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Preparation and determination of the physical and chemical properties of the nano-gel film of bacterial cellulose

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Currently, updated compounds of natural origin are becoming more and more popular as an alternative to synthetic materials. In this work, the physicochemical properties of the nanogel film of bacterial cellulose obtained by culturing the symbiosis of acetic acid bacteria *Gluconacetobacter xylinus C-3* and the Hestrin-Schramm culture medium were investigated. The sorption properties in relation to goose fat were determined. It was found that the process of desorption of goose fat from the obtained nanogel film of bacterial cellulose into the buffer solution is completed after 24 hours, which indicates the optimal desorption rate for applications with prolonged action, namely, anti-burn action. The pH value of human skin changes from slightly acidic to neutral, and this confirms the possibility of manufacturing transdermal therapeutic systems based on nanogel film of bacterial cellulose impregnated with oil solutions of drugs. Thus, the prospects of using nanogel films of bacterial cellulose for creating transdermal therapeutic systems are evaluated.

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Keywords: Bacterial cellulose, Nanogel film, Transdermal therapeutic systems, Goose fat, Gluconacetobacter xylinus C-3

1. Introduction

The skin is the largest and outermost organ that covers the entire body, forming 8% of the body weight [1]. It is responsible for the body's physical defense and sensitivity, serves as a barrier to microbes and UV radiation, and regulates biochemical, metabolic, and immune functions such as temperature, water loss (preventing dehydration), and vitamin D3 synthesis [2]. When the skin barrier is breached through wounds, a series of complex physical and chemical processes occur in an attempt to repair and regenerate the damaged tissue [3]. The first modern wound dressing was produced in the mid-1980s, and it was characterized by its ability to maintain a moist environment and absorb fluids, and played an important role in reducing infection and stimulating wound healing/treatment [4]. This is due to the fact that the optimal dressing is able to maintain high humidity at the wound site, as well as remove excess exudate, it is non-toxic, does not cause allergies, provides oxygen exchange, can protect against invasion of microorganisms, is convenient and economical [5]. Modern dressings are designed as carriers for the delivery of therapeutic agents to the wound site, and they take a variety of forms, including hydrogels, films, sponges, foam, and more recently, nanofiber mats [6]. Nanofiber dressings have attracted a lot of attention in the field of biomedicine, tissue engineering. They are able to control drug delivery due to their complex structure. Currently, there is a great demand for materials that are more environmentally friendly and can be processed at the nanoscale [7]. With this in mind, biomass-based polymers such as cellulose and its derivatives have become the subject of scientific attention due to their intrinsic properties [8].

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Cellulose, one of the most common natural polymers on Earth, with relatively simple extraction, excellent biocompatibility, non-toxicity, and biodegradability, has been considered as a de facto option for wound dressing formulations, either as an additive or as a primary substrate [9]. The acquired data were very promising, with excellent effects recorded for cell adhesion and growth [10].

Nanocellulose is one of the cellulose-based materials that has aroused great interest in the last few decades in the promising biomedical field. Its biocompatibility, non-toxicity, biodegradability, water absorption, optical transparency, and good mechanical properties attract researchers from all fields [11]. Among the many available types of nanocellulose, bacterial cellulose (BC) has already been successfully applied, having begun its commercialization in wound dressings in 1980 by Johnson & Johnson (New Brunswick, USA). The nanofibrillar structure of BC causes a large specific surface area and a microporous structure [12]. Its wet films have a high water retention capacity (up to 99%), a high sorption capacity for liquids, as well as vapor and gas permeability. Due to these unique properties, natural BC is considered an extremely promising material for the needs of the medical industry, because it can be used as a dressing material, a temporary skin substitute for the treatment of wounds and burns, a matrix for transdermal therapeutic systems (TTS) [15]. In addition, medical devices based on them are much cheaper. Many studies have been conducted to create bioactive dressings that can resist the growth of pathogens that are resistant to microbes, without compromising the healing process [16].

The aim of the work was to study the physical and chemical properties of the purified nanogel film of BC obtained during the cultivation of *the Gluconacetobacter xylinus C-3* strain and to evaluate the possibility of using nanogel film of BC as a matrix for TTS containing fat-soluble drugs.

2. Experimental

2.1. Synthesis of nanogel film of BC and its purification

To prepare *the Hestrin-Schramm* nutrient medium, 2% glucose, 0.5% peptone, 0.5% yeast extract, 0.27% sodium phosphoric acid, substituted without water, 0.15% citric acid were placed in a 2 L Erlenmeyer flask at a temperature of 90 ºC with an exposure for 10-15 minutes. After complete dissolution, the volume was brought to 1 L. The medium was prepared in boiled tap water with a total hardness of 4.5 mmol / dm^3. The culture media was sterilized in an autoclave for 30 minutes at a temperature of 120 ºC. The prepared nutrient medium was cooled to room temperature (25 ºC) and transferred to a fermentation tank, after which 50 ml of 5-7 day culture of the *Gluconacetobacter xylinus C-3* strain was added. Biosynthesis was carried out for 7 days at a temperature of 30 ºC under static conditions.

For cleaning, a film of BC *Gluconacetobacter xylinus C-3* is placed in a 0.1% NaOH solution and boiled for 30 minutes at a temperature of 80-100°C. At the end of the synthesis, the polymer films are removed from the liquid and washed with distilled water. After that, the film is treated for 15 minutes at a temperature of 80-100ºC in a solution of 0.5% acetic acid to remove the coloring matter of the polymer. Then the film is washed with distilled water until neutral pH 6-7 and dried at 40-60°C to constant weight. A block diagram of the biochemical production of BC is shown in Fig. 1.

Biochemical reactions of cellulose synthesis using *Gluconacetobacter xylinus C-3* is a multi-stage process involving a large number of individual enzymes and a complex of catalytic and regulatory proteins. The process involves the formation of uridine pyrophosphate glucose, which is a precursor to the formation of cellulose, followed by the polymerization of glucose into a b-1-4 glucan chain and the resulting chain, which forms a ribbon structure of cellulose chains formed by hundreds or even thousands of individual cellulose chains, their extrusion outside the cell and self-assembly into fibrils. The synthesis of cellulose in *G. Xylinus* is closely related to catabolic oxidation processes and consumes up to 10% of the energy obtained as a result of catabolic reactions. The production of BC does not interfere with other anabolic processes, including protein synthesis. *Gluconacetobacter xylinus* follows either the pentose phosphate cycle or *the Krebs cycle* in combination with gluconeogenesis [14].

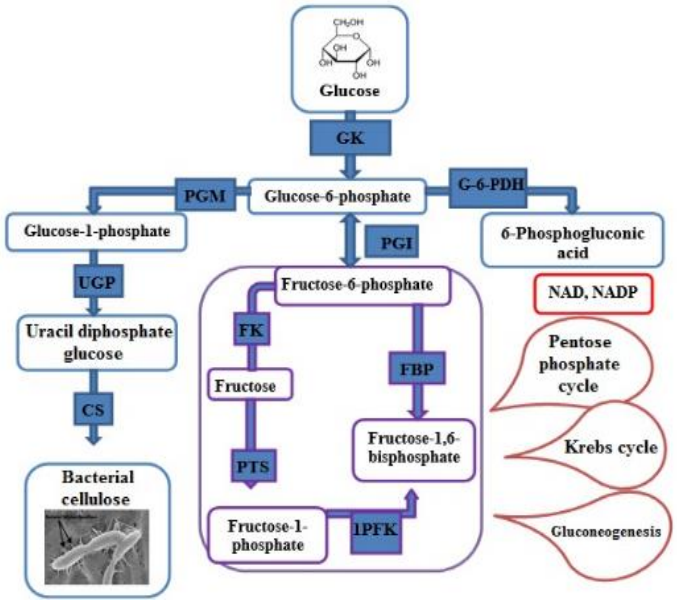

Fig. 1. Biochemical preparation of a nanogel film of bacterial cellulose. Marking abbreviations: CS-Cellulose-synthase, UPG-Uridine Pyrophosphate Glucose, PGM- Phosphoglucomutase, FK – Fructokinase, FTS - phospho-transferasa system, FPK - fructose pyruvate kinase, Fructose - 1,6-bisphosphate, GK – Glucokinase, IPG – Isomerism of phosphogluconate, G-6-PDG - Glucose-6-phosphate dehydrogenase, NAD - Nicotinamide adenine dinucleotide, NADP-Nicotinamide Adenine Dinucleotide Phosphate.

2.2. Characteristics of membranes

2.2.1. Determination of the physicochemical properties of nanogel film of BC

To determine the physicochemical properties of the obtained nanogel film of BC, standard methods were used. The results are shown in Table 1.

Table 1. Characteristics of bacterial cellulose nanogel-film.

No	Determination methods	Results
1	Swelling, %	99.7
2	Humidity, % (State Standard 16932-93)	4.0
3	Ash content, % (State Standard 595-79)	1.8
4	Wettability, g (State Standard 595-79)	195.8
5	Average degree of polymerization, units (State Standard 9105-74)	950.0
6	Determination of sheet density, g / m^2 (State Standard 11720-76)	180.4
7	Mass fraction of α-cellulose, % (State Standard 16932-71)	98.6
8	Tensile strength, average unit (State Standard 14236-81)	44.61

2.2.2. X-ray diffractogram of nanogel film of BC

X-ray diffraction patterns of nanogel film of BC were obtained on a DRON-4 diffractometer in digital form using copper radiation. The sampling modes are as follows: the voltage on the X-ray tube is 35 kV, the tube current is 20 mA, the step of the goniometer is 0.05° 2θ, and the intensity measurement time at a point is 1.5 sec. During the survey, the sample rotated in its own plane at a speed of 60 rpm. The phase analysis was performed using the PCPDFWIN and Search Match programs with the PDF-2 diffractometric data base. The measurement results were digitally processed in the ORIGIN program.

2.2.3. Electron microscopy

The structure of samples of nanogel films of BC was investigated on a scanning electron microscope JSM-6510 LA, in the Kazakhstan-Japan Innovation Center, in the laboratory of engineering profile "Electron Microscopy". For SEM analysis, dried samples were sprayed with silver using an Emitech K575X setup (10 mA, 2 x 40 seconds).

3. Results and discussion

A nanogel film of BC was obtained in the form of pellicles that floated on the surface of *the Hestrin-Schramm* medium during the cultivation of *Gluconacetobacter xylinus C-3*. After drying, a film was obtained in a dry state, namely, a film of BC (BCF). After the synthesis process, the physical and chemical characteristics of the film were investigated. In addition, during the study of *the Gluconacetobacter xylinus C-3 strain*, it was found that it releases amyloytic enzymes into the culture fluid as a result of vital activity. Therefore, it is possible to carry out cellulose biosynthesis not only on hexoses, but also to use disaccharides or oligosaccharides as a carbon source, which can be obtained by hydrolysis of low-grade plant cellulose [17].

3.1. Determination of the physicochemical properties of BCF

The physicochemical properties of BCF produced are presented in Table 1. It should be noted that the use of an acetic acid solution at the stage of BCF purification significantly increases its ash content. The high values of swelling and wettability of BCF, as well as satisfactory low adhesion to the wound surface, makes it possible to justify the choice of BCF as the basis for matrix-type TTS.

3.1.2. X-ray diffraction pattern of BCF nanogel

On the diffraction pattern of the BCF nanogel sample of interference peaks corresponding to crystalline cellulose, three lines are observed, indicating the presence of an additional substance of the crystal structure, which may be related to another intermediate product of the BCF nanogel preparation. The dimensions of the crystallites were estimated by substituting the width at half the height in the Scherer equation [18]. The obtained values are shown in Table 2. The d_1 values for algal BCF nanogels are rich in the triclinic phase Iα. Figure 2, from which d_1 is calculated, shows that d_1 consists of the reflection of the (100) triclinic and (110) monoclinic phases, and d_{100} of the triclinic phase is more than d_{110} of the monoclinic phase. The d_3 values decrease with increasing crystallite sizes, as indicated earlier in [19]. Perhaps the reason for this ratio of interplane distances is the difference between the packing of cellulose chains in the triclinic and monoclinic phases.

Table 2. Interplanar distances and sizes of BCF crystallites.

Interplanar distances d (Å)			Crystallite sizes d (Å)		
d_1	d_2	d_3	c_1	c_2	c_3
6.15	5.31	3.95	52	79	58

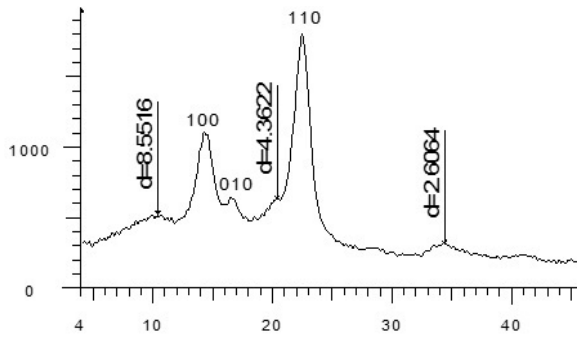

Fig. 2. X-ray structural spectrum of the BCF nanogel.

3.1.3. Adsorption and desorption properties of the BCF nanogel

Liquid phase ratio (animal goose fat) and the solid BCF was equal to 1:25. The obtained samples of BCF in a disc-shaped form with a diameter of 5 mm and a thickness of 7 microns were conditioned for 3 hours at a relative humidity of 70% and a temperature of 25ºC, then weighed to an accuracy of 0.0001 g and placed in an adsorbate for 5 hours at a temperature of 30ºC.

After the end of the exposure, the BCF nanogel was removed and placed on a porous plate for 30 minutes. Then it was dried in a drying cabinet at a temperature of 60 ºC to a constant mass. The amount of adsorbate was calculated as the mass difference between the impregnated BCF and the carrier before adsorption. The mass of the sample before adsorption is 0.1 g, after 0.0578 g.

The arithmetic mean of parallel measurements is 10 times. To determine the rate of adsorption, a study of the kinetics was carried out (Fig.3). The process is almost completed within 3 hours, and the nature of the change in the adsorption value indicates a multilayer adsorption on the surface of the BC.

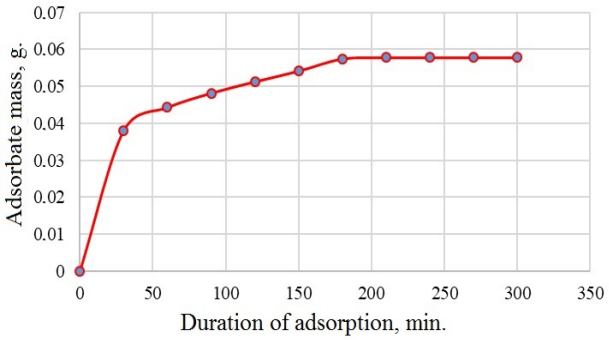

Fig. 3. Kinetics of goose oil adsorption by the BCF nanogel.

To determine the strength of the binding of adsorbate molecules to the surface of the BCF nanogel, the desorption of these substances under the influence of buffer solutions with a pH interval of 2-8 was investigated. Samples were placed in buffer solutions with pH 2; 3.5; 6; 7.2; 8 and with a ratio of solid and liquid phases equal to 1:50 at a temperature of 40 °C. Solutions with pH 2 and pH 3.5 were prepared using glycine buffer, solutions with pH 7.2 and pH 8 - phosphate buffer, distilled water was used as a solution with pH 6. It should be noted that the interaction of the components of the buffer solution with the adsorbate does not occur due to the large hydromodulus and low temperature for chemical reactions. The obtained samples were placed in a container with a buffer solution. After the end of exposure, they were removed and placed on a porous plate for 30 min. After that, they were dried in a drying oven at 60°C to constant weight.

The amount of desorption substance was calculated as the difference between the masses of the impregnated BCF and the sample after desorption and the arithmetic mean of the parallel measurements performed 10 times.

The results of the study of the desorption properties of BCF nanogel (Fig.4) showed that the adsorbate is desorbed faster in a solution with a pH of 6. The process of desorption of goose fat from the film into the buffer solution ends after 24 hours. This indicates the optimal desorption rate for applications of prolonged action, namely, anti-burn action. The pH value of human skin varies from slightly acidic to neutral, and this confirms the possibility of TTS manufacturing based on BCF nanogel impregnated with oil solutions of medicines.

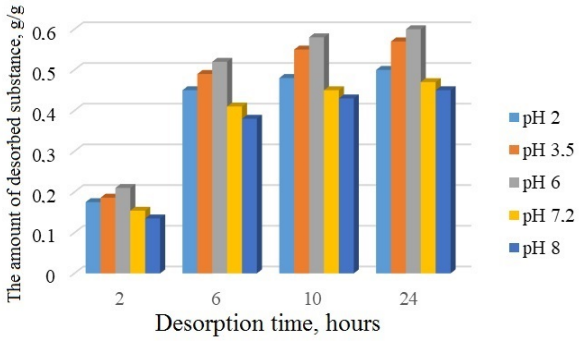

Fig. 4. Kinetics of desorption of goose fat from BCF.

3.1.4. Electron microscopy

The study of the microstructure of BCF nanogel by scanning electron microscopy is shown in Figure 5, where differences in the morphology of the BCF nanogel surface as a result of the addition of goose fat are visible. It is noted that the films have a nanostructure, with a visually smooth surface. With sufficient moisture, such a film will be removed from the wound easily and painlessly, without injuring the fresh epithelium.

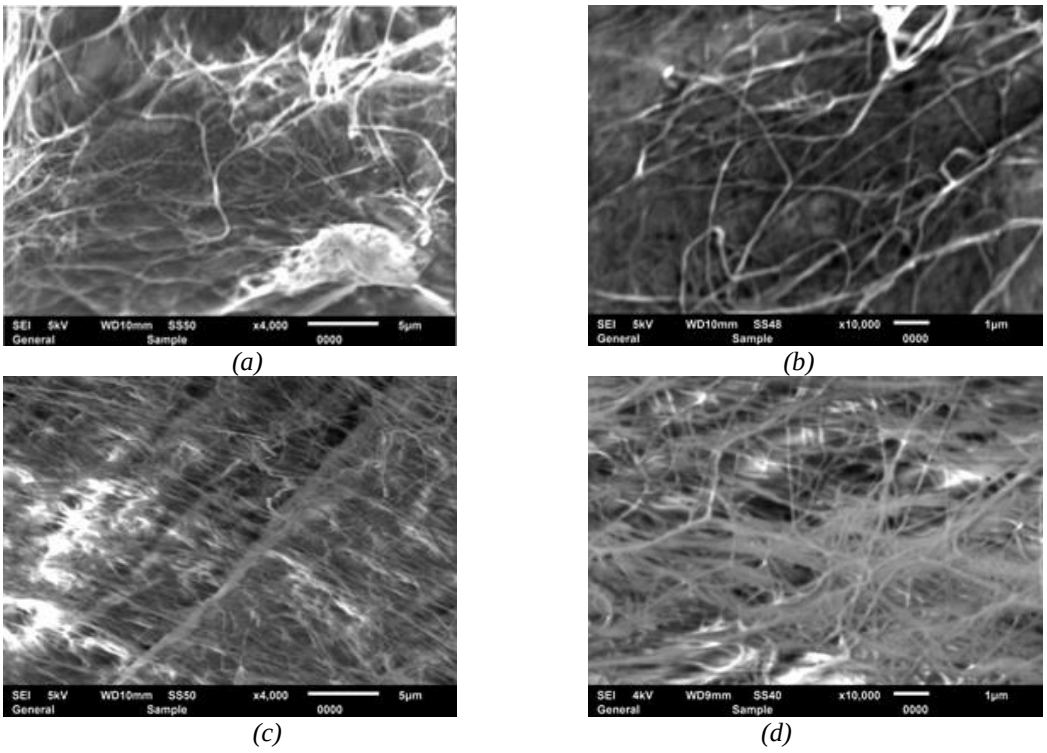

Fig. 5. SEM images of BCF nanogel: before oil impregnation (a, b), after oil impregnation (c, d).

The structure of the BCF nanogel is formed by microfibrils and has the form of a grid of irregular structure. The parallel arrangement of BC molecules in crystalline microfibers, guaranteed by the conditions of extracellular polymer biosynthesis, ensures the minimum thickness of microfibrils in a single fiber. The SEM method was used to determine the thickness of a single fiber - it is in the range of 50-100 nm. In addition, it is obvious that there is a uniform density of the distribution of the fibers of the frame, which ensures high strength of the film. After applying the fat, the surface of the BCF nanogel became smooth. It can be seen that the pores are covered with fat cells, the fiber thickness has become much thinner than the original BCF nanogel.

Thanks to this structure, when in contact with the surface of the wound, it is possible to gradually release them.

4. Conclusions

The physicochemical properties of β-1,4-glucone obtained by culturing *Gluconacetobacter xylinus C-3* were studied. A method for cleaning the resulting film from non-cellulose impurities has been developed. The microstructure of BC films was studied by scanning electron microscopy, the crystal structure was determined by X-ray phase analysis, and the sorption properties were investigated. The possibility of using BCF nanogels in the preparation of transdermal therapeutic systems (TTS) as a fat-soluble drug has been established. The use of drugs in the form of gel films reduces their toxicity and side effects on the body. Also, medicinal gel films can be stored for up to 2 years, and this is also their valuable quality. In this regard, the creation of a new domestic drug in the form of gel films for the treatment of burns is relevant and promising.

References

[1] P. Ghaffari-Bohlouli, F. Hamidzadeh, P. Zahedi, M. Shahrousvand, M. Fallah-Darrehchi, Journal of Biomaterials Science Polymer Edition **31**(2), 219 (2020).
[2] W. K. Son, J. H. Youk, W. H. Park, Carbohydrate Polymers **65**(4), 430 (2006).
[3] R. J. Moon, A. Martini, J. Nairn, J. Simonsen, J, Youngblood, Chemical Society Reviews **40**(7), 3941 (2011).
[4] R. S. Ambekar, B, Kandasubramanian, European Polymer Journal **117**, 304 (2019).
[5] H. P Felgueiras, M. A. Teixeira, T. D. Tavares, N. C. Homem, A. Zille, M. T. P. Amorim, Journal of Applied Polymer Science **137**(18), 48626(2020).
[6] H. Li, G. R. Williams, J. Wu, Y. Lv, X. Sun, H. Wu, L. M. Zhu, International Journal of Pharmaceutics **517**(1-2), 135 (2017).
[7] S. A. Majd, M. R. Khorasgani, S. J. Moshtaghian, A. Talebi, M. Khezri, International Journal of Biological Macromolecules **92**, 1162 (2016).
[8] A. N. Nakagaito, S. Iwamoto, H, Yano, Applied Physics A **80**(1), 93 (2005).
[9] Y. Li, M. Lin, J. W. Davenport, The Journal of Physical Chemistry C **115**(23), 11533 (2011).
[10] S. W. Ko, J. P. E. Soriano, A. R. Unnithan, J. Y. Lee, C. H. Park, C. S. Kim, International Journal of Biological Macromolecules **110**, 531 (2018).
[11] F. E. Che Othman, N. Yusof, A. A. Raffi, H. Hasbullah, F. Aziz, W. N. W Salleh, A. F. Ismail, Malaysian Journal of Analytical Science **21**(2), 365 (2017).
[12] A. Gupta, D. J. Keddie, V. Kannappan, H. Gibson, I. R. Khalil, M. Kowalczuk, C. Martin, X. Shuai, I. Radecka. European Polymer Journal **118**, 437 (2019).
[13] A. Gupta, S. M. Briffa, S. S. H. Gibson, V. Kannappan, G. Adamus, M. Kowalczuk, C. Martin, I. Radecka, Biomacromolecules **21**(5), 1802 (2020).
[14] A. Jaworek, A. Krupa, M. Lackowski, A.T. Sobczyk, T. Czech, S. Ramakrishna, S. Sundarrajan, D. Pliszka, Fibres and Textiles in Eastern Europe **17**(4), 77 (2009).
[15] M. Golizadeh, A. Karimi, S. Gandomi-Ravandi, M. Vossoughi, M. Khafaji, M. T. Joghataei, F. Faghihi, Carbohydrate Polymers **207**, 796 (2019).
[16] A. Awal, M. Sain, M. Chowdhury. Composites Part B: Engineering **42**(5), 1220 (2011).
[17] R. Poonguzhali, S. K. Basha, V. S. Kumari, International Journal of Biological Macromolecules **112**, 1300 (2018).
[18] H. Yamamoto, F. Horii, H. Odani. Macromolecules **22**(10), 4130 (1989)
[19] E. M. Debzi, H. Chanzy, J. Sugiyama, P. Tekely, G. Excoffier. Macromolecules **24**(26), 6816 (1991).