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Light therapy for mechanical damages

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UV-VIS spectroscopy was used to study components of human tissue. Phenylalanie, dixydroxyphenylalanine, adenosine 5'-triphosphate and albumin all undergo changes under the influence of light and temperature. Characteristic bans for these materials were hightlighted and the relation to different solutions was compared. The role of UV-VIS spectroscopy for measuring tissue damages is discussed.

Keywords: UV-VIS spectroscopy, amino acids, ATP, albumin, NIR

1. Introduction

Already in ancient times light was utilised for therapeutic purposes. However, the wide application of this strong resource to diagnostic [1] and therapeutic purposes [2–5] was restricted until 1960's. Since that time, new techniques have been developed and utilised and are continually being developed. The large interest in using laser light in medicine stems, first of all, from its unique properties. The features of laser radiation can be classified as follows: monochromacy, time and space coherence, linear polarisation, only slight divergence, a large power density, and also the possibility of being used in a continuous stream or in impulses. The tissue absorption coefficient and wavelength depend on the choice of laser beam penetration depth as well as an adequate wavelength range.

The influences of laser light on tissues characteristics were divided into two groups:

- high-energy lasers,
- low-energy lasers.

The second group relates to lasers used in therapy (biostimulation, physical therapy and so on) and in diagnosis. Especially, He–Ne [6] in visible region and Ga–As [7] in infrared have widely been used in the treatment of variety of diseases, particularly involving mechanical damages. The biomedical effects of low-energy laser irradiation have recently become the focus of extensive studies [8–10], but nobody knows exactly what kinds of molecular changes and processes occur within biological systems. It is necessary to know the effects of light–tissue interactions. Of numerous effects that low-energy laser irradiation has on living organisms thermal and photochemical effects are most important.

In our experiment, we studied the effect of light and temperature (and occasionally only light) on tissue models like amino acids, polyamino acids and proteins. UV-VIS spectroscopy was chosen because it allows for the identification of these substances.

2. Materials and methods

Reagents. DL-phenylalanine, DL-3, 4-dihydroxyphenylalanine, adenosine 5'-triphosphate, and albumin were obtained from the Sigma Chemical Co. (St. Quentin Fallavier, France).

Treatments. For our experiments, those substances were dissolved in the following solutions: 2.5 mM citrate buffer, 1/15 M phosphate buffer, 0.9% NaCl and 99.8% spectrally pure methanol. The buffers were diluted in distilled water (which was boiled for half an hour to 100 °C) to eliminate all traces of carbon dioxide. Also in our experiment, we used rigid concentrations of materials added to the solutions: 0.5×10^{-4} M phenylalanine (pH = 6.5), 0.5×10^{-4} M dixydroxyphenylalanine (pH = 6.8), 0.2×10^{-4} M adenosine 5'-triphosphate (pH = 7.2) and 10^{-7} M albumin.

Experiment sorts. Two sorts of research were carried out. The first related only to the solutions temperature. In the second, the above solutions were first exposed to NIR, and then heated.

Heating. Each solution was placed into the ultrathermostat, model UTU-2/77, which contained water heated to 40 °C, 50 °C and 70 °C. Samples of each solution were removed in 5, 15 and 30 minute intervals, and then rapidly water-cooled to room temperature.

NIR exposure. A simple near infrared light source with a 700–2000 nm wavelength was used. Half the samples were placed in a special exposure vessel, and then enclosed in a closed house. Next they were exposed to a halogen lamp for half an hour at a constant air humidity and a temperature of 20 °C, maintained by a water-cooling system and an air blast chilling system, and were constantly mixed at 1000 turns/minute by a magnetic mixer, model BMM 21. The cooling systems were used for the purpose of eliminating any temperature rise during the irradiation treatment.

Measurement. Spectra were obtained by using a SPECORD UV VIS spectrometer with an UV spectral range of 50 000–28 000 cm^{-1}.

pH determination. pH of each solution (except those involving methanol) was measured at room temperature by means of pH-meter, model N 517, after each spectrometer measurement.

3. Results and discussion

UV spectroscopy can be used to identify tissue materials. Our results will be explained using the sample of DL-3,4-dihydroxyphenylalanine; all other materials studied behaved in exactly the same way. Figure 1 shows the results of dissolving DL-3, 4-dihydroxyphenylalanine in methanol; the spectrum of our irradiated control sample is shown, as well as the spectrum from a dark sample, for comparison's sake.

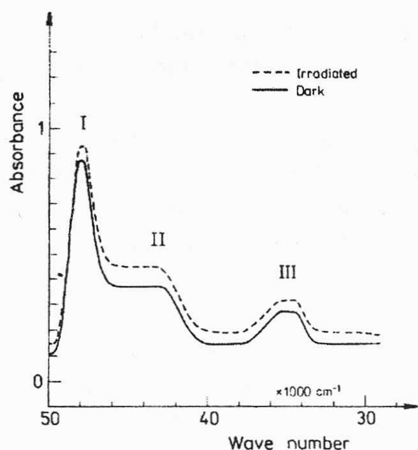

Fig. 1. UV spectra of DL-3, 4-dihydroxyphenyl -alanine dissolved in methanol for the dark and the irradiated samples

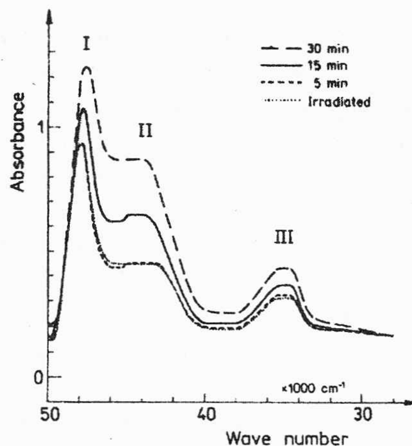

Fig. 2. UV spectra of DL-3, 4-dihydroxyphenyl -alanine dissolved in methanol for the samples irradiated at 70 °C: control one, 5, 15, 30 min

The most basic findings (Figs. 2–4) allow us to conclude that increased temperatures affect the intensity and position of each band. First, the second absorption band grew in intensity, though that growth took place at the cost of the first absorption

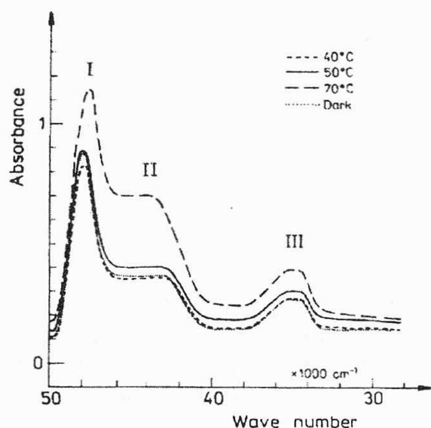

Fig. 3. UV spectra of DL-3, 4-dihydroxyphenyl -alanine dissolved in methanol for the dark samples heated for 30 min at 40, 50, 70 °C

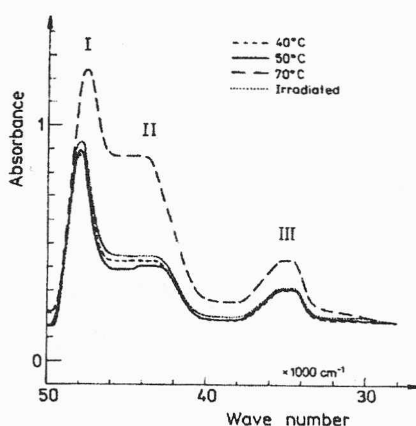

Fig. 4. UV spectra of DL-3, 4-dihydroxyphenyl -alanine dissolved in methanol for the irradiated samples heated for 30 min at 40, 50, 70 °C

band. Moreover, in the area of basic characteristics, the first absorption band indicated a movement towards longer waves. The observed changes are dependent on temperature and also on time of heating. The longer the specimen was heated, the bigger the changes that occurred. The most distinct changes occurred after 30 minutes: the NaCl spectra of our dark samples were similar at 40 °C and 50 °C, as op-

posed to the measurements at 70 °C, where perceivable and fundamental changes were observed. For both the methanol mixtures and the buffers, the largest wave intensity leap occurred between 50 °C and 70 °C.

The band intensities of the irradiated samples compared to the dark one also increased, the biggest increase in band intensity appeared in the same second absorption band (see Figs. 3, 4). The irradiated samples demonstrated the same dependence on temperature and time. The differences between only heated samples and irradiated and heated samples are as follows:

- The processes of irradiation depend on both time of heating and temperature level (see Fig. 5). It seems important that the most obvious changes are found above 50 °C.
- NIR exposure accelerates the changes occurred after heating (see Figs. 3, 4).

In the heated sample, there appears a maximum absorption shift in the first band, which indicates proton transition. The presence of the second band is connected with chemical reaction. Protonation explains the weakened hydrogen bonds under NIR radiation. Free carbonyl and amino groups create a new hydrogen bond between two neighbouring molecules. The process is more effective for less polar environment, e.g. for methanol. In contrast, strong hydrogen bonds are in the buffers, the observable effects are weaker. Exactly the same happens with proteins [11].

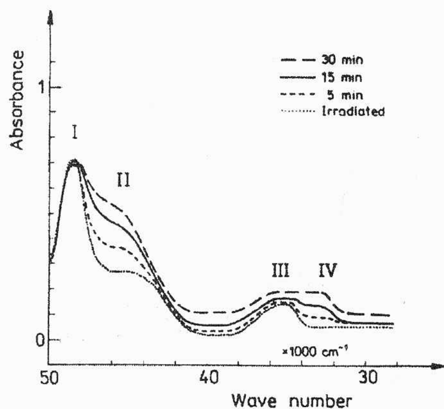

Fig. 5. UV spectra of DL-3, 4-dihidydroxyphenylalanine dissolved in citrate buffer for the irradiated samples heated for 5, 15, 30 min at 70 °C; IV – additional absorption band

Free proteins like albumin are able to join the surrounding cell membranes, which causes accelerated growth. The positive reaction took place when wounds were healing. However, there may be some unprofitable results from heating. For example, a quantity of proteins may increase in side cell membrane, resulting in the death of those damaged cells [11].

It seems that molecular mechanisms of light- and high-temperature therapies, resulting in a gain or loss of protons during the reactions, change pH of environment, and finally lead to protein agglomeration. Both of those processes can prove useful or harmful. It is therefore important to know exactly what is the molecular level of different therapies applied.

References

[1] ZENG H., LUI H., MCLEAN D.I., MACAULAY C., PALCIC B., Proc. SPIE, 1995, Vol. 2628, 281–285.
[2] STRĘK W., PODBIELSKA H., KUZMIN A.N., GES J.A., RYBATSEV G.I., KOROBKO I.YU., Proc. SPIE, 1995, Vol. 2628, 269–272.
[3] BASFORD J.R., MALANGA G.A., KRAUSE D.A., HARMSEN W.S., Arch. Phys. Med. Rehabil., 1998, Vol. 79, Iss. 3, 249–254.
[4] USUBA M., AKAI M., SHIRASAKI Y., Lasers Surg. Med., 1998, Vol. 22, Iss. 2, 81–85.
[5] SKINNER M.G., LIZUKA M.N., KOLIOS M.C., SHERAR M.D., Phys. Med. Biol., 1998, Vol. 43, Iss. 12, 3535–3547.
[6] BASFORD J.R., DAUBLE J.R., HALLMAN H.O., MILLARD T.L., MOYER S.K., Lasers Surg. Med., 1990, 10, 35–39.
[7] BULLOW P.M., JENSEN H., DANNESKIOLD-SAMOSE B., Scand. J. Rehab. Med., 1994, 26, 155–159.
[8] GOATS G.C., FLETT E., HUNTER J.A., Stirling, 1994, 82, 311–320.
[9] USUBA M., AKAI M., SHIRASAKI Y., Lasers Surg. Med., 1998, 22, 81–85.
[10] SIEROŃ A., CIEŚLAR G., ADAMIAK M., *Magnetoterapia i laseroterapia. Podstawy biofizyczne. Oddziaływania biologiczne. Zastosowania kliniczne.* Śląska Akademia Medyczna, Katowice, 1994.
[11] KOTER M., *Termoczułość błony plazmatycznej*, Wydawnictwo Uniwersytetu Łódzkiego, Łódź, 1994.