatoninin kontrollü salım için, üretilen boncukların taşıyıcı olarak kullanılabileceği anlaşılmaktadır.

**Anahtar kelimeler:** Melatonin, aljinat, gam arabik, boncuk, kontrollü ilaç salınımı.

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