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Production and Characterization of Colon Targeted pH Sensitive Macrospheres and Investıgation of Release Kinetics

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Abstract

The gastrointestinal track has different pH values at different sections. Thus, it is not easy to carry a drug to the colon for absorption. pH sensitive polymeric macrosphere drug carriers have important advantages such as being able to be taken orally, targeting the active ingredient to the desired area and dosing the active ingredient at the desired concentration for a long time in the target area. In this contex pH sensitive sodium alginate-gelatin macrospheres were produced by the dispersion phase gelling and cross-linking (complex coacervation) process method then loaded with *Sternbergia lutea* extract in this study. The macrosphere extract release kinetics were investigated for different pH medias that simulates different sections of the gastrointestinal track. As a result, the produced drug carrier macrospheres released the active ingredient at the colon pH (pH 7.0) while at lower pH values did not show a significant extract release. Therefore, it was reported that the produced macrospheres have potential to be used for colon diseases treatments.

Keywords: Colon targeted drug delivery, macrosphere, controlled release, release kinetics, pH sensitive

1. INTRODUCTION

The colon is a suitable site for the systematic absorption of a drug or active ingredient due to its long drug residence time, less enzyme activity than the upper gastrointestinal tract and pH value of approximately 7.0 [1, 2]. However, it is not always easy to carry the active ingredient up to the colon and absorb it in the colon, due to the different pH values of different sections of the gastrointestinal tract [3]. Polymeric sphere based controlled drug release systems are systems with important advantages such as being able to be taken orally, targeting the active ingredient to the desired area and dosing the active ingredient at the desired concentration for a long time in the target area [4-7]. Thus, orally ingested macrospheres can be used to transport the active ingredient to the colon and provide local treatment in colon diseases [8, 9]. However, macrosphere carriers can be degradate along the gastrointestinal tract leading the active ingredient at the desired dosage cannot reach the colon [10, 11]. Orally available pH sensitive macrospheres are one of the solutions that can be used to overcome this disadvantage [12, 13]. In this

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context, the production of pH sensitive sodium alginate macrospheres in calcium chloride ($CaCl_2$) solution was carried out by electrospray collection method in the study conducted by the Z. Yao et. al. [14] and it was reported that the active ingredient release from the macrospheres was very low at pH 1.7 but increased towards pH 7.0.

In the present study, pH sensitive macrospheres were produced using natural sodium alginate and gelatin polymers. With the use of two polymers, it is aimed to obtain a system with higher targeting performance, thanks to the relatively high interaction between negative and positive charged groups at pH 3.0. Active ingredient of a plant origin was loaded into the produced macrospheres and it was investigated whether the active ingredient could be delivered to the colon at the desired dosage and released long-term in the colon.

2. MATERIALS AND METHODS

2.1. Materials

Sodium alginate (Sigma, medium viscosity) and gelatin (Huaxuan, 80-120 bloom) polymers used for macrosphere production were commercially supplied. *Sternbergia lutea* (*S. lutea*), which is used to obtain herbal active ingredients, from the Aegean Region of Turkey was commercially supplied in dried form. All other reagents used were of analytical grade.

2.2. Methods

2.2.1. Plant extract preparation

Extraction of *S. lutea* flowers was started with shredding the plants into small pieces and placing them in a mouth closed ethanol containing flask. The extraction was carried out three times at room temperature for 48 hours for each extraction. After all extraction steps, the mixture was filtered, and ethanol solution was separated. The total ethanol solution obtained was treated by using a rotary evaporator for removing ethanol 72 hours at 45 ^{o}C. Then the extract was taken into a beaker and subjected to freeze drying in the lyophilizer. The obtained *S. lutea* extract was stored at -20 ^{o}C until use [15-17].

2.2.2. Macrosphere production

Macrospheres were produced by the dispersion phase gelling and cross-linking (complex coacervation) process method. The method used was created by modification of the literature studies [18-25] and used in our previous studies [16].

First of all, 4% sodium alginate and 1.5% gelatin solutions were prepared and the solutions were homogenized with the help of an ultrasonic homogenizator. Then, these two solutions were mixed and a homogeneous alginate-gelatin mixture was obtained. Pre-prepared 0.035 g of *S. lutea* extract was added to the alginate-gelatin mixture and homogenization was achieved with mechanical mixing and ultrasonic homogenization. The pH value of this mixture was adjusted to pH 7.0 with the use of 0.1 M HCl and 0.1 M NaOH. 5 mL mixture was injected using a disposable plastic syringe with 22-gauge needle. At this stage care was taken that the mixture to be air bubble free. The syringe was placed to a push-pull syringe pump at a speed of 0.1 mLh^{-1} and the mixture was dropped into a beaker that contains 0.20 M $CaCl_2$ (pH=3.0, ionic strength=0.1) as curing solution in it. During the dropping procedure the macrospheres formed instantaneously while $CaCl_2$ solution was continued to be stirred at a speed that is slow enough for not to shred the macrospheres and fast enough for not to allow them stick to each other. The formed macrospheres were filtered and washed with deionized water for 2 times, taken into falcon tubes and kept for 2 days at -80°C for lyophilization. Then the spheres were fiberized in the freeze-drying unit and stored in desiccator.

2.3. Characterization of macrospheres

2.3.1. Morphology studies

The produced macrospheres were first gold-coated in a cryopreparation chamber and morphology of the macrospheres were investigated via scanning electron macroscopy (SEM, Quanta 250) studies.

2.3.2. In vitro extract release kinetics investigation

The release kinetics of the macrospheres were investigated for the buffer solutions that simulate gastrointestinal track's different section pH values (pH of 1.3, 3.0, 5.0, 6.0 and 7.0) since they are produced to be taken orally and targeted to the colon during application.
0.05 g of lyophilized macrosphere were taken into 100 mL of buffer solutions for release kinetics investigation studies. The beakers were placed into an incubator shaker at 200 cpm and 37°C simulating human body temperature. 3 mL of the solutions were taken from the buffer solutions at various time intervals. An UV spectrometer (Shimadzu-UV-1601) was used to record the absorbance values of the solutions in the 200-800 nm wavelength. The cumulative extract release from the macrospheres was calculated by using the calibration equation that is determined by using UV scan results of 15 standard calibration solutions that are prepared in different concentrations. The standard solutions of *S. lutea* extract were prepared with the concentrations in the range of 0.01-0.15 $mgmL^{-1}$. The absorbance values of the standard solutions were recorded with respect to concentrations and the calibration curve is obtained. The calibration equation was obtained by using the calibration curve and the extract concentrations of the solutions with respect to time were calculated by using the equation and absorbance values.
The results were given in terms of percent cumulative release (CR%) that is defined as the percentage ratio of the instantaneous amount of extract released at a certain time of incubation to the initial amount of extract loadings. The initial amount of loaded extract was accepted as the maximum released amount of the extract.

2.3.3. Swelling behavior investigation

The solution sorption capacity tests of the macrospheres were carried out by using 100 mg of dry macrospheres. The macrospheres were swelled for 24 h to reach equilibrium swelling. The swelled macrospheres were taken from the solutions at different time intervals and blotted with filter paper to take surface liquid before weighted. The swelling tests were carried for for 4 times. The percent swelling (S%) of the macrospheres was then calculated by using Equation 1.

$$S\% = \frac{(W_e - W_0)}{W_0} \times 100\% \quad (1)$$

where W_e denotes the weight of the gel macrospheres at equilibrium swelling and W_0 is the dry weight of the macrospheres.

3. RESULTS AND DISCUSSION

The sodium alginate aqueous solution electrical charge differs with respect to pH of media. Mainly, below pH 7.0 it has a negative charge while above pH 7.0 it has no charge. Thus, the crosslinking between sodium alginate and gelatin could not be occur at the pH values above 7.0. However, when the mixture is added to the solution with low pH value gelatin was crosslinked with sodium alginate molecules by gaining positive charge. $CaCl_2$ was also used to obtain more stable structure of the macrospheres. Each of the Ca^{2+} cations of the $CaCl_2$ caused ionic interaction with negatively charged alginate anions, causing cross-linking. The produced macrospheres are shown in Figure 1.

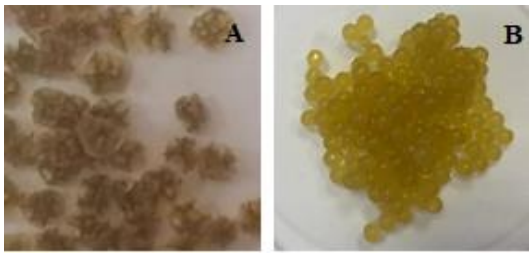

Figure 1 The produced macrospheres (A: Dry, B: Wet)

3.1. Morphology of the macrospheres

The surface morphology of macrospheres was determined by SEM and represented by the particle size and a characteristic shape. The SEM macrographs of loaded and unloaded macrospheres taken at different magnifications are shown in Figure 2.

The macrospheres did not possed proper spherical shape after drying while proper spherical shapes are observed before drying and during swelling. The surfaces of the macrospheres were slightly rough and extract particles were also present on the surface of the macrospheres. The diameter of the macrospheres were in the range of 1.2-1.4 mm.

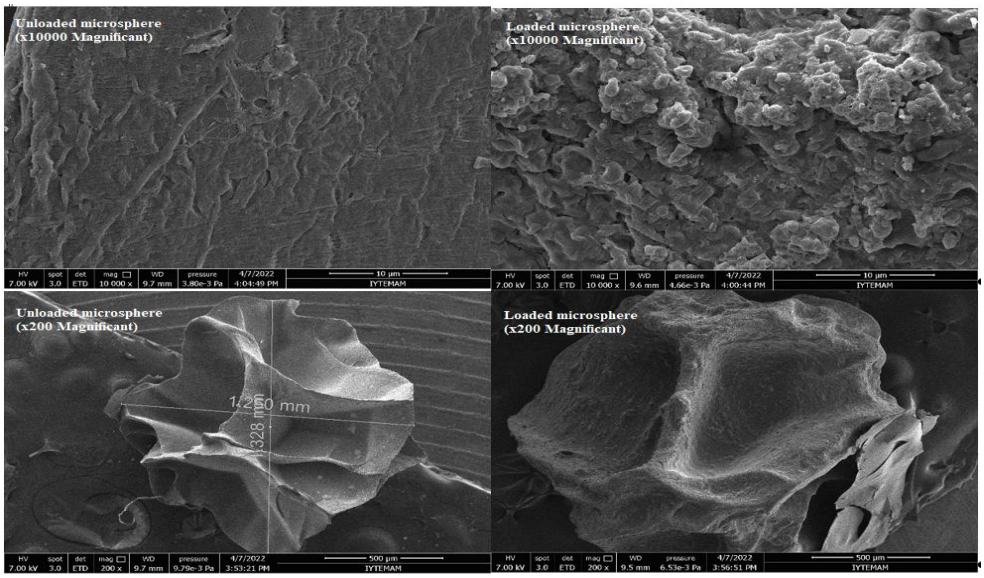

Figure 2 SEM images of produced macrospheres

3.2. In vitro exract release kinetics

In vitro extract release kinetics were investigated for the buffer solutions with differen pH values. The measurements were done for 480 minutes, CR% values were calculated by using pre-prepared calibration curve and results are given in Table 1.

The results are piloted as CR% with respect to time and given in Figure 3. As it is seen from Table 1 and Figure 3 the extract release was very low at the pH values under 7.0 while at pH 7.0 a rapid extract release was observed approximately in 300 minutes. Approximately 60% of the extract content was release in 180 minutes at pH 7.0.

After determination of CR% values with respect to time the kinetic release model of extract release of produced macrospheres were studied by fitting the experimental data to zero-order, first-order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models at pH 7.0 media. The data was 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 [26, 27] and given in Figure 4-8.

Table 1 CR% values of the macrospheres at different pH values

Time (min)	CR %				
	pH 1.3	pH 3.0	pH 5.0	pH 6.0	pH 7.0
5	0.00000000	0.00000049	0.00000052	0.00000139	0.75674259
15	0.00000004	0.00000594	0.00003972	0.00001269	2.22216165
25	0.00000011	0.00000877	0.00005496	0.00001919	4.46037276
35	0.00000033	0.00001129	0.00006607	0.00002817	7.68461721
45	0.00000076	0.00001385	0.00007324	0.00003242	11.30058034
60	0.00000105	0.00001777	0.00008219	0.00003658	14.99282525
90	0.00000200	0.00002322	0.00009266	0.00004164	19.85479550
120	0.00000298	0.00002662	0.00010391	0.00004800	27.78215802
150	0.00000348	0.00003123	0.00011405	0.00005939	39.52295194
180	0.00000440	0.00003425	0.00012663	0.00006879	61.60389020
240	0.00000459	0.00003505	0.00017379	0.00007223	80.15732572
300	0.00000644	0.00003736	0.00025639	0.00008247	98.91430800
360	0.00000755	0.00003874	0.00039552	0.00009375	100.00000000
420	0.00000850	0.00003994	0.00060703	0.00010408	100.00000000
480	0.00000924	0.00004079	0.00090126	0.00012205	100.00000000

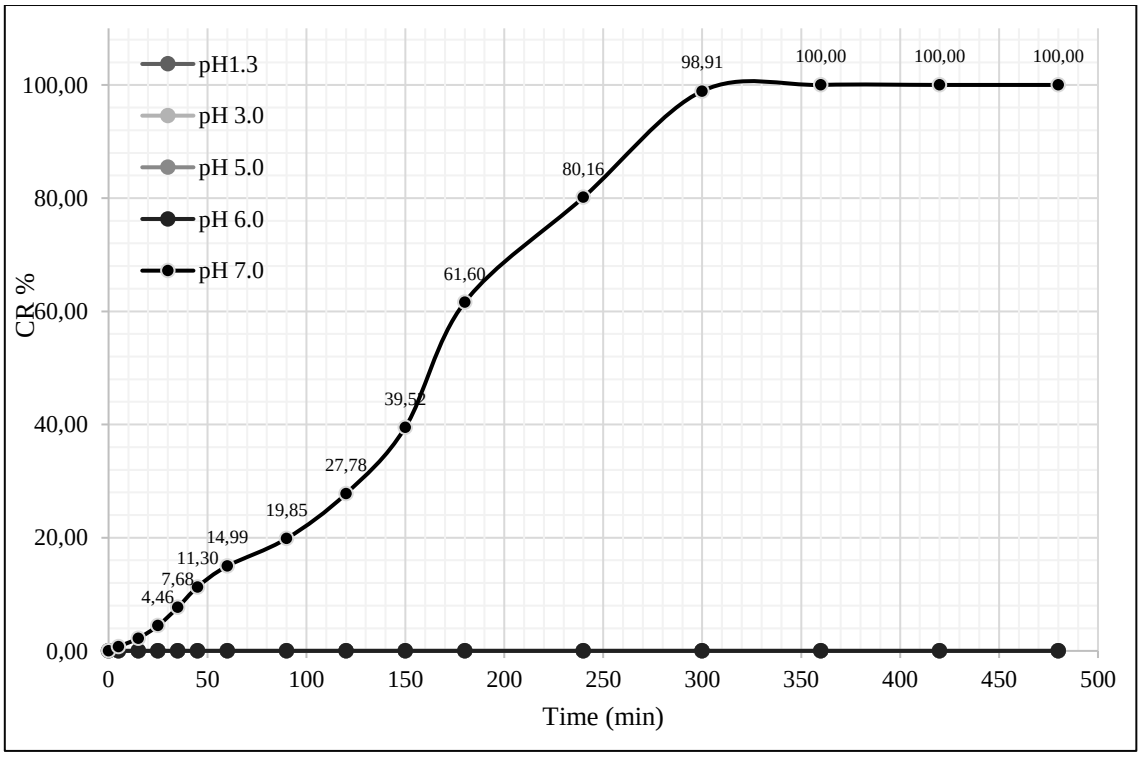

Figure 3 CR% values of the macrospheres with respect to time

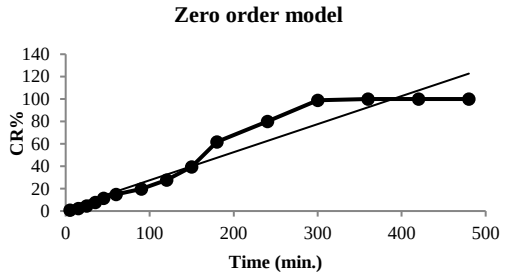

Figure 4 Kinetic model fitted to the release data of the macrospheres for zero order

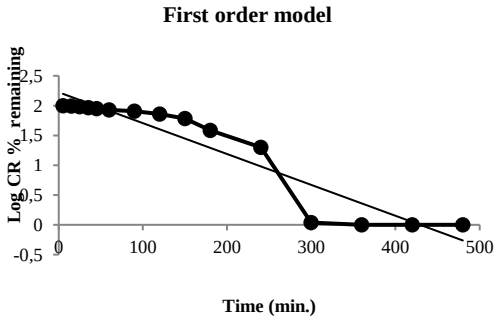

Figure 5 Kinetic model fitted to the release data of the macrospheres for first order

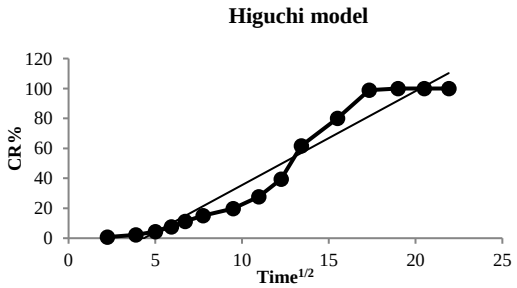

Figure 6 Kinetic model fitted to the release data of the macrospheres for Higuchi

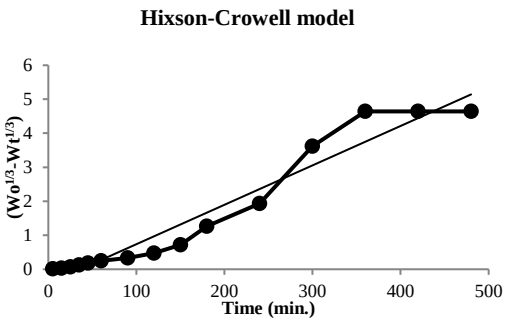

Figure 7 Kinetic model fitted to the release data of the macrospheres for Hixson-Crowell

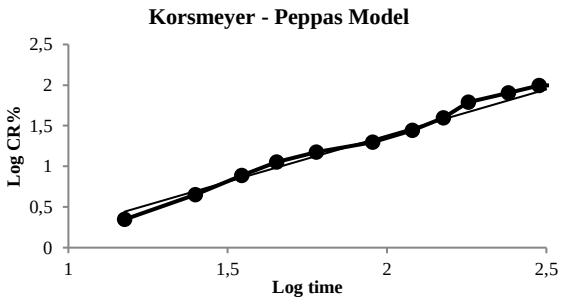

Figure 8 Kinetic model fitted to the release data of the macrospheres for Korsmeyer-Peppas

The model plots were fitted to the model equations and the regression coefficients (R^2), release rate constants (K) and release exponent (n) of Korsmeyer-Peppas model are given in Table 2 for pH 7.0 media. The Korsmeyer-Peppas model n value calculation was done for first 60% extract release.

Kinetic model studies showed that the best fit model for extract release at pH 7.0 was Korsmeyer-Peppas model with the regression coefficient of 0.9797 and Hixson-Crowell model was the second-best fit model with the regression coefficient of 0.9447.

The n value is indicative of the mechanism of transport of extract through the polymer and used to characterize the release mechanism of extract from the macrospheres. The n value was 1.2002 for the produced macrospheres which is higher than 1 and indicates drug transport mechanism as Super case II transport and release mechanism as relaxation/erosion. Case-II relaxational release is the drug transport mechanism associated with stresses and state-transition in hydrophilic glassy polymers which swell in water or biological fluids. This term also includes polymer disentanglement and erosion.

Table 2 Release kinetic parameters of the macrospheres

Model	**Model Equation**	**Regression coefficient (R^2)**	**Release rate constant (K)**
Zero Order	$Q_t=Q_0+K_0t$ Where; Q_t: Amount of drug dissolved at time Q_0: Initial amount of drug K_0: Zero order release rate constant	0.9268	0.2506 ($mgmin^{-1}$)
First Order	$\ln(m_0-m_t)=\ln(m_0)-K_1t$ Where; C_0: Initial concentration of drug t: Time K_1: First order release rate constant	0.8958	0.0052 (min^{-1})
Higuchi	$Q=K_Ht^{1/2}$ Where; Q: Cumulative amount of drug release at time t K_H: Higuchi release rate constant t: Time	0.9430	6.2767 ($mgmin^{-1/2}$)
Hixson-Crowell	$W_0^{1/3}-W_t^{1/3}=kt$ Where; W_0: Initial amount of drug W_t: Remaining amount of drug at time t K: Release rate constant	0.9447	0.0116 ($mg^{1/3}min^{-1}$)
Korsmeyer–Peppas	$M_t/M=K_mt^n$ Where; M_t: Mass of drug released at time M: Total mass of drug in dosage form K_m: Release rate constant n: Release exponent t: time	0.9797	0.8935 (min^{-n}) n=1.2002

As expected and aimed, the extract release of the macrospheres were insignificantly low at low pH values since the ionic interactions in the structure of macrospheres do not undergo a significant change at low pH values and the deformation of macrospheres remained at minimum levels at low pH values.

With the pH being 7.0, the ionic bonds in the structure disappeared in addition to the conformational structure changes on the macrosphere surface as a result of the displacement of the polymer chains at this pH value. The macrospheres rapidly swelled and 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 macrospheres have been degraded in a short time. The amount of extract that macrospheres can release has reached its maximum value after 300 minutes.

3.3. Swelling behavior

Swelling behaviour of the macrospheres were invesigated by using swelling media of pH 7.0 since the significant extract relase was observed at this pH. Figure 9 shows the swelling behaviour of the macrospheres with respect to time. The rapid degredation of the macrospheres in first 500 minutes was compatible with the release kinetics of the macrospheres. The equilibrium percent swelling was determined as aproximately 6500 %.

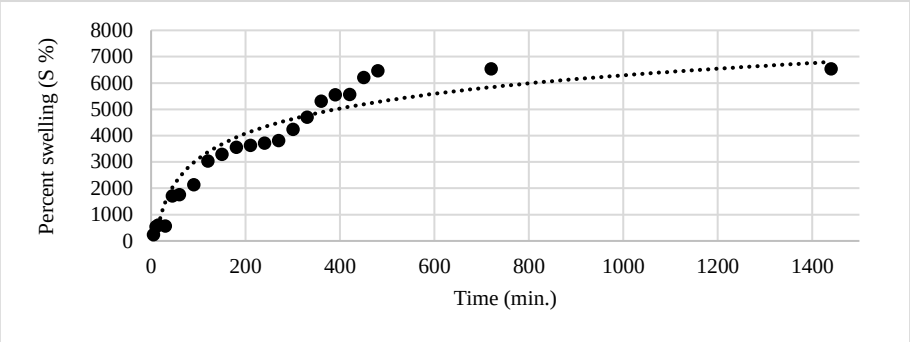

Figure 9 S% values of the macrospheres at pH 7.0

4. CONCLUSION

In this study, the pH sensitive sodium alginate-gelatin macrospheres were produced by the dispersion phase gelling and cross-linking (complex coacervation) process method and the prepared *S. lutea* extract was loaded to the macrospheres. The equilibrium percent swelling of the macrospheres was determined as aproximately 6500 %. Extract release kinetics of the loaded macrospheres were investigated in different solutions with different pH values that represents sections of the gastrointestinal track. When all the results obtained were evaluated, it was understood that the produced drug carrier macrospheres within the scope of the study released the active ingredient at the colon pH while at lower pH values that simulates different sections of the gastrointestinal track did not showed a significant extract release. The amount of extract that macrospheres can release has reached its maximum value after 300 minutes.

Kinetic model studies also showed that the best fit model for extract release at pH 7.0 was Korsmeyer-Peppas model Therefore, they have the potential to be used as colon targeted drug carrier.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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