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The Synthesis of a Controlled Release System Based on MMT Added IPN Type Hydrogels

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Anahtar Kelimeler

Hidrojel
Jelatin
MMT
Kontrollü İlaç Salım
Naproksen

Graphical/Tabular Abstract (Grafik Özet)

The aim of this study is to develop a controlled Naproxen releasing system for anti-inflammatory treatments. Interpenetrating polymer network (IPN) type of hydrogels were synthesized by using alginate and gelatin. The effect of MMT in the IPN structure on Naproxen releasing was investigated. The release kinetics of Naproxen are match the Fickian diffusion. / Bu çalışmanın amacı, anti-inflamatuar tedaviler için kontrollü bir Naproksen salım sistemi geliştirmektir. Aljinat ve jelatin kullanılarak iç içe geçmiş polimer ağı (IPN) tipi hidrojeller sentezlenmiştir. IPN yapısındaki MMT'nin Naproksen salımına etkisi araştırılmıştır. Naproksen'in salım kinetiği Fickian difüzyonuyla eşleşmektedir.

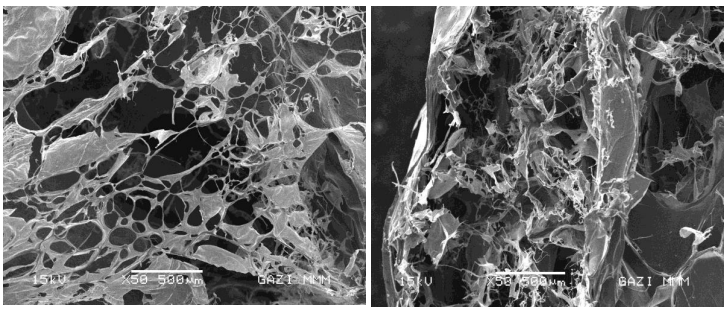

Figure A: *SEM micrographs of $(AG)_2$ and $(AG)_2$-MMT hydrogel strips / **Şekil A:** $(AG)_2$ and $(AG)_2$-MMT hidrojel şeritlerine ait SEM mikrografları*

Highlights (Önemli noktalar)

- *In this study, a controlled Naproxen releasing system was developed for anti-inflammatory treatments. / Bu çalışmada anti-inflamtuar tedaviler için kontrollü Naproksen salım sistemi geliştirilmiştir.*
- *The hydrogel formation, swelling/degradation and releasing properties were investigated after the addition of MMT to the IPN structure. / MMT'nin IPN yapıya eklendikten sonra hidrojel oluşum, şişme/bozunma ve salım özelliklerine etkisi incelenmiştir.*
- *The swelling profiles of the hydrogels were compatible with their releasing behaviors. / Hidrojellerin şişme profilleri salım davranışlarıyla uyumludur.*
- *The release kinetics of Naproxen through the hydrogels are match the Fickian diffusion. / Hidrojellerden Naproksen salım kinetikleri Fickian difüzyonuyla eşleşmektedir.*

Aim (Amaç): *The aim of this study is to release an anti-inflammatory drug from hydrogels synthesized using natural polymers and clay. / Bu çalışmanın amacı doğal polimerler ve kil kullanarak sentezlenen hidrojellerden anti-inflamatuar ilaç salımının sağlanmasıdır.*

Originality (Özgünlük): *The combination of natural polymers and clay has not been reported in the literature for naproxen release. / Naproksen salınımı için literatürde doğal polimerler ve kil kombinasyonu rapor edilmemiştir.*

Results (Bulgular): *The results of swelling/degradation tests and SEM micrographs of the hydrogels are consistent with the release results. / Hidrojellerin şişme/bozunma testlerinin sonuçları ve SEM mikrografları salım sonuçlarıyla tutarlıdır.*

Conclusion (Sonuç): *Our study showed that these synthesized hydrogels including or not MMT can be promising candidate of drug delivery for Naproxen. / Çalışmamız, MMT içeren veya içermeyen bu sentezlenmiş hidrojellerin, Naproksen için umut vaad eden bir ilaç salım adayı olabileceğini gösterdi.*

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Abstract

The purpose of our study is to develop a controlled Naproxen releasing system for anti-inflammatory, analgesic treatments. Interpenetrating polymer network (IPN) type of hydrogels were obtained by using natural polymers alginate and gelatin. The hydrogels were prepared by combining alginate and gelatin in the presence of glutaraldehyde as crosslinker of gelatin. Montmorillonite (MMT) clay was used as a nano-dimensioned and layered dopant. The prepared hydrogels were characterized via hydrogel formation, swelling/degradation measurements, Fourier Transform Infrared Spectroscopy (FT-IR), and Scanning Electron Microscopy (SEM) analysis. Swelling tests presented that the presence of MMT in the polymer network decreases the swelling. From the SEM micrographs, it can be seen that MMT added hydrogel has smaller and more pore than the other. The release studies of Naproxen were maintained in Britton-Robinson Buffer (BRB) solution (pH=7.4) and at 37°C. It is observed that the swelling profiles of the hydrogels were compatible with the releasing behaviors. The release kinetics of Naproxen are match the Fickian diffusion.

MMT Katkılı IPN Tipi Hidrojel Temelli Kontrollü Salım Sisteminin Sentezi

Makale Bilgisi

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Öz

Çalışmamızın amacı antiinflamatuar, analjezik tedaviler için kontrollü bir Naproksen salınım sistemi geliştirmektir. İç içe geçmiş polimer ağı (IPN) tipi hidrojeller, doğal polimerler aljinat ve jelatin kullanılarak elde edildi. Hidrojeller, aljinat ve jelatinin, jelatinin çapraz bağlayıcısı olarak glutaraldehit varlığında birleştirilmesiyle hazırlandı. Nano boyutlu ve katmanlı katkı maddesi olarak Montmorillonit (MMT) kili kullanıldı. Hazırlanan hidrojeller, hidrojel oluşumu, şişme/bozulma ölçümleri, Fourier Dönüşümü Kızılötesi Spektroskopisi (FT-IR) ve Taramalı Elektron Mikroskobu (SEM) analizi ile karakterize edildi. Şişme testleri, polimer ağındaki MMT varlığının şişmeyi azalttığını ortaya koydu. SEM mikrograflarından MMT katkılı hidrojelin diğerine göre daha küçük ve daha fazla gözeneklere sahip olduğu görülmektedir. Naproksen'in salım çalışmaları Britton-Robinson Tampon (BRB) çözeltisinde (pH=7,4) ve 37°C'de sürdürüldü. Hidrojellerin şişme profillerinin salım davranışlarıyla uyumlu olduğu gözlendi. Naproksen'in salım kinetiği Fickian difüzyonuyla eşleşmektedir.

1. INTRODUCTION (GİRİŞ)

Hydrogels are composed of three dimensionally crosslinked of hydrophilic polymer networks that have capable of absorption of liquids. The amount of water that they able to absorb is between 10 and 1000 times than their own dry weight. In addition, the swelling capacities are affected by their content, composition ratio, temperature, pH or nature of solutions etc. They can compose by interpenetrating of at least two polymers in the presence of crosslinkers. These polymers can be from natural or synthetic origin and crosslinked by chemically, physically or enzymatically [1]. Interpenetrating polymer networks (IPN) are called semi-IPN when one of the polymers in network is crosslinked. If both polymers are crosslinked, they called full-IPN. The combination of polymers that have different properties in one network provides advantages to usage in wide range of areas such as food industry, water purification and dye removing, cosmetics,

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agriculture and biomedical applications (biosensors, pharmaceuticals, drug delivery) [2].

Controlled release phenomenon aims to distribute an active agent at a certain rate and time. This release is regulated by diffusion, erosion, swelling, magnetic and mechanically controlled release systems. Hydrogel forms such as matrix, encapsulation or ink are used as a controlled release material nowadays. Pore sizes and hydrophilicity of hydrogels are the major parameters in order to control of releasing system. Therefore, nature of polymers, crosslinking process, thermal or pH dependent properties arrange the releasing [3]. The natural polymers are quietly advantageous materials for controlled release systems.

Alginate is a linear polysaccharide that is commonly used due to the ability of water absorption. Besides its superior properties such as biodegradable, biocompatible and non-toxic nature, it has low bioadhesivity and inertness. However, when the weak properties of alginate are enhanced by combining them with other materials, there are areas of use in pharmaceuticals, wound healing, drug delivery system, cell therapy [4].

Gelatin is a type of protein obtained by hydrolysis of collagen. It is a natural polymer with a high charge density including amino, hydroxyl, and carboxyl groups. Its biodegradability, water absorption capacity and low cost make it a preferred choice to bioengineering and food industry [5].

The use of clays in controlled drug release systems have a great attention with the back to nature. The clays have advantages such as their high surface area, cation exchange capacity, microporosity, and strong hydrogen bond interactions. MMT belongs to smectite clay group. The smectite clay is a layered alumino silicates that composed of 2:1 ratio of two tetrahedral sheets of $[SiO_4]4-$ and one octahedral sheet of $[AlO_3(OH)_3]^{6-}$. Molecules or cations can intercalate into the interlayer space of the (001) plane of MMT. Thus, the space of interlayer in MMT can arrange the hydrophilicity of the structure it is in. Its combination with polymer networks becomes biodegradable, biocompatible nanocomposites [6,7].

In this study, we reported a combining of the natural amphoteric gelatin and anionic alginate polymers and MMT clay. The effect of MMT on swelling/degradation properties of the hydrogels were examined. Naproxen is well known non-steroidal anti-inflammatory drug (NSAID) that have effect of anti-inflammatory treatment. The controlled release of Naproxen was performed through the hydrogels and determined the release kinetic parameters.

2. MATERIALS AND METHODS (MATERYAL VE METOD)

2.1. Experimental Equipment (Deneysel Ekipman)

Sodium alginate, gelatin (powder), ethylene glycol dimethacrylate (EGDMA), glutaraldehyde (25% aqueous solution), $CaCl_2$, montmorillonite and Naproxen were provided from Sigma-Aldrich. The Britton-Robinson Buffer (BRB) solution was prepared as in the literature [8]. The mixture of H_3BO_3 (Merck), H_3PO_4 and CH_3COOH (Sigma-Aldrich) solutions has been titrated to targeted pH with 0.2 M NaOH.

2.2. 'One Pot Method' Preparation of the IPN Type Hydrogels (Deney Tasarımı)

In order to obtain the IPN type hydrogels, alginate solution (5%) and gelatin solution (10%) were prepared, separately. The alginate and gelatin solutions were mixed at 1:3, 1:1 and 3:1 ratios, respectively and stirred until they were dispersed in each other. Glutaraldehyde, as crosslinker of gelatin, was added the homogenous solutions. Then mixtures were poured to mold rapidly. The mold was allowed to stand at 37°C for 1h to curing. This process provides the formation and crosslinking of hydrogel physically and chemically. When gelation was completed, obtained hydrogels were sliced and washed distilled water to remove unreacted components. Semi-IPN type hydrogels that obtained by crosslinking of one of the polymers were kept at room temperature, then dried at 37°C until reach to the constant weight. They were named as $(AG)_1$, $(AG)_2$ and $(AG)_3$ hydrogel strips, respectively by ratios.

In the synthesis of MMT clay added (AG) hydrogels, MMT was included at the mixing stage of the polymer solutions. After the MMT was well dispersed in the mixture, glutaraldehyde was added and the mixture was poured to mold rapidly. The obtained MMT added semi-IPN hydrogels were dried in the same way and names as $(AG)_1$-MMT, $(AG)_2$-MMT and $(AG)_3$-MMT hydrogel strips. The predetermined amounts of all reactants are given in Table 1.

2.3. Characterization (Karakterizasyon)

Yield of Hydrogel Formation (Hidrojel Oluşum Verimi)

Yields of hydrogel formation (HF) of the strips were gravimetrically calculated. The obtained hydrogel strips were dried until reach to the constant weight. Then they were kept in water bath for 48h to remove unreacted or excessive components. After the washed hydrogel strips were taken from the bath, they were dried at room temperature, then at 37°C and weighed again. In order to determine of HF, below formula was followed:

$$HF\ (\%) = \frac{m}{m_0} \quad (1)$$

where m0 and m are the weight of dried hydrogel strips before and after washing, respectively. All measurements were carried out in triplicate.

Table 1. Amount of the reactants used to synthesize the hydrogel strips and yield of HF (Hidrojel şeritlerin sentezi için kullanılan reaktantların miktarı ve hidrojel oluşumu (%))

Hydrogel	Alginate (5%), mL	Gelatin (10%), mL	MMT (w/w) (%)	Hydrogel Formation (%)
$(AG)_1$	20	60	-	85.2
$(AG)_2$	40	40	-	90.5
$(AG)_3$	60	20	-	95.8
$(AG)_1$-MMT	20	60	1.0	88.2
$(AG)_2$-MMT	20	20	1.0	92.9
$(AG)_3$-MMT	60	20	1.0	97.5

*3.0 mL of Glutaraldehyde (25%, v/v) was used in each syntheses.

FT-IR Measurements (FT-IR Ölçümleri)

FT-IR spectra of the hydrogel strips and MMT were determined by Thermo Scientific Nicolet İS5 spectrometer. The spectra were obtained at a resolution of 4 cm−1 after 128 scans.

Swelling Tests (Şişme Testleri)

Swelling tests of the hydrogels were gravimetrically performed at three stages. At the first stage, dry samples were placed to swell in BRB solution (pH=7.4) at 37°C. Swollen hydrogels were taken from the BRB, weighed at certain intervals and then placed into the bath again. Equation (2) was used for all calculations of swelling rates (S%):

$$S(\%) = \frac{Mw - Md}{Md} x\ 100 \quad (2)$$

where Mw and Md is the hydrated (swollen) and the dehydrated (dry) weight of the hydrogel strips. Time dependence swelling rates were continued for 24 hours until reach constant weight for each samples.

In the second stage, temperature dependence of swelling rates at different temperatures between 4°C and 60°C was carried out. The swelling tests were performed in the same way above mentioned for 24h.

In the third stage, pH dependence of swelling profiles of the hydrogels was investigated. The dried samples were allowed to swell at 37°C for 24 hours at various pH ranges from 2 to 12.

Degradation Tests (Bozunma Testleri)

The hydrogels dried to constant weight were put to swell in BRB buffer (pH=7.4) at 37°C. The swollen strips were taken from the BRB solution end of the 24h. This weight of most swollen state of strips was noted as Mm. After that the hydrogels were put into the BRB solution again and weighed at determined intervals for 30 days. Degradation rates (%) were calculated by the Equation (3):

$$Degradation\ (\%) = \frac{M_m - M_t}{M_m} X\ 100 \quad (3)$$

where Mm and Mt is the weight of hydrogels at the maximum swollen state and at the time t, respectively. All experiments were performed in three times.

SEM Observations (SEM Gözlemleri)

Firstly, the hydrogels were allowed to swell to equilibrium in BRB solution at 37°C and then they were put in a deep freezer at -20°C for 24 h and then replaced into a freeze dryer at −85°C for 24 h (Christ-Alfa 2–4 Model, Martin Christ GmbH). They were coated with 200 Å Au. The surface morphology of the hydrogel strips was observed via SEM (JEOL JSM 6060 LV).

Drug Loading and Release Studies (İlaç Yükleme ve Salım Çalışmaları)

Naproxen loaded IPN hydrogel strips were obtained by directly adding Naproxen (10 mg per strip) at the stage of mixing of polymer solutions. Likewise, hydrogel preparing process as mentioned above, the wet hydrogel strips were taken from the molds and drug loaded hydrogels were dried at room temperature, then at 37°C.

The release studies of Naproxen from the hydrogel strips were investigated in 100 mL of BRB solution by a spectrophotometer (Unicam UV-2100 Haverhill, MA). The specific wavelength of the drug was determined at $\lambda max = 260$ nm. Aliquots of 0.5 mL were drawn from the BRB solution at various time intervals such as 60, 120, 180, 240 and 300 minutes and added with an equivalent volume of BRB. Thus, sink conditions were provided during the drug release [9]. All measurements were repeated in triplicate. The percentage of cumulative drug release was calculated by the Equation (4):

$$\text{Cumulative Release (\%)} = \frac{Wt}{W_{total}} \times 100 \qquad (4)$$

where Wt and Wtotal is the weight of the released drug at any time and the initial total weight of Naproxen loaded into the matrix of hydrogel strips, respectively.

3. RESULTS (BULGULAR)

Hydrogel Formation (Hidrojel Oluşumu)

A series of (AG) hydrogel strips prepared by combining two natural polymers to provide controlled release of naproxen. The properties of the hydrogels like durability after swelling and yields of hydrogel formation were evaluated. In this study, we aimed to form semi-IPN hydrogels between alginate and gelatin. Thus, while alginate provided strength to the hydrogel structure, gelatin contributed to the swelling values and flexibility of the network structure. The $(AG)_1$ hydrogel had very tight matrix due to the ratio of alginate volume in mixture (3:1) therefore the swelling values of this hydrogel was the lowest. Since the $(AG)_3$ hydrogel structure contained three times more gelatin volume (1:3), it maximized the swelling values. Unfortunately, it was not suitable for controlled release because of very loose matrix. For this reason, we choose $(AG)_2$ hydrogel among the (AG) hydrogel strips and prepared MMT added $(AG)_2$ hydrogel strips.

FT-IR Measurements (FT-IR Ölçümleri)

The FT-IR spectra of MMT clay, alginate and gelatin hydrogels, the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips are given in Figure 1. The curve given at Figure 1 (a) shows the three peaks belongs to MMT clay that indicate Al-OH stretching at 3624 cm^{-1}, deformation vibration of the hydroxyl groups at 1636 cm^{-1} and Si-O stretching vibration at 1013 cm^{-1} [10]. The bands at 1632 cm^{-1}, 1533 cm^{-1} and 1234 cm^{-1} indicates the amide I, II and III bands of gelatin at Figure 1 (d). In addition, at the curve (d), the broad peak at 3291 cm^{-1}, 3074 cm^{-1}, 2935 cm^{-1} and 1446 cm^{-1} indicates to N-H stretching, aliphatic C-H stretching and bending vibrations, respectively [11]. At the curve in Figure 1 (e), a broad peak at 3368 cm^{-1} is assigned to the stretching vibration of hydroxyl groups of alginate hydrogel. Additionally, the peaks at 1619 cm^{-1} and 1429 cm^{-1} are belong to asymmetric and symmetric carboxyl groups, at 1016 cm^{-1} is attributed to cyclic ether bridge of Alginate [12]. It is observed the specific peaks at 822 cm^{-1} and 942 cm^{-1} indicate Gluronic (G) and Manuronic (M) acid functional groups of alginate.

In general, the characteristic peaks of gelatin were obviously observed both curves of the hydrogel strips that given at Figure 1 (b) and (c). It can be interpreted, as the amount of gelatin in polymeric mixture are high, the character of gelatin is dominant in hydrogel strips. The difference between the curves of $(AG)_2$ and $(AG)_2$-MMT hydrogels is the peak intensity at 1028 cm^{-1}. The intensity of peak at 1028 cm^{-1} is higher in the curve of X (c) belongs to MMT added $(AG)_2$ hydrogel strip. The difference is originated from the MMT clay in the matrix of $(AG)_2$-MMT hydrogel.

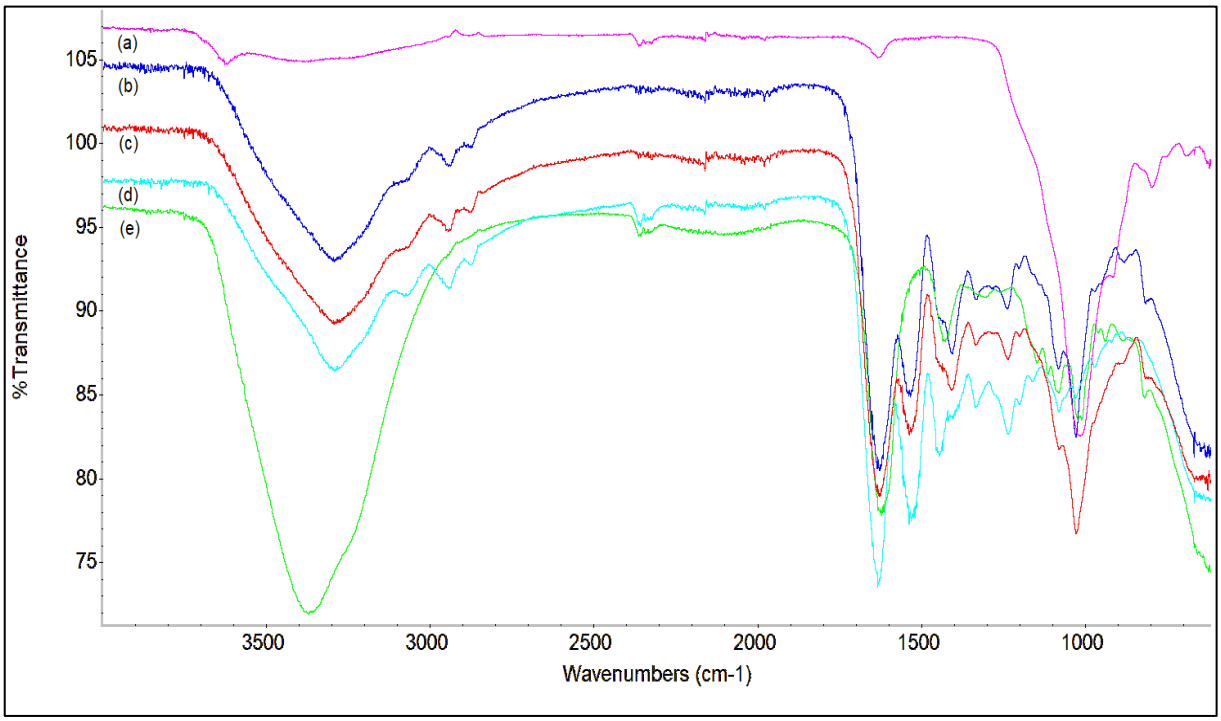

Figure 1. FTIR spectrum of a) MMT clay, b) $(AG)_2$, c) $(AG)_2$-MMT, d) gelatin and e) alginate hydrogels
((a) MMT kili, b) $(AG)_2$, c) $(AG)_2$-MMT, d) jelatin ve e) aljinat) hidrojellerin FT-IR spektrumları)

Swelling Behaviors of the Hydrogels (Hidrojellerin Şişme Davranışları)

Figure 2 shows the changing of swelling values with time at pH=7.4 and 37°C. Swelling values increased at first and then stabilized after 12 h. While alginate and gelatin hydrogels had low swelling percentages, (AG) series that combining of them with different ratio had higher swelling values. When preparing the (AG) hydrogels, the glutaraldehyde that gelatin crosslinker was only used. Alginate chains, which are free in the semi-IPN hydrogel matrix, prevent the crosslinking of gelatin chains. Thus, swelling values are higher than alginate and gelatin hydrogels. It is observed that from the Figure 2 as the gelatin ratio in the hydrogel matrix was increase, the swelling values increase.

As can be seen from Figure 3, MMT added homopolymer alginate and gelatin hydrogels (A-MMT and G-MMT) and hybrid $(AG)_2$ hydrogel has lower swelling values than without MMT added hydrogels that presented in Figure 2. It can be interpreted that MMT acted as a crosslinker in the polymer matrix and enabled semi-IPN hydrogels to be full-IPN.

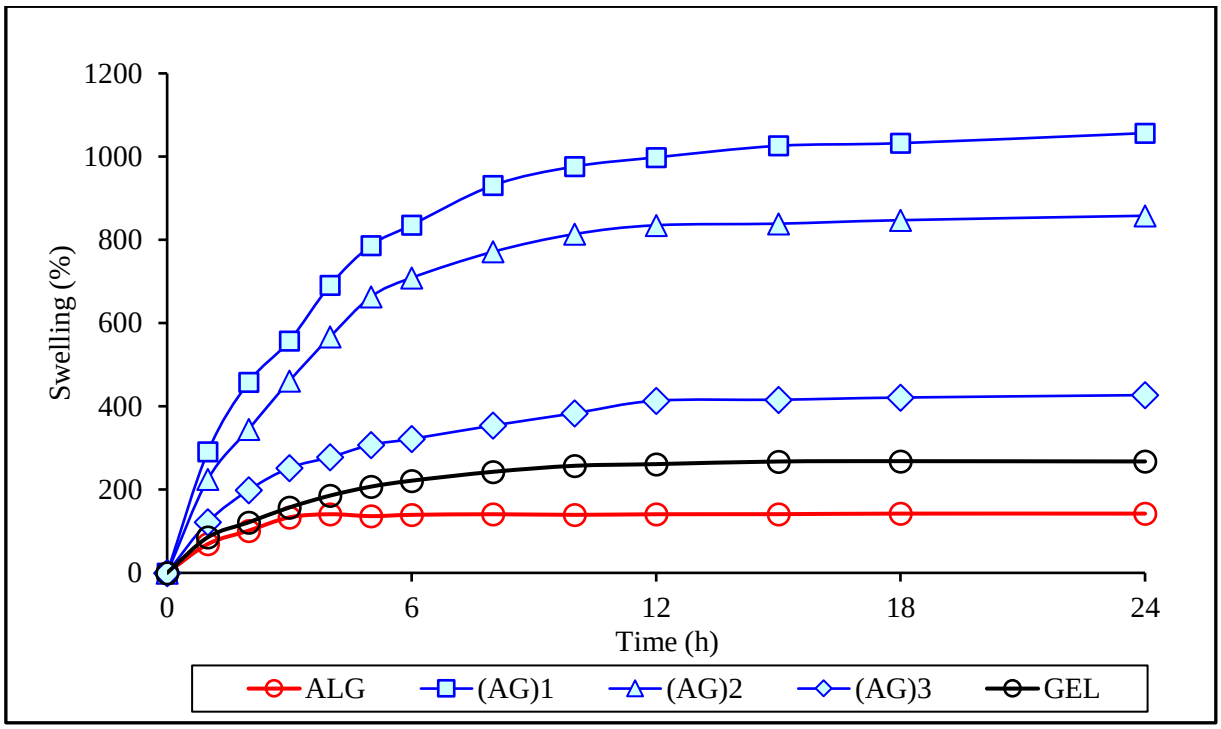

Figure 2. The change of S% with time for the hydrogel strips (Hidrojel şeritlerin şişme değerlerinin zamanla değişimi)

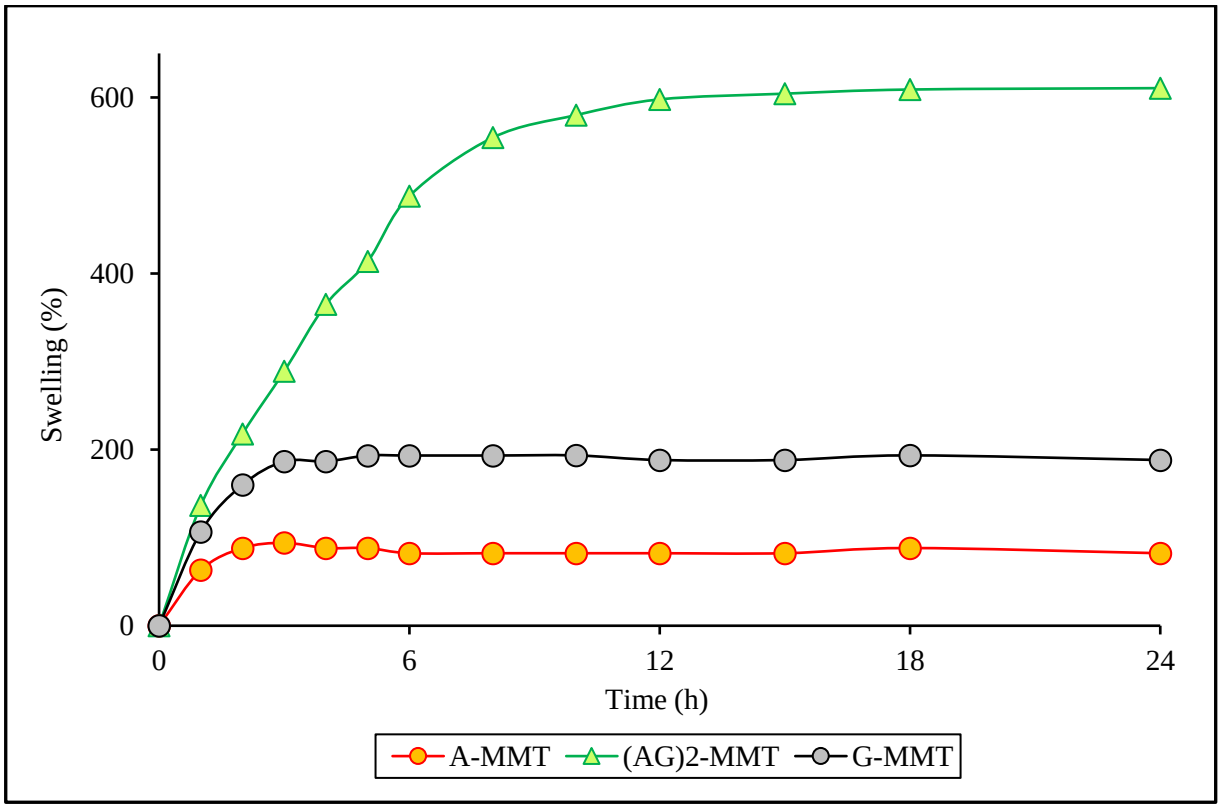

Figure 3. The change of S% with time for the MMT added hydrogel strips (MMT katkılı hidrojel şeritlerin şişme değerlerinin zamanla değişimi)

Figure 4 shows the change of swelling with temperature at pH=7.4 for 24 h. We can say that the hydrogel strips were not affected by the changes of temperature. The temperature dependent swelling

values of the hydrogels are very close to time dependent swelling values. Compared to (AG) and $(AG)_2$-MMT, the swelling value of the MMT added hydrogel strip have almost no fluctuations. It is stated that MMT nanosheets contributes to composites that containing gelatin by its own superior thermal properties [13].

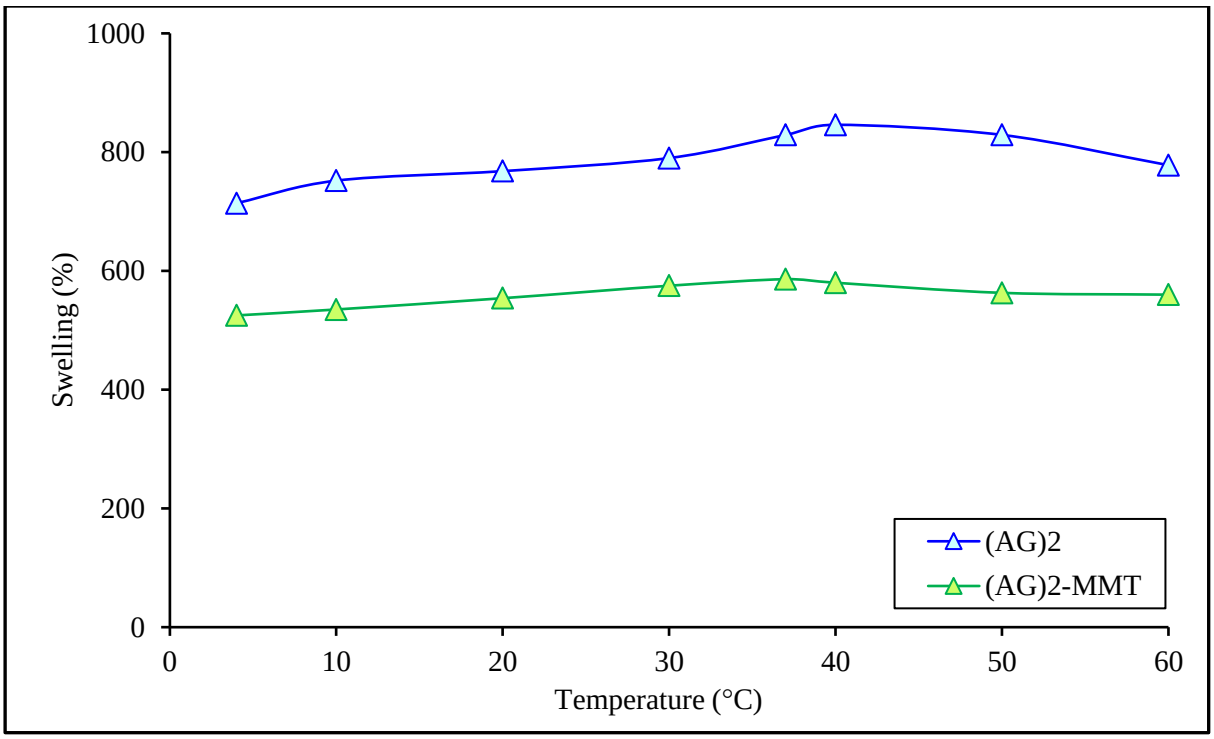

Figure 4. The change of S% with temperature for the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips ($(AG)_2$ and $(AG)_2$-MMT hidrojel şeritlerinin şişme değerlerinin sıcaklıkla değişimi)

The changes of swelling values with pH at 37°C for 24 hours is presented in Figure 5. $(AG)_2$ hydrogel strips contain free anionic alginate and crosslinked amphoteric gelatin chains. The anionic carboxylate ends of alginate ($-COO^-$) and the amine ends of gelatin ($-NH_2$) are converted to neutral carboxylic acid groups (-COOH) and ammonium ($-NH_3^+$) at acidic pH values. This prevents the polymer network to swelling at these pH values. The carboxylate groups are dominant in swelling at alkaline pH values and the swelling is affected positively. The isoelectric point (pI) value of gelatin is between 4.68–5.26 [14]. The ionization of carboxylic acid and amine groups is equal in amino acid structures such as gelatin at the pI. Therefore, swelling is minimum. It can be said that the increase in swelling values between pH from 4 to 8 and being the maximum swelling are due to the carboxylate groups belonging to the free alginate chains in the matrix. This can be interpreted as anionic alginate dominating the amphoteric gelatin in the hydrogel structure.

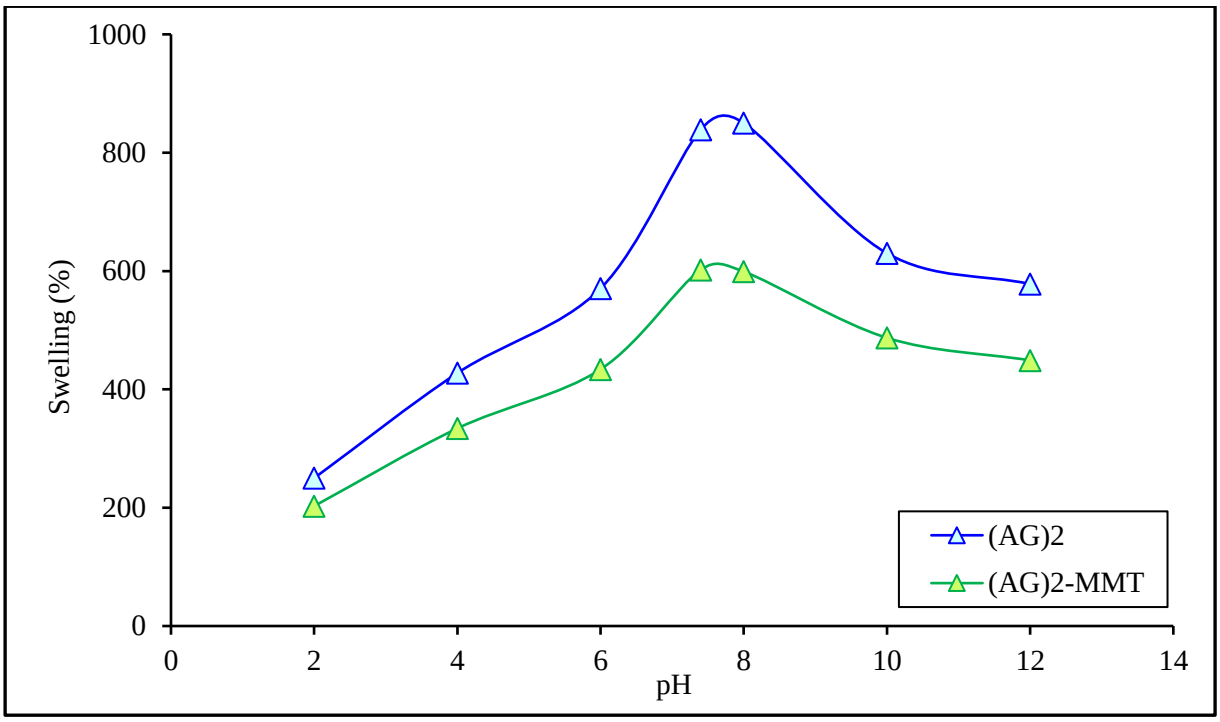

Figure 5. The change of S% with pH for the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips ($(AG)_2$ and $(AG)_2$-MMT hidrojel şeritlerinin şişme değerlerinin pH ile değişimi)

Degradation Behaviors of the Hydrogels (Hidrojellerin Bozunma Davranışları)

The degradation of hydrogel strips was investigated in BRB solution for 30 days. The rates of degradation were calculated by the Equation (3) and the degradation profiles were presented gravimetrically in Figure 6. It is observed that the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips have lost nearly 50% and 30%, respectively. Overall, the hydrogels exhibited a slight degradation profile. While gelatin chains are crosslinked in the $(AG)_2$ hydrogel matrix, the alginate chains are in free form. Considering the time dependent swelling values, as the hydrogel structure swells, uncrosslinked gelatin and free alginate chains may be removed from the matrix. This situation is limited by the presence of MMT in the structure and can be interpreted as providing strength to the structure due to hydrogen bond interactions and intercalation properties of MMT.

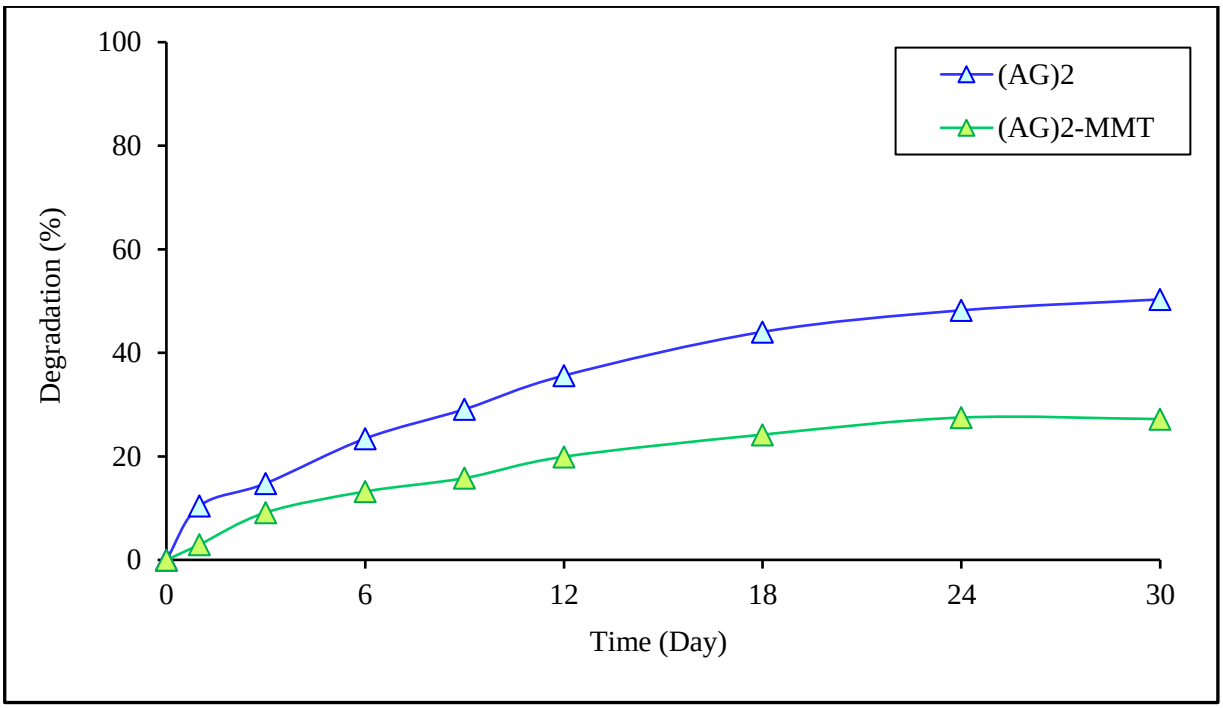

Figure 6. The degradation profiles of the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips (t=37°C and pH=7.4) ($(AG)_2$ and $(AG)_2$-MMT hidrojel şeritlerinin bozunma profilleri)

SEM Observations (SEM Gözlemleri)

The morphologies of $(AG)_2$ and $(AG)_2$-MMT hydrogel strips were observed through a scanning electron microscope and SEM micrographs were presented in Figure 7. SEM results showed porosity of the hydrogels. These porous structures enable the water absorption. The absorbed water inside the matrix interacts with hydrophilic groups and this promote the swelling and hence drug releasing [15]. As can be seen that from the micrograph (b), the presence of MMT in the hydrogel matrix caused an effect of second crosslinker. The structure of $(AG)_2$-MMT strip have smaller pores. Similar SEM results in the literature confirm the effect of MMT to the pores in the hydrogel matrix [6]. So, it can be said that MMT added hydrogel became a full-IPN type hydrogel.

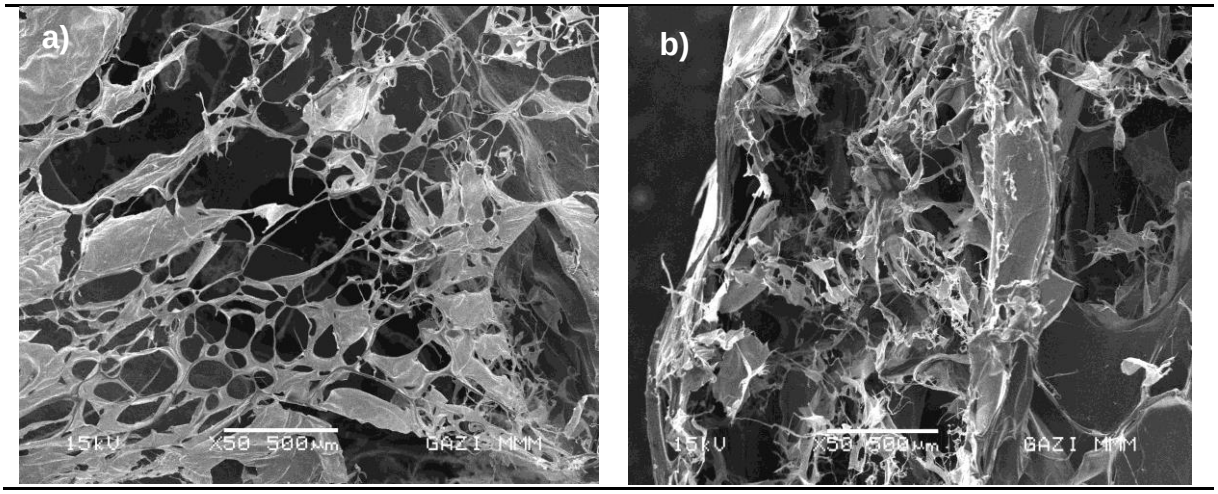

Figure 7. SEM micrographs of the hydrogel strips a) $(AG)_2$ (x50) and b) $(AG)_2$-MMT (x50) ((a) $(AG)_2$ (x50) ve b) $(AG)_2$-MMT (x50) hidrojel şeritlerinin SEM mikrografları)

Drug Release Kinetics (İlaç Salım Kinetikleri)

The cumulative release profiles of Naproxen through $(AG)_2$ and $(AG)_2$-MMT hydrogel strips were presented in Figure 8. As can be seen from the figure, the values of Naproxen release are 61% and 47% for $(AG)_2$ and $(AG)_2$-MMT hydrogel strips, respectively. The half of all releasing amount were nearly released at the first 6 hours. The rates of release decreased after the first 12 hours and remained stable in 24 hours for both. In the literature, the therapeutic range of Naproxen has been reported as 3-9 µg/mL [16]. At the end of 48h, the release of Naproxen from the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips had reached 0,88 and 0,68 µg/mL, respectively. At the stage of the synthesis, 10 mg of Naproxen had loaded to hydrogel mixture. So, if the amount of Naproxen to load is increase, it can be reach at requested period according to the amount and usage [17].

The $(AG)_2$ hydrogel strip has higher release values than the $(AG)_2$-MMT hydrogel as it has larger pores in its matrix as seen from the SEM micrographs. As mentioned above, MMT makes a secondary crosslinker effect through hydrogen bond interactions within the polymer matrix. Hence, MMT added $(AG)_2$ hydrogel strips have lower swelling and drug releasing values as have tighter matrix. Also, the releasing profiles of the hydrogels were accord with their swelling behaviors because the swelling parameters directly affect the release behaviors. Similar results have been reported on drug release from MMT added alginate composite beads [18].

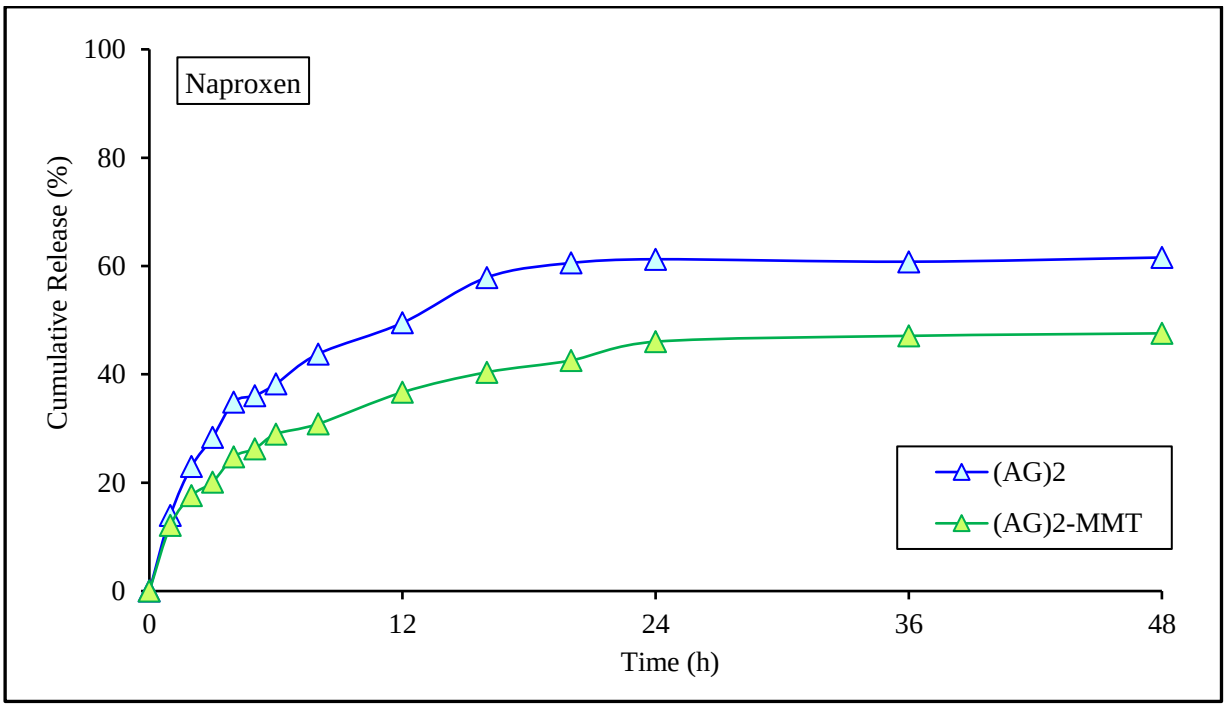

Figure 8. Release profile of Naproxen from the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips strips ($(AG)_2$ and $(AG)_2$-MMT hidrojel şeritlerinden Naproksen salım profili)

The releasing data of the hydrogels was calculated by different empirical kinetic models to determine the kinetics related to diffusion type of system. The fitted results that given in Figure 9 were matched the Peppas model. Equation (5) given below was used to express drug release parameters [32]:

$$\frac{M_t}{M\infty} = k.t^n \qquad (5)$$

M_t: is the cumulative release at time t,
M_∞: the maximum cumulative released drug,
k: a characteristic constant correlated to the structure of the matrix,
n: the diffusional exponent characteristic of the release mechanism.

The n and k values of $(AG)_2$ and $(AG)_2$-MMT were defined by Equation (5) and presented in Table 2. When n<0.45, the drug release mechanism is

Fickian diffusion; when 0.45<n<0.89, it is non-Fickian diffusion, and when n>0.89 Case II or Super Case II mechanism. The characteristic parameter n indicates that the release mechanism of Naproxen from $(AG)_2$ and $(AG)_2$-MMT hydrogel strips is Fickian diffusion (R^2: 0.9936 and R^2: 09951, respectively). In summary, the hydrogels prepared in our study can accomplish treatment of Naproxen by controlled release of drugs.

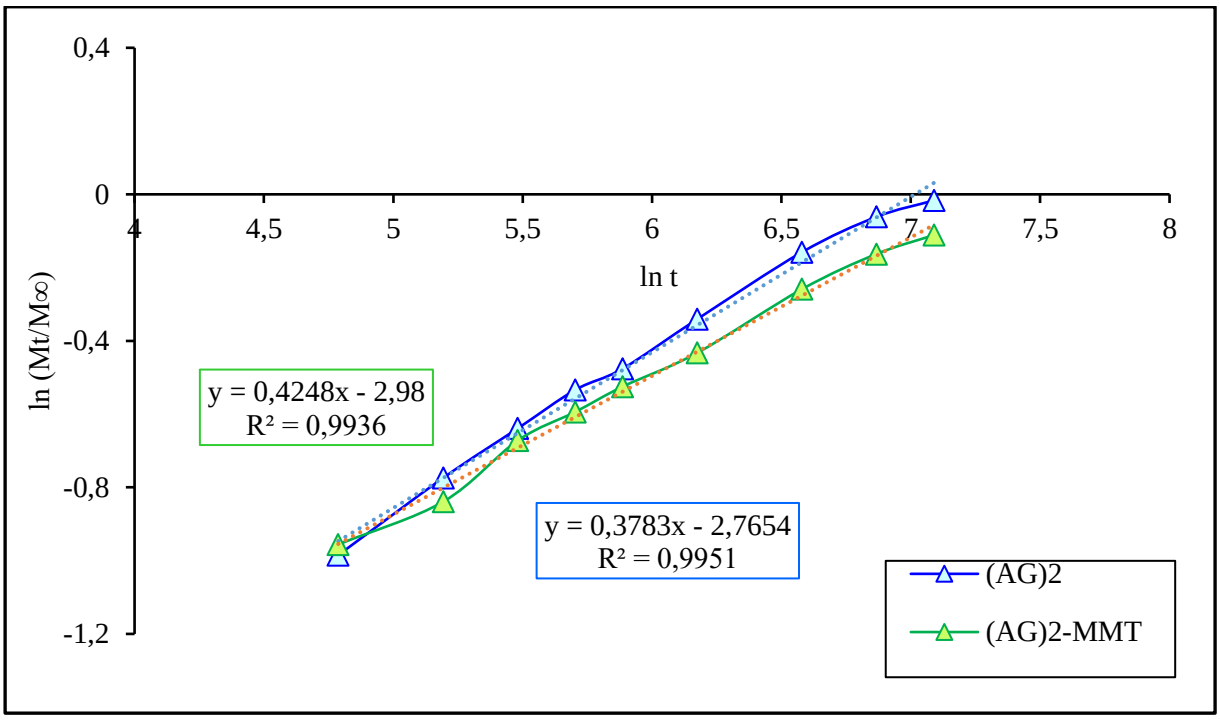

Figure 9. Release kinetics of Naproxen from the $(AG)_2$ and $(AG)_2$-MMT hydrogel strips ($(AG)_2$ and $(AG)_2$-MMT hidrojel şeritlerinden Naproksen salım kinetikleri)

Table 2. Release parameters of drug through the hydrogel strips (R^2: Deterministice coefficient) (Hidrojel şeritlerden ilaç salım parametreleri)

Hydrogels	**Release of Naproxen**		
	n	**k**	**R^2**
$(AG)_2$	0.42	5.08×10^{-2}	0.9936
$(AG)_2$-MMT	0.37	6.29×10^{-2}	0.9951

4. CONCLUSIONS (SONUÇLAR)

Synthesis and characterization of a series hydrogels that combining natural alginate and gelatin polymers with MMT clay was presented in this paper. Based on swelling values of the hydrogel, the proper hydrogel $(AG)_2$ to releasing studies was selected. MMT added $(AG)_2$ hydrogel strips were synthesized and determined the swelling/degradation properties for all hydrogels. The hydrogels were evaluated by the data from the results of HF, swelling and degradation and characterized by SEM and FT-IR. Then the controlled release studies were carried out. Naproxen that has an anti-inflammatory effect was used for the controlled release. It is found that the release mechanism of the hydrogels suited Peppas model. This mechanism and SEM micrographs confirmed the effect of MMT in the matrix on porous structure and releasing. The amount of Naproxen cumulative release of $(AG)_2$ and $(AG)_2$-MMT hydrogels are 61% and 47%, respectively. Our study showed that these synthesized hydrogels including or not MMT can be promising candidate of drug delivery for Naproxen.

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DECLARATION OF ETHICAL STANDARDS (ETİK STANDARTLARIN BEYANI)

The author of this article declares that the materials and methods they use in their work do not require ethical committee approval and/or legal-specific permission.

Bu makalenin yazarı çalışmalarında kullandıkları materyal ve yöntemlerin etik kurul izni ve/veya yasal-özel bir izin gerektirmediğini beyan ederler.

AUTHORS' CONTRIBUTIONS (YAZARLARIN KATKILARI)

Evrim SEVER: She conducted the experiments, analyzed the results and performed the writing process.

Deneyleri yapmış, sonuçlarını analiz etmiş ve maklenin yazım işlemini gerçekleştirmiştir.

Mehlika PULAT: She contributed with her knowledge and experience at every stage of the study.

Çalışmanın her aşamasında bilgi ve tecrübeleriyle katkı sağlamıştır.

CONFLICT OF INTEREST (ÇIKAR ÇATIŞMASI)

There is no conflict of interest in this study.

Bu çalışmada herhangi bir çıkar çatışması yoktur.

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