Mugla Journal of Science and Technology

PRODUCTION OF *GANODERMA LUCIDUM* EXTRACT LOADED GELATIN-SODIUM ALGINATE MICROSPHERES, INVESTIGATION OF RELEASE KINETICS AT DIFFERENT pH VALUES AND EVALUATION OF KINETIC MODELS

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Received: 07.02.2022, Accepted: 09.05.2022 Research Article
*Corresponding author DOI: 10.22531/muglajsci.1069305

Abstract

In this study, pH sensitive microsphere polymeric drug carriers were produced by using biodegradable natural gelatin and sodium alginate polymers. Microspheres were loaded with prepared Ganoderma lucidum extract that is a medicinal mushroom and has the potential to be used in several diseases' treatment. Extract release kinetics of the microspheres were examined by spectrophotometric method by using an UV spectrometer. Buffer solutions with different pH values were used as release medium for examination of drug release kinetics of the produced microspheres. The Ganoderma lucidum release of microspheres was presented in terms of percent cumulative release (CR%) defined as the percentage ratio of the instantaneous amount of Ganoderma lucidum released at a certain time of incubation to the initial amount of Ganoderma lucidum loadings. As a result, it was seen that the release of the extract accelerated as pH of the release medium increased and the fastest extract release was observed in the pH 7. The release kinetic models of the microspheres were examined. The release kinetics of microspheres fitted Higuchi model for pH 1.3, pH 5.0 and pH 6.0 and first-order model for pH 3.0 and pH 7.0.

Keywords: *Ganoderma lucidum*, drug carrier, controlled release, pH sensitive polymer, kinetic release model

GANODERMA LUCIDUM EKSTRAKTI YÜKLENMİŞ JELATİN-SODYUM ALJİNAT MİKROKÜRELERİN ÜRETİMİ, FARKLI pH DEĞERLERİNDE SALIM KİNETİKLERİNİN İNCELENMESİ VE KİNETİK MODELLERİNİN TESPİTİ

Özet

Bu çalışmada, biyolojik olarak parçalanabilen doğal jelatin ve sodyum aljinat polimerleri kullanılarak pH'a duyarlı mikroküre polimerik ilaç taşıyıcıları üretilmiştir. Mikrokürelere, hazırlanan tıbbi bir mantar olan ve çeşitli hastalıkların tedavisinde kullanılma potansiyeline sahip Ganoderma lucidum özütü yüklenmiştir. Mikrokürelerin ekstrakt salma kinetiği UV spektrometresi kullanılarak spektrofotometrik yöntemle incelenmiştir. Üretilen mikrokürelerin ilaç salım kinetiğinin incelenmesi için salım ortamı olarak farklı pH değerlerine sahip tampon çözeltiler kullanılmıştır. Mikrokürelerin Ganoderma lucidum salınımı, belirli bir inkübasyon zamanında salınan anlık Ganoderma lucidum miktarının Ganoderma lucidum yüklemelerinin ilk miktarına yüzde oranı olarak tanımlanan kümülatif salınım yüzdesi (CR%) cinsinden sunulmuştur. Sonuç olarak, salım ortamının pH'ı arttıkça ekstrakt salınımının hızlandığı ve en hızlı ekstrakt salınımının pH 7'de gerçekleştiği görülmüştür. Mikrokürelerin salım kinetik modelleri de incelenmiştir. Mikrokürelerin salım kinetiği, pH 1.3, pH 5.0 ve pH 6.0 için Higuchi modeline ve pH 3.0 ve pH 7.0 için birinci dereceden modele uymuştur.

Anahtar Kelimeler: *Ganoderma lucidum*, ilaç taşıyıcı, kontrollü salınım, pH duyarlı polimer, kinetik salım modeli

Cite

Eren, B. E., Gönen, H., Çiçek, H., (2022). "Production of Ganoderma Lucidum Extract Loaded Gelatin-Sodium Alginate Microspheres, Investigation of Release Kinetics at Different pH Values and Evaluation of Kinetic Models", Mugla Journal of Science and Technology, 8(1), 41-50.

1. Introduction

Ganoderma lucidum

Ganoderma lucidum (*G. lucidum*), a medicinal mushroom belonging to the family of *Ganodermataceae* from the *Basidiomycetes* mushroom class, has been used in Asia and recently in western communities since 2000s [1]. Mushrooms usually contain 90% water. The remaining 10% part consists of 26-28% carbohydrates, 3-5% crude

fat, 59% crude fiber, 7-8% crude protein and 1.8% ash for *G. lucidum* [2].

It is known that *G. lucidum* has immune modulating, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidative, radical cleansing, anti-allergic and anti-aging properties thanks to the bioactive ingredients it contains, and there are studies in the literature on its properties. In particular, triterten and polysaccharide ingredients are examined in terms of anti-cancer and anti-tumor properties. In one of these studies, it was reported that polysaccharides and triterpenes in *G. lucidum* have chemopreventive and/or tumoricidal effects. It was found as a result of the study that it has inhibitory effect on angiogenesis and metastasis in tumor animal models [3]. Extracts from *G. lucidum* have a carcinostatic effect on various cancer cells such as breast, pancreas, lung, skin and prostate and there are many mechanisms of anti-cancer activities of *G. lucidum* extracts. The mechanism of direct reduction of cell proliferation is one of them [4]. Studies have shown the use of *G. lucidum* extracts in the treatment of cancer cells, leads to the regulation of cell cycle-related proteins resulting in cell cycle arrest [5]. Some researchers have identified that it provides apoptosis of cancer cells as a result of the application of *G. lucidum* triterpenes [6]. *G. lucidum* triterpene extracts have been reported to reduce tumor growth in mice with colon cancer in other study [7].

Given the studies conducted, *G. lucidum* has the potential to improve treatment regimens and the quality of life of patients suffering from several diseases. For this reason, in this study, it is aimed to produce *G. lucidum* extract loaded microsphere drug carriers that provide controlled drug release and examine release kinetics in release mediums with different pH values.

Controlled Release Systems and Drug Carrier Microspheres

Controlled release systems can keep the plasma concentration of the active substance in the desired region for the desired time as well as target the active substance to the desired region. In controlled drug release systems, natural and synthetic polymers are generally used to both form the carrier matrix and control the rate of release from the system. Many of the polymer-based controlled drug releasing systems are used by placing them inside the body. Therefore, the polymers used should be compatible with the biological environment.

Polymer based microspheres are widely used as controlled or delayed drug release systems. Microspheres are monolithic microcarriers with different physicochemical properties and dimensions, carrying the active substance in the form of particles at the molecular level. In recent years, biodegradable polymer microspheres have been widely studied as drug carriers. The advantages of such systems are that they can be taken orally or by injection and made suitable for the desired release profiles [8]. The primary features that the microspheres should carry are; releasing the active substance in the targeted area in a controlled manner without changing the structure and activity, enabling the use of a low dose of active substance, reducing toxicity, being biocompatible/biodegradable and having non-toxic degradation products [9].

The drug type, features, usage (oral, parenteral etc.), dosage and active substance release duration have to be taken into consideration for a proper microsphere polymer matrix selection. Natural polymers are preferred for microsphere production because they can control their size distribution, provide high loading capacity for water-soluble drugs, be metabolized and have stable structures [9].

Preparation of microspheres by gelling and crosslinking in dispersing phase is a method generally used to obtain microsphere carriers by using natural polymers. In this method, the natural polymer is dissolved in the aqueous phase then the drug is dissolved in this phase. The mixture, which carries the drug and polymer, is dispersed in the oil phase containing suitable emulsifiers. The system is first cooled, then heated, or crosslinking agents are added to gel, denature or crosslink the polymer. The oil is removed from the medium, the microspheres are washed and the solvent is removed. Afterwards, microsphere is obtained by drying [10]. The release of active ingredient from microspheres during usage is realized by surface wear, total sphere dispersion, microsphere hydration, diffusion and desorption of the active ingredient, infiltration events [11].

The oral usage is the most preferred way for delivering therapeutic active substances to the body for local treatment of organ-specific diseases. However, some of the dosage forms administered by the oral way do not always provide the desired therapeutic response at the desired organ because of being sensitive to pH. The pH of the gastrointestinal tract is acidic in the stomach, and the pH increases in the small intestine and colon. This pH variety in different segments of the gastrointestinal tract can be used in the development of organ-specific drug delivery systems. For example, the colon is a very favorable area for increasing the systemic absorption of active substances, since the drug has a long residence time, the enzyme activity is less than the upper parts of the gastrointestinal system and the pH is close to neutral pH (about 5.5–7). Thus, it is important to design pH sensitive drug carrier systems and understand release kinetics to develop targeted drug carriers.

In this study, sodium alginate and gelatin were used to obtain a pH sensitive controlled release system that could carry *G. lucidum* extract to the colon. Sodium alginate is a structural polysaccharide found in brown seaweed species and is a naturally derived biopolymer from algae. Gelatin is a biocompatible and biodegradable natural polymer obtained by hydrolysis of collagen, which is an essential component of animal skin, bone and connective tissue, under controlled conditions. Gelatin is a heat-reversible gel-making protein and can be used in encapsulation both alone and in combination with other

compounds. Due to its amphoteric nature, it has the feature of being used with anionic polysaccharides such as gellan gum. These hydrocolloids can be mixed in a reaction higher than pH 6 because both carry a net negative charge and each pushes the other. However, when the net charge of the gelatin is set below the pH isoelectric point, it becomes positive and the negatively charged gels cause a strong interaction with the gum.
Sodium alginate was used in a study on loading *G. lucidum* into pH-controlled microspheres and microspheres were collected in a $CaCl_2$ solution by electrospray. When the release kinetics of the spheres were examined, it was found that the release amount was very low at pH 1.7 and increased considerably at pH 7 [12]. Differently, the interaction between positive and negative groups that will occur at pH 3 between two polymers (gelatin and sodium alginate) was used for microsphere production in this study. Thus, a controlled release system has been obtained, which is more compatible with the extract.

2. Experimental

2.1. Materials

Sodium alginate (Sigma, medium viscosity) and gelatin (Huaxuan, 80-120 bloom) natural polymers were commercially supplied and used to form the matrix structure of the microspheres by cross-linking with each other. In order to increase the stability of microspheres that will be formed in the water environment, calcium chloride ($CaCl_2$) solution, whose ionic strength is adjusted with sodium chloride (NaCl), has been used as crosslinker agent.

G. lucidum used in the study was picked from its natural growing region of Dalaman/Mugla/Turkey sweetgum trees.

2.2. Preparation of *G. lucidum* Extract

The mushrooms were first cleaned, washed, dried and shredded with the aid of a laboratory shredder for the preparation of *G. lucidum* extract. Shredded mushrooms were placed in an ethanol containing flask and the first step extraction was carried out at room temperature with the mouth closed for 48 hours. The mixture was then filtered and ethanol solution was separated. After the same process was repeated two more times, the total ethanol solution obtained was taken into the rotary evaporator and ethanol was removed for 72 hours. The extract was then taken into a beaker and subjected to freeze drying in the lyophilizer. After that, the extract was kept with open mouth for 72 hours in a fume hood. The obtained dark viscous *G. lucidum* extract was immediately capped and stored at +8°C until use.

2.3. *G. lucidum* Extract Loaded pH-sensitive Microsphere Production

Biodegradable, pH-sensitive and *G. lucidum* extract loaded microsphere carriers were produced via dispersion phase gelling and cross-linking (complex coacervation) processes. The steps applied for the production of are given below. The method used was created by examining the literature studies [13-20].

- 0.15 M $CaCl_2$ solution was prepared. The ionic strength of the solution was adjusted to 0.1 using NaCl and its pH was adjusted to 3.0 using 0.1M HCl and/or 0.1M NaOH.
- 3% sodium alginate solution and 1% gelatin solution were prepared with the help of sonicator and these two solutions were mixed.
- 0.5 g of *G. lucidum* extract was added directly to 8 mL of sodium alginate-gelatin solution mixture and a homogeneous mixture was obtained by stirring and using sonicator. The pH of this mixture was adjusted to 7.0 by adding 0.1M HCl and/or 0.1M NaOH.
- The mixture was dropped into the $CaCl_2$ solution being mixed with a magnetic stirrer by means of a syringe at a height of 10 cm, right angle and as slowly as possible to form microspheres.
- Stirring continued for 5 minutes more in the magnetic stirrer. Then the microspheres were filtered and washed three times with distilled water.
- The microspheres were taken into falcon tubes and kept for 2 days at -80°C for lyophilization.
- At the end of this period, the spheres taken from the freezer were fiberized in the freeze-drying unit.

The different steps of the production process of *G. lucidum* loaded microspheres are seen in Figure 1. Empty microspheres without *G. lucidum* loading were also prepared by following the above steps without extract addition.

Figure 1. Different steps of *G. lucidum* loaded microsphere production process and produced microspheres.

2.4. Investigation of Drug Release Kinetics of Microspheres and Kinetic Modelling

Drug release kinetics of the produced microspheres were examined for 5 different pH environments by using buffer solutions in the pH of 1.3, 3.0, 5.0, 6.0 and 7.0 values.

After preparation of the buffer solutions 0.05 g of lyophilized *G. lucidum* loaded microspheres were transferred in a beaker containing 100 ml buffer. Beaker was placed in an incubator shaker working at 200 cpm(rpm?) and 37°C. Samples were taken from the beaker in certain time intervals (0, 5, 10, 25, 35, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480 min) and absorbance of the solution was read spectrophotometrically. UV scanning was performed in the range of 200-800 nm. Absorbance values and spectra were recorded.

The same procedure was also carried for unloaded microspheres to determine net absorbance value of the extract by taking the difference of absorbance values of loaded and unloaded microspheres in each time interval.

Then 20 standard solutions of *G. lucidum* extract were prepared between 0.01-0.20 gmL^{-1} concentrations in order to determine amount of extract release from the microspheres. UV scanning of these standard solutions were held for 200-800 nm. The obtained absorbance values were plotted against the concentration of the standards and the calibration curve/equation were obtained. The concentration of *G. lucidum* released solutions and mass of released *G. lucidum* from loaded microspheres were calculated by using the obtained calibration equation.

The *G. lucidum* release of microspheres was presented in terms of percent cumulative release (CR%) defined as the percentage ratio of the instantaneous amount of *G. lucidum* released at a certain time of incubation to the initial amount of *G. lucidum* loadings.

After determination of CR%, the data was fitted to linearized drug release kinetic models of zero order, first order, Hixson-Crowell, Higuchi and Korsmeyer-Peppas. Regression coefficients were determined to obtain the best fit model for the data.

3. Results and Discussion

The sodium alginate aqueous solution was negatively (-) charged below pH 7 and had no charge above pH 7. Therefore, at pH 7 and above cross-linking reactions between sodium alginate and gelatin did not occur. However, by adding the mixture to the solution with low pH, gelatin gained positive (+) charge and it was cross-linked with sodium alginate molecules located around it. $CaCl_2$ was used to obtain more stable structure of the microspheres. Each of the Ca^{2+} cations of the $CaCl_2$ caused ionic interaction with negatively (-) charged alginate anions, causing cross-linking, Figure 2. The lyophilized/dried microspheres were deformed and swollen in the buffer solutions that simulate the digestive system as it was expected. Extract was released from the openings formed between the chains that diverged from each other due to the movements of the polymer chains.

In order to examine the release kinetics, UV spectrum of G. lucidum extract standard solutions was obtained firstly. The absorbance peak for G. lucidum was seen at the wavelength of approximately 256.0 nm. Therefore, the calibration curve was obtained by plotting the absorbance values at this wavelength against the concentration of the standards and is given in Figure 3.

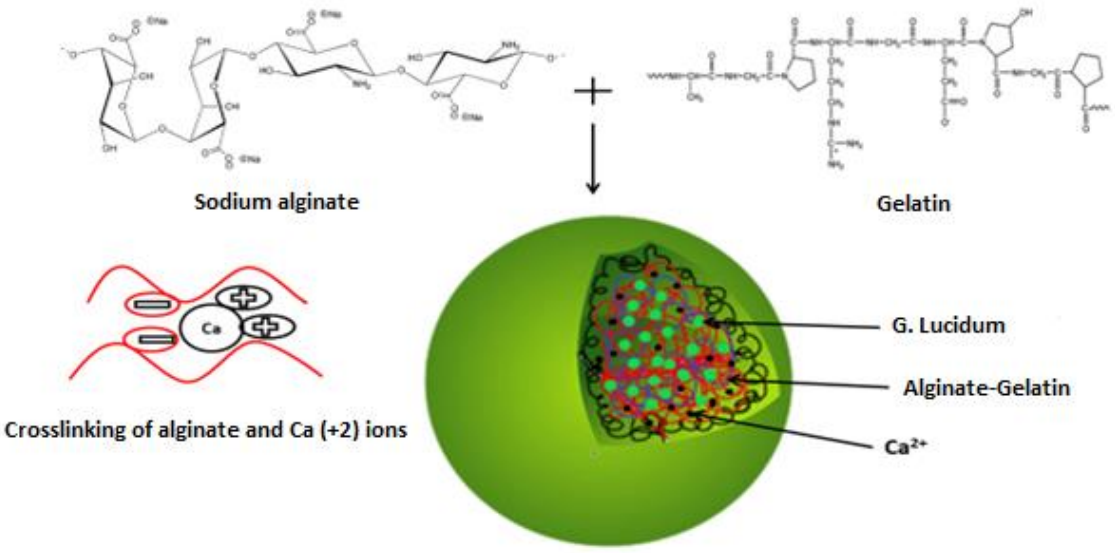


Figure 2. Microsphere structure.

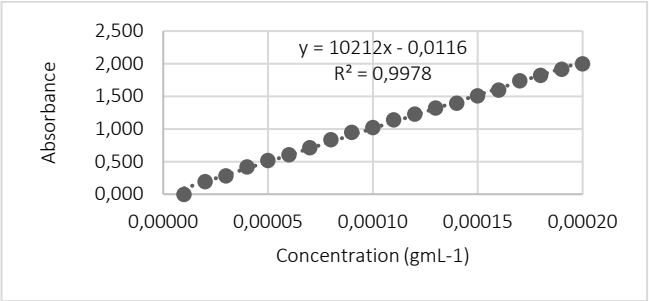


Figure 3. Calibration curve obtained for use in emission kinetics studies.

In order to find out the amount of *G. lucidum* release from microspheres, the possible absorbance values that might come from unloaded microspheres have been examined, but no peaks were found in the UV spectra of the unloaded microspheres. For this reason, it was accepted that the absorbance values of loaded spheres came only from *G. lucidum*.

Then, the absorbance values of *G. lucidum* were determined spectrophotometrically in buffer solutions with different pH values that simulated the digestive system and the released *G. lucidum* concentrations were calculated using the obtained calibration curve. Recorded absorbance and calculated concentration values are given in Table 1.

Table 1. *G. lucidum* absorbance and concentration values with respect to time at different pH values.

	Time (min)	Absorbance (256 nm)	Concentration (gmL^{-1})	Percent cumulative release (CR%)
pH 1.3	5	0.135	0.000014	8.51
	15	0.247	0.0000254	15.43
	25	0.318	0.0000322	19.56
	35	0.381	0.000038	23.09
	45	0.434	0.0000436	26.49
	60	0.448	0.000045	27.34
	90	0.565	0.0000565	34.33
	120	0.641	0.0000638	38.76
	150	0.713	0.0000709	43.07
	180	0.755	0.0000751	45.63
	240	0.837	0.0000831	50.49
	300	0.894	0.0000887	53.89
	360	0.929	0.0000921	55.95
	420	0.959	0.000095	57.72
	480	0.975	0.0000961	58.38
pH 3	**Time (min)**	**Absorbance (256 nm)**	**Concentration (gmL^{-1})**	**Percent cumulative release (CR%)**
	5	0.130	0.0000139	8.44
	15	0.213	0.000022	13.37
	25	0.309	0.0000313	19.02
	35	0.400	0.0000403	24.48
	45	0.449	0.0000451	27.40
	60	0.528	0.0000528	32.08
	90	0.664	0.0000661	40.16
	120	0.745	0.000074	44.96
	150	0.816	0.000081	49.21
	180	0.860	0.0000853	51.82
	240	0.919	0.0000911	55.35
	300	0.949	0.0000941	57.17
	360	0.945	0.0000945	57.41
	420	0.979	0.000097	58.93
	480	0.985	0.0000976	59.30
pH 5	**Time (min)**	**Absorbance (256 nm)**	**Concentration (gmL^{-1})**	**Percent cumulative release (CR%)**
	5	0.123	0.000013	7.90
	15	0.251	0.000026	15.80
	25	0.385	0.000039	23.69
	35	0.480	0.000048	29.16
	45	0.567	0.000057	34.63
	60	0.667	0.000064	38.88
	90	0.826	0.000082	49.82
	120	0.915	0.000091	55.29
	150	0.924	0.000092	55.89
	180	0.986	0.000098	59.54
	240	1.039	0.000102	61.97
	300	1.064	0.000105	63.79
	360	1.085	0.000107	65.01
	420	1.110	0.000109	66.22
	480	1.127	0.000111	67.44
pH 6	**Time (min)**	**Absorbance (256 nm)**	**Concentration (gmL^{-1})**	**Percent cumulative release (CR%)**
	5	0.152	0.0000132	8.02
	15	0.287	0.0000257	15.61
	25	0.416	0.0000383	23.27
	35	0.564	0.0000482	29.28
	45	0.736	0.0000566	34.39
	60	0.868	0.0000664	40.34
	90	1.000	0.000082	49.82
	120	1.090	0.0000907	55.10
	150	1.146	0.0000917	55.71
	180	1.177	0.0001157	70.29
	240	1.214	0.00012	72.90
	300	1.227	0.0001212	73.63
	360	1.253	0.0001239	75.27
	420	1.277	0.000126	76.55
	480	1.278	0.0001268	77.04
pH 7	**Time (min)**	**Absorbance (256 nm)**	**Concentration (gmL^{-1})**	**Percent cumulative release (CR%)**
	5	0.143	0.0000153	9.30
	15	0.320	0.0000324	19.68
	25	0.554	0.00005538	33.65
	35	0.694	0.000069	41.92
	45	0.918	0.000091	55.29
	60	0.963	0.0000954	57.96
	90	1.272	0.0001256	76.31
	120	1.322	0.0001305	79.28
	150	1.468	0.0001448	87.97
	180	1.533	0.0001512	91.86
	240	1.554	0.0001533	93.13
	300	1.590	0.0001568	95.26
	360	1.613	0.000159	96.60
	420	1.633	0.000161	97.81
	480	1.673	0.0001646	100.00

Extract CR (%) values at different pH are then plotted and given in Figure 4.

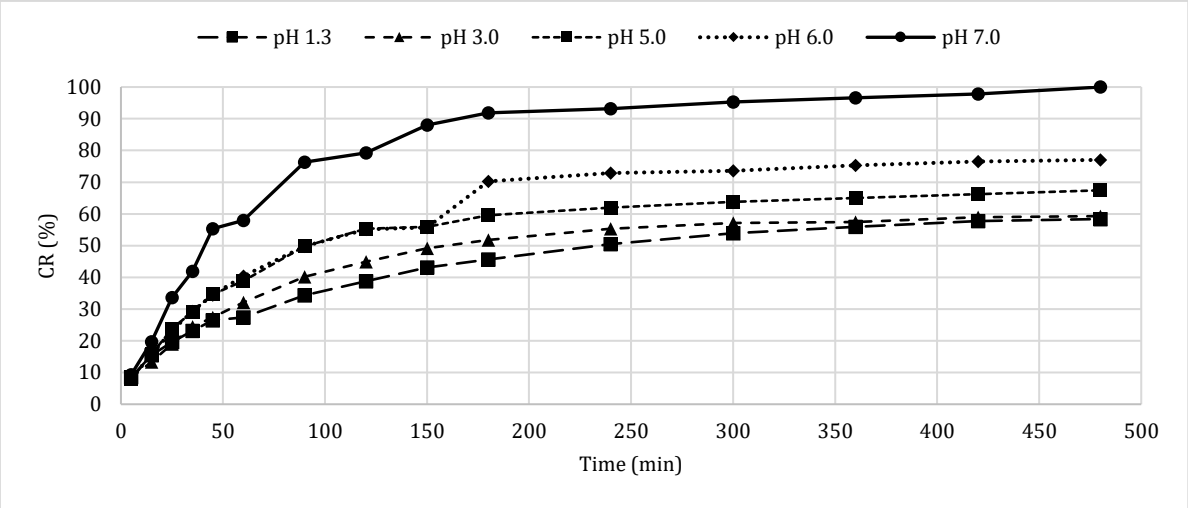


Figure 4. Extract concentrations released from microspheres at different pH values.

At pH 1.3, *G. lucidum* extract, which is embedded in the microsphere structure, started to be released into the solution with the conformational change of polymer chains due to swelling. However, since the ionic interactions do not undergo a significant change below pH 3.0, the release amount at pH 1.3 was low. The amount of extract released from the microspheres that were shaken for 8 hours at a constant temperature increased over time as expected. Towards the end of the period, this increasing rate decreased and no significant increase was observed in the amount of extract released. Thus, the amount of extract that microspheres can release has reached its maximum value after 8 hours.

As expected, the amount of released *G. lucidum* extract to the pH 3.0 environment, which is a relatively low pH value, did not increase much compared to pH 1.3. Since the ionic interactions in the structure of microspheres do not undergo a significant change at low pH values, the deformation of microspheres remained at minimum levels in pH 3.0. As time passed, the amount of extract released from the microspheres increased as the microspheres swelled but increasing behaviour was stopped towards the end of this period. This situation showed that the amount of extract that microspheres can release has reached its maximum value after 8 hours.

In parallel with the increase in the pH, the amount of extract released at pH 5.0 started to increase. The amount of extract release increased as the ionic bonds in the structure began to decrease in addition to the conformational structure changes on the microsphere surface as a result of the displacement of the polymer chains at this pH value. The reduction of ionic bonds caused more *G. lucidum* extract releasing.

When the amount of extract released in the pH 6.0 buffer solution is considered, it was seen that the extract tended to release rapidly. Compared to the release amounts in pH 3.0 and pH 5.0 buffer solutions, the aforementioned pH effect appeared to be valid at pH 6.0 as well. Especially since ionic interactions have decreased significantly and lost their effect near pH 6.0, the release has been rapid.

With the pH being 7.0, the release of *G. lucidum* extract from the microspheres occurred very rapidly. It is known that the produced microspheres are degraded after a certain period of time at pH 7.0. The reason for this is the disappearance of ionic bonds in the microsphere structure at pH 7.0 and rapidly swelling by getting solution from the surface of the microspheres. As a result, the chains forming crosslinks between the main polymer chains have moved and during this movement, the bonds between main chains have been broken and the microspheres have been degraded in a short time. The amount of extract that microspheres can release after 8 hours has reached its maximum value.

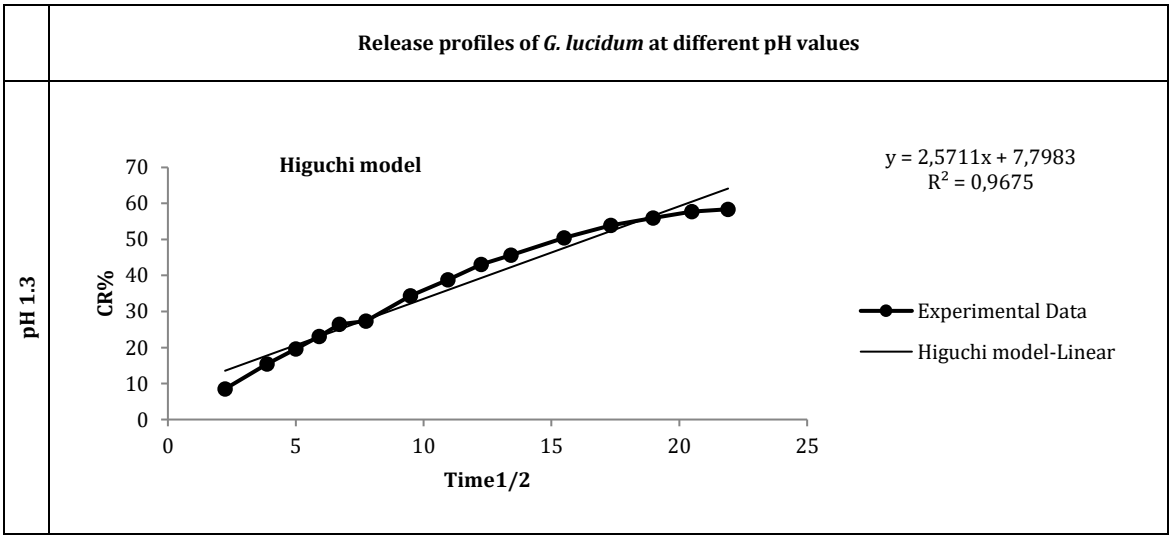
The kinetic model of *G. lucidum* release of produced microspheres were studied by fitting the experimental data to zero-order, first-order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models. The data is plotted as cumulative amount of drug released versus time for zero-order model, log cumulative percentage of drug remaining versus time for first-order model, cumulative percentage drug release versus square root of time for Higuchi model, cube root of drug percentage remaining in matrix versus time for Hixson-Crowell model and log cumulative drug released versus log time for Korsmeyer-Peppas model [21, 22]. The calculated regression coefficients, release rate constants and release exponents are given in Table 2 for different pH environments for the models where m_0 is the amount of the drug in the formulation before dissolution and m_t is the amount of the drug released over time t, M_t/M_∞ is the fraction of drug released at time.

According to the given regression coefficients in Table 2, release kinetics of microspheres best fitted to Korsmeyer-Peppas model for all pH values. The release exponent n is found by using the portion of the release curve only that gives $M_t/M_\alpha < 0.6$ for Korsmeyer-Peppas model [21, 22]. Thus, beside Korsmeyer-Peppas, the second-best fit models and kinetic parameters are given in Table 2. Higuchi model for pH 1.3, pH 3.0, pH 5.0 and pH 6.0 and first-order model for pH 7.0 are given as the second-best fit models. The second-best model fitted release profiles are given in Figure 5, too.

The first order relationship is generally used for porous matrices loaded with water soluble drugs. The first order rate law is predicted by using dissolution mechanism for alone and in combination. The Higuchi relationship is generally used for transdermal systems and matrix tablets loaded with water soluble drugs. The matrix releases the solid drug by simultaneous penetration of the surrounding liquid, dissolution of the drug, and leaching out of the drug through interstitial channels or pores for Higuchi model [23, 24].

Table 2. Kinetic evaluation of *G. lucidum* release data of microspheres.

Release environment pH value	**Regression coefficient (R^2)**	**Best fit models and linear fit equations**	**Release rate constant (k)**	**Release exponent (n)**
pH 1.3	Zero-order: 0.8542 First-order: 0.9146 **Higuchi: 0.9675** Hixson-Crowell: 0.8961 **Korsmeyer-Peppas Model: 0.9862**	**Higuchi Model:** $m_t=k_H t^{1/2}$ y = 2.5711x + 7.7983 **Korsmeyer-Peppas Model:** $M_t/M_\infty = kt^{1/2}$ y = 0.418x + 0.6972	Higuchi Model: 2,5711 mg.min$^{-1/2}$	0.418
pH 3.0	Zero-order: 0.7563 First-order: 0.8237 **Higuchi: 0.9092** Hixson-Crowell: 0.8022 **Korsmeyer-Peppas Model: 0.9595**	**Higuchi Model:** $m_t=k_H t^{1/2}$ y = 2.6841x + 9.2023 **Korsmeyer-Peppas Model:** $M_t/M_\infty = kt^{½}$ y = 0.4423x + 0.6741	Higuchi Model: 2.6841 mg.min$^{-1/2}$	0.4423
pH 5.0	Zero-order: 0.7070 First-order: 0.8021 **Higuchi: 0.8741** Hixson-Crowell: 0.7715 **Korsmeyer-Peppas Model: 0.9795**	**Higuchi Model:** $m_t=k_H t^{1/2}$ y = 2.9372x + 12.699 **Korsmeyer-Peppas Model:** $M_t/M_\infty = kt^{½}$ y = 0.5726x + 0.5457	Higuchi Model: 2.9372 mg.min$^{-1/2}$	0.5726
pH 6.0	Zero-order: 0.7739 First-order: 0.8712 **Higuchi: 0.9183** Hixson-Crowell:0.8426 **Korsmeyer-Peppas Model: 0.9847**	**Higuchi Model:** $m_t=k_H t^{1/2}$ y = 3.64x + 8.7987 **Korsmeyer-Peppas Model:** $M_t/M_\infty = kt^{½}$ y = 0.5949x + 0.5174	Higuchi Model: 3.64 mg.min$^{-1/2}$	0.5949
pH 7.0	Zero-order: 0.6711 **First-order: 0.9669** Higuchi: 0.8463 Hixson-Crowell: 0.9138 **Korsmeyer-Peppas Model: 0.9894**	First Order Model: $\ln(m_0-m_t)=\ln(m_0)-k_1t$ y = -0.0039x + 1.8374 **Korsmeyer-Peppas Model:** $M_t/M_\infty = kt^{½}$ y = 0.7775x + 0.4173	First Order Model: 0.0039 min^{-1}	0.7775



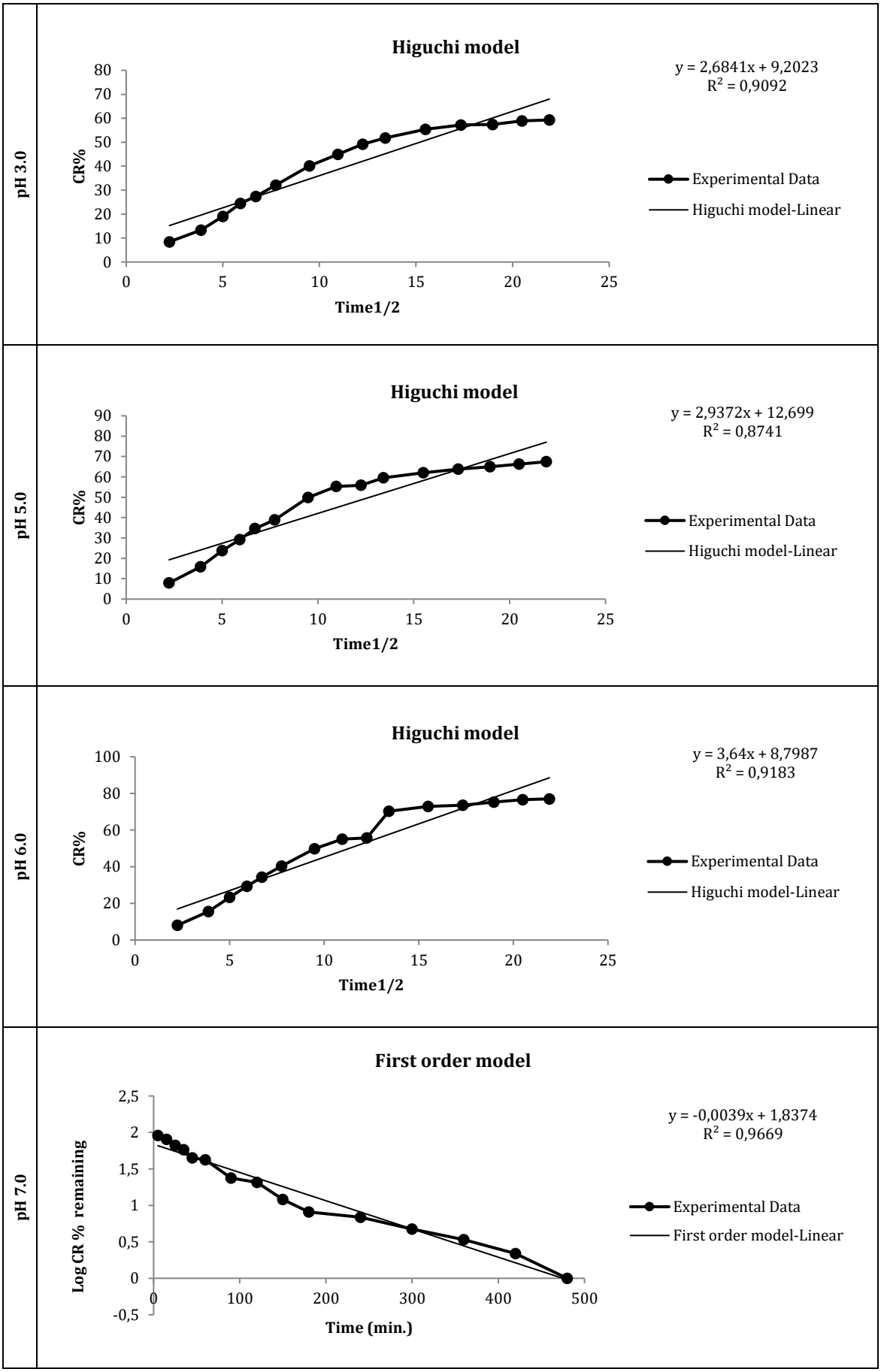


Figure 5. Release profiles of *G. lucidum* at different pH values fitted to most suitable kinetic models

The release exponents of the microspheres are found under 5 for pH1.3 and pH 3.0 release kinetics. This value showed that for low pH values drug release mechanism was fiction diffusion. The release exponents of microspheres are found between 0.5 and 1 values for higher pH values that shows the drug release mechanism is anomalous (non-Fickian) [25, 26].

4. Conclusion

In this study, gelatin, and sodium alginate-based microsphere drug carriers were produced and loaded with *G. lucidum* extract. Extract release kinetics of the microspheres were examined at different pH conditions. As a result, it was seen that the release of the extract accelerated as pH of the release medium increased and the fastest extract release was observed in the pH 7. The release kinetics of microspheres fitted Korsmeyer-Peppas model when only the data that gives $Mt/M\alpha < 0.6$ values are considered. The best fit models are found Higuchi model for pH 1.3, pH 5.0, pH 3.0 and first-order model for pH 7.0 when all data is considered.

5. Acknowledgment

The authors would like to acknowledge the financial assistance of Mugla Sitki Kocman University Scientific Research Project Office through the 19/082/01/1/2 June 2020.

6. References

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