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Effects of Conformation and Molecular Weight on The Surface Energy and Mucoadhesion Properties of Calcium Alginate Gels

Konformasyon ve Molekül Ağırlığının Kalsiyum Aljinat Jellerinin Yüzey Enerji ve Mukoz Yapışma Özellikleri Üzerindeki Etkisi

Research Article

Elif Dikicier and Murat Şen*
Hacettepe University, Department of Chemistry, Polymer Chemistry Division,Beytepe, Ankara, Turkey

ABSTRACT

In this study, the effects of the ratio of guluronic acid (G) to mannuronic acid (M) (G/M) and molecular weight of sodium alginate on the mucoadhesion and surface energy (γ) properties were investigated. Sodium alginate fractions were prepared by radiation induced degradation and calcium alginate gels were prepared in the form of three-dimensional structure by using an in-situ gelling method. In order to determine the free energy of bioadhesion, the contact angles of paraffin, ethylene glycol and water on the surface of gels were measured. Based on the contact angles, the γ components of the Liftshitz-van der Waals and the Lewis acid base interactions were calculated. It was found that CaAlg gels would lead to a thermodynamically favorable bioadhesion process, a decrease in the molecular weight of sodium alginate could increase the bioadhesion of CaAlg polymers, and that the bioadhesion of these gels was independent of G/M.

Key Words
Alginate, bioadhesion, gluronic acid / mannuronic acid ratio, gelation

ÖZET

Bu çalışmada, guluronik asitin (G) mannuronik asite (M) oranının (G/M) ve sodyum aljinatın molekül ağırlığının mukoz yapışma ve yüzey enerjisi (γ) özellikleri üzerindeki etkisi incelenmiştir. sodyum aljinat fraksiyonları radyasyonla bozunma yöntemi ile hazırlanmıştır. Kalsiyum aljinat jelleri in-situ jelleşme yöntemi kullanılarak üç boyutlu formda hazırlanmıştır. Biyoyapışma serbest enerjisini belirlemek amacıyla etilen, parafin ve suyun jel yüzeyindeki temas açıları ölçülmüştür. Temas açılarına bağlı olarak, Liftshitz-van der Waals ve Lewis asit-baz etkileşimlerinin yüzey enerji bileşenleri hesaplanmıştır. Sonuç olarak, CaAlg jellerinin termodinamik olarak istemli biyoyapışma işlemi yürüttükleri, sodyum aljinatın molekül ağırlığının azalmasıyla CaAlg polimerlerinin biyoyapışmasının arttığı ve de bu jellerin biyoyapışmasının G/M oranından bağımsız olduğu görülmüştür.

Anahtar Kelimeler
Aljinat, biyoyapışma, guluronik asit/mannuronik asit oranı, jelleşme

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Correspondence to: Murat Şen, Hacettepe University, Department of Chemistry, Polymer Chemistry Division, Beytepe, 06800, Ankara, Turkey
Tel: +90 312 297 79 89 Fax: +90 312 297 79 89 E-Mail: msen@hacettepe.edu.tr

INTRODUCTION

Alginate is one of the natural polysaccharides and found in substantial amounts in brown seaweeds. Alginates are unbranched binary co-polymers of (1-4)-linked residues of β-D-mannuronic acid (M) and α-L-guluronic acid (G) which have found wide applicability in food, textile and pharmaceutical industries.

Alginate can be ionically cross-linked by various divalent ions [1]. Currently Ca^{2+} is preferred to crosslink alginate for biomedical applications because of the mild reaction conditions compared to the cellular toxicity of both Ba^{2+} and Sr^{2+} [2,3]. Two methods of gelation have been extensively described and used to create alginate hydrogels: diffusion gelling and in-situ gelling. A common use of alginates is in the form of gel beads. Calcium alginate gel beads are used for cell encapsulation, drug delivery, and other biomedical applications [4]. Diffusion gelling is an ideal approach that is widely used for rapidly encapsulating cells in microspheres of alginate. In this method calcium ions diffuse through the liquid alginate boundary, cross-linking alginate strands as the ions move through the volume of alginate. However, cross-links are not uniformly distributed throughout the gel.

On the other hand, in recent years there has been a very big interest in the preparation of homogeneous alginate gels in different forms by using in-situ gelling methods. In this method $CaCO_3$ is mixed with alginate to create a homogeneous mixture. D-Glucono-d-lactone (GDL) is added to acidify the solution and release calcium ions, making them available for cross-linking. The resultant hydrogel has a uniform distribution of cross-links [5].

Bioadhesion is the special case of adhesion in which the substrate is skin or a mucous membrane. Bioadhesion is crucially important for pharmaceutical applications to design and develop biomaterials with optimal mucoadhesive properties. In the past few decades, several theories have been developed to describe the adhesion phenomena. Such theories include the electronic theory, [6] the adsorption theory, [7] the wetting theory, [8] and the diffusion theory [9].

Thermodynamic analysis has been also used for the identification of the driving force of mucoadhesion. An acid-base (AB) approach, presented by van Oss et al. [10], is one of the best techniques describing the interactions at interfaces. By introducing AB interaction into the surface energy (γ) analysis, thermodynamic properties of surfaces can be more precisely characterized using the γ parameters related to Lifshitz–van der Waals (LW) interaction, Lewis acid, and Lewis base. Rillosi and Buckton [11, 12] and Xiang et al. [13] found a good correlation between the values of thermodynamic properties or the total free energy of adhesion and the measured force of mucoadhesion.

The interfacial interactions can be characterized using the γ analysis. When AB interaction is included in the γ analysis, the γ consists of two components: AB interactions and LW interactions [12].

$$\gamma = \gamma^{LW} + \gamma^{AB} \tag{1}$$

In this equation, LW represents the nonpolar Liftshitz-van der Waals interaction, and AB represents Lewis acid-base interaction. γ^{AB} consists of two components: an electron acceptor "γ^{+}" (Lewis acid) and an electron donor "γ^{-}" (Lewis base).

$$\gamma^{AB} = 2\sqrt{\gamma^{+}.\gamma^{-}} \tag{2}$$

When a liquid (l) is contacted on the surface of a solid (s), the AB component and LW component across the interface can be calculated as follows:

$$\begin{aligned}\gamma_{sl}^{AB} &= 2(\sqrt{\gamma_s^{+}\gamma_s^{-}} + \sqrt{\gamma_l^{+}\gamma_l^{-}} - \sqrt{\gamma_s^{+}\gamma_l^{-}} - \sqrt{\gamma_s^{-}\gamma_l^{+}} \\ &= 2(\sqrt{\gamma_s^{+}} - \sqrt{\gamma_l^{+}})(\sqrt{\gamma_s^{-}} - \sqrt{\gamma_l^{-}})\end{aligned} \tag{3}$$

$$\gamma_{sl}^{LW} = (\sqrt{\gamma_s^{LW}} - \sqrt{\gamma_l^{LW}})^2 \tag{4}$$

The free energy of adhesion across the interface (ΔG^{a}_{sl}) can be determined by measuring the contact angle (θ) of the liquid on the surface of the solid:

$$\begin{aligned}\Delta G_{sl}^{a} &= -\gamma_l(1+\cos\theta) \\ &= -2(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^{+}\gamma_l^{-}} + \sqrt{\gamma_s^{-}\gamma_l^{+}})\end{aligned} \tag{5}$$

If liquids form nonzero contact angles on the surface of a solid, the following general contact angle equations can be obtained for three different liquids on the same solid surface [13].

$$\gamma_{l_1}(1+\cos\theta_1) = 2(\sqrt{\gamma_s^{LW}\gamma_{l_1}^{LW}} + \sqrt{\gamma_s^{+}\gamma_{l_1}^{-}} + \sqrt{\gamma_s^{-}\gamma_{l_1}^{+}}) \quad (6)$$

$$\gamma_{l_2}(1+\cos\theta_2) = 2(\sqrt{\gamma_s^{LW}\gamma_{l_2}^{LW}} + \sqrt{\gamma_s^{+}\gamma_{l_2}^{-}} + \sqrt{\gamma_s^{-}\gamma_{l_2}^{+}}) \quad (7)$$

$$\gamma_{l_3}(1+\cos\theta_3) = 2(\sqrt{\gamma_s^{LW}\gamma_{l_3}^{LW}} + \sqrt{\gamma_s^{+}\gamma_{l_3}^{-}} + \sqrt{\gamma_s^{-}\gamma_{l_3}^{+}}) \quad (8)$$

where γ_l is the surface free energy of the liquid, l_1, l_2, and l_3 refer to liquid 1, liquid 2, and liquid 3, respectively, θ_i (i=1, 2, 3) is the contact angle of the liquid drops on the solid surface, and γ_l^{LW} and γ_s^{LW} are the nonpolar Lifshitz–van der Waals components of the liquid and the solid, respectively. γ_s^+ and γ_l^+ are the electron acceptor surface free energy components of the solid and the liquid, respectively. γ_s^- and γ_l^- are the electron donor surface free energy components of the solid and the liquid, respectively. For a nonpolar liquid (paraffin) γ_s^{LW} can be obtained from the following equation [14]:

$$\gamma_s^{LW} = \gamma_{l_1}^{LW}\frac{(1+\cos\theta_1)^2}{4} \quad (9)$$

As a sum of equations (7), (8) and (9) the values of γ_s^+ and γ_s^- can be calculated;

$$\sqrt{\gamma_s^{+}} = \frac{A\sqrt{\gamma_{l_3}^{+}} - B\sqrt{\gamma_{l_2}^{+}}}{2\sqrt{\gamma_{l_2}^{-}\gamma_{l_3}^{+}} - 2\sqrt{\gamma_{l_2}^{+}\gamma_{l_3}^{-}}} \quad (10)$$

$$\sqrt{\gamma_s^{-}} = \frac{B\sqrt{\gamma_{l_2}^{-}} - A\sqrt{\gamma_{l_3}^{-}}}{2\sqrt{\gamma_{l_2}^{-}\gamma_{l_3}^{+}} - 2\sqrt{\gamma_{l_2}^{+}\gamma_{l_3}^{-}}} \quad (11)$$

The constants A and B can be expressed by the Equations 12 and 13;

$$A = \gamma_{l_2}(1+\cos\theta_2) - 2\sqrt{\gamma_s^{LW}\gamma_{l_2}^{LW}} \quad (12)$$

$$B = \gamma_{l_3}(1+\cos\theta_3) - 2\sqrt{\gamma_s^{LW}\gamma_{l_3}^{LW}} \quad (13)$$

In a ternary system formed by placing a material (1) (adhesive material) on the surface of another (2) (mucous membrane), which is put into a liquid (3) (saline or water), the free energy of the adhesion can be expressed by the following equations [11]:

$$\begin{aligned}\Delta G^{LW} &= \gamma_{12}^{LW} - \gamma_{13}^{LW} - \gamma_{23}^{LW}\\ &= (\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}})^2\\ &-(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}})^2\\ &-(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}})^2\end{aligned} \quad (14)$$

$$\begin{aligned}\Delta G^{AB} &= \gamma_{12}^{AB} - \gamma_{13}^{AB} - \gamma_{23}^{AB}\\ &= 2\begin{bmatrix}\sqrt{\gamma_3^{+}}(\sqrt{\gamma_1^{-}} + \sqrt{\gamma_2^{-}} - \sqrt{\gamma_3^{-}})\\ +\sqrt{\gamma_3^{-}}(\sqrt{\gamma_1^{+}} + \sqrt{\gamma_2^{+}} - \sqrt{\gamma_3^{+}})\\ -\sqrt{\gamma_1^{+}\gamma_2^{-}} - \sqrt{\gamma_1^{-}\gamma_2^{+}}\end{bmatrix}\end{aligned} \quad (15)$$

Finally, from the sum of ΔG^{LW} and ΔG^{AB} the total free energy of adhesion can be obtained:

$$\begin{aligned}\Delta G^{TOT} &= \Delta G^{LW} + \Delta G^{AB}\\ &= \gamma_{12} - \gamma_{13} - \gamma_{23}\\ &= \gamma_{12}^{LW} - \gamma_{13}^{LW} - \gamma_{23}^{LW} +\\ &2\begin{bmatrix}\sqrt{\gamma_3^{+}}(\sqrt{\gamma_1^{-}} + \sqrt{\gamma_2^{-}} - \sqrt{\gamma_3^{-}})\\ +\sqrt{\gamma_3^{-}}(\sqrt{\gamma_1^{+}} + \sqrt{\gamma_2^{+}} - \sqrt{\gamma_3^{+}})\end{bmatrix}\end{aligned} \quad (16)$$

A negative value of ΔG indicates the spontaneous formation of adhesion between the surfaces of Material (1) and (2) when they are immersed in Liquid (3).

MATERIALS AND METHODS

Chemicals

Sodium alginates (LF 120, LF 200, LF 240) were obtained from FCM Biopolymers Company, Norway, Calcium carbonate and D-glucono-δ-lactone (GDL) were obtained from Aldrich

Preparation of NaAlg Fractions

The initial molecular weights of NaAlgs, the ratio of G to M (G/M), and the abbreviations used for these polymers are given in Table 1. For the preparation of different molecular weight NaAlg fractions the polymer samples were placed in tightly closed containers, and irradiated at the required doses (2.5, 5, 10, 15 kGy) in a Gammacell 220 type ^{60}Co-gamma irradiator at room temperature in air. Molecular weights of the irradiated NaAlgs are given in Table 2. Preparation of NaAlg fractions by radiation-induced degradation, and the effect of G/M on the chain scission yield of this polymer were detailed in our previous study [15].

Preparation of Alginate Gels

A $CaCO_3$-GDL system was used to achieve a controlled gelation and to produce homogenous alginate gel plates. Calcium carbonate was used as a source of calcium ions to initiate gelation and GDL was used as a weak acid for the activation of calcium ions from $CaCO_3$. To achieve a neutral pH value, the molar ratio of $CaCO_3$ to GDL was maintained at 0.5. A molar ratio of calcium ion to GDL of 0.18 (basic) was designated as 1 X. In order to form uniform gels 1.75 X were prepared from 1.5% sodium alginate solution as follows: 0.105 g of sodium alginate was dissolved in 7 mL of deionized water and 0.0158 g $CaCO_3$ was put into the sodium alginate solution and the mixture was vortexed for 60 seconds. 0.056 g of GDL was dissolved in 0.02 mL of deionized water and added into alginate solution immediately and vortexed for 45 seconds. Within the next 24 hours calcium alginate gels were obtained [5]. These gels were prepared in tubes to form circular discs. The tubes were capped and stored at low temperature. Through the same method calcium alginate gels were prepared from irradiated sodium alginates.

Controlling the Molecular Weight of Sodium Alginates

In a Waters Breeze model Gel Permeation Chromatograph, a solution of $NaNO_3$ (0,1 M) was used as the eluting solvent. Water2000–1000–500 hydrogel columns were used for molecular weight analyses, and universal calibration was constructed by using narrow-molecular weight poly(ethylene oxide) standards. K and a constants for NaAlg were taken as $7.3.10^{-5}$ and 0.92, respectively [16].

Table 1. Initial molecular weights and G/M of NaAlgs used.

Polymer	$\overline{M}_n$	$\overline{M}_w$	G/M
LF120	340×10^3	670×10^3	70/30
LF200	310×10^3	690×10^3	50/50
LF240	275×10^3	500×10^3	45/55

Table 2. Weight and number average molecular weight of the irradiated NaAlgs.

Polymer	Dose (kGy)	$\overline{M}_n$	$\overline{M}_w$
LF120	2.5	308.1×10^3	506.6×10^3
LF200	2.5	292.7×10^3	538.9×10^3
LF240	2.5	257.6×10^3	425.4×10^3
LF120	5.0	285.7×10^3	460.3×10^3
LF200	5.0	267.6×10^3	489.4×10^3
LF240	5.0	242.3×10^3	406.5×10^3

Table 3. γ components of various materials at 20°C (mJ/m^2)

Solvent	γ	γ^{LW}	γ^{AB}	γ^+	γ^-
Paraffin	28.9	28.9	0	0	0
Ethylene glycol	48.0	29.0	19.0	1.92	47.0
Water	72.8	21.8	51.0	25.5	25.5
Mucin	46.2	6.92	39.28	49.17	7.84
Saline	71.1	33.72	37.97	27.36	13.18

Contact Angle Measurements

Contact angle measurements were conducted using a Krüss DSA100 contact angle instrument. The contact angles were measured applying three different solvents, paraffin, ethylene glycol and water, on the surfaces of the unirradiated and the irradiated calcium alginate gels, which were cut into slices of approximately the same thickness (Figure 1). All measured contact angles were the average of four measurements. The free energy of bioadhesion was calculated according to the Equations (1)-(13). The γ components were shown in Table 3.

RESULTS AND DISCUSSION

Contact Angle Measurements

The $CaCO_3$-GDL system produced uniform, trans-

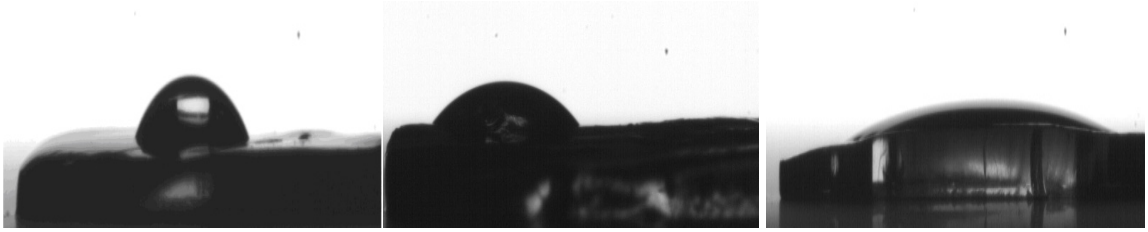

Figure 1. Photographs showing interactions of different solvents with CaAlg gels (a) paraffin (b) ethylene glycol (c) water.

Figure 2. CaAlg gels in disc form and their slices made from 1.5% alginate solution and 1.75 X $CaCO_3$.

parent and three-dimensionally defined calcium alginate (CaAlg) gels with unirradiated and 2.5, 5.0 kGy-irradiated NaAlgs (Figure 2). The CaAlg gels were prepared with 10 and 15 kGy-irradiated NaAlgs. However, they were unstable, and crumbled to fragments upon lightly tapping. This behavior was attributed to inhomogeneous cross-link formation within the gel structure and relatively low molecular weight of these fractions (Figure 3).

The contact angle (θ) is the angle at which a liquid/vapor interface meets the solid surface. In order to get an idea on the γ of the CaAlg gels prepared, contact angles of different liquids with gels were determined. The contact angle values of paraffin, water and ethylene glycol drops on the surface of CaAlg are given in Table 4. The hydrophilic property is characterized by the contact angle of the surface. If the liquid is very strongly attracted to the solid surface, the droplet will completely spread out on the surface and the contact angle will be low. If the liquid is less strongly attracted to the surface, the fluid will form a compact droplet and the contact angle will be larger. The contact angle of 90° or greater characterizes a surface as hydrophobic and one less than 90° as hydrophilic [17]. As shown in Table 4, the contact angle for water, a polar liquid, was measured to be 13.2, and 39.8 for paraffin, a nonpolar liquid. According to these values it was seen that the hydrophilic character of CaAlg is greater. Furthermore, the contact angle values increased upon irradiation at a dose of 2.5 kGy or upon a decrease in the molecular weight of the polymer, and did not change significantly as the irradiation dose increased (Table 4). Table 4 also indicated that the contact angle values of CaAlgs were independent of the G/M of the polymer.

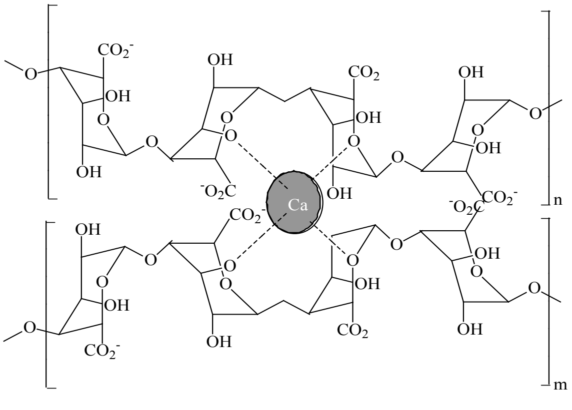

Figure 3. Structure of calcium alginate gels.

Table 4. Contact angle values of CaAlg gels (°).

	Unirradiated NaAlg			2.5 kGy-Irradiated NaAlg			5.0 kGy-Irradiated NaAlg		
Solvent	LF120	LF200	LF240	LF120	LF200	LF240	LF120	LF200	LF240
Paraffin	39.8	39.3	38.1	47.3	48.5	48.6	48.1	47.5	48.4
Ethylene glycol	19.6	20.5	19.0	28.5	29.3	29.1	28.8	28.9	28.2
Water	13.2	13.3	13.5	16.1	17.8	18.8	17.3	17.8	17.4

Table 5. γ components of CaAlg gels at 20 °C (mJ/m^2).

	Unirradiated NaAlg				
	γ	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}
LF120	45.48	22.60	22.88	1.82	71.90
LF200	45.08	22.73	22.35	1.73	72.20
LF240	45.54	23.07	22.47	1.77	71.34
	2.5 kGy-irradiated NaAlg				
LF120	42.79	20.30	22.44	1.67	75.37
LF200	42.38	19.97	22.41	1.68	74.73
LF240	42.52	19.94	22.58	1.73	73.69
	5.0 kGy-irradiated NaAlg				
LF120	42.56	20.10	22.46	1.69	74.65
LF200	42.24	20.28	21.96	1.65	74.42
LF240	42.93	20.00	22.93	1.77	74.30

Table 6. Variation of the total free energy of adhesion, and their ΔG^{AB}, ΔG^{LW} components with irradiation for CaAlg's (mJ/m^2).

Polymer	LF120			LF200			LF240		
Irradiation dose	ΔG^{AB}	ΔG^{LW}	ΔG^{TOT}	ΔG^{AB}	ΔG^{LW}	ΔG^{TOT}	ΔG^{AB}	ΔG^{LW}	ΔG^{TOT}
Unirradiated	-23.72	-6.69	-30.41	-23.85	-6.60	-30.45	-23.64	-6.38	-30.02
2.5 kGy	-24.54	-8.23	-32.77	-24.40	-8.50	-32.90	-24.10	-8.52	-32.67
5.0 kGy	-24.38	-8.41	-32.79	-24.36	-8.28	-32.64	-24.25	-8.48	-32.73

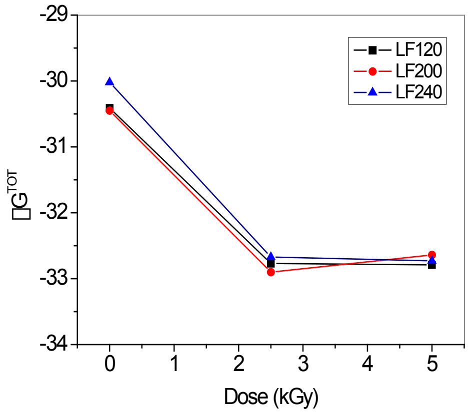

Figure 4. Effect of irradiation dose on the total free energy of interaction in polymer/mucin/saline system.

γ and Free Energy of Adhesion

Contact angles also provide information about the γ and free energy of adhesion. The influence of irradiation or molecular weight and G/M on the adhesion could be identified by contact angle measurements. The γ components for CaAlg were calculated by using equations (1)-(10). When the Lewis acid (γ^+) and the Lewis base (γ^-) components of (γ^{AB}) were compared, it was seen that CaAlg had a highly basic character (CaAlg, unirradiated, LF120; $\gamma^- = 71.90$ mJ/m^2 > $\gamma^+ = 1.82$ mJ/m^2). It was clearly seen from Table 5 that irradiation had a reducing effect on the value of γ^+. In contrast, it had a significantly increasing effect on the value of γ^-. These decrease and increase were attributed to the formation of more electron donor groups due to the scission of glycosidic bonds during irradiation. Table 5 revealed that although there was not a big difference between γ^{LW} values of 2.5 kGy- and 5.0 kGy-irradiated polymers, the irradiated polymers had significantly lower γ^{LW} values than those of the unirradiated ones. However, γ^{AB} values of the unirradiated polymers were very close to those of the irradiated ones.

The γ^{LW} component exhibited a nonpolar behavior whose contribution to the total γ was lower than that of the γ^{AB} component for all irradiated samples. The decrease in the γ^{LW} values reflected in a decrease in the total γ of the polymers with increasing irradiation dose, or a decrease of the molecular weight. Table 5 also indicated that G/M did not have a significant effect on the total γ of the unirradiated and irradiated NaAlgs.

The free energy of Lewis acid-base interactions, the free energy of Lifshitz-van der Waals interactions, and the free energy of bioadhesion were estimated calculating interfacial free energy of a polymer-mucin-saline system. Materials that form the ternary system were indicated as Material (1): CaAlg polymer, Material (2): Mucin, Material (3): Normal Saline. The calculated values are given in Table 6.

The negative value of ΔG indicated the spontaneous formation of adhesion. The ΔG^{AB} values of the irradiated polymers and the unirradiated polymers were not significantly different from each other. On the other hand, ΔG^{LW} values of the polymers decreased as the irradiation dose increased. According to the Equation (16), the total free energy of adhesion (ΔG^{TOT}) was a combination of LW and AB interactions. In the irradiated polymers, the significant difference in ΔG^{LW} values resulted in more negative ΔG^{TOT} values (Figure 4). Therefore, ΔG^{AB}, ΔG^{LW}, and ΔG^{TOT} values indicated that the LW interactions were critical for a favorable bioadhesion.

CONCLUSION

The γ analyses performed indicated that the molecular weight of NaAlg is a factor that could affect the bioadhesion properties of CaAlg gels whereas the G/M appeared to be an insignificant parameter on same. It could therefore be concluded that the AB interactions could drive the spontaneous formation of adhesion at all irradiation dose levels, and that the LW interactions could increase with irradiation dose, which would result in more negative total free energy ΔG^{TOT}. Therefore, the bioadhesion of CaAlg polymers would increase with decreasing molecular weight of sodium alginate, and these gels could lead to a thermodynamically favorable bioadhesion process.

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