SWELLING STUDIES OF COMPOSITE HYDROGELS BASED ON BACTERIAL CELLULOSE AND GELATIN

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This paper describes the swelling study in various pH media of a monolayer and multilayer composite hydrogels based on bacterial cellulose and gelatin (BC-G). Some considerations on the swelling mechanisms were given. Obtained results indicate that the swelling of BC-G hydrogels was influenced by the concentration of gelatin, pH, and by the existence of a coating layer. Swelling mechanism for all samples follow a pseudo-Fickian diffusion.

Keywords: bacterial cellulose, gelatin, swelling, network parameters

1. Introduction

In recent years, the research of bacterial cellulose as biomaterial in various biomedical applications has gain a lot of interest. Bacterial cellulose or biocellulose (BC), also known as microbial cellulose, is a polysaccharide synthesized by various species of bacteria, such as *Aerobacter*, *Acetobacter*, *Achromobacter*, *Agrobacterium*, *Alacaligenes*, *Azotobacter*, *Rhizobium* and *Sarcina*. It has the same chemical structure to plant cellulose, but with a significantly different fibrous nanostructure which confers superior physical and chemical properties. BC has excellent elasticity, biocompatibility, purity, good mechanical properties, high hydrophilic nature and high liquid loading capacity [1-3]. A method to further improve BC properties is to make composites. In this purpose, BC was mixed with different substances like poly (vinyl alcohol) [4,5], gelatin [6, 7], chitosan [8], starch [9], alginate [10,11], acrylic acid [12,13]. Composites based on BC were investigated for various pharmaceutical and biomedical applications such as tissue regeneration or replacement, wound dressings, artificial skin, film coating, and drug release [14-16].

Also, the use of gelatin in biomedical field is very attractive, since it is a non-toxic, biodegradable, low cost, non-immunogenic, and water soluble polymer. Gelatin has been used as wound dressing, sealant for vascular prosthesis, and in drug delivery systems [6,7,17,18].

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By combining bacterial cellulose with gelatin can result hydrogels, three-dimensional polymeric networks capable to absorb and retain large amounts of biological fluids. Generally, hydrogels are widely used in biomedical field due to their remarkable properties such as high water content, elasticity, biocompatibility, non-toxicity etc. Swelling behavior of hydrogels plays an essential role in biomedical applications and is mainly related to the network elasticity, the presence of hydrophilic groups (-OH, -COOH, -CONH$_2$, -SO$_3$H) in polymer chains, the extent of crosslinking, and porosity of the polymer [19]. Also, swelling kinetic is a very important property for practical applications of hydrogels, for example at hydrogels used as wound dressings a fast and large water uptake capacity to absorb the wound exudates is needed.

Although several papers related to composite hydrogels based on BC and gelatin were published [6,7,20,21], to our best knowledge detailed investigations of swelling and water uptake of these composites have not been yet reported in literature.

In the present work, bacterial cellulose-gelatin hydrogels were prepared by impregnation method with various gelatin concentrations. These hydrogels were characterized with respect to their network parameters. The effect of composition, pH, and coating on swelling behavior were investigated. The mechanism of swelling was postulated.

2. Experimental part

2.1. Materials
BC membranes (99% water content) were produced in the Mass Transfer Laboratory of the Chemical and Biochemical Engineering Department of POLITEHNICA University of Bucharest. All other chemicals used were analytical grade.

2.2. Preparation of BC membranes
BC membranes were prepared using *Acetobacter sp.* strain, isolated from traditionally fermented vinegar in laboratory, in a modified Hestrin–Schramm medium containing fructose (2 %) in static culture, as described elsewhere [22].

2.3. Preparation of BC-Gelatin composite hydrogels
A gelatin solution (concentrations 1%, 5%, and 10% respectively) was prepared by dissolving gelatin powder (food use) in distilled water at 50°C under continuous stirring for about 3 h. Wet BC membranes were superficially wiped with filter paper to remove free surface water. Then BC was immersed in gelatin prepared solutions and kept seven days at 50°C in laminar flow to insure gelatin penetration in cellulose pores. After polymer adsorption the composites

were dried at room temperature (samples were encoded BC-G1%, BC-G5%, and BC-G10%).

Multilayer composites (encoded mBC-G5% and mBC-G10%) were prepared from previously obtained composites by coating with a BC membrane layer on both sides. After coating the composites were dried at room temperature several days. The sample BC-G1% was not coated with BC because this sample was very thin and fragile.

2.4. Swelling studies

Hydrogels were kept in 20 mL distillated water, 20 mL buffer solution of pH 1.2 or 20 mL buffer solution of pH 7.4 at room temperature (25°C) for 72 h to reach swelling equilibrium. At predetermined moments, hydrogels were removed from solutions, wiped with filter paper for free water removal and then weighed.

The degree of swelling (SR, g/g) and the equilibrium degree of swelling (SRE, g/g) were calculated using the following expressions:

$$SR = \frac{W_t - W_d}{W_d}$$
(1)

$$SRE = \frac{W_\infty - W_d}{W_d}$$
(2)

where W_t is the weight of swollen hydrogel at time t, W_d is the weight of dried hydrogel and W_∞ is the weight of hydrogel at swelling equilibrium.

Swelling experiments were performed in triplicate and average results were reported.

2.5. Characterization of BC-Gelatin hydrogels composites

The hydrogels densities were determined with pycnometric method using a nonpolar solvent (n-hexane) for dry hydrogels and water for swollen hydrogels. The procedure was as follows: the sample was measured (m_s); the weighing bottle was measured empty (m_0); the weighing bottle was filled with the solvent and measured (m_1); the sample was placed in the weighing bottle and solvent was slowly added and the weight of the bottle with the sample and filled with solvent was measured (m_2).

The dry hydrogel density (ρ_P) and the swollen hydrogel density (ρ_S) were calculated using the following equation:

$$\rho_P(\rho_S) = \frac{m_s}{m_1 - m_2 + m_s} \rho_L$$
(3)

where ρ_L is the liquid solvent density, m_s is the weight of dried hydrogel (for dry hydrogel density), respectively the weight of swollen hydrogel (for swollen hydrogel density).

Hydrogels were characterized by determining polymer volume fraction in the swollen state and the Flory-Huggins polymer-water interaction parameter using observed swelling data.

The polymer volume fraction in the swollen state ($v_{2,s}$) shows the amount of liquid that can be imbibed in hydrogels and can be calculated using equation 4. The Flory-Huggins polymer-water interaction parameter (χ) can be expressed as equation 5 [23].

$$v_{2,s} = Q^{-1} = \frac{1/\rho_P}{Q_m/\rho_L + 1/\rho_P}$$

(4)

$$\chi = \frac{1}{2} + \frac{v_{2,s}}{3}$$

(5)

where Q is the volumetric swollen ratio and Q_m is the mass swollen ratio.

3. Results and Discussion

3.1. Swelling behavior

Swelling is an important step to understanding the structure of hydrogels and is affected by several factors, such as: network density, hydratability of materials, ionic strength, pH, temperature etc. The dynamic swelling behavior of BC-G hydrogels is shown in Fig. 1. It was observed that for all samples swelling increases with time, first rapidly and then slowly, reaching a constant value called swelling equilibrium after 24 h. As presented in Fig. 1a, the gelatin concentration in composites affects water uptake. The increase in gelatin concentration enhances the crosslinking density and diminishes the network voids leading to a decrease in water uptake. Therefore, the highest SR value (SR = 39.42 g/g) is observed for the composite with the lowest gelatin concentration. These results are according to other literature studies [21].

In Fig. 1b and 1c is presented liquid uptake for BC-G composites using pH 7.4 and pH 1.2. These values were selected because pH 1.2 simulates the condition of gastric fluid and pH 7.4 simulates the condition of intestinal fluid. The sample BC-G1% was disintegrated at contact with pH medium so swelling profile in pH 1.2 and in pH 7.4 could not be obtained. In acidic environment, BC-G hydrogels showed slightly higher swelling compared to the basic environment. These results were attributed to the polyelectrolyte character of gelatin. Gelatin contains amino groups in its structure, which are protonated in acidic media, causing electrostatic repulsions leading to an increase of swelling properties. In basic medium, amino groups are deprotonated, the electrostatic repulsions are reduced, leading to a decrease of swelling [24].

The effect of coating on swelling was also studied. As stated in literature, coating plays an important role on swelling due to diminish the contact area of

matrix to the dissolution medium. In all media multilayer**,** composites present lower SR values than monolayer composites. These types of results have been previously mentioned [25].
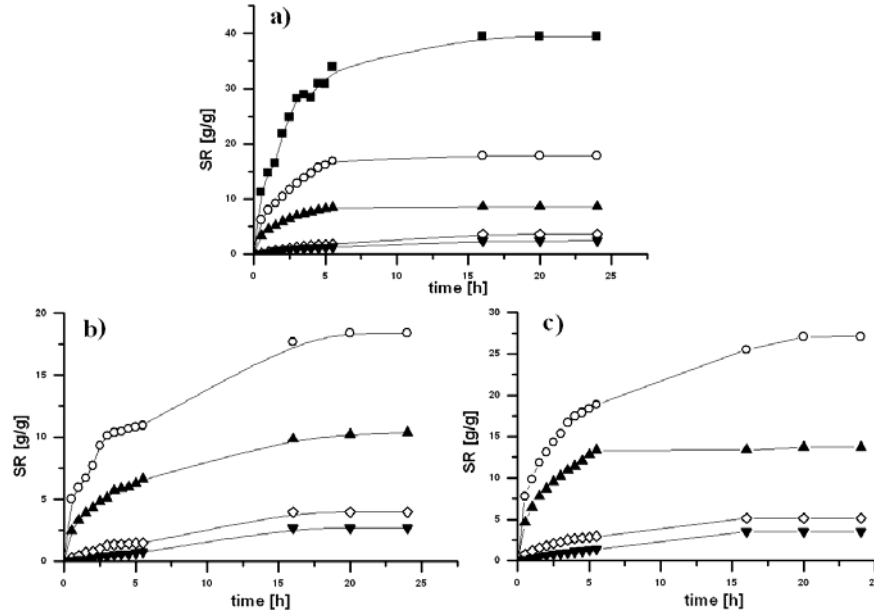

Fig 1. Swelling behavior of BC-G composite hydrogels in distilled water (a), in buffer of pH 7.4 (b), in buffer of pH 1.2 (c): ■ - BC-G 1%, ○ - BC-G 5%, ▲ - BC-G 10%, ◊ -mBC-G 5%, ▼ - mBC-G 10%.

3.3. Swelling kinetics

In order to study the effect of pH and composition of hydrogels on the kinetics of liquid uptake of hydrogels, swelling data were fitted using two models, model I (equation 6) and model II (equation 7) [26, 27]:

$$F = \frac{W_t}{W_\infty} = k \cdot t^n$$

(6)

where F is the amount of liquid fraction at time t, k is the characteristic constant of the hydrogel, and n is the characteristic exponent of the transport mechanism.

$$\frac{t}{SR} = A + Bt$$

(7)

where A is the reciprocal of the initial swelling rate of the hydrogel and B is the inverse of maximum (or equilibrium) swelling of hydrogel. ($B = 1/SR_{max}$)**.**
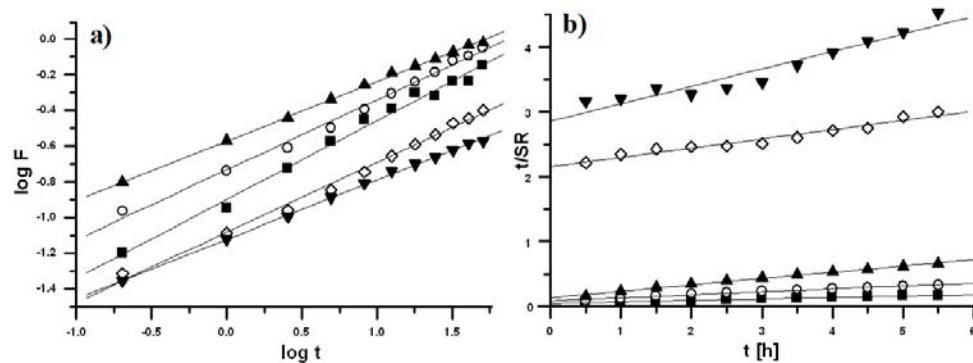
Fig 2. Plot of *log F* vs. *log t* (a) and *t/SR* vs. *t* (b) for BC-G composite hydrogels in distilled water: ■ - BC-G 1%, ○ - BC-G 5%, ▲ - BC-G 10%, ◊ - mBC-G 5%, ▼ - mBC-G 10%.

Fig 3. Plot of *log F* vs. *log t* (a) and *t/SR* vs. *t* (b) for BC-G composite hydrogels in buffer of pH 7.4: ○ - BC-G 5%, ▲ - BC-G 10%, ◊ - mBC-G 5%, ▼ - mBC-G 10%.
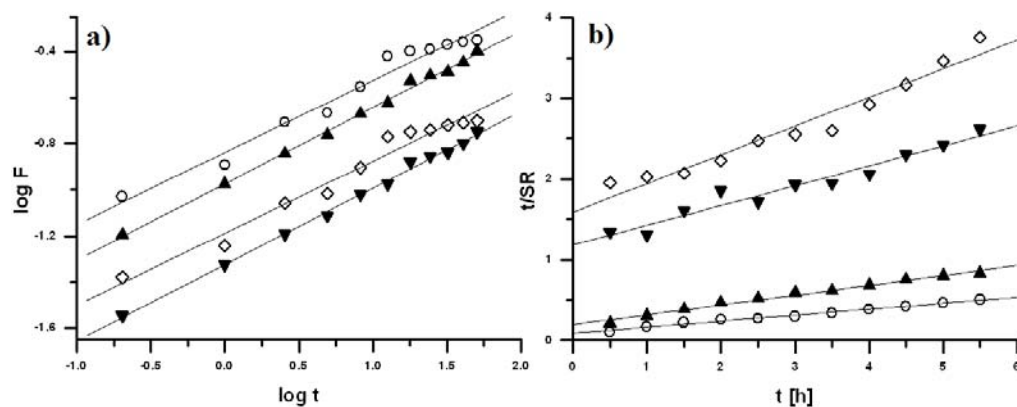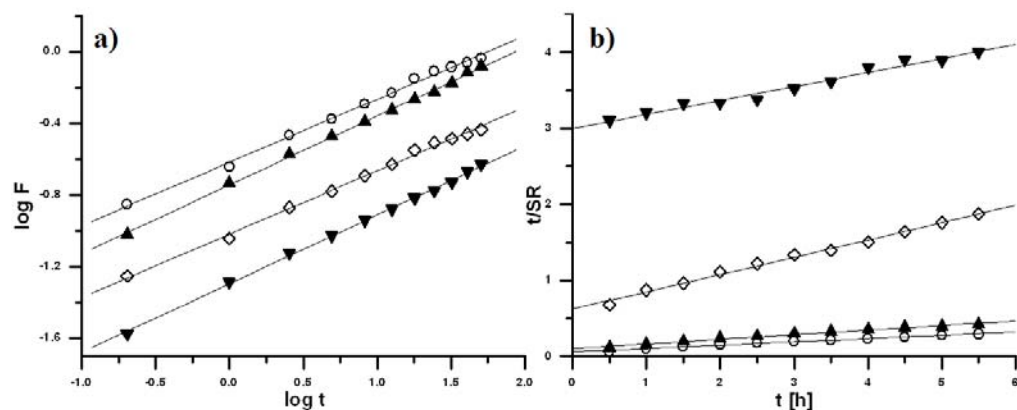

Fig 4. Plot of *log F* vs. *log t* (a) and *t/SR* vs. *t* (b) for BC-G composite hydrogels in buffer of pH 1.2: ○ - BC-G 5%, ▲ - BC-G 10%, ◊ - mBC-G 5%, ▼ - mBC-G 10%.

Model I is the power law model, known also as Peppas model, common used for describing the swelling mechanism. The value of n provides information about the kinetic mechanism of swelling. For $n < 0.5$, liquid uptake follows pseudo-Fickian diffusion and for $0.5 < n < 1$, liquid uptake conforms to an anomalous diffusion (non-Fickian diffusion). The parameters n and k were calculated by plotting $log F$ against $log t$ as the slope and the intercept of the straight lines fitted to the data (Fig. 2a, Fig. 3a, Fig. 4a). It can be observed from table 1 that multilayer samples have n and k values smaller than monolayer composites. Also, all samples present n values lower than 0.5, thus indicates that the solvent transport mechanism follows pseudo-Fickian diffusion.

Model II represents the second order kinetic used to explain the swelling of hydrogels. The linear regression of the swelling curves fitted to model II is presented in Fig. 2b, Fig. 3b, Fig. 4b. In table 1 are presented obtained values for A and SR_{max}. It can be observed that the experimental values of maximum equilibrium swelling ratio SRE and the values predicted by model II are in reasonable agreement.

For all samples the correlation coefficients R^2 are higher than 0.95 indicating a good agreement between experimental data and models. Also, model I presents better correlation values than model II.

Table 1

Swelling kinetic parameters of BC-G composite hydrogels using Models I and II

Sample code	Model I			Model II			
	k	n	R^2	A	SR_{max}	SRE	R^2
Sample in distilled water							
BC-G 1%	0.407	0.442	0.995	0.044	44.189	39.42	0.985
BC-G 5%	0.481	0.391	0.997	0.085	21.659	17.84	0.987
BC-G 10%	0.565	0.332	0.999	0.135	10.287	8.560	0.996
mBC-G 5%	0.339	0.390	0.997	2.154	5.017	3.230	0.979
mBC-G 10%	0.326	0.331	0.999	2.8544	3.723	2.920	0.959
Sample in buffer of pH 7.4							
BC-G 5%	0.432	0.312	0.986	0.087	13.408	18.39	0.993
BC-G 10%	0.377	0.332	0.998	0.191	8.165	10.38	0.993
mBC-G 5%	0.305	0.310	0.986	1.179	4.066	3.970	0.975
mBC-G 10%	0.266	0.331	0.998	1.589	2.811	2.675	0.975
Sample in buffer of pH 1.2							
BC-G 5%	0.538	0.353	0.998	0.058	23.063	27.074	0.996
BC-G 10%	0.475	0.383	0.999	0.101	16.628	13.690	0.992
mBC-G 5%	0.361	0.351	0.992	0.622	4.398	5.100	0.997
mBC-G 10%	0.274	0.380	0.999	2.995	4.252	3.468	0.988

4. Network parameters

The densities of dry and swollen hydrogels were measured in n-hexan and respectively in water, and the results are listed in Table 2.

The densities of dry hydrogels increased with the increase of gelatin content. However, the difference between the various hydrogels is not significant. The densities of the swollen hydrogels were slightly different from those of dry hydrogels. Because all the synthesized hydrogels are capable of absorbing a lot of water, all the densities of swollen hydrogels are very close to water density.

The two key network parameters in describing hydrogel structure, the polymer volume fraction in the swollen state and the Flory-Huggins polymer-water interaction parameter are presented in Table 2.

It can be observed that the polymer volume fraction in the swollen state values ($v_{2,s}$) and the Flory-Huggins polymer-water interaction parameters (χ) are increasing with the increase of the gelatin content. A higher value of χ means a weaker interaction between polymer and water. Therefore the weaker interaction is observed at hydrogels with high gelatin content.

Table 2

Densities and network parameters of BC-G composite hydrogels

Sample code	ρ_P	ρ_S	$v_{2,s}$	χ
Sample in distilled water				
BC-G 1%	0.917	0.899	0.027	0.508
BC-G 5%	1.042	1.011	0.051	0.517
BC-G 10%	1.267	1.030	0.085	0.528
Sample in buffer of pH 7.4				
BC-G 5%	1.042	1.011	0.057	0.049
BC-G 10%	1.267	1.030	0.071	0.070
Sample in buffer of pH 1.2				
BC-G 5%	1.042	1.011	0.046	0.034
BC-G 10%	1.267	1.030	0.0544	0.054

6. Conclusions

Composite hydrogels based on bacterial cellulose and gelatin were prepared and characterized by determining network parameters. Swelling studies in distilled water, buffer solutions of pH 7.4 and pH 3.0 of BC-G monolayer and multilayer composits were performed. Liquid uptake mechanism was investigated using the Peppas model. Obtained results revealed that swelling was influenced by crosslinking density, polyelectrolyte character of gelatin**,** and by the existence of a coating layer. All samples presented *n* values lower than 0.5 indicating that swelling mechanism was gouverned by pseudo-Fickian diffusion.

Also, it was observed that the polymer volume fraction in the swollen state and the Flory-Huggins polymer-water interaction parameter increased with the increase of the gelatin content. Therefore**,** the sample with higher gelatin content displayed the weaker interaction between polymer and water.

R E F E R E N C E S

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