Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2013; 38 (4) ; 468–474
doi: 10.5505/tjb.2013.28247

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Aralık, 2013 © TurkJBiochem.com

[Published online 30 December, 2013]

Bacteriorhodopsin embedded in gelatin and polyvinyl alcohol films as recording materials for holographic memories

[Jelatin ve polivinil alkol filmlere holografik hafızalar için kayıt edici materyal olarak bakteriorodopsin yerleştirilmesi]*

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Registered: 6 April 3013; Accepted: 13 October 2013

[Kayıt Tarihi: 6 Nisan 2013; Kabul Tarihi: 13 Ekim 2013]

ABSTRACT

Objectives: Bacteriorhodopsin is a heptahelical membrane protein found in the archaean *Halobacterium salinarum*. The performance of bacteriorhodopsin film was evaluated as a holographic optical memory.

Material and method: Bacteriorhodopsin was immobilized on polymeric films with different weight/volume ratios of polyvinyl alcohol and gelatin. The effect of different concentrations of phosphate buffer (from 0.001 to 0.1 M) on performance of the polyvinyl alcohol / gelatin composite films was studied.

Results: The results indicate that the best quality of the film was obtained in 0.001 % (w/v) of polyvinyl alcohol and gelatin mixture and 0.1 M phosphate buffer. For the control, bovine serum albumin was immobilized on polyvinyl alcohol/gelatin films instead of bacteriorhodopsin. The films based on bacteriorhodopsin were illuminated by two orthogonal beams, green laser (vertical) and red laser (horizontal) for 10 minutes, then their absorbance spectrum was measured. The green and red lasers were irradiated to the bacteriorhodopsin film for writing data which lead to absorption peaks about 380 nm (Q state) and 640 nm (O state). Finally, the decrease in absorption at 380 nm was monitored after 15 minute radiation of 1 watt blue LED utilized to erase the data.

Conclusion: As a conclusion, this matrix is suitable for immobilization of bacteriorhodopsin protein and can be used in optical memory devices.

Key Words: Optical memory, bacteriorhodopsin film, laser beam, protein immobilization, bioelectronics

Conflict of Interest: The authors have declared that no conflict of interest exists.

ÖZET

Amaç: Bakteriorodopsin, arkebakteri *Halobacterium salinarum* da bulunan yedi sarmallı zar proteinidir. Bakteriorodopsin filmlerin, holografik optik hafıza olarak performansı değerlendirildi.

Gereç ve Yöntemler: Bakteriorodopsin, polimerik filmler üzerinde farklı ağırlık/hacim oranlarındaki polivinil alkol ve jelatin ile hareketsiz hale getirildi.

Değişik konsantrasyonlardaki fosfat tamponunun (0.001 den 0.1 M a kadar) polivinil alkol / jelatin bileşimli filmlerin performansı üzerine etkileri çalışıldı.

Bulgular: En iyi kalitedeki filmin 0.1 M fosfat tamponu ve % 0.001(w/v) polivinil alkol ve jelatin karışımından elde edildiği gösterildi. Kontrol olarak sığır serum albumini, bakteriorodopsin yerine polivinil alkol ve jelatin filmler üzerinde hareketsiz hale getirildi. Bakteriorodopsin kökenli filmler 10 dakika boyunca yeşil (dikey) ve kırmızı (yatay) lazer olan iki dik açılı ışın ile aydınlatıldı ve daha sonra absorbans spektrumu ölçüldü. 380 nm (Q durum) ve 640 nm (O durum) de absorpsiyon piklerine öncülük eden yeşil ve kırmızı lazerler, verileri yazmak için bakteriorodopsin filme ışınlandı. Son olarak, 380 nm'de verileri silmede kullanılabilecek, 1 vat mavi LED ışımasından 15 dakika sonra absorpsiyonda azalma gözlendi.

Sonuç: Sonuç olarak bu anayapı bakteriorodopsin proteininin hareketsiz hale getirilmesi için uygundur ve optik hafıza cihazlarında kullanılabilir.

Anahtar Kelimeler: Optik hafıza, bakteriorodopsin film, laser ışını, protein immobilizasyonu, biyoelektronikler

Çıkar Çatışması: Yazarlarin çıkar çatışması bulunmamaktadır.

Introduction

The research in the area of data storage devices has lead to the proposal of three-dimensional optical memories. Three-dimensional optical memories are claimed to have higher storage capacity than two-dimensional memories. Holographic memories read and write information by using two orthogonal laser beams to address an irradiated volume (l-50 μm^3) within a much larger volume of a non-linear photochromic material [1, 2]. These systems contain photosensitive optical material that undergoes some irreversible or reversible change and record the interference of two light sources. Merely the reference beam utilized to create the hologram can read the recorded information. To acquire a reading, the laser beam must be focused into the photosensitive material at the same angle it was applied for recording. The laser beam hits the data storage material and results in initiation of immediate chemical reaction in it. This alteration in structure of recording material change the amount and wavelength of reflected light [3, 4].

Among the photoactive proteins, bacteriorhodopsin (bR) has been investigated as a volume holographic data storage material. Bacteriorhodopsin is the light-harvesting protein contained in the purple membrane of the archeon *Halobacterium salinarum* [5, 6]. Bacteriorhodopsin was discovered in the early 1970s by D. Oesterhelt and W. Stoeckenius [7]. The bR protein consists of 248 amino acids, arranged in seven α-helical bundles inside the lipid membrane [8]. The bacteriorhodopsin core photocycle contains BR, K, L, M, N and O intermediate states and the Q intermediate is formed in branched photocycle of bacteriorhodopsin. Each intermediate has a distinct absorbance maximum; the most studied are BR (570 nm), O (640 nm), and Q (380 nm). Furthermore, both the structure–function relations of bacteriorhodopsin, and ways to modify bR structure and thus its functions, have been studied intensively [7]. There are some unique properties, such as stability against thermal, chemical, and photochemical degradation that make bacteriorhodopsin as an active element in biological device applications [9].

Optical devices and memories that consist of bR films for information processing have been developed due to their high response characteristics and excellent stability in respect of optical and photoelectrical properties [10-12]. The absorption of a photon results in conversion of all-trans to 13-cis photoisomerization of the BR's retinal chromophore. The conformational changes of bacteriorhodopsin are obtained from the changes of the BR's absorption spectrum. These characteristic changes in the absorption spectra could be used to create biological holographic memory. Upon absorption of green light by the retinal, the structure of bacteriorhodopsin altered from the bR native state (BR) to form O intermediate. After a red light pulse, the O intermediate converted to P intermediate, which quickly reverts to Q state, which is stable for long periods of time. The absorption maximum of Q intermediate is about 380 nm that can be recycled back to the bR resting state (570 nm) through illumination with blue light. The speed of BR writing depends on kinds of photocycle states used. Usually the BR's initial state and another relatively stable intermediate (M) state are used [7, 9, 13].

The films based on bacteriorhodopsin have been used to create biosensors and optical memories. Physical and chemical properties of film-forming materials are essential for their successful implementation in devices. The polymeric material is not inert and ineffective. Their functional groups can interact with proteins and change the optical properties of them. For example, chemical additives [14], cations [15], metal ions [16], ammonium ions and amines [17], all have demonstrable effects on the spectral and the photocycle parameters. Undoubtedly, the effect of experimental conditions such as pH changes on protein structure is not forgotten [18].

In this study, bacteriorhodopsin was immobilized on thin films of different weight/volume ratios of gelatin (GE) and polyvinyl alcohol (PVA), and their absorption spectra were measured. The bacteriorhodopsin based polymeric films were illuminated by the green and red laser beams to investigate the bR writing ability. Finally, a 1 watt blue LED was used to convert back the P and Q intermediates into bR resting state for erasing the encoded data.

Materials and methods

Materials

Bacteriorhodopsin, gelatin, polyvinyl alcohol, and HCL were purchased from Sigma, bovine serum albumin and phosphate buffer were provided from Merck. All other materials not specifically indicated were obtained from Sigma or other reputable sources.

Preparation of bacteriorhodopsin films

Bacteriorhodopsin films in polyvinyl alcohol and gelatin matrices were prepared by using the following procedure. First of all, the polymeric matrix with different weight/volume (w/v) ratios of polyvinyl alcohol and gelatin were obtained by dissolving GE and PVA powder in the double distilled water for 15 minutes before heating and stirring the mixtures at 60°C for 40 minutes. In this research, 0.01, 0.05 and 0.001 % (w/v) of polyvinyl alcohol and gelatin solutions were prepared. The PVA–GE films based on bacteriorhodopsin were obtained from 50 µL of the bR suspension (2 mg/mL) and investigated [19].

Optimization of bacteriorhodopsin films

The 0.1, 0.01 and 0.001M phosphate buffer solutions (PBS) were prepared by using Na_2HPO_4 and KH_2PO_4. Polyvinyl alcohol-gelatin solutions of 0.001% (w/v) were prepared with the three different concentrations of phosphate buffer to optimize thin film performance

for chosen parameters. The films based on bR in the polyvinyl alcohol-gelatin matrix were optimized as a three-dimensional optical memory in phosphate buffer solution and absorbance spectrum recorded at the wavelengths between 200 and 700 nm.

Measurement

In order to evaluate the photo-activity of bacteriorhodopsin, pH changes were measured in the presence of light (using an inoLab pH meter and 200 Watt lamp from a 30 cm). The ratio of the absorbance at 280 and 570 nm (A280/A570) is used to assess the quality of film. In this study, the quality of bacteriorhodopsin films was determined by recording the absorption spectrum by Unicom UV 300 and Ultrospec 2100 pro UV-Vis spectrophotometers in the wavelengths interval of 200 to 700 nm. Then, the ratio of absorbance at 570 and 280 nm was obtained. The solutions of bacteriorhodopsin dissolved in polyvinyl alcohol and gelatin matrices, were applied in a cuvette and a thin slide (1 mm thickness) on an area about 15 mm long and 4 mm wide. The film immobilized on the cuvette was dried for 24 hours in the fridge temperature. Also, the gelatin and polyvinyl alcohol films based on bovine serum albumin were used as control. We used the casting method to immobilize bR films on the slide. A drop of the film-forming solution was placed onto a horizontally fixed cleaned glass substrate. After deposition of a drop of solution, the formed film was dried through evaporation of water for 24 hours using a desiccator to increase the evaporation rate of water from the sample [20].

The illumination was done by the helium-neon laser (5mW max output) at 632.8 nm as the red laser and a pointer laser at 532 nm, 5mW/cm2 as the green laser. The irradiation of green and red laser pulses on the bR films surface were carried out in two ways. In the first, the polymeric films were illuminated with green light for 5 min. Then, red light was applied for 5 and 15 min. In the second, the films based on bacteriorhodopsin were illuminated by two orthogonal beams, green laser (vertical) and red laser (horizontal) for 10 minutes. We also used a 1W blue LED to convert back the formed intermediates into bR resting state. Therefore, the bR film was lighted with a blue light for 15 min. The photo induced absorption changes were measured successfully. All experiments were performed at room temperature (25°C).

Results and Discussion

Optimization of condition affects the properties of bacteriorhodopsin film

The photochemical activity of wild-type bacteriorhodopsin and bR film

The photochemical activity of wild-type bacteriorhodopsin and bR film were investigated by evaluation of pH changes induced by variation of light intensity and the light-induced changes of both bacteriorhodopsin protein and bR film were observed. Fig. 1 and Fig. 2 show light-induced pH changes of bR and bR film, respectively. The pH change is an important factor in assessing the activity of bacteriorhodopsin. Results show the activity of the light-driven proton pump. Bacteriorhodopsin pumps protons across a membrane using the energy of light. In the *Halobacterium salinarum*, proton pumping of bacteriorhodopsin forms pH gradient across the membrane. The pH gradient can be used to synthesize ATP from inorganic phosphate and ADP [8].

The effect of PVA-GE concentrations on wavelength of maximum absorbance

UV-Vis absorption spectra of bacteriorhodopsin films with different concentrations of polyvinyl alcohol and gelatin were investigated. In high concentration of PVA-GE, wavelength of maximum absorbance (λmax) was lower due to decrease of film optical transparency. The best results were obtained with 0.001 % (w/v) of polyvinyl alcohol and gelatin gels (Fig. 3).

A combination of gelatin and polyvinyl alcohol can be an appropriate candidate for biochemical applications. In addition, hydrophilic nature, good biocompatibility, and consistency of PVA-GE film make it superior to other materials for use in protein-based holographic memories [19].

The effect of buffer concentrations on bacteriorhodopsin film

The bR films were constructed in various phosphate buffer solutions. The films were tested by comparing the spectral properties of the products to explore the best production method or principle. Here, we employed three different concentrations of phosphate buffer to optimize the thin films. Bacteriorhodopsin protein was immobilized in the film solutions of 0.001% (w/v) prepared with different concentrations of phosphate buffer. The rate of absorption at wavelengths between 200 nm and 700 nm shows a sensitivity of thin films. The sensitivity of bacteriorhodopsin film is a key parameter to improve the film quality and the holographic memory performance [21]. The UV-Vis absorption spectra of bacteriorhodopsin films were measured at three different concentrations of 0.1, 0.01 and 0.001 M of phosphate buffers. The highest absorption spectrum was related to the 0.1 M concentration of phosphate buffer (Fig. 4). The concentration of 0.1 M of phosphate buffer could improve the bacteriorhodopsin film optical properties. The film prepared in 0.1 M phosphate buffer has less amount of water in comparison with the films prepared in 0.01 and 0.001 M phosphate buffer. Increasing the humidity causes decreasing of the quality of the films. In addition, the concentration of bR in 0.1 M phosphate buffer is more than 0.01 and 0.001 M phosphate buffer.

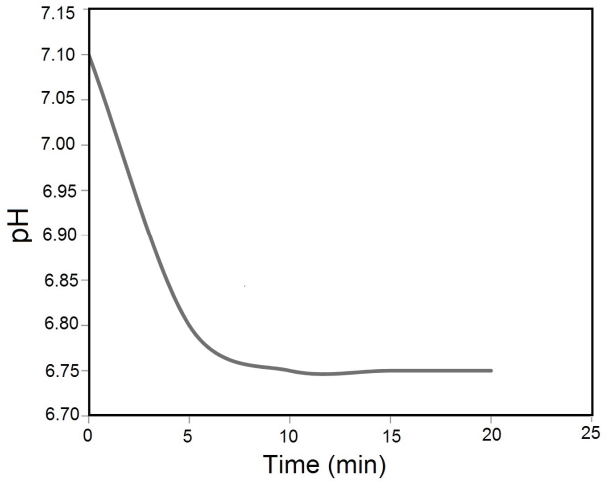

Figure 1. Light-induced pH changes of 50 µL Wild-type bR suspended in 80 mM MgCl2 and 3M KCL.

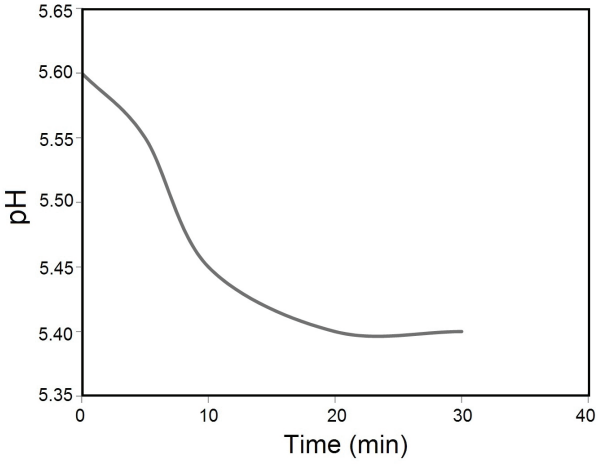

Figure 2. pH changes on illumination of polyvinyl alcohol and gelatin (50 ml) film based on bacteriorhodopsin (50 µL).

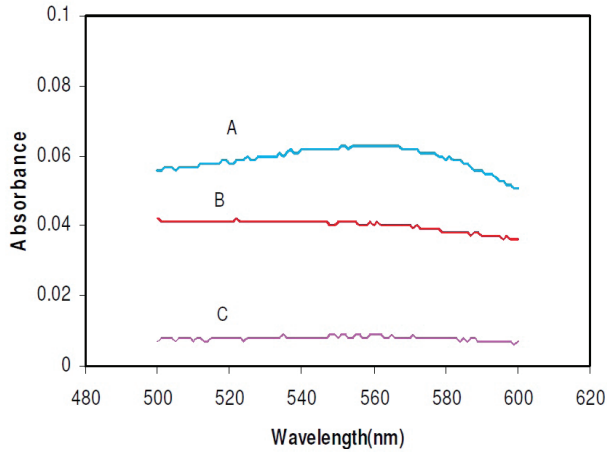

Figure 3. UV–vis absorption spectra of bR in (A) 0.001% (w/v), (B) 0.05% (w/v) and (C) 0.01 % (w/v) of polyvinyl alcohol and gelatin gels.

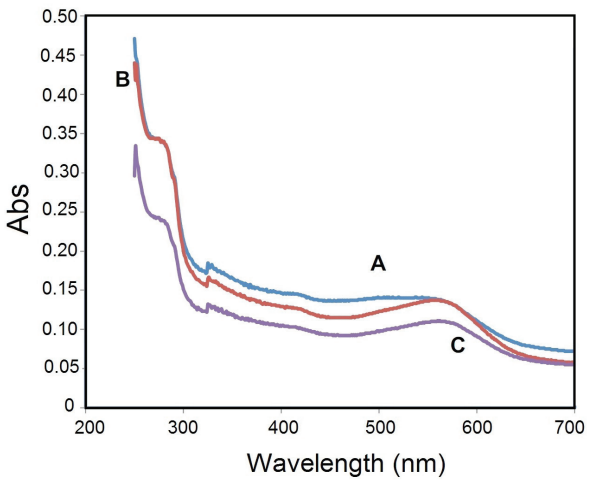

Figure 4. Light absorption spectra of bR films at (A) 0.1, (B) 0.01 and (C) 0.001 M phosphate buffers.

According to the direct relationship between the absorbance and the concentration, the light absorption of film produced using 0.1 M phosphate buffer is increased.

Measurement

The relative optical quality of bacteriorhodopsin film can be estimated, using an A280/A570 ratio. In the present work, the absorption ratio (A280/A570) was 3. A280 and A570 indicate the absorptions of the aromatic amino acid residues in the bacteriorhodopsin, and the absorption of the protonated Schiff base retinal, respectively. The ground state bR has a broad absorption band around 570 nm. The bacteriorhodopsin film within the cuvette is illuminated by a green laser flash for 5 min. The bR photocycle was initiated with the green light and M and Q states were observed. This process is referred to paging, and involves initiating the bactriorhodopsin photocycle with a photon of green light. Figure 5 displays the maximum absorption peak at the wavelengths 380 and 410 nm following green laser excitation. Then, a red laser was irradiated to the bR film for 5 and 10 min and the UV-Vis spectra were recorded between 370 nm and 390 nm. The maximum absorption was found at 380 nm which correspond to the stable Q state (Fig. 6). Exposure to red light from O state makes the structure of the bacteriorhodopsin to state P then spontaneously to state Q, which is a very stable intermediate. The film of bacteriorhodopsin was lighted with a vertical green laser pulses and a horizontal red laser beams for 10 and 15 min. The absorption peaks were observed at about 380 nm and 640 nm and the Q and O states were detected (Fig. 7). In the holographic memory based on

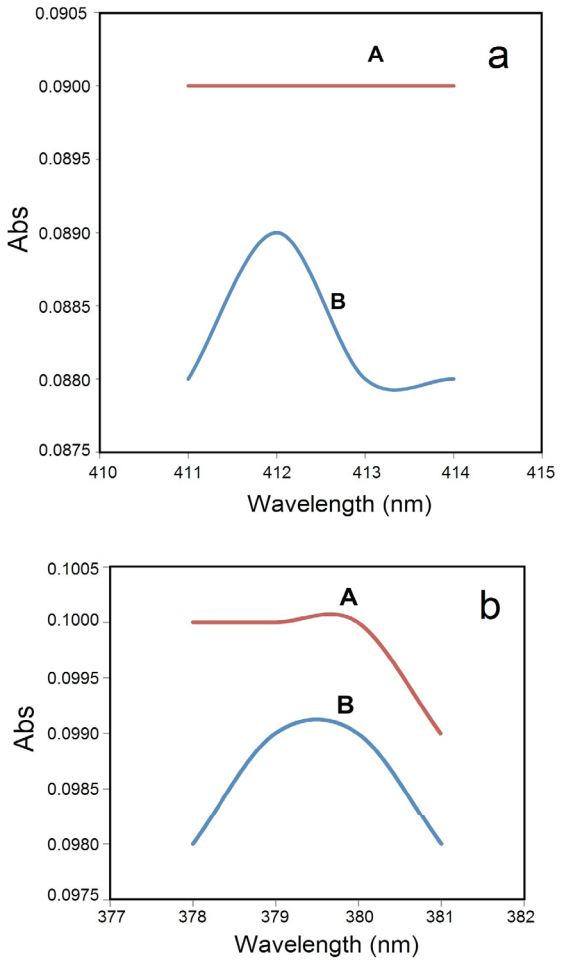

Figure 5. UV–vis absorption spectra of bR film (A) without any light and (B) in the presence of green light at wavelengths (a) 411–413 nm and (b) 378–381 nm, for 5 min.

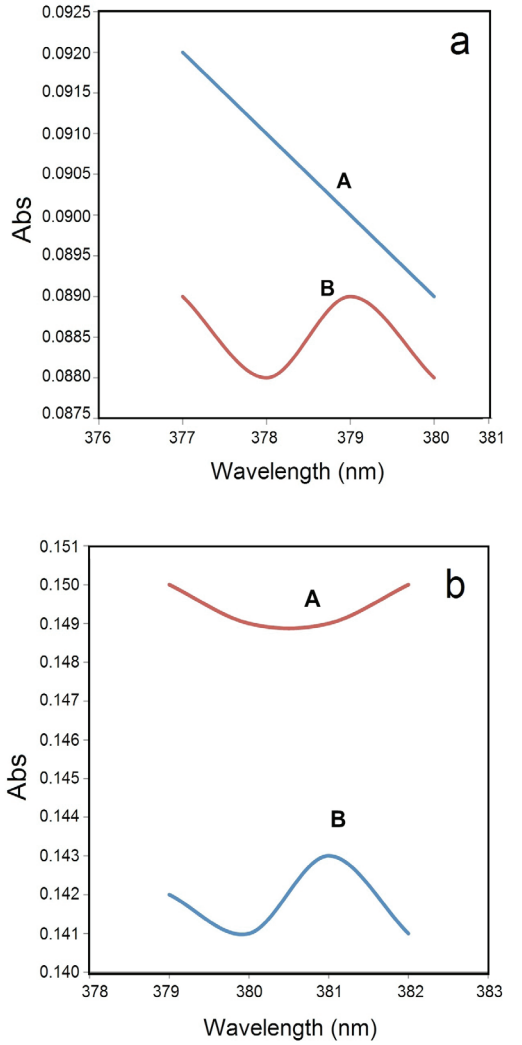

Figure 6. UV–vis absorption spectra of bR film, (A) without any light and (B) in the presence of red light for (a) 5 min and (b) 15 min.

bacteriorhodopsin, the Q state indicates a 1-bit and the ground state bR indicates a 0-bit. Hence, the bit 1 (Q state) was created by green and red light irradiation.

The P and Q intermediates absorb the blue light to return to their ground state bR. Thus, erasing the data was done via blue light (1W LED, 15 Min). The decrease in absorption at 380 nm was monitored for a 15 minute radiation which is related to the loss of the Q state and return to steady-state (Fig. 8).

The writing and erasing was performed 59 times and a very stable bacteriorhodopsin film was shown that did not lose writing capability. For the control, bovine serum albumin was used instead of bacteriorhodopsin. Bovine serum albumin was immobilized on thin films of gelatin and polyvinyl alcohol. The absorption spectra were measured before and after immobilization of this protein on the film. The green and red lights were irradiated for 5 min. The absorption spectra of film based on bovine serum albumin point to protein degradation under laser irradiation. The absorption spectra of the film based on BSA are illustrated in Figure 9.

Conclusions

This paper has reported on the use of bacteriorhodopsin embedded in gelatin and polyvinyl alcohol for holographic memories. In this report, high quality bacteriorhodopsin films have obtained with polyvinyl alcohol and gelatin solutions of 0.001 % (w/v) prepared with 0.1M phosphate buffer. Maximum absorbance was obtained at 570 nm and 280 nm which point to the excellent optical quality and low light scattering of bacteriorhodopsin films. Also, the phosphate buffer solutions were applied to optimize sensitivity of bR film. We have shown that green and red laser irradiation leads to absorption peaks about 380 nm (Q state) and 640 nm (O state). Finally, we have proved that the blue light could be used to erase the recordings (the absorbance decreasing at 380 nm). As a conclusion, this matrix is suitable for immobilization of bacteriorhodopsin protein and can be used in optical memory devices.

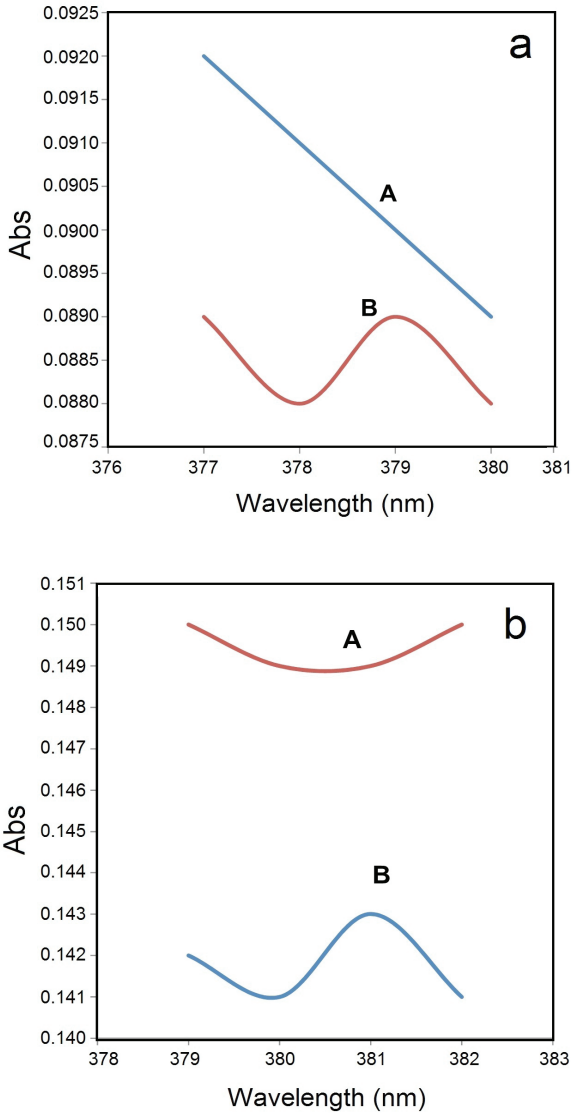

Figure 7. UV–vis absorption spectra of bR film (A) without any light and (B) in the presence of red and green lights at wavelengths (a) 638–642 nm and (b) 378–381 nm for 10 min.

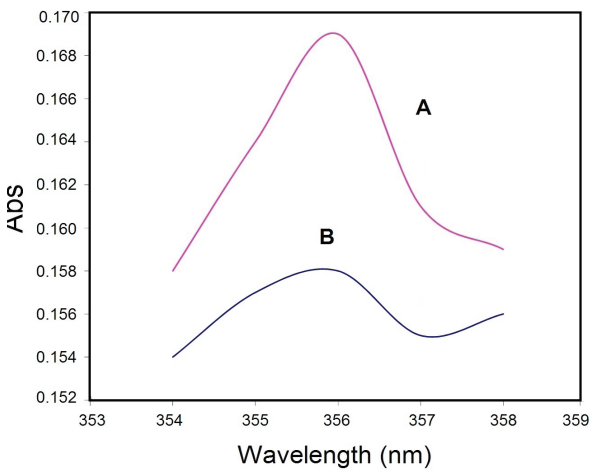

Figure 8. Light absorption spectra of bR films, (A) without any light and (B) in the presence of blue light for 15 min

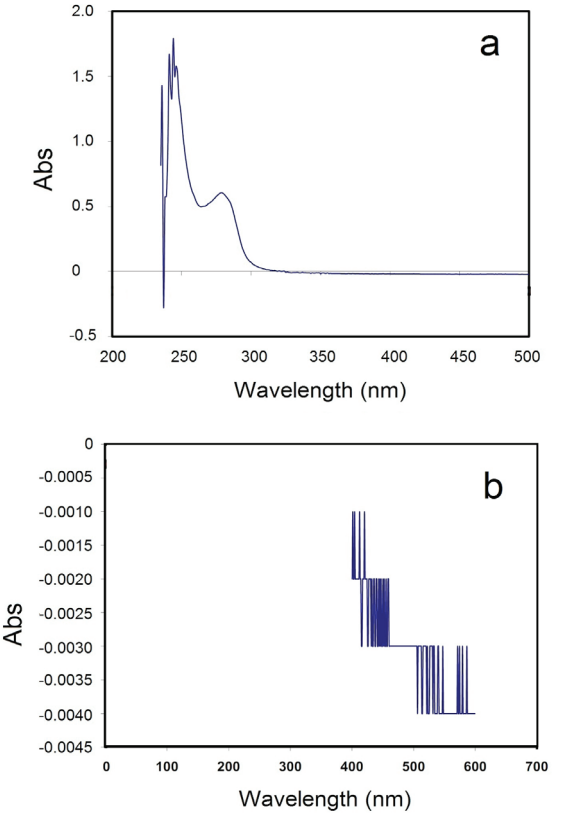

Figure 9. UV–Vis absorption spectra of BSA film (a) without any light and (b) in the presence of red lights for 5 min

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