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# MAGNETIC NANOPARTICLE EMBEDDED STIMULI RESPONSIVE HYDROGELS AS ANTI-INFLAMMATORY DRUG CARRIERS

## *(UYARANLARA KARŞI DUYARLI, MANYETİK NANOPARÇACIK İÇERİKLİ HİDROJELLERİN ANTİ-ENFLAMATUAR İLAÇ TAŞIYICISI OLARAK KULLANIMI )*

**Sibel BARBAROS[1], Tuğba TECİM[2], Rükan GENÇ[3]**

## ABSTRACT

In this study, magnetic alginate beads were successfully synthesized by integrating superparamagnetic iron oxide nanoparticles ($Fe_3O_4$) in sodium alginate microbeads during the synthesis. The as-obtained dried samples were analyzed by means of their water detention capacity and drug encapsulation efficiency. Further, an anti-inflammatory drug (Cefazolin), mostly used for the treatment of joint inflammations after surgery, was used as a model drug in order to evaluate the stimuli-responsive properties of macrocomposites under magnetic field for the development of on-site drug delivery system. To do so, their drug release kinetics at changing environmental conditions, such as pH, temperature, and magnetic field were investigated and compared with bare alginate beads.

**Keywords:** Magnetic nanocomposites, Stimuli-responsive hydrogels, Alginate hydrogel, Drug delivery

## *ÖZ*

*Bu çalışmada, manyetik aljinant taneleri, Süperparamanyetik demir oksit ($Fe_3O_4$) nanoparçacıkların, sentez sırasında sodyum aljinat mikrotaneleri içerisine entegre edilmesi ile sentezlenmiştir. Elde edildiği haliyle kurutulan örnekler, su tutma kapasiteleri ve ilaç enkapsülasyon verimliliği açısından analiz edilmiştir. Ayrıca, genellikle ameliyat sonrası eklem iltihaplanmalarının tedavisinde kullanılan bir anti inflamatuar ilaç (Sefazol), manyetik alan altında mikrokompozitlerin uyaranlara duyarlılık özelliklerinin değerlendirilmesi için model ilaç olarak kullanılmıştır. Bunun için, pH, sıcaklık ve manyetik alan gibi değişen ortam koşullarındaki ilaç salım kinetikleri incelenmiş ve boş aljinat taneleri ile karşılaştırılmıştır.*

***Anahtar Kelimeler:*** *Manyetik nanokompozit, Uyaran duyarlı hidrojel, Aljinant hidrojel, İlaç taşıma*

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## 1. INTRODUCTION

Statistics show that more than 100 million people worldwide suffer from osteoarthritis (OA) and in recent years increased number of people with age over 65 years has been reported as they were affected by knee OA [1]. Mostly OA patients in their late ages choose knee replacement due to the high success rates in restored quality of life after the surgery. However, statistics showed that almost 10% of the total knee replacement procedures ends up with implant revision due to implant failure. Implant failure due to uncontrollable inflammation during the wound healing process is one of the major issues that must be solved. Locating biomaterials with the ability to control the release of anti-inflammatory molecules during implantation or directly on inflammation site could increase the possibility to achieve control over inflammation. However, depending on where the inflammation occurs, physicochemical properties of the biomaterial (natural or synthetic) and macrostructure as filler influence the therapeutic effectiveness of the anti-inflammatory agent which incorporated into the biomaterial [2].

Alginate as a natural polysaccharide is non-toxic, biocompatible, biodegradable and renewable and due to this it has received increasing attention in different fields. Among their potential use in food technology [3], biosensors [4], biocatalysis [5] and waste-water treatments [6], its physically stable, highly porose hydrogels formed in the presence of calcium chloride have been widely used in biomedical applications as support material or molecular carrier [2].

Parallel to the increased knowledge on nanomaterials, the design of smart composite materials combining the basic features of functional nanoparticles and biopolymers have extended the potential of these matrixes for their use in the fields requiring stimuli-responsive materials [7]–[11]. Using hybrid polymer/nanoparticle systems one can achieve controlled release of the drug by external stimuli, for example, UV or IR triggered photorelease, hypothermia triggered or magnetically triggered systems. No different than other metals, iron oxide shows outstanding magnetic and thermal properties at sizes below 100 nm and there is an increasing trend of the development of new materials by incorporating magnetic nanobeads to biopolymers. For example, magnetically controlled water-soluble chitosan (WSC) particles which physical cross-linked with sodium alginate dipped into ferrous chloride has been used as an adsorbent for different dyes and hemoglobin [12]. In the same manner, polysaccharide/biopolymer based hydrogels encapsulating magnetic nanoparticles have been used fort he release of several bioactive molecules including insulin [13], dopamine [11], vitamins [3] and so on.

The anti-inflammatory drug Cefazolin sodium (Figure 1) is a broad spectrum antibiotic which often used to reduce inflammation in cartilage and mostly requires delivery together within a polymer support. When incorporated within an a biodegradable hydrogel made up of P(FAD: SA, 50:50 w/w) polyanhydride, Park. et al. demonstrated the slow release of cefazolin in situ [14]. In another study, cefazolin sodium and gentamicin sulfate has been grafted to biodegradable polymers (poly(dl-lactide):co-glycolide) and the long-term in vitro drug release have been monitored for over 30 days on S. aureus (ATCC65389) by using an antibiotic disk diffusion method which revealed an enhanced bacterial inhibition compared favorably with no treatment and free cefazolin [15]. To the best of our knowledge, there is no report on a magnetically controlled drug delivery system for cefazolin for their use to prevent or heal the implant rejection driven inflammations.

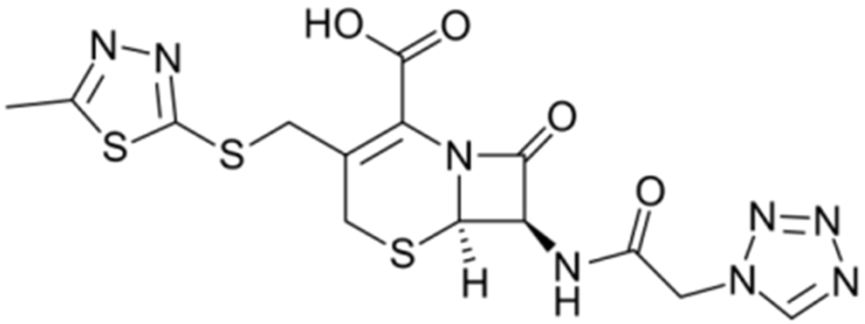

**Figure 1.** Chemical structure of Cefazolin

In this report, superparamagnetic nanoparticles (SPIONs) were incorporated inside alginate capsules, and a magnetically switchable system for controlled release of cefazolin as a model anti-inflammatory drug for the development of an on-site drug delivery system was achieved by an external magnetic field. Moreover, we have showed that use of metallic nanoparticles as filler can also slow biodegradation of alginate.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

All chemicals used were of analytical grade and used without further purification. Cefazolin (Sefazol) was obtained from a local pharmacy, sodium alginate was obtained from Sigma-Aldrich, Hydrogen peroxide (30%), and sulfuric acid, ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous sulfate ($FeCl_2 \cdot 7H2O$), sodium hydroxide, ammonium hydroxide (30 %), calcium chloride were purchased from Sigma-Aldrich.

### 2.2. Preparation of Magnetic Nanoparticles

Superparamagnetic nanoparticles $Fe_3O_4$ (SPIONs) were synthesized by a co-precipitation method using ammonia as precipitation agent. Magnetic nanoparticles were synthesized by co-precipitation method with slight changes. Basically, keeping 10 mL of $NH_4OH$ (Sigma-Aldrich, 30-33% $NH_3$ in $H_2O$) was added dropwise to previously prepared iron chloride solutions (Iron(III) chloride hexahydrate/Iron(II) chloride (purchased from Sigma-Aldrich) mixture from prepared with 0.5 $Fe_2$+/$Fe_3$+ molar ratios. After the dark black solution was obtained, reaction temperatures increased to 90 C° and let for oxidation for over an hour. Magnetic particles formed were collected with a strong magnet and washed three times with pure water to eliminate unreacted salts. Further, particles were either washed with HCl (1M) to enhance their stability when not used and separate by a magnet and wash with distilled water. Particle size distribution was then analyzed with Malvern Zeta Sizer. Morphological analysis of the samples was performed with ZEISS Supra 55 model FE-SEM. Each sample was freeze-dried on a glass slide and further coated with 1 cycle of platinum.

### 2.3. Synthesis of Magnetic Alginate Beads

Bare and magnetic alginate beads encapsulating cefazolin (10% w) were prepared following the standard procedure using calcium chloride as a cross-linking agent. Changing alginate concentrations 1.0 to 4.0 % w/v were prepared and mixed with superparamagnetic particles (SPIONs) (10 mg/mL alginate) of 12 nm in size (Figure 2). Once a homogenous mixture was obtained, macroparticles were formed by adding this mixture to $CaCl_2$ solution (%2.0) dropwise by a 10 mL syringe. Particles were then washed three times with $CaCl_2$ solution and further dried at room temperature for overnight.

### 2.4. Determination of Swelling Value at Equilibrium (%)

Dried samples were weighed and soaked in water and in different periods, beads were taken, further dried with a tissue paper and weighed until a steady state swelling value (S) reached. S value was calculated from Equation 1.

$$S=\frac{w\infty-wo}{wo}\times 100 \quad (1)$$

where; S is defined as swelling value at at equilibrium, $wo$ is the weight of the hydrogel in the begining and $w\infty$is the weight of the hydrogel at equilibrium swelling obtained after a long period of swelling.

### 2.5. Encapsulation of Cefazolin into Alginate Magnetic Beads

After macrobeads were prepared, the beads were taken out and the remaining solution was analyzed by UV spectroscopy at 280 nm. Cefazolin concentration was calculated using previously obtained a calibration curve. The encapsulated amount of cefazolin (%) was determined by Equation 2.

$$\text{Encapsulated Cefazolin (\%)}\ \frac{Co-Ct}{Co}\times 100 \quad (2)$$

where; $Co$ stands for initial cefazolin concentration and $Ct$ is the cafeazolin concentration at washing collected at washing step.

### 2.6. Cefazolin Release from Magnetic Beads

0.2 g/mL of macrobeads were prepared in PBS solutions (10x) at pre-decided pHs. Experiments conducting magnetic field was generated inducing an oscillating magnetic field that results in 1800 G for a sample located in 0.5 cm distance. At predetermined time intervals, the magnetic field was stopped and the liquid sample was withdrawn and replaced with fresh PBS (10x). The cefazolin content of the withdrawn sample was determined spectrophotometrically at 280 nm. UV–vis measurements were performed using a with Analytic Jena, Secord 210 Plus model. The temperature was controlled by placing the samples in a water jacket.

## 3. RESULTS AND DISCUSSIONS

In this study, cefazolin (Scheme 2) as the model anti-inflammatory drug was incorporated in macrobeads prepared from sodium alginate/magnetic nanoparticles and under external stimuli, temperature, pH and magnetic field, magnetic particle based changes in drug release profile was reported as compared with bare alginate beads. To do so, bare and magnetic alginate beads encapsulating cefazolin (10% w) were prepared following the standard procedure [5]. Changing alginate concentrations 1.0 to 4.0 % w/v were prepared and mixed with superparamagnetic particles (SPIONs) (10 mg/mL alginate) of 12 nm in size (Figure 2). Once a homogenous mixture was obtained, macroparticles were formed by adding this mixture to $CaCl_2$ solution (%2.0) dropwise using a 10 mL syringe. Particles were then washed three times with $CaCl_2$ solution and further dried at room temperature for overnight. As depicted in Figure 1a and b, homogenous particulates were achieved for both cases where for 2.0% alginate concentration, mean size of the magnetic alginate beads decreases in the presence of magnetic nanoparticles which is expected since magnetic nanoparticle surface with acidic groups alters the electrostatic interaction between each component which results in tighter macrobeads (Alginate-SPION) (Figure 3a). However, at lower alginate

concentrations, macrobeads were found to be agglomerated and degraded so we continued our studies with 2.0 % and 4.0 % w/v of alginate. Figure 3b, shows the magnetic property of the prepared beads in the presence of a magnet and the SEM image of the magnetic nanoparticles located as clusters on the porous structure of the alginate beads.

Encapsulated amount of the cefazolin was measured indirectly by collecting the samples from washing step and measuring the absorbance at 285 nm at spectrophotometer. As depicted in Figure 2b, encapsulation capacities were enhanced when SPIONs were incorporated in both alginate concentrations where 4.0 % alginate has 50% higher encapsulation capacity compared to 2%. Loading cefazolin into Alg and Alg-SPON beads at pH 7.4 were 51.32±2.05 % and 68.49±2.37 % for 2% Alginate and 72.35±4.34 % and 85.56±3.02% for 4% Alginate which is highly satisfactory compared to the reported values for other compounds [11,13].

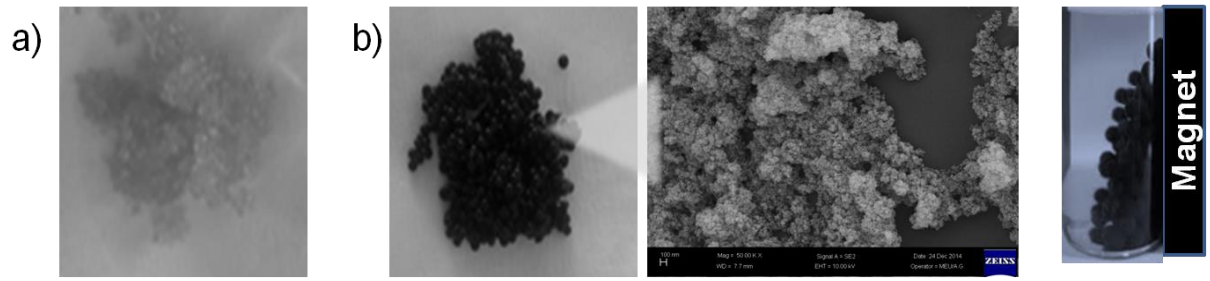


**Figure 2**. Photograph of Cefazolin encapsulating a) alginate macrobeads (2%) and superparamagnetic nanoparticle (SPION) embedded alginate macrobeads. FE-SEM image of SPION clustered on alginate bead surface and image demonstrating the magnetic properties of the resulting macrobeads

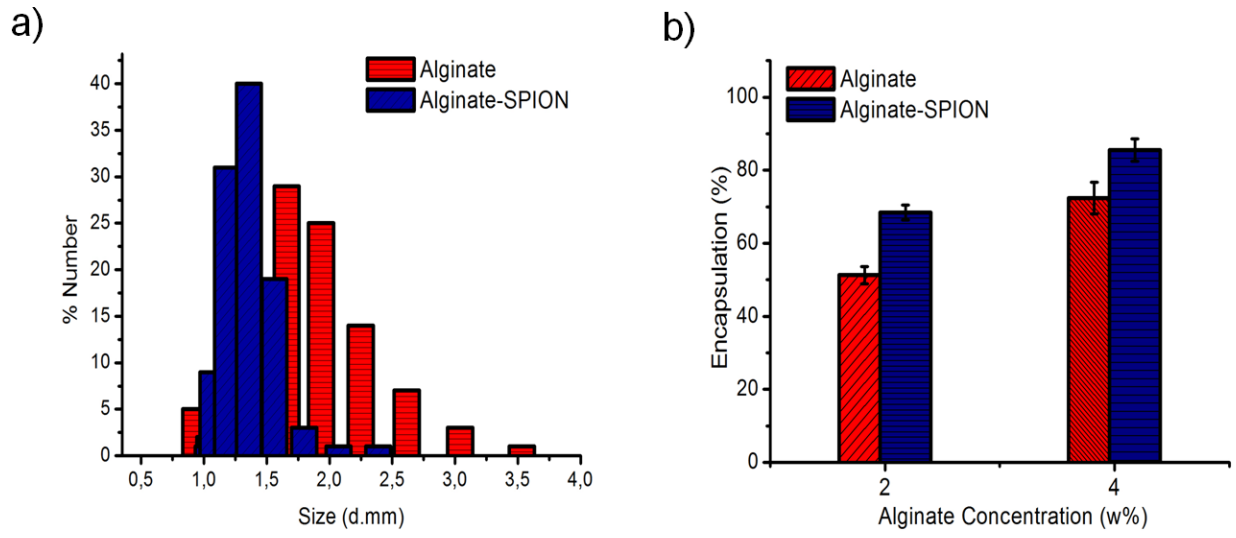


**Figure 3.** a) Size distribution and b) Cefazolin encapsulation efficiency of as prepared alginate macrobeads (2%) w/two superparamagnetic nanoparticles

Swelling is another important parameter that changes the biodegradation and drug release character of the polymer matrix when it faced to biological environment. Effects of three main

parameters, pH, temperature and magnetic field on the swelling value of magnetic beads at equilibrium are shown in Figure 4. Regardless of magnetic field, there is a slight increase in swelling for all types of particles due to the increased temperature of 25 °C to 37 °C. ALG-SPION magnetic beads prepared at 2% of alginate were not stable at pH 7.4 as they degraded after 24 hours which needed for calculating the swelling value of the hydrogels at equilibrium. Overly swelled beads can also be seen in figure 4b that framed with red color. However, for the magnetic particles prepared with 4% alginate, the effect of magnetic field is more significant at pH 7.4 where the swelling increased 1/3 to ½ depending on the temperature (Figure 4a and Figure 4b). At pH 5.5 swelling is lower and beads are more stable in both alginate concentrations. Although, pH and temperature tailored release of the alginate content is known phenomenon, altered release in the presence of the magnetic field could initiate the development of new filler materials.

The release performances of hydrogels in varying polymer concentrations are characterized in vitro at varying conditions such as pH, temperature, and magnetic field. In order to probe temperature effect, the experiments are held with particles in 2% and 4% concentrations and experiments were run for 5 hours. Figure 5 summarizes the effect of three environmental parameters and shows whether those parameters have a synergetic effect or not. Considering the cumulative (%) cefazolin releases from magnetic alginate beads at 25 °C and 37 °C without a magnetic field, we can see the similar pattern shown in swelling values that Alg-SPION (%2) particles show higher release rate at pH 7.4 at both temperatures. Increased temperature effects Alg-SPION (%4) at this temperature. Under applied magnetic field, it is observed that 4 % Alg-SPION complex has the lower release performance at both 25 °C and 37 °C while no additional pH effect perceived at these temperatures compared to non-magnetic conditions. However, for Alg-SPION (%2) particles, the release rate increases at both pHs, and cefazolin release at pH 5.5 reaches to release rate at pH 7.4 at both temperatures. This means that magnetic beads have the capacity to heat up and vibrate even at room temperature, which as a result disturb the polymeric infrastructure and results in a faster drug release compared to the bare alginate macrobeads which showed in all conditions as low as 20 % release of the drug.

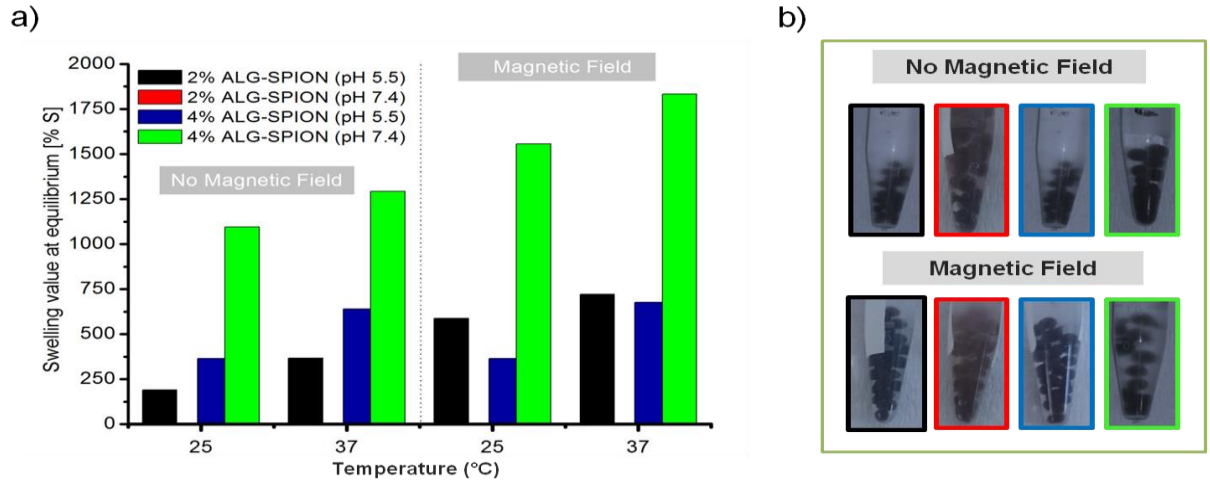


**Figure 4.** a) Effect of pH, temperature and magnetic field on the swelling value of the hydrogel at equilibrium depending on the initial alginate concentration of the ALG- SPION macrobeads and b) images depicting the effect of swelling after 24 hours at 37 °C (Frame colour of each image corresponds to legend color of the samples depicted in the graph. Experiments were carried out with 0.2 g/1 mL hyrdogel

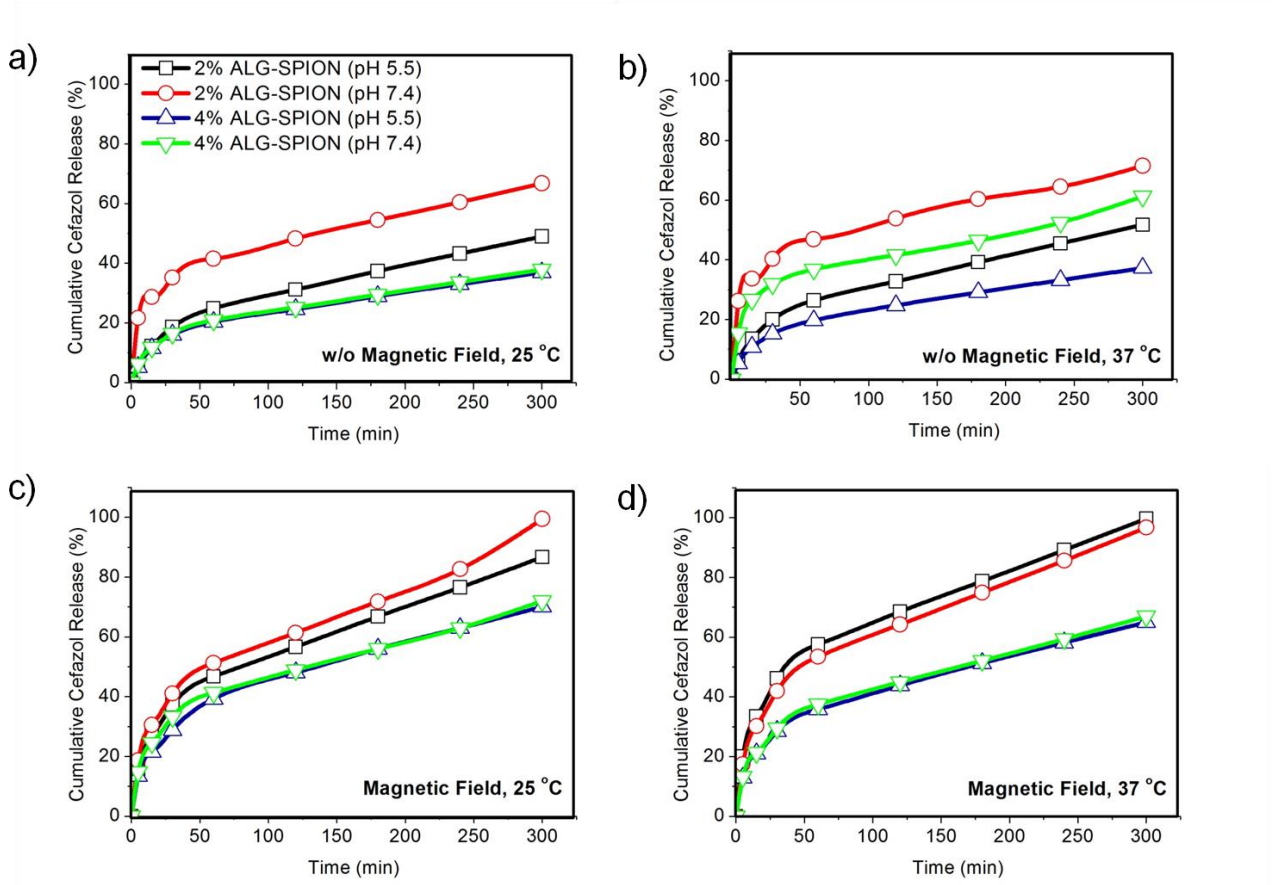


**Figure 5.** Cefazolin release profile of the ALG-SPION magnetic beads at changing environmental parameters (pH, temperature, and magnetic field)

## 4. CONCLUSIONS

In this report, superparamagnetic nanoparticles (SPIONs) were incorporated inside alginate capsules, and a magnetically switchable system for controlled release of cefazolin as a model anti-inflammatory drug for the development of an on-site drug delivery system was achieved by an external magnetic field. Moreover, we observed and reported a synergetic effect of the magnetic field with temperature and the pH of the environment where 2% alginate macrobeads with SPIOs showed a higher response to the environmental stumili. Considering the results, magnetic alginate beads could have potential as on-site drug delivery system for an anti-inflammatory drug that designed to be controllable by an external magnetic field and can be used as drug loaded filler to prevent or heal the knee replacement driven inflammations.

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## CV/ÖZGEÇMİŞ

**Sibel Barbaros; Master Student (Yüksek Lisans Öğrencisi)**
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**Tuğba TECİM; Master Student (Yüksek Lisans Öğrencisi)**
Tuğba has her bachelor degree from Yıldız Technical University Chemical Engineering department. She studied

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**Rükan GENÇ; Asst. Professor (Yrd.Doç.Dr.)**
Asst. Prof. Rükan Genç obtained her bachelor degree from Bioengineering (Ege University, Turkey) in 2005. In 2011, she received her Ph.D. in Chemical and Process Engineering from Rovira i Virgili University (Spain). She pursued her postdoctoral research first in the Institute of Materials Science and Nanotechnology (UNAM), Bilkent University and further at Chemistry Department of the Middle East Technical University. She is Assistant Professor in Chemical Engineering Department of Mersin University and leading Functional Nanomaterials Research Group (FUNGROUP) with ongoing projects focused on the use of biological materials and lipids as functional moieties to design multifunctional hybrid materials for their use in biomedical technologies, in bioanalysis and catalysis. She is currently serving as Assistant Director of Mersin University Advanced Technology Education, Research, and Application Center (MEITAM).

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