OPTICAL CLEARING OF THE RAT SKIN *EX VIVO* BY GLYCEROL-DMSO SOLUTION IN THE SPECTRAL RANGE OF 350-2500 NM

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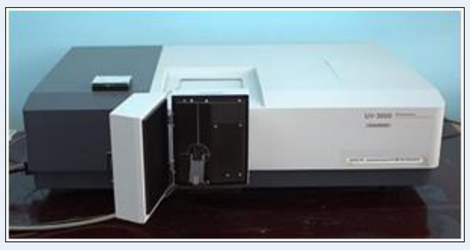
The goal of the study was to measure total transmission and diffuse reflectance spectra of skin samples and to obtain spectra of the reduced scattering of samples before and after immersion in glycerol-DMSO solution. DMSO was chosen as an optical clearing enhancer. The study were performed in a wide spectral range 350-2500 nm. The study shows the optical clearing effect on the rat skin *ex vivo* in a wide spectral range.

MATERIALS AND METHODS

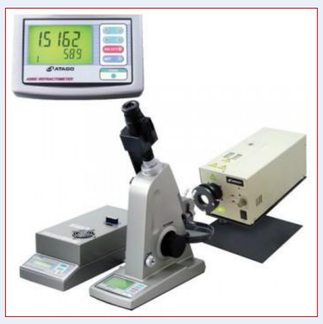
The experimental study was performed for ex *vivo* skin of white Wistar rats. Recording of diffuse reflectance and total transmission spectra of samples was carried out using a UV-3600 spectrophotometer with an integrating sphere (Shimadzu, Japan) in the spectral range of 350-2500 nm with a step width of 2 nm before and after 3 hours of immersion in aqueous 70%-glycerol solution with 10% of DMSO.



UV-3600 spectrophotometer (Shimadzu, Japan)

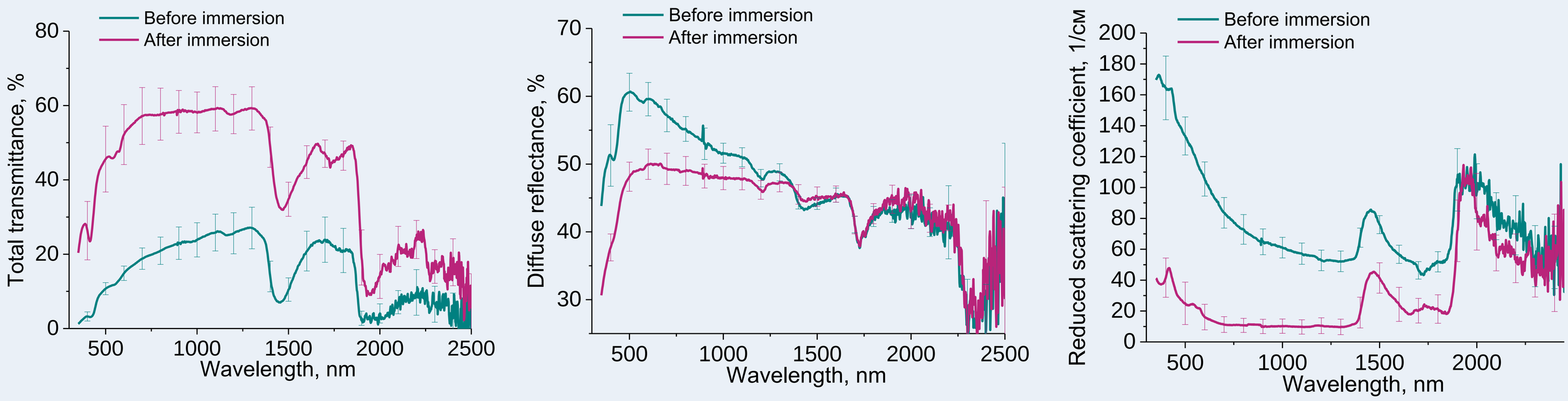
The measured total transmission and diffuse reflectance spectra of samples were used in calculating the spectral dependences of the reduced scattering coefficient of the samples using the algorithm [1] described in [2].

The refractive indices (RI) of skin before and after optical clearing were measured using a multi-wavelength Abbe refractometer DR-M2/1550 at a wavelength of 589 nm as 1.4660 and 1.4584 respectively. [3].



Abbe Multiwave Refractometer DR-M2/1550

RESULTS



a b c

Total transmittance (a) diffuse reflection (b) and reduced scattering (c) of the skin samples before and after immersion in aqueous 70%-glycerol solution with 10% of DMSO

The thickness of skin samples before and after immersion in aqueous 70% solution of glycerol with 10% of DMSO was 0.82±0.11 mm and 0.77±0.08 mm respectively.

The efficiency of optical clearing of skin samples was estimated as the ratio of the difference between the values of the reduced scattering coefficient of the sample, measured before μ'_{s_0} and after μ'_{s_oc} immersion in glycerol-DMSO solution, to the initial μ'_{s_0} value:

$$OC_{eff} = \frac{\mu'_{s\_0} - \mu'_{s\_oc}}{\mu'_{s\_0}}$$

The efficiency of optical clearing of skin samples

Wavelength, nm	415	548	700	1000	1460	1700	1950
OC_{eff}, %	71±6	81±8	87±5	82±8	44±12	50±17	2±20

Refractive index (RI) of aqueous 70%-glycerol solution with the addition of 10% DMSO at different wavelengths in the range from 480 to 1550 nm

Wavelength, nm	480	486	546	589	644	656	680	930	1100	1300	1550
RI	1.4507	1.4505	1.4466	1.4450	1.4428	1.4422	1.4404	1.4369	1.4338	1.4297	1.4245

CONCLUSION

The transmittance of skin increased significantly after exposure of the tissue to the solution. In the visible wavelength range, the transmission of the sample increased by approximately 35±5%, and in the IR range, by 30±5%. Noises are observed in the long-wavelength region are associated with the low sensitivity of the setup. Reduction in skin reflectance was obtained in the short wavelength range up to 1200 nm. A significant decrease in the reduced scattering coefficient (2-7 times) in almost the entire spectral range is demonstrated. Hemoglobin absorption peaks become more pronounced in the short-wavelength range (415-580 nm). Decrease of the reduced scattering coefficient at a wavelength of 1450 nm is demonstrated in the water absorption band, which indicates tissue dehydration.

References:

1. Inverse Adding-Doubling https://omlc.org/software/iad/index.html
2. S. Prahl, Everything I think you should know about Inverse Adding-Doubling. *Oregon Medical Laser Center, St. Vincent Hospital*, 1-74 (2011).
3. D. Zhu, E. Genina, V. Tuchin (Eds.), Tissue optical clearing: new prospects in optical imaging, CRC Press (imprint of Taylor & Francis Group, LLC). 1st Edition 2022, 682 p.

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