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Manufacturing of chitosan based drug release systems from tamarind shells for long-term and effective methylprednisolone sodium succinate release

İdil Karaca Açarı*

Bioengineering Department, Faculty of Engineering and Natural Sciences, Malatya Turgut Ozal University, 44210, Malatya, Turkey

E-mail: idil.karaca@ozal.edu.tr

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In this study a microparticular drug delivery system containing tamarind shell and chitosan has been developed to realize the controlled release of methylprednisolone sodium succinate an important corticosteroid. Drug-loaded tamarind shells are coated with chitosan for a *p*H-controlled release. The release kinetics of the system have been examined in detail at three different *p*H ranges (3, 5 and 7.4). In our *p*H sensitive release systems a slow release up to 39.02%±0.81 at *p*H=7.4 at 72 hours. But we see that there is a faster release at 70.58±1.73% at *p*H=5 and 96.68±1.70% at *p*H=3 in 72 hours. Rapid release in acidic media depends on the solubility of the chitosan structure in acidic environments. It is believed that the *p*H sensitive carrier system obtained within the scope of the study will have a high potential for use in many biomedical application areas (wound surface application, etc.).

Keywords: Chitosan, Drug delivery systems, Methylprednisolone sodium succinate, Tamarind shell particles

Drug delivery systems provide predetermined and reproducible controlled drug release for a long-term treatment locally or systemically at certain time intervals. The purpose of controlled drug release systems is to improve the performance of drug therapy. This mechanism increases therapeutic activity and reduces side effects by reducing toxicity due to dose frequency, dose reduction, and overdose during treatment[1]. Biodegradability, biocompatibility, availability in nature and use of natural materials as drug release vehicles have become increasingly important[2]. Among these natural materials, natural polymers containing polysaccharides (carbohydrates) are a group of useful biomaterials that are widely used in numerous biomedical applications. In the past few decades, different natural polysaccharides have been widely used in the development of various drug delivery systems due to their distinct properties such as biodegradability, non-toxicity, biocompatibility, good mechanical properties, high mucoadhesiveness and swelling properties. Important natural polysaccharides widely used as excipients in foods and medicines; chitosan, alginates, gellan gum, gum arabic, okra gum, sterculia gum, pectin, starches tamarind gum (TG) are examples[3-5]. The reasons for the use of these biopolymers in systems related to drug encapsulation, transport and controlled release in the body are that they can be easily absorbed in the body after carrying and releasing the drug, and thus can be quickly eliminated from the body[6]. Microparticles with almost unlimited application in drug delivery systems; It is used as a component in many advanced materials and composites, in the health and personal care industries and in many research and development applications[7]. As drug delivery systems, porous microparticles are particularly suitable for providing delayed or controlled drug release of oral formulations with very low risk of dose dumping. Porous materials are designed for use in a variety of medical and pharmaceutical applications, such as drug delivery systems, agents in medical imaging, immunotherapy, cancer therapy, wound dressing, tissue engineering, biosensors, and cardiac prosthetics[8]. Due to the micropores they have in their structure, they are important for the transport, absorption and controlled release of many important drugs and therapeutic agents.In addition, it is known that they are widely used in diagnosis and treatment in medical and biomedical applications[9]. Natural cellulose-based structures are frequently used for the development of biocompatible microparticle structures due to their high biocompatibility. Cellulosic particles obtained by grinding cellulose wastes, ground tree shell, fruit seed peels, stem structures of plants such as corn and sunflower are especially important in terms of drug absorption. Within the scope of the study, the outer shell part of tamarind (TS) was preferred as a

porous microparticle. The tamarind tree (*Tamarindus indica*, family: Fabaceae) is locally known as the imli (Hindi) tree. This evergreen tree is also grown in almost all parts of India and other Southeast Asian countries[10]. Tamarind consists of different parts. We can give examples of these parts, the outer shell part, the gum part and the core part. It is known that every part of tamarind tree is used in the food industry, textile industry, chemical industry or pharmaceutical industry[11]. Within the scope of this study, a slower and longer lasting methylprednisolone sodium succinate (MPSS) release was targeted. Methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$, 11β, 17a, 21-trihydroxy-6a-methylpregna-1,4-diene-3,20-diketone-21-sodium succinate) has strong anti-inflammatory and immunosuppressive effects like other corticosteroids. Methylprednisolone sodium succinate has a wide range of applications. For instance we can give allergic conditions, collagen disease, brain and spinal edema, gastrointestinal disorders, neoplastic diseases, nervous system disorders, rejection of transplanted organs, respiratory tract diseases, acute spinal cord injuries[12,13]. In this studycore-shell microparticle structure was preferred for a slower and longer lasting methylprednisolone sodium succinate release. The core microparticle structure is formed in the outer skin of the tamarind fruit as a porous and easily grindable structure.Thanks to its porous structure, it quickly absorbs and releases target drug structures. The outer surface of tamarind microparticles was coated with chitosan (CS) in order to prolong the release and prevent the uncontrolled dispersal of the absorbed drug molecules from the structure.Thus, a core-shell microparticular release system containing chitosan as an outer shell was obtained. The chitosan in the shell part also enables a controlled release of the changing pore structure at different *p*Hs. For this reasona *p*H-controlled core-shell microparticle structure was developed in order to release the corticosteroid MPSS which has many uses.

Experimental Section

Chitosan (low molecular weight)organic solvents and acetic acid used in the study were obtained from sigma aldrich company. The tamarind structure used in the production of microparticles was obtained from local markets, the shell parts were removed, dried in an oven for 1 day and ground and sieved with a sieve below 25 microns. Methylprednisolone sodium succinate was obtained as a corticosteroid drug and was purified by removing the solvent by solvent extraction method. In the drug release step, a cellulose membrane of 43mm × 27mm, which is also a sigma aldrich brand, was used.

Instrumentation

First of all, FTIR spectrophotometer was used for the characterization of drug-loaded tamarind structures obtained within the scope of the study. Perkin Elmer 283 model IR spectrophotometer was used for FTIR analysis of the structures. 4 cm^{-1} spectral sensitivity and 400-4000 cm^{-1} wavelength range were used for FTIR analysis. Thermal analyzes were made with Shimadzu TGA-50 and Shimadzu DSC-60 model thermogravimetric analyzers. The thermal stability, decomposition temperatures and decomposition energies of the structures obtained in these analyzes were determined by TGA techniques. TGA analyzes were performed at 10°C/min heating rates and 10 mg sample amounts in a constant air atmosphere. TGA thermograms were taken between room temperature and 700°C. DSC analyzes were carried out by taking 5 mg sample amounts at 10°C/min heating rates and in a constant air atmosphere. DSC thermograms were taken between room temperature and 400°C. Microscope images of the pure tamarind shell structure were taken with a Soif metallurgical upright microscope at 50, 100 and 200 times magnification. The surface properties and morphologies of the obtained drug-loaded tamarind structures both were examined with SEM (LEO Evo-40 VPX) device. Solvent removal in the experimental processes was carried out using Freeze Dryer brand BK-FD12S (-80°C) model lyophilizer device.

Synthesis of methylprednisolone sodium succinate (MPSS)-loaded structures

In the process of obtaining the microparticles, the tamarind shell was first left aside. The removed crust part was dried in the oven for 1 day and ground well. The ground fraction was sieved through a ~25 micron mesh diameter sieve. Sieved shell samples (0.2 g) were taken and the surface pores of cellulosic structure were opened in 20 mL of distilled water at 40°C for a specified time. 10% of the total mass of MPSS was added to this solution, in which the pores were opened, and it was stirred continuously at 40°C overnight. The outer shell of the prepared drug-loaded core microparticles was modified with chitosan in order to prevent uncontrolled dispersal of drug molecules from the structure. For this modification, 0.2 g of chitosan was dissolved in 10 mL (2% w/v HAc) aqueous solution[14,15] at room

temperature for 24 h by stirring. The resulting chitosan solution was added to the core microparticular structure and mixed for 24 h at room temperature to obtain the core-shell microparticular structure.

MPSS release studies

First of all, the wavelength at which MPSS absorbs maximum was determined. Then, the drug released from the release system obtained within the scope of the study at the determined wavelength was followed.

Determination of maximum absorption wavelength for MPSS

Before monitoring the MPSS release from the core-shell microparticle structure prepared by UV spectrophotometer, the wavelength at which MPSS absorbs maximum was determined. For this, MPSS samples prepared at different concentrations were scanned in the range of 200-800 nm.

MPSS release from core-shell microparticular structure

In order to monitor MPSS delivery in microparticular structures with UV spectrophotometer, MPSS calibration graph was drawn first. Delivery studies in microparticle structure were carried out by using dialysis membrane.The release was monitored for 72 h in PBS solutions prepared with various *p*H values (3, 5 and 7.4) at 37°C with continuous mixing at 120 rpm. An equal volume of fresh medium was left in the system in each sampling. Measurements were made with UV spectrophotometer in the solution taken from the environment at certain time intervals. Kinetic models were then studied to determine the % cumulative drug release and drug release from dosage forms in which the drug amount was plotted as a function of time or time. All calculations are given with standard deviation values.

Results and Discussion

Characterization of MPSS-loaded core-shell microparticular structures

Within the scope of the study, tamarind shell based drug release systems were prepared that can provide controlled and long-term MPSS release. A *p*H sensitive drug delivery system was created with a chitosan-based coating on tamarind drug combinations in order to ensure structural diversity and MPSS regularity in these delivery systems. The FTIR spectra of the developed drug delivery systems and pure tamarind, chitosan and MPSS structures are given in Fig. 1. First of all, the FTIR spectrum of the pure tamarind shell structure was interpreted for the first time in our study. When looking at the FTIR spectrum, the peaks showing the classical cellulosic structure are clearly seen. Especially at 3000-3600 cm^{-1}, OH vibrations in the carbohydrate groups of the tamarind shell structure are evident[16]. C-H stretching vibrations due to CH_2 and CH units are observed at 2850-2920 cm^{-1} (Ref. 17). The etheric stretching vibrations in the structure are evident and clear at approximately 1037 cm^{-1}. C-C aliphatic stretching

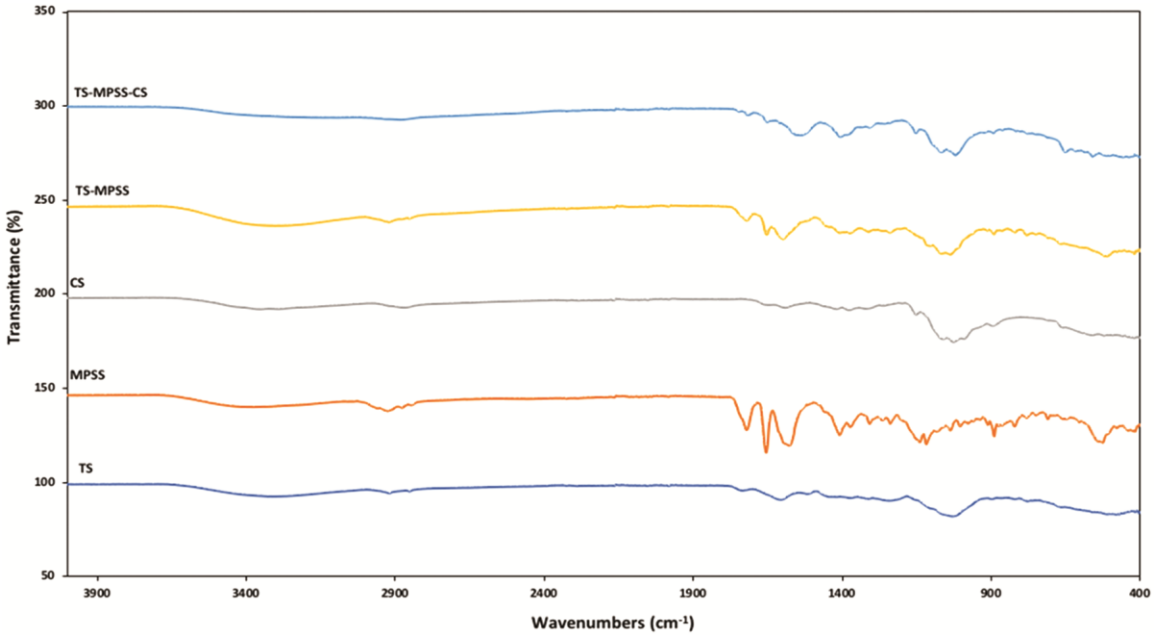

Fig. 1 — FTIR spectrum of core-shell microparticle structures loaded with pure tamarind shell (TS), chitosan (CS), methylprednisolone sodium succinate (MPSS) and MPSS.

vibrations confirm the presence of carbohydrates in the structure at approximately 1520 cm^{-1}.The related tamarind shell structure is a suitable matrix for the absorption of corticosteroid-based drugs such as MPSS due to its carbohydrate groups.It facilitates the absorption of MPSS thanks to its porous structure and surface affinity. In the FTIR spectrum of the chitosan structure used in the study, C-O-C etheric stretching vibrations at approximately 1050 cm^{-1} are seen as a band peak. In addition, NH_2 and OH groups in the structure give a wide band peak in the range of 2900-3650 cm^{-1}. C-N stretching vibration at 1420 cm^{-1} originates from C-NH_2 groups. All bands are also found in the spectrum of chitosan samples reported by others[18,19]. The MPSS used in the study has a more complex spectrum structure. Acidic, esteric and ketonic carbonyl groups in the structure were seen as 3 different stretching vibrations at the values of 1580 cm^{-1}, 1720 and 1685 cm^{-1}, respectively. Aliphatic C-H stretching vibrations at 2917 and 2962 cm^{-1}, C-C stretching vibrations at 1460 cm^{-1}, esteric carbonyl peaks at 1115 cm^{-1} clearly confirm the structure. In the FTIR spectrum of MPSS loaded tamarind shell microparticles, prominent tamarind shell peaks and esteric, etheric and acidic carbonyl groups belonging to methylprednisolone sodium succinate groups are clearly seen. All these peaks show that the desired drug is clearly bound to the structure.

In the FTIR spectra of the chitosan coated structures, it is clearly seen that the carbonyl groups originating from methylprednisolone sodium succinate are partially screened and the chitosan structure is more prominent. In particular, the etheric stretching vibrations in the chitosan structure are quite evident. Surface hydroxyls originating from the chitosan structure appeared much more prominently in the range of 2950-3650 cm^{-1}. According to the spectrum in Fig. 2, it is clearly seen that the desired methylprednisolone sodium succinate loaded structures are obtained. The microscope images of the pure tamarind shell structure are given in Fig. 2 with 50, 100 and 200 times magnification.

In these figures, the particulate structure and porous texture of the drug-loaded tamarind shell structures are clearly demonstrated.In order to show this porous surface more clearly, SEM images in Fig. 3 are given. In SEM images, surface images of pure tamarind shell structure, drug loaded tamarind shell structures and chitosan coated core-shell structures are given as 5000, 10000 and 20000. When the pure tamarind shell structure is examined, it is seen that there is a very high porosity and regular

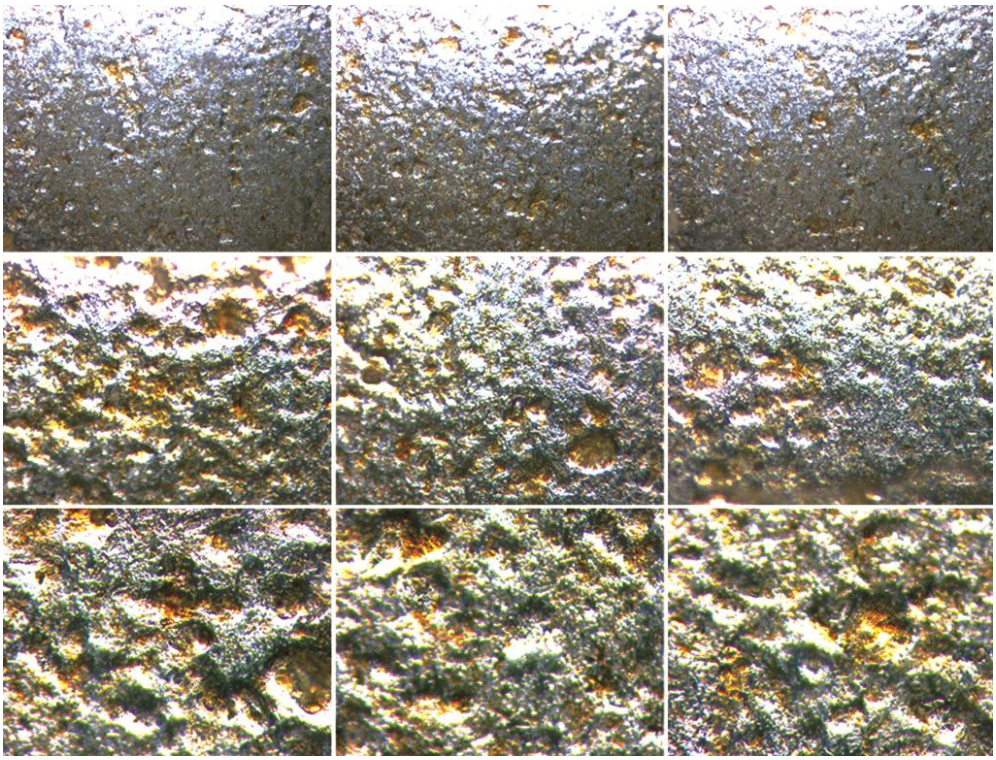

Fig. 2 — Optical microscope images of pure TS structure at different magnifications taken from three different regions.

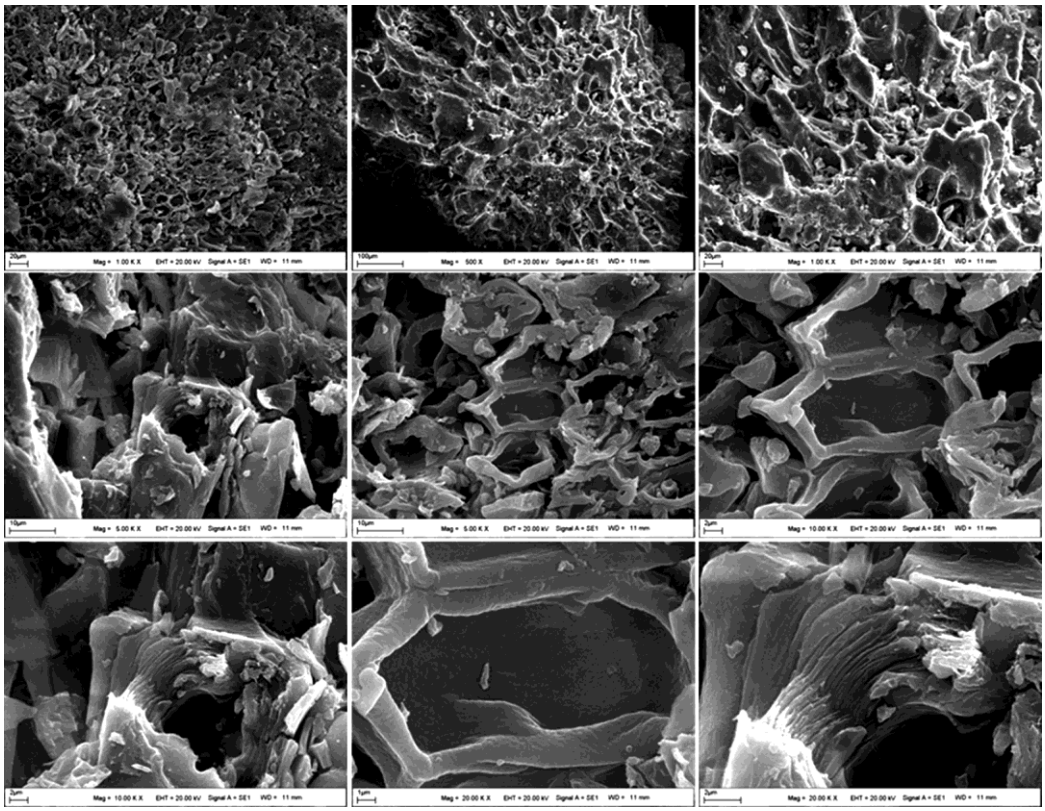

Fig. 3 — SEM images of the pure TS structure at different magnifications.

cavities. SEM images of tamarind shell structures, especially at low magnifications, draw an ideal absorbent image.

After loading the drug on this fractal structure, it is seen that the porous structure partially disappears and the pores are filled with MPSS structures.When the relevant SEM images are examined, cavities dominate in partial areas, but flat areas are seen in places.In this image, it is seen that the surface exhibits a smoother morphology, especially due to the chitosan coated on the outer surface after the chitosan coating. Related SEM images show that methylprednisolone sodium succinate groups are adsorbed regularly as targeted on tamarind shell microparticles with porous structure, and at the same time, chitosan binds to the outer layer of this structure in Fig. 4. It has been proven that the prepared chitosan-coated drug delivery system is achieved as intended. Such structures must show structural stability for sterilization purposes, especially during and after application. Thermal analyzes were carried out to determine both this structural stability and the amount of drug bound to the structure.

TGA thermogram given in Fig. 5(A) and DSC thermogram given in Fig. 5(B) were applied for each sample as thermal analysis.In the TGA thermograms in Fig. 5(a), three basic mass losses are seen in the pure tamarind shell structure. The first of these mass losses is the removal of structural moisture, which is observed in the range of 80-100°C and corresponds to a mass loss of approximately 12%. The second mass loss is the mass loss resulting from the breaking of side groups and etheric stretching vibrations in cellulosic structures. It is around 60% and has been seen in the range of 180-400°C. The final mass loss is around 400-500°C and is due to thermal degradation of the entire polymeric structure. When MPSS was added to this structure,4different mass losses occurred.The 1st, 3rd and 4th mass losses are due to identical properties with the mass losses in the pure tamarind thermogram. The second mass loss occurs in the range of 90-110°C and is a mass loss of approximately 12% due to the presence of the drug attached to the structure.After the chitosan is coated on this structure, 4 basic mass losses are seen again, and the 1st and 2nd mass loss values are very close to each other. The reason for this is that the moisture groups attached to the chitosan groups move away from the structure in a wider range. However, in this structure, there is also a significant loss of mass in the

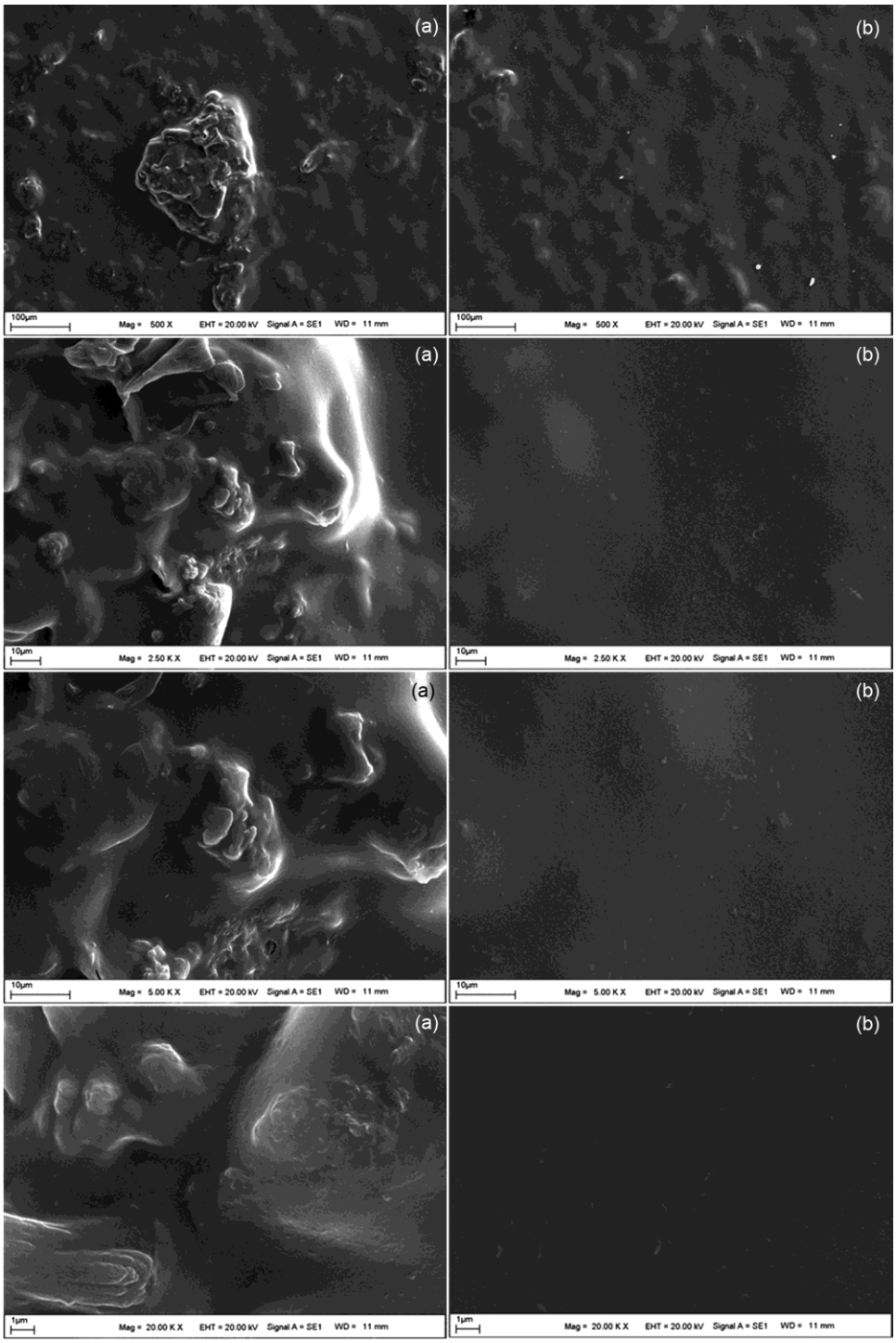

Fig. 4 — SEM images of methylprednisolone sodium succinate loaded structures (a; TS-MPSS, b; TS-MPSS-CS).

range of 97-110°C due to the degradation of the drug in the structure. These TGA thermograms show that the tamarind shell structure is structurally stable up to 100°C as a pure absorbent, while drug-loaded tamarind microparticles can be easily used up to approximately 95°C in both formulations. DSC thermograms were taken to show that the related drug structure is more specific. Related DSC thermograms are given in Fig. 5(B). DSC thermogram of pure tamarind shell structure shows two basic exotherms up to 400°C.While the exotherm between 50-140°C is caused by the removal of moisture, the large exotherm

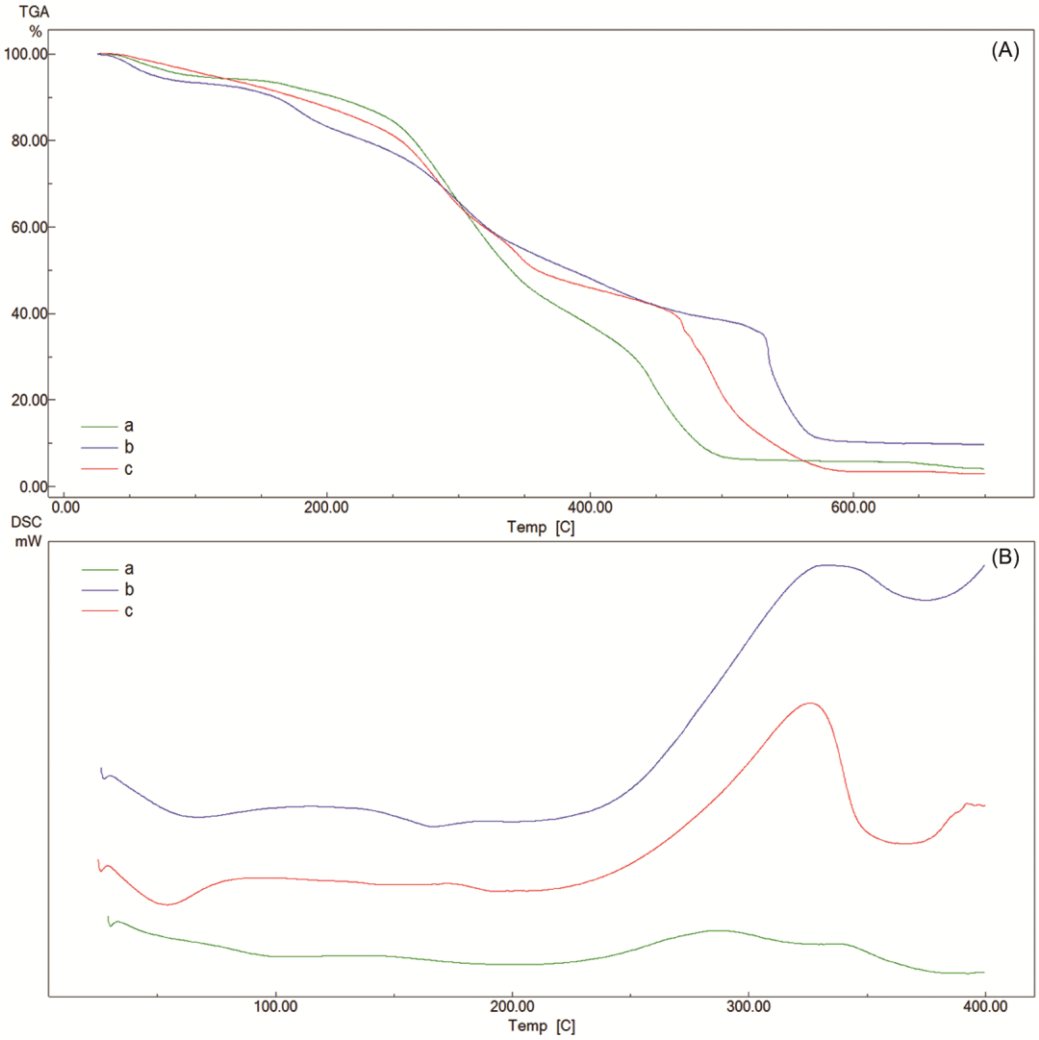

Fig. 5 — (A) TGA thermograms of TS (a), TS-MPSS (b) and TS-MPSS-CS (c) structures and (B) DSC thermograms of TS (a), TS-MPSS (b) and TS-MPSS-CS (c) constructs.

between 200-375°C is caused by the degradation of the cellulosic structure.In the drug formulation, MPSS degradation peak is observed between these two exotherms between 150-200°C and the presence of this peak proves that the drug is adhered to the structure. When chitosan is coated on this structure, the relevant peak is seen more clearly and clearly around 100 and 200°C, and a degradation peak between 300-400°C is observed not only from the tamarind structure but also from the chitosan structure. All these findings show that the desired triple combination is achieved.

MPSS drug release and kinetics results

Within the scope of the study, MPSS release kinetics of the targeted tamarind shell-based core-shell microparticle structure were studied. For this, firstly, the wavelength at which MPSS gave maximum absorbance was determined. The wavelength was determined as 260 nm as a result of scanning. Calibration graph was drawn for MPSS at the determined wavelength and was found to be linear.

% Cumulative drug release graph for MPSS

The % cumulative drug release of MPSS drug at different *p*H conditions (3, 5 and 7.4) is given in Fig. 6. According to this,in the first hour we observed that the release was 18.28±1.31% at *p*H=3, 13.23±1.38% at *p*H=5 and 10.83±0.35% at *p*H=7.4. When we look at the 12th h, we see that the release is 91.05±1.42% at *p*H=3, 33.15±1.44% at *p*H=5 and 27.62±0.66% at *p*H=7.4.When we look at the 72 h, it is 96.68±1.7% at *p*H=3, 70.58±1.73% at *p*H=5, and

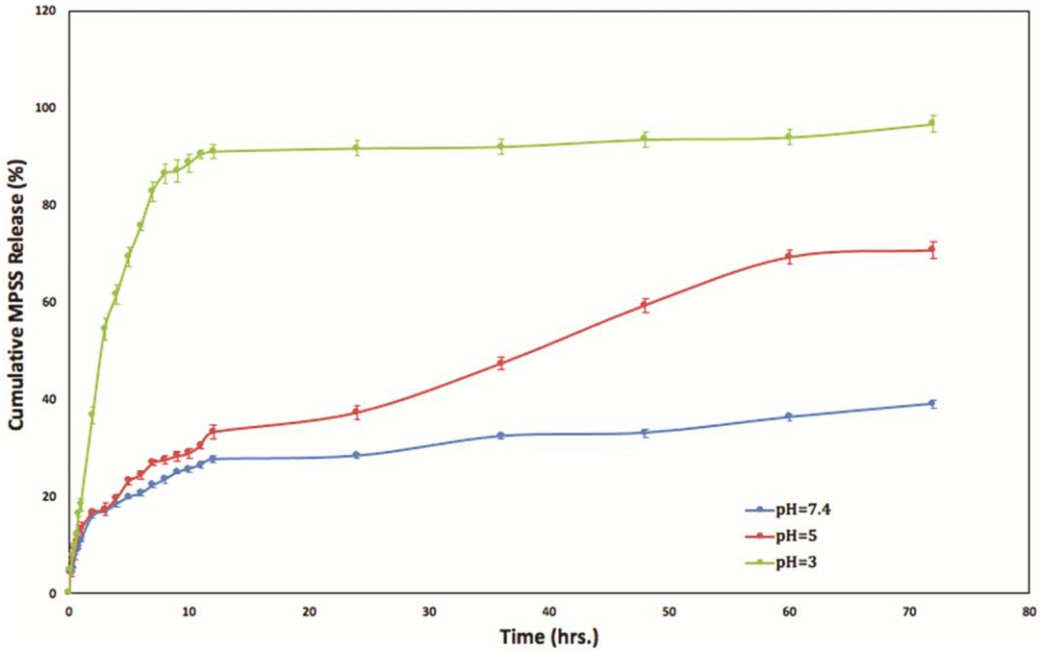

Fig. 6 — % Cumulative drug release graph for MPSS at different *p*Hs.

finally 39.02±0.81% at *p*H=7.4. As a result, we observed that the MPSS drug release of the tamarind-based core-shell microparticular release system is slower at increasing *p*H.

A very limited number of studies have been found in the literature for MPSS. Karabey-Akyurek *et al.* in their study, MPSS loaded nanoparticles, MPSS loaded nanoparticles dispersed in fibrin gel and MPSS dispersed delivery in fibrin gel were studied. The delivery times were followed between the 1st and 8th hours. The slowest release was observed in the nanoparticle structures dispersed in the fibrin gel. They observed ~30% in the 1st hour and ~35% in the 8th hour[20]. Pritchard *et al.* have synthesized thiol-acrylate poly(ethylene glycol)hydrogel structures for MPSS. They studied the release of MPSS from hydrogels at different concentrations. They concluded that the release was high as expected at high concentration[21]. When one looks at the literature, the *p*H sensitive release system obtained in this study within the scope of the study has the feature of being the first in the literature because it is both tamarind shell based and obtained from natural sources for MPSS release. In addition, in this *p*H sensitive release systems, a very slow release of up to 39.02%±0.81% at *p*H=7.4% at 72 h, and a rapid release of 96.68±1.70% at *p*H=3 is seen. It can be attributed that the rapid release in the acidic environment to the solubility of the chitosan structure in acidic environments. Since the outer part of the core-shell microparticle release system obtained in the study is coated with chitosan, it can be interpreted that the pores of the system expand and release the drug faster in acidic environments.

Release kinetics for MPSS drug

Kinetic models were developed to determine drug release from dosage forms where the amount of drug released (Q) was plotted as a function of time, t, or time as a function of Q=f (t). Some kinetic explanations of the dissolved drug amount (Q) as a function of time are zero order, first order, Hixson–Crowell, Higuchi, Korsmeyer–Peppas models. The Higuchi model describes drug release from a matrix system. The Hixson-Crowell cube root law describes the release from systems where there is a change in the surface area and diameter of particles[22]. The kinetic approach of the study was studied in zero order, first order, Higuchi and Korsmeyer–Peppas models for all pH. Kinetic studies are given in Figs 7 (a), (b) and (c).

When the regression coefficients of the models are compared (R^2), it is seen that the highest value at *p*H=3 and *p*H=7.4 is in the Korsmeyer-Peppas model. In the Korsmeyer-Peppas model, the release exponent (n) indicates the drug release mechanism from the designed matrix. Accordingly, the drug transport mechanism at *p*H=3 is supercase II transport.

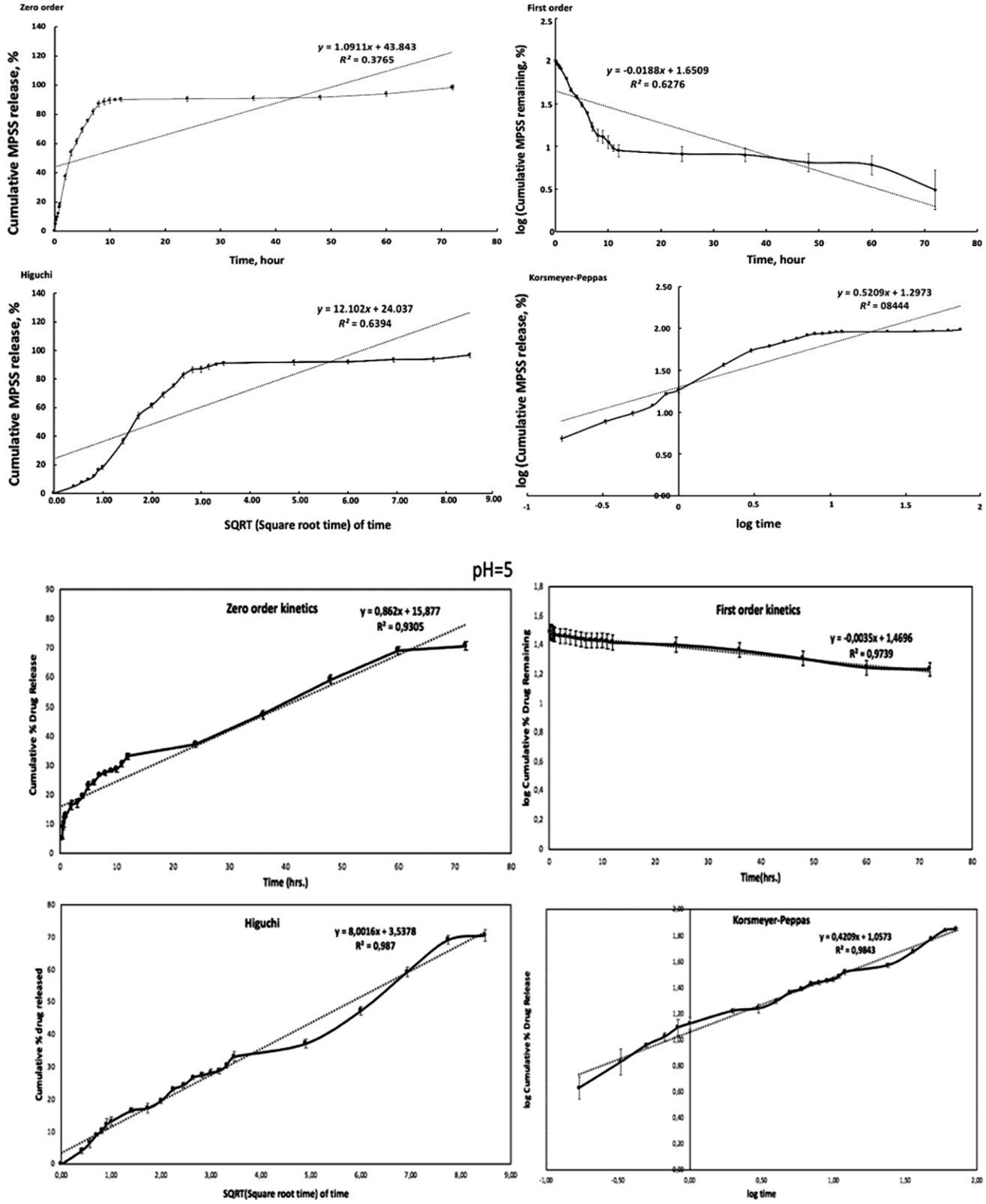

Fig. 7 — (a) Kinetic models for MPSS at *p*H=3; (b) Kinetic models for MPSS at *p*H=5

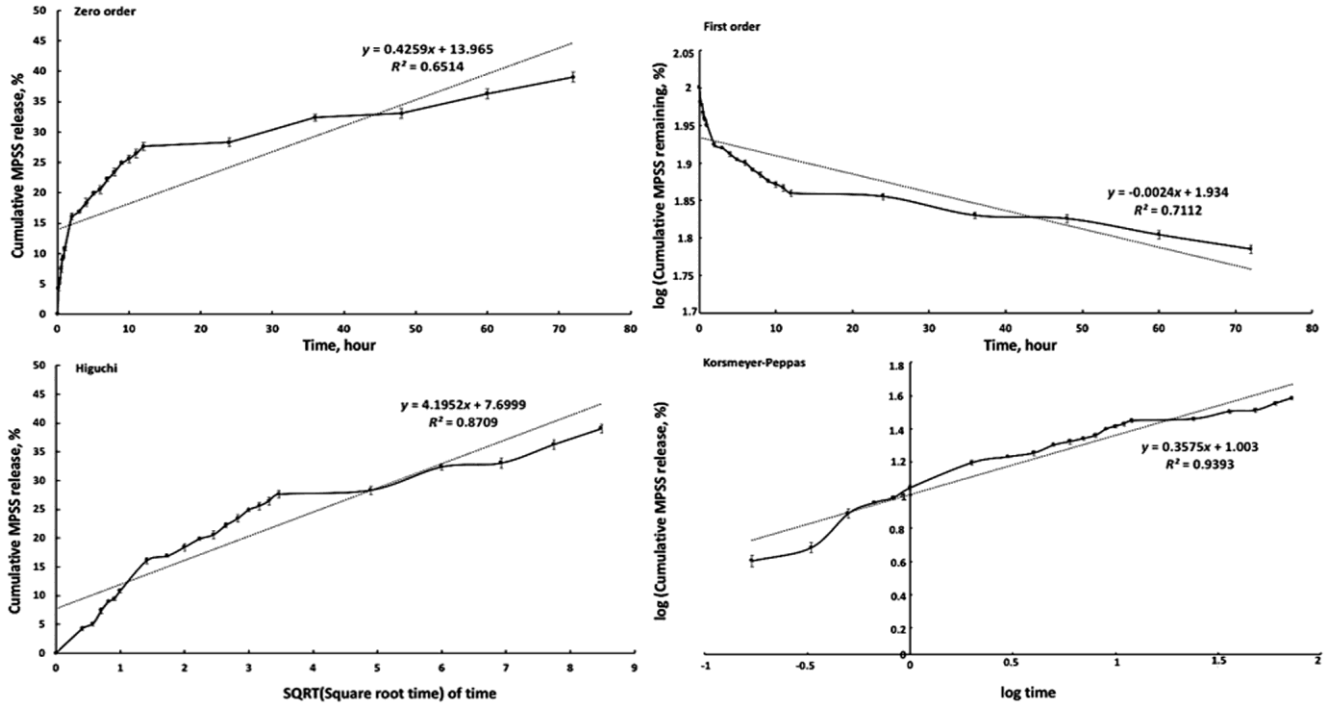

Fig. 7 — (c) Kinetic models for MPSS at *p*H=7.4

Accordingly, the drug release mechanism is zero-order release. In other words, it can be said that the prepared carrier system is a system that does not decompose and releases the drug slowly at *p*H=7.4 the drug transport mechanism is case II transport[23-25]. When we look at the R^2 values in the delivery kinetics performed at *p*H= 5 the closeness of the Higuchi and Korsmeyer-Peppas kinetic models draws attention. It is higher in the Higuchi model. The Higuchi equation is used for diffusion-controlled release of drugs from insoluble and non-biodegradable matrices[26]. As can be seen from these results, it is believed that the targeted pH-controlled release systems can be produced from cellulosic materials based on the shell parts of tamarind and will easily find use in the field of release systems to be used in wound dressing materials.

Conclusion

Methylprednisolone sodium succinate is a widely used corticosteroid based drug. Due to the nature of this drug, the dose taken in long-term treatments can cause serious side effects. For this reason, the design of drug carrier structures that will provide regular and long-term release of corticosteroid-based drugs is very important in terms of drug effectiveness. Therefore, within the scope of the related study natural release systems were prepared from the outer shell of the natural tamarind fruit in order to achieve this aim.There are many publications in the literature regarding gum and kernel parts as drug delivery systems[3,10,11]. However, the use of the outer shell part in drug release was tried for the first time in this study. In order to prolong the release in tamarind shell and to develop a *p*H sensitive system coating with chitosan whose *p*H sensitivity has been proven by the literature was preferred. Thus, a core-shell microparticular release system based on tamarind shell has been obtained. The resulting structures are characterized by TGA, DSC and FTIR spectra. Surface properties and morphologies are examined with optical microscope and SEM images. MPSS delivery is observed at various *p*H (3, 5 and 7.4). As a result, the designed release system achieved slower and prolonged MPSS drug release at high pH. It is expected that the *p*H-controlled release system will be the focus of attention for wound surface treatments where *p*H sensitivity is very important.

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