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Evaluation of Immobilized Bacteriorhodopsin's Function by Laser Irridiation

A. Molaeirad[a,]*, M. Besharati Vineh[b], M. Alijanianzadeh[a] and M. Khayati[a]

[a]*Department of Bioscience and Biotechnology, Malek-Ashtar University of Technology, Tehran, Iran*

[b]*Department of Biology, College of Basic Science, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran*

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ABSTRACT

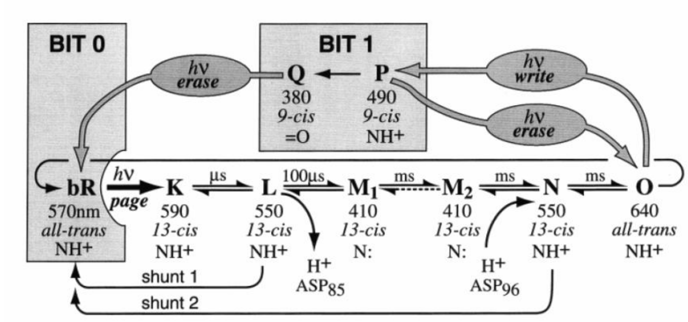
Bacteriorhodopsin (BR) is a retinal protein that is a light-driven proton pump and has an important role in photosynthesis in archaebacterium Halobacterium salinarum. The BR molecule absorbs light and photochemical changes occur in it, and different intermediates will be produced in its photochemical cycle that some of them like P and Q intermediates have a long half-life. There have been many efforts to immobilize BR for constructing data storage devices. In this study the BR suspension-contained film has been immobilized on a polycarbonate and the effects of green and red lasers on it (in different times and temperatures) have been investigated by UV spectrophotometer method, The modified surfaces were characterized by ATR-FTIR and AFM techniques. The results show that with used condition for immobilization of BR on polycarbonate, the 0 and 1 bite that relate to BR and P, Q intermediate, are formed. The red and green lasers convert BR to O and, P or Q intermediates respectively that could be used instead of 0 and 1 bites in popular compact disks.

Keywords: Bacteriorhodopsin, Polycarbonate, Laser, Immobilization

INTRODUCTION

Bacteriorhodopsin (BR) is a retinal protein that is a light-driven proton pump and has an important role in photosynthesis in archaebacterium *Halobacterium salinarum* [1]. Structural analysis of BR and purple membrane (PM) has shown a detailed molecular picture of light-dependent proton transport across the cell membrane [2,3]. BR is extracted from this bacterium in the form of purple membrane (PM) patches, which consist of BR and lipids only. The BR molecules are arranged in a two dimensional hexagonal crystalline lattice inside the PM. The BR molecule absorbs light and photochemical changes occur in it. Its photocycle is shown in Scheme 1 [4]. As it is indicated in the scheme, in this cycle different intermediates are formed. Each intermediate is shown by a single letter code and indices represent the maximum wavelength absorption of them. In this cycle, first by photoreaction, the B_{570} is converted to J_{625} that is the short-living state and proceeds to the K state. In this stage, the all-trans retinal isomerizes to 13-C is retinal. At the end of the cycle the B_{570} will be formed again. The retinal conformation of B_{570} and O_{640} is the same and is all-trans but all other intermediates have 13-*cis* retinal conformation. The result of these conformational changes is the transportation of a proton across the BR [5]. Because of its photochromic properties, chemical and thermal stability, BR is a candidate~~s~~ for technical applications such as data storage or optical devices [6,7]. BR has a good cross-section for absorption of two photons [8]. The reason for studying two-photon-induced processes in BR is twofold. First, molecular properties that could not study by classical single-photon excitation, can be analyzed [9]. Second, two-photon-induced processes can be done with extremely high spatial resolution [10,11], which makes them attractive for data storage. In order to use BR in data storage devices it is necessary to immobilize it. There have been some attempts to immobilize BR on different materials until now [12-15]. In this work, we combine structural studies with scanning electron microscopy (SEM), atomic force microscopy (AFM), Fourier transform infrared spectroscopy (ATR-FTIR spectroscopy) and UV spectrophotometer to analyze structural changes in immobilized BR on polycarbonate surface upon two-photon induced photobleaching (TPP).

*Corresponding author. E-mail: molaeirad@gmail.com



Scheme 1

MATERIALS & METHODS

Materials

Wild-type BR, polyvinyl alcohol (PVA), triethanolamine (TEA), KCl, $MgCl_2$-$2H_2O$, HNO_3 and gelatin (GE) were purchased from Sigma. Methanol and HCl were purchased from Merck.

Methods

BR films preparation. BR films were prepared according to previously described method [16]. First, suspension of BR (3.2 mg ml^{-1}) was prepared with tri-distilled water. The produced suspension was sonicated for 20 min. Then for film preparation, 1% (w/v) PVA powder and GE were dissolved in 19.8 ml tri-distilled water and the produced solution was stirred at 60 °C for 40 min. At the end the film with 1% (w/v) PVA was prepared. Next, BR suspension was soaked with film and a 0.4 M aqueous solution of triethanolamine (TEA) was added to the film-forming suspension to give TEA/BR molar ratio of 250:1 (which give maximal photosensitivity).

Physical immobilization. The physical immobilization of BR with PVA-GE film on the polycarbonate surface was done. BR suspension-contained film was immobilized on the polycarbonate surface with two methods (Spin coating & Casting) and allowed to be dried [17,18].

BR activity measurement and photon induction. For measuring the BR activity, KCl (3 M) and $MgCl_2$ (80 mM) solutions were used. In pH = 7.1, BR contained film was added to this solution. For excitation of proton pump activity of BR, a lamp with 200 W power in a distance of 30 cm from the sample was used and pH changes were measured for 30 min.

After preparation of BR-suspension-contained film and its physical immobilization on polycarbonate surface, two orthogonal beams were used as data lasers: a green laser with wavelength of 630 nm and 60 mW power (vertical) for paging action and, a red laser with a wavelength of 680 nm and 50 mW power (horizontal).

UV-Vis spectroscopy. UV-visible absorption spectra were obtained with Kuhner Lab-Therm, unicum uv-300.

Atomic force microscopy (AFM). The physical immobilization of BR on the polycarbonate surface was done. The resulting changes in BR structure was analyzed by Atomic Force Microscopy DS 95-200E model

Fourier transform infrared (FTIR) spectroscopy. The physical immobilization of BR on the polycarbonate surface was done. The resulting changes in BR structure was analyzed FTIR NEXUS 870 model.

Spin coating. The spin coating of BR contained film was done by spin coating SC409 model made in Iran [17,18].

RESULTS

This research was done in several steps:

Evaluation of laser effect (green and red) on BR suspension-contained film and immobilized BR on solid polycarbonate surface.

Figures 1a and b show the absorption spectra of BR suspension-contained film and BR suspension-contained film immobilized on polycarbonate surface respectively after green and red laser radiation at 25 °C. In both states the concentration of BR is 3.2 mg ml^{-1}.

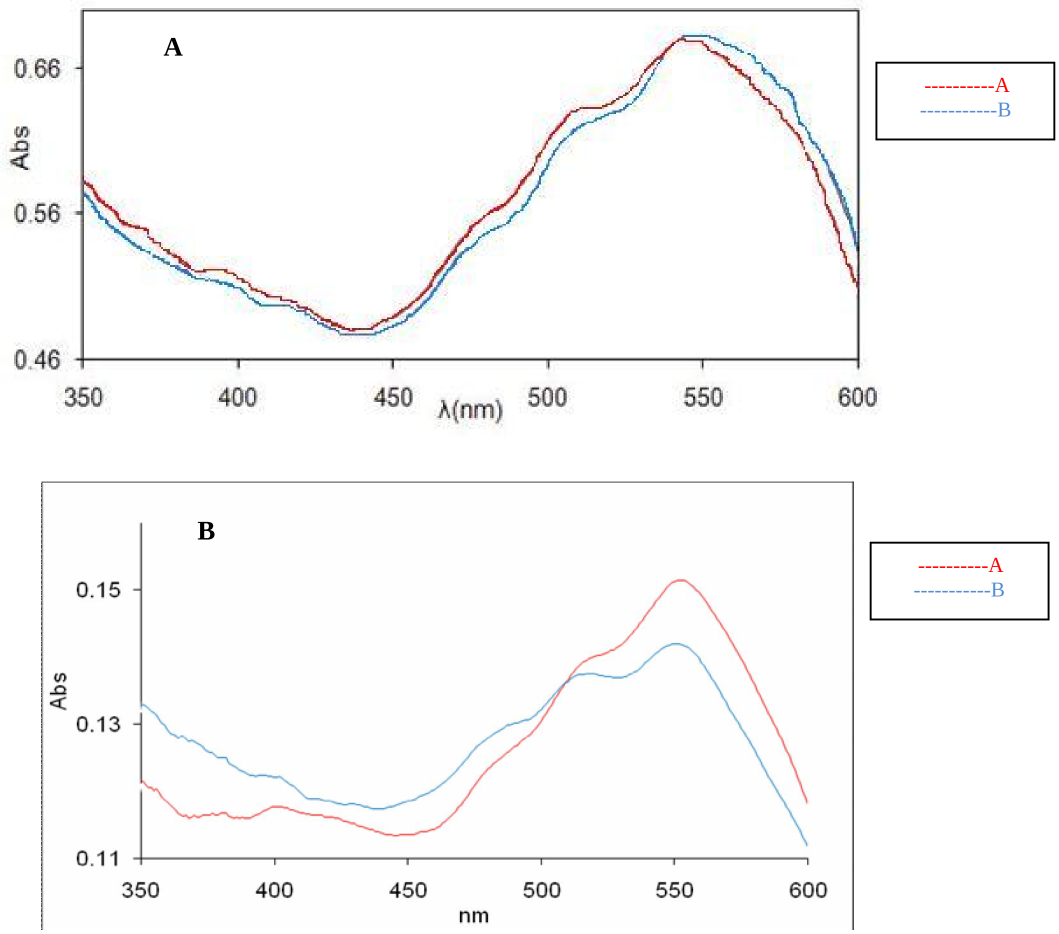


Fig. 1. a) the absorption spectra of BR suspension and b) BR suspension contained film immobilized on polycarbonate surface contained film after green and red laser radiation at 25 °C. The concentration of BR is 3.2 mg ml^{-1}. (A and B spectrum are before and after laser radiation, respectively).

Figure 2 shows absorption spectra of immobilized BR on polycarbonate in different times (from 4-28 min) and at room temperature. This is done every 4 min. As it is shown in Fig. 2, the same changes as Fig. 1 are occurred here.

Figures 3 and 4 show the effect of different temperatures on absorption spectra of laser-radiated BR suspension-contained film and immobilized BR on polycarbonate, respectively. As it is shown in two Figs. 3 and 4 no changes occur in the absorption intensities at 490 and 570 nm before 70 °C and the same changes as Fig. 1 to 5 occur in these wavelength absorption intensities after 70 °C.

For studying the BR activity, the effect of temperature on the activities of BR suspension-contained film in two forms of solution and immobilized on polycarbonate, were investigated and the results are depicted in Figs. 5a and 5b, respectively. In all temperatures, the initial pH values were fixed in 7.1 and after 5 min the data were measured. The figures results show that BR suspension-contained film has maximum activity in lower temperatures but BR suspension immobilized on polycarbonate has maximum activity in higher temperatures.

Also the results of green and red laser radiation on BR

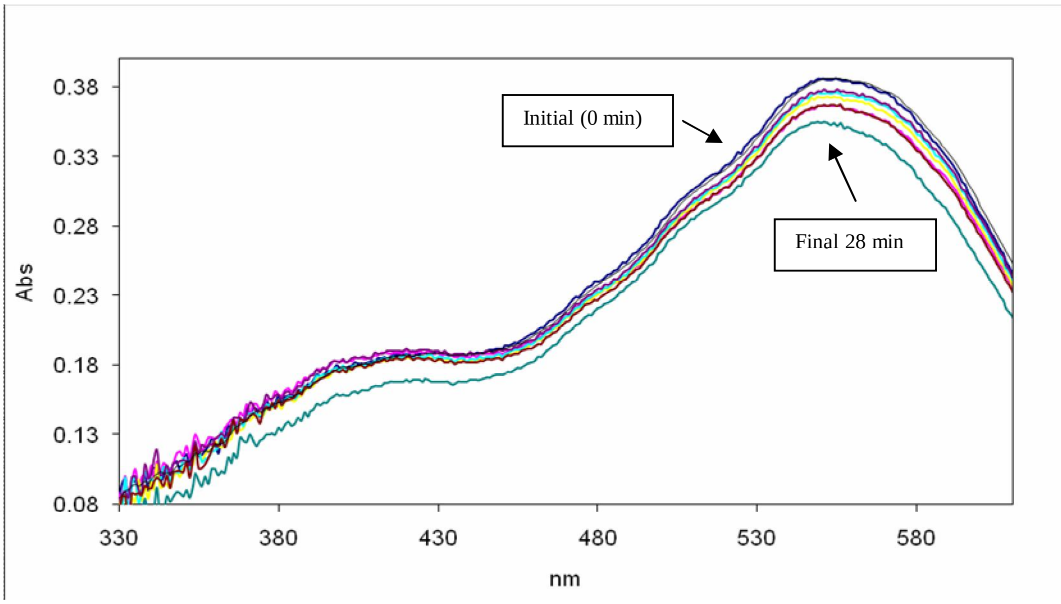


Fig. 2. Absorption spectra of immobilized BR on polycarbonate in different times (from 4-28 min) (every 4 min) at room temperature.

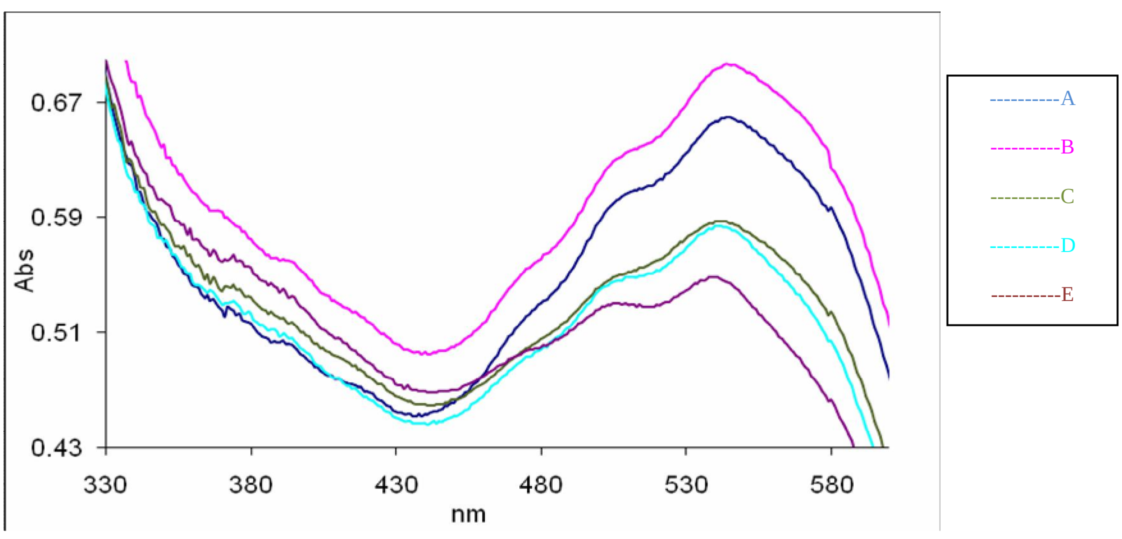


Fig. 3. Effect of different temperatures on absorption spectra of laser radiated BR suspension contained film. (A spectrum is in room temperature before laser radiation, B, C, D and E ones are BR spectra after laser radiation at 70, 80, 87 and 93 °C, respectively.

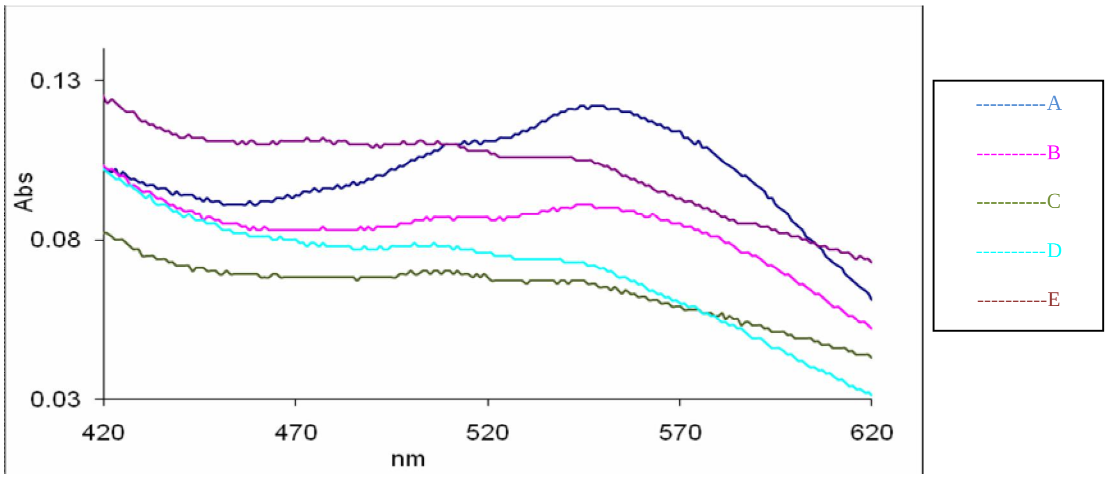
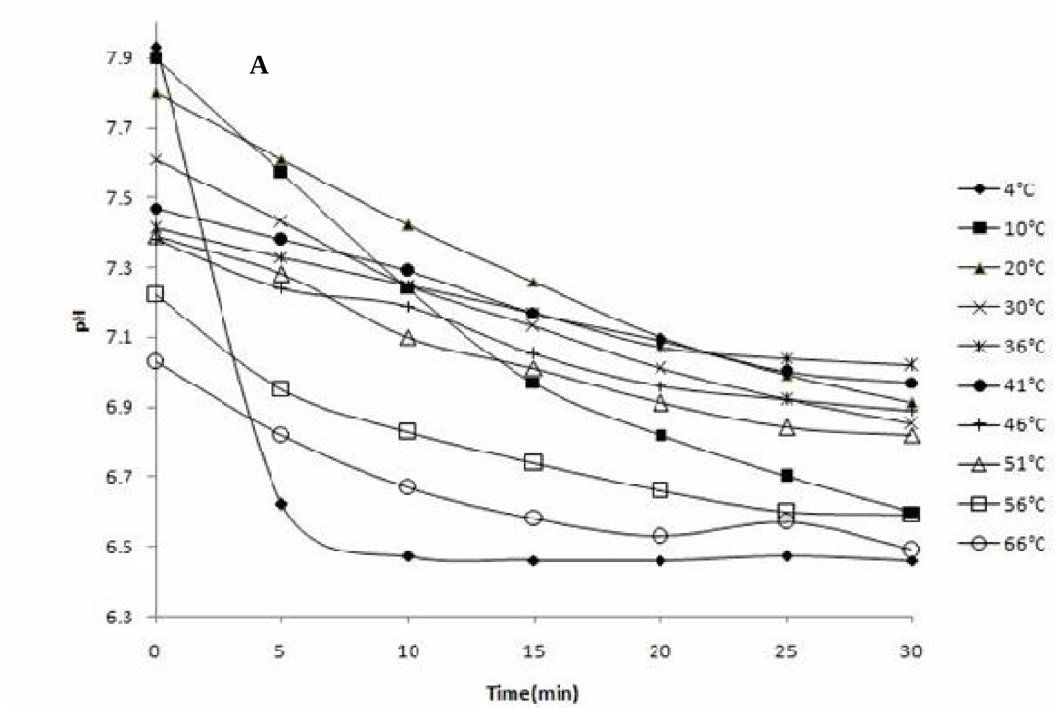


Fig. 4. Effect of different temperatures on absorption spectra of laser radiated BR suspension contained film immobilized on polycarbonate. (A spectrum is in room temperature before laser radiation, B, C, D and E ones are BR spectra after laser radiation at 25, 50, 75 and 85 °C, respectively.

suspension-contained film immobilized on grooved polycarbonate surface were investigated by ATR-FTIR aperture. These results is shown in Fig. 6. In this state the imine group (3377 cm^{-1}) is converted to amine (1663 cm^{-1}) group. In the final step the morphology of BR immobilized polycarbonate surface was studied by AFM technique. In this step change that occurs in BR suspension immobilized on polycarbonate surfaces after radiation by green and red lasers was investigated by AFM microscope (Fig. 7). Panel A shows polycarbonate surface before laser radiation, Panel B shows surface after laser radiation. The image of AFM microscopy show that after laser radiation the structure of BR has been changed.

DISCUSSIONS

The results show that with used condition for immobilization of BR on polycarbonate, the 0 and 1 bite that relate to BR and P, Q intermediate, are formed. AFM images show that BR is effectively attached to the polycarbonate surface after physical immobilization, Also the images of this show that structure of BR changes with this laser beams. The goal of this work was to produce P and Q intermediates with a new lasers beam and new surfaces, and the results confirmed that it is done. The result of Fig. 2 shows that after some minutes, a new peak appears in 350 up to 490 nm region, but the intensity of peak at 570 nm reduces. As well from results of Figs. 5a and b we can conclude that, 1) BR suspension-contained film in both solution and immobilized states, doesn't lose activity at high temperatures, and 2) in the BR suspension-contained film in solution state the maximum activity is in low temperatures but in immobilized state the maximum activity is in high temperatures. BR is a trans-membrane protein so by increasing its flexibility, its activity will be decreased. When protein is in the solution, with increasing temperature, the lipids around it will be removed and the flexibility of protein will be increased so the protein conformation will partially be disrupted and activity will be decreased. But when the protein is immobilized on polycarbonate grooves, with increasing temperature, the flexibility of protein will not be changed. On the other hand due to the fact that the protein is thermophile, the increasing temperature doesn't affect its activity, and as a result, these two factors are keeping BR structure intact and increasing its activity. So the immobilized BR film activity in these conditions increases with elevating temperature in comparison with BR film solution that its activity decreases with elevating



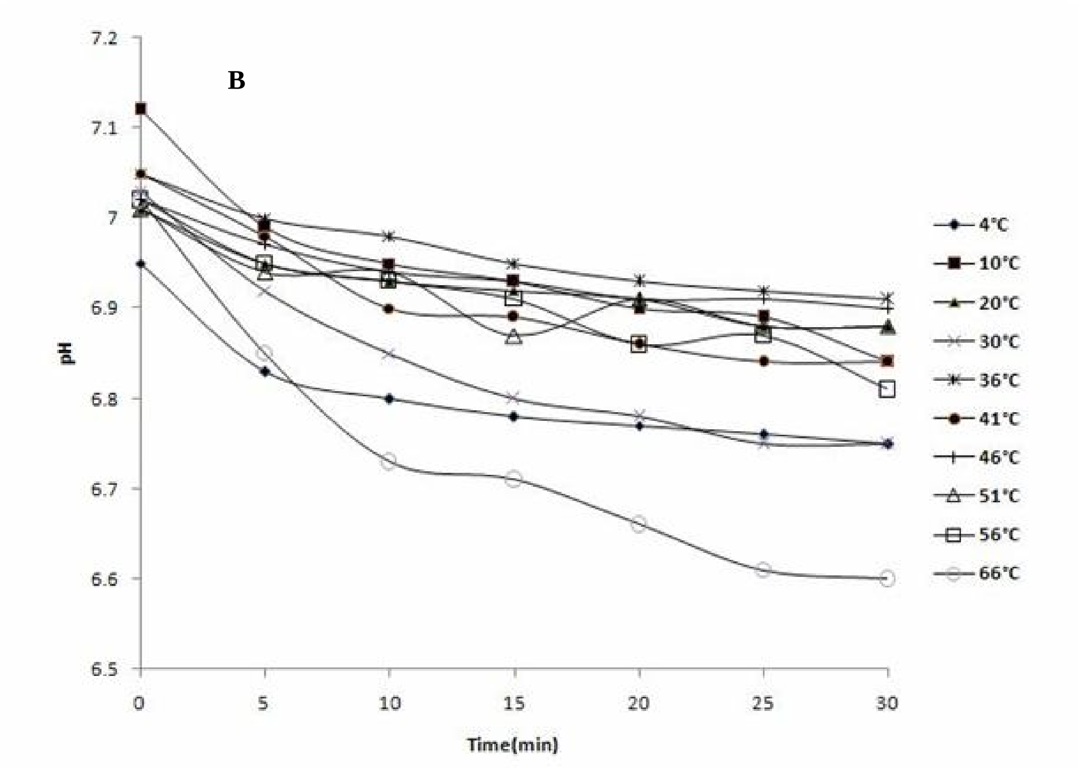


Fig. 5. a) Effect of temperature on the activity of BR suspension contained film in form of solution and b) BR suspension contained film immobilized on polycarbonate. (◆, ■, ▲, ×, ✳, ●, +, △, □ and ○ are 4, 10, 19, 29, 36, 41, 46, 51, 56 and 66 °C respectively).

Fig. 6. ATR-FTIR spectrum of BR suspension contained film immobilized on polycarbonate after 10 min of laser radiation.

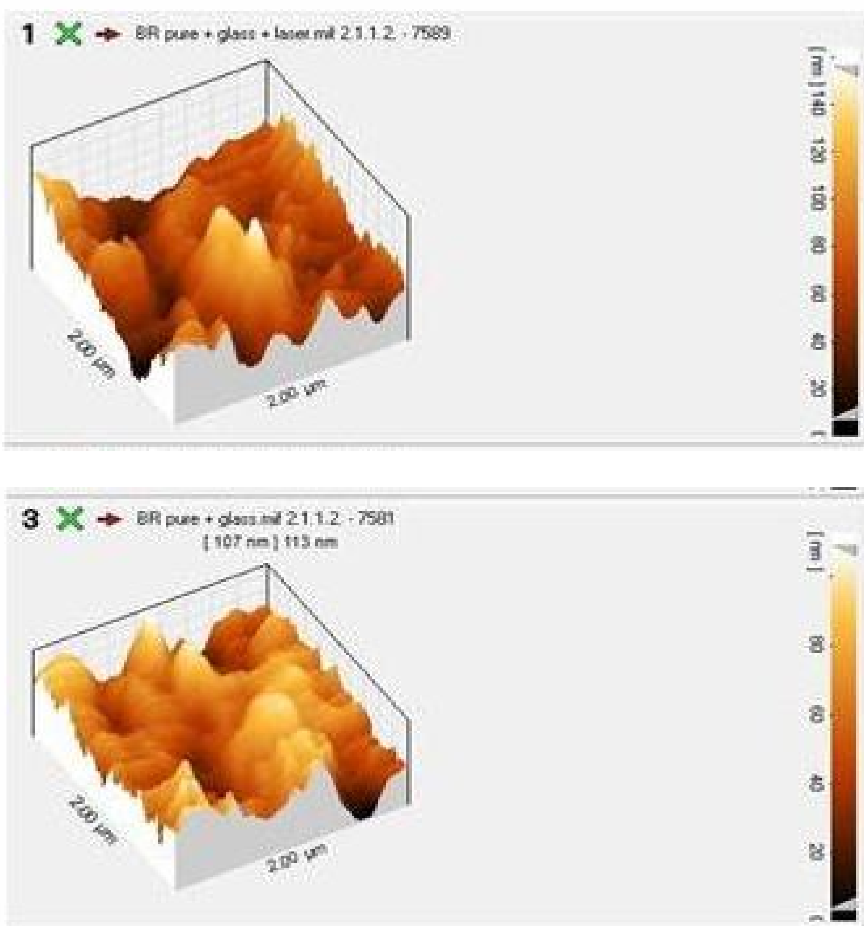


Fig. 7. Atomic force microscopy (AFM) image with resolution of 2 µm of BR suspension immobilized on polycarbonate after 10 min of laser radiation.

temperature.

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