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IMPROVE OPTICAL CLEARING OF SKIN *IN VITRO* WITH PROPYLENE GLYCOL AS A PENETRATION ENHANCER

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In order to enhance the optical clearing effect of topically applied optical clearing agents (OCAs), we evaluated the effect of propylene glycol (PG) as a chemical penetration enhancer (PE) on optical clearing of skin *in vitro* by observation and measurement of optical-transmittance and diffuse-reflectance spectra. Three OCAs, i.e., glycerol, D-sorbitol and PEG400, and two other penetration enhancers, Azone and Thiazone, were used in this study. The results indicated that the decrease of reduced scattering coefficient caused by OCA/PG was larger than that by pure OCA, and the change by OCA/water was the least after the same treatment time. There were significant differences for the reduced scattering coefficient at 630 nm after 120 min application of agents between OCA and OCA/PG. The efficacy of optical clearing caused by OCA/PG depended on the OCA itself. When PEG400 was mixed with three different PEs, we found the optical clearing were different. The penetration enhancing ability of PG was much better compared to Azone, and suboptimal to Thiazone. Also, this study provides evidence for the use of PG as a PE in order to improve skin optical clearing.

Keywords: Skin; penetration enhancer; propylene glycol; optical clearing agent.

1. Introduction

Optical diagnostic and therapeutic techniques have become a hotspot in the area of life science and technology. However, because of the limited penetration depth of visible and near infrared light caused by the high scattering of biological tissue, the clinical application of optical techniques is extremely restricted. Tissue optical clearing technique proposed by Tuchin[1,2] can reduce the optical scattering and improve light penetration depth in tissues by introducing hyperosmotic, high-refractive-index chemical agent, i.e. Optical Clearing Agent (OCA) into tissue. It provides new opportunity for the development of biomedical optical diagnosis and therapy.

Numerous work has been conducted on optical clearing of different tissues, among which skin is the most attracted one.[3–6] However, due to the resistance of the outermost layer of the skin called stratum corneum (SC), it is difficult for OCAs to penetrate into the dermis, which makes the efficacy of optical clearing with topically applied agents suboptimal. To overcome the barrier function of the SC, a number of physical and chemical methods have been proposed to accelerate the penetration of OCAs into dermis and improve the skin optical clearing effect. Physical methods, including the use of sandpaper,[7] lattice of islets of damage,[8] microneedles,[9] etc. were attempted to breach the SC barrier. Unfortunately, these methods are

harmful to skin, and the results are hard to be quantified and the repeatability is poor.

Compared to the invasive physical methods, a non-invasive way is to incorporate a chemical penetration enhancer (PE) in the transdermal formulation to reduce the SC barrier and enhance the permeability of drugs into skin.[10,11] In fact, chemical penetration enhancer has been commonly used for the enhancement of drug penetration into tissue in clinical medicine. Currently, it was introduced into the research area of skin optical clearing. Jiang *et al.* combined DMSO or oleic acid with propylene glycol (PG) and Xu *et al.* mixed Azone with glycerol or PG, respectively,[12,13] then they applied the mixtures topically on porcine skin *in vitro* and found that all the mixtures were able to improve the optical clearing effect of skin effectively compared to single agent. However, although PG was always used as an OCA in tissue optical clearing research, its effect is not as ideal as glycerol or PEG400, for example, when applied with the same concentration.[14] In fact, PG was also considered to be a useful penetration enhancer in clinical application, especially for alcohol soluble drugs.[11,15,16]

Hence, in our study we concentrated on the evaluation of PG as a PE mixed with several commonly used alcohols (OCAs) for the enhancement of skin optical clearing *in vitro*. Further, we compared the penetration enhancing capability of PG with other PEs (Azone, Thiazone).

2. Materials and Methods

2.1. *Chemical agents*

In this study, three penetration enhancers, Propylene Glycol (PG), Azone, and Thiazone were chosen. Among them, Azone is commonly used and widely recognized as chemical skin penetration enhancer.[13,17] Whereas Thiazone (chemical name of 1,2-benzisothiazole-3 (2H)-2-butyl-1,1-dioxidea), a derivative of Azone, is a new chemical PE designed by Applied Chemistry Institution of Beijing Normal University in China, and has a penetration enhancing effect three times higher than Azone.[18−21] PG is what we mainly want to study about as a PE.

The previous study shows that the optical clearing effect of skin induced by alcohols is better than other agents.[14] The more hydroxyl groups alcohol contains, the better the optical clearing effect of the skin is.[14] Meanwhile, the solubility of OCA and PG was also considered. Hence, we chose three multihydric alcohols as OCAs, i.e., D-sorbitol (hexahydric alcohol), glycerol (trihydric alcohol), and polyethylene glycol (PEG400, dihydric alcohol) (Qiangsheng Chinese Chemicals, Limited, China), which are all mixable with PG.

In order to compare how the chemical penetration enhancer PG improves different OCAs' optical clearing effect of skin *in vitro*, the OCAs, the OCAs with water or PE as ratio of 19:1 were designed and listed in Table 1. In the table, the numbers represent the percentages of chemical substance and water. Among them, pure D-sorbitol is solid, and its saturated solution is only 70%. Hence, we use 70% D-sorbitol as pure OCA to mix with water or PG in the volume ratio of 19:1.

2.2. *Preparation of skin samples and experimental protocol*

Fresh porcine skin was obtained from an accredited abattoir and the fat tissue beneath dermis

Table 1. Agents used in this study.

Solutions	G	D-sorbitol	PEG	Azone	Thiazone	PG	H_2O	RI*
Glycerol(100%)	100							1.471
Glycerol/water(19:1)	95						5	1.458
Glycerol/PG(19:1)	95					5		1.468
D-sorbitol(70%)		70					30	1.466
70%D-sorbitol/water(19:1)		65					35	1.458
70%D-sorbitol/PG(19:1)		66.5				5	28.5	1.462
PEG400(100%)			100					1.469
PEG400/water(19:1)			95				5	1.460
PEG/Azone(19:1)			95	5				1.461
PEG/Thiazone(19:1)			95		5			1.462
PEG/PG(19:1)			95			5		1.470
PG(100%)						100		1.433

*RI represents refractive indices of agents measured by Abbe Digital Refractometer.

was carefully removed. The skin samples, 3 cm × 3 cm, were sealed to prevent natural dehydration and stored at 4°C for no longer than four hours before use. In this work, all samples ($n = 64$) were divided into 12 groups, which included 11 experimental groups (agents listed in Table 1) and a control group (saline). Five to six samples for each group were used to perform an experiment with the same agent. The thickness of sample was measured with a micrometer. The native thickness was about 1.38 ± 0.21 mm.

A commercially available spectrophotometer (Lambda 950, PerkinElmer, USA) with an integrating sphere of 150 mm in diameter and an entrance-port and an exit-port of 1 inch in diameter was applied to measure the transmittance and the reflectance of the sample. A section of prepared porcine skin sample was placed between two glass slides, and this component was placed in the apertures of the integrating sphere for measurements. A beam of 15 mm × 7 mm irradiated on the skin sample, and the spectra of transmittance or reflectance could be obtained (as reference). Then a chemical solution listed in Table 1 or saline was topically applied onto the epithelium surface of the sample, and the spectra and thickness were obtained at the time points of 15, 30, 60, and 120 min, respectively. In order to eliminate the mirror reflection, the solution on the sample was removed just before acquiring the spectrum and added again right after the measurement. The scanning wavelength range of the measurement was 400–1000 nm with 10 nm interval.

Apart from spectroscopic measurements, direct observation of the optical clearing process on the skin sample was done with a camera (Sony DSCT-200, Japan). Photos of skin sample placing on a specially designed background (Logo of CBMP) were taken at the time intervals for spectroscopic measurements. All photos were taken under the same illumination condition in a bright room and the distance from skin sample to camera was about 10 cm.

2.3. *Quantitative analysis*

With the new IAD program (2007) developed by Dr Prahl from Oregon Medical Laser Center[22,23] we calculated the optical properties of skin sample at different time intervals based on the measurements of transmittance, reflectance spectra and the thickness of sample. The parameters used in the calculation were as follows: default anisotropy factor was 0.7, and the number of quadrature point was 8. Since a dual beam system was used and the integrating sphere properties are unknown, the program makes no corrections for the integrating sphere. Reduced scattering coefficient (μ_s') is a good indicator for the optical clearing effect because the introduction of OCA into tissue mainly affects the scattering rather than the absorption. Due to the reduction of light scattering, the tissue becomes more cleared. Hence, the relative change in μ_s' after the application of agents was calculated according to:

$$\Delta\mu_s' = \frac{\mu_{s\ \text{treated}}' - \mu_{s\ \text{native}}'}{\mu_{s\ \text{native}}'}, \tag{1}$$

where the subscripts "native" and "treated" refer to the samples before and after the application of agent at the different time intervals, respectively.

In addition, we also carried out a statistical analysis to compare the differences between the reduction of μ_s' caused by agents and saline at special wavelength (630 nm). A t-test was used to compare the significance between paired conditions. The hypothesis that samples from two sets of data could come from the same population, i.e., the expected value of one set is equal to the expected value of another set, is tested.

3. Results and Analysis

3.1. *OCA-induced optical clearing of skin*

In order to directly observe the optical clearing of skin caused by OCAs, photos of background trough skin with different OCAs treatment are taken at time points of 0 (native state), 15, 30, 60, and 120 min. The typical results are shown in Figs. 1(a) to 1(e) represent the groups of Glycerol, D-sorbitol, PEG400, PG and saline, respectively and each group includes OCA, OCA/water and OCA/PE with the same volume ratio of 19:1 except for PG and saline group. The PG and saline group were set as control groups, from Fig. 1(d) and Fig. 1(e) we can see that saline almost induced no change and no clearing effect to the skin, while PG, whose refractive index is 1.43, induced a clearing effect at 120 min. From each row, we can see that, with continuous treatment, the skin samples become clearer and clearer, and finally we are able to see the background partly or totally except for

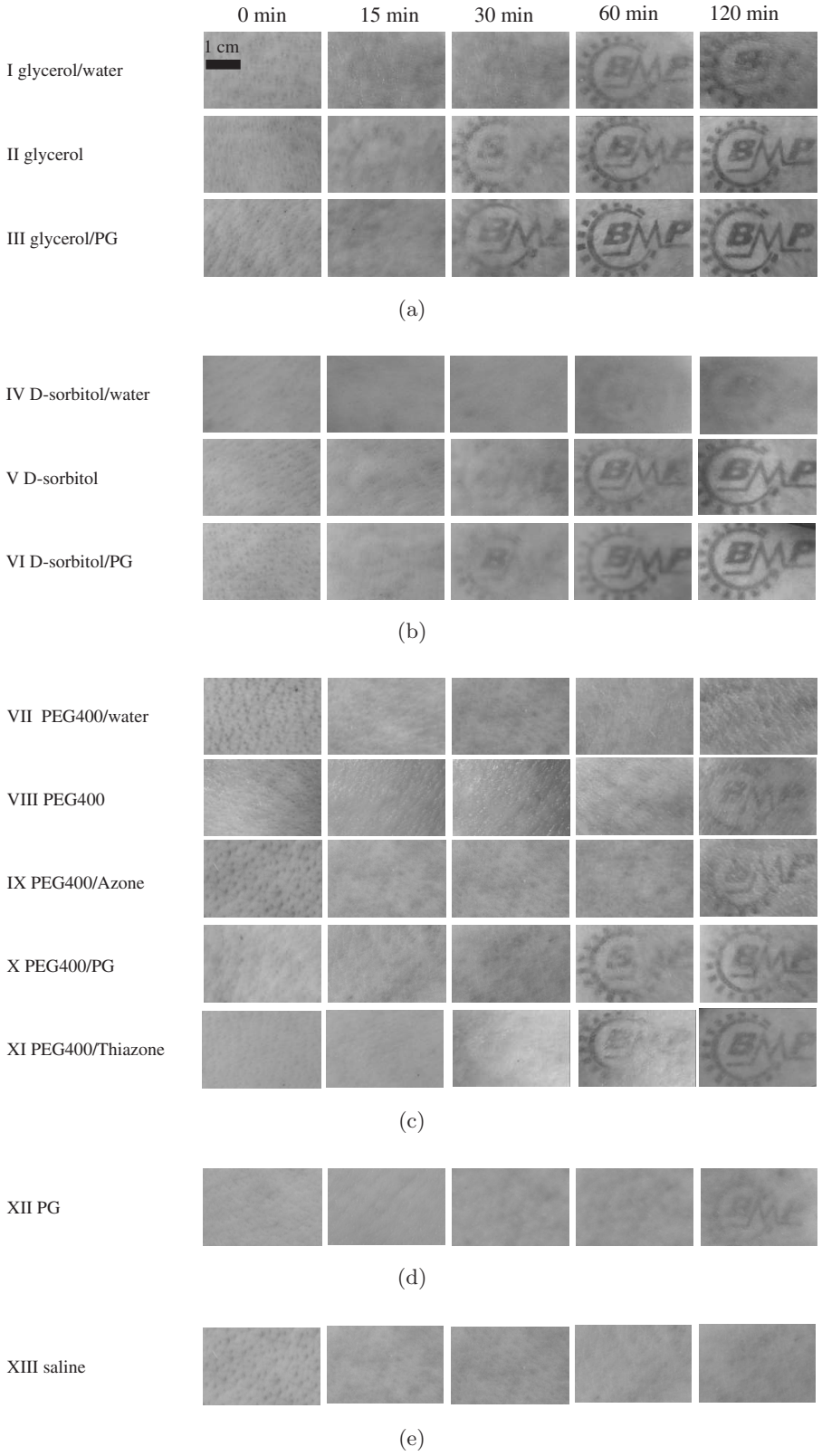


Fig. 1. Changes of skin samples after treatments with different agents. Among them, (a) the group of glycerol, (b) the group of 70%D-sorbitol, (c) the group of PEG400, (d) pure PG, (e) saline.

the group of saline. Furthermore, at the same treatment time, the optical clearing effect varies for different agents as well as for different groups.

Among each group, obvious discrepancy can be found between the samples treated by pure OCA, with water or with PE. From Fig. 1(a) glycerol group, we can see that, in row III which shows the sample treated by glycerol/PG, background can be identified at 30 min and it can be seen more and more clearly with increasing time; row II which represents the sample treated by glycerol only, turned clear more or less at 30 min; whereas at the same time point of 30 min, row I which was sample treated by glycerol/water, was still very turbid and the background could not be identified.

In D-sorbitol group [Fig. 1(b)], because the saturated concentration of D-sorbitol is 70%, so this was considered to be the highest concentration. It was then mixed with water and PG (V/V 19:1). The agent with PG induced the best clearing effect, enabling us to observe the pattern underneath the skin at around 30 min; the effect of 70% D-sorbitol/water was the worst.

PEG400 can be mixed with different PEs, and Fig. 1(c) shows the clearing effects of PEG400 group. The results indicate that (i) treatment with PEG400/water could not induce good clearing effect even at 120 min, and (ii) pure PEG or PEG/Azone could only induce optical clearing at 120 min. In contrast, PEG/PG or PEG/Thiazone treatment enabled us to see the pattern at 60 min, and more clearly at 120 min, but the difference between these two agents was not obvious.

By comparing the effect of the three groups, we can observe that, for pure OCAs, glycerol induced the best clearing effect, followed by 70% D-sorbitol, while PEG400 was the worst. If water was added into the OCAs, the optical clearing effect turned to be worse but the order for the three agents was the same as pure OCAs; however, when PG was added into the OCAs, the clearing effect turned to be better and the order did not change, i.e., glycerol/PG induced the best clearing effect, 70% D-sorbitol/PG the second, and PEG400/PG the worst.

3.2. *Changes in transmittance, reflectance and reduced scattering coefficient spectra during optical clearing process*

Figure 2 shows the typical measured optical transmittance and reflectance spectra over 400–1000 nm at time intervals of 0, 15, 30, 60, and 120 min for glycerol group. From the figures we can find that the optical transmittance increases and reflectance decreases against treatment time. This indicates that more light penetrates the skin and the depth of penetration was enhanced. However, differences

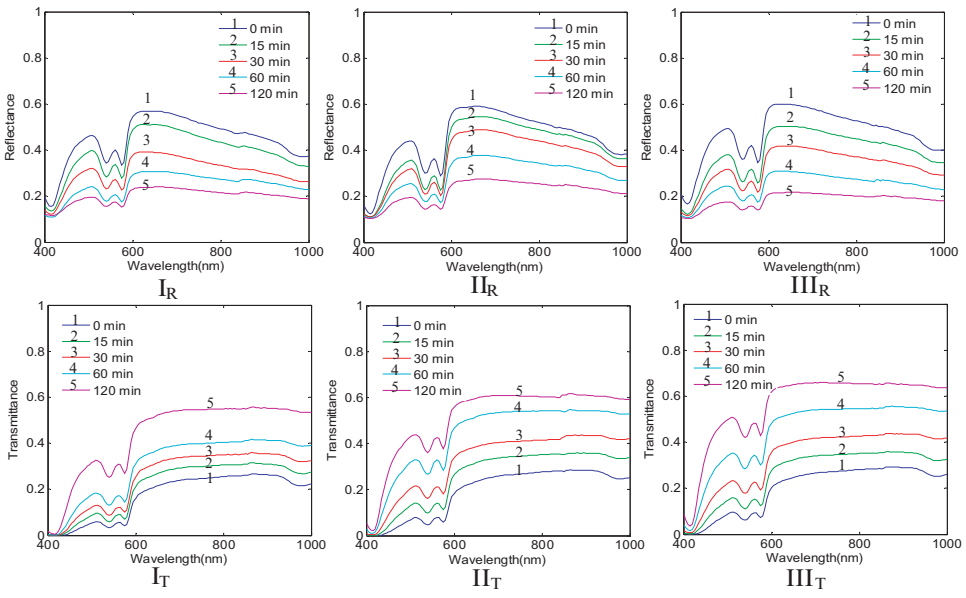


Fig. 2. Typical measured optical reflectance and transmittance spectra over 400–1000 nm for fresh porcine skin samples. I, II, and III represent treatments with glycerol/water, glycerol and glycerol/PG, respectively; top row shows reflectance spectra and bottom row shows transmittance spectra.

Table 2. Changes of skin thickness after agent application for different time interval.

Agent group	Agent	Change of thickness (mm)				
		0 min	15 min	30 min	60 min	120 min
Glycerol group	Glycerol/water	1.36 ± 0.08	1.24 ± 0.06	1.18 ± 0.04	1.14 ± 0.06	1.08 ± 0.04
	Glycerol	1.34 ± 0.10	1.20 ± 0.08	1.12 ± 0.08	1.08 ± 0.04	1.04 ± 0.06
	Glycerol/PG	1.32 ± 0.08	1.16 ± 0.08	1.10 ± 0.06	1.04 ± 0.04	1.00 ± 0.06
D-sorbitol group	70%D-sorbitol/water	1.40 ± 0.06	1.28 ± 0.10	1.20 ± 0.08	1.16 ± 0.06	1.08 ± 0.06
	70%D-sorbitol	1.38 ± 0.10	1.26 ± 0.06	1.18 ± 0.10	1.10 ± 0.06	1.04 ± 0.08
	70%D-sorbitol/PG	1.40 ± 0.10	1.22 ± 0.10	1.12 ± 0.08	1.06 ± 0.08	1.02 ± 0.08
PEG400 group	PEG400/water	1.34 ± 0.08	1.28 ± 0.08	1.24 ± 0.06	1.18 ± 0.06	1.12 ± 0.06
	PEG400	1.42 ± 0.06	1.32 ± 0.10	1.26 ± 0.06	1.20 ± 0.04	1.14 ± 0.04
	PEG400/Azone	1.44 ± 0.06	1.30 ± 0.06	1.24 ± 0.08	1.18 ± 0.06	1.12 ± 0.04
	PEG400/Thiazone	1.38 ± 0.10	1.30 ± 0.04	1.20 ± 0.06	1.12 ± 0.08	1.06 ± 0.08
	PEG400/PG	1.36 ± 0.08	1.28 ± 0.08	1.20 ± 0.06	1.14 ± 0.08	1.10 ± 0.06
control	PG	1.40 ± 0.04	1.34 ± 0.08	1.28 ± 0.06	1.22 ± 0.04	1.16 ± 0.06
	Saline	1.38 ± 0.06	1.38 ± 0.08	1.36 ± 0.08	1.36 ± 0.06	1.34 ± 0.06

can be found between changes caused by the three agents. The changes in both reflectance and transmittance induced by glycerol/PG are larger than those of pure glycerol, and the glycerol/water induced the least changes. The measurements match well with the results from above pictures shown in Fig. 1.

Although OCA treatment can induce increase in transmittance and decrease in reflectance, these changes could be affected by the thickness of samples. Hence, it would be more persuasive to use the change of tissue optical scattering property to evaluate the optical clearing effect of different agents. We calculated the reduced scattering coefficient spectra from the measured reflectance and transmittance spectra, as well as the thickness of skin with IAD program. The change of thickness is presented in Table 2; all the thicknesses were average values of 5 to 6 measurements with micrometer and given as mean with standard deviation. From the table, we can see that the initial thicknesses were between 1.3 mm and 1.5 mm and they decreased according to the treatment time.

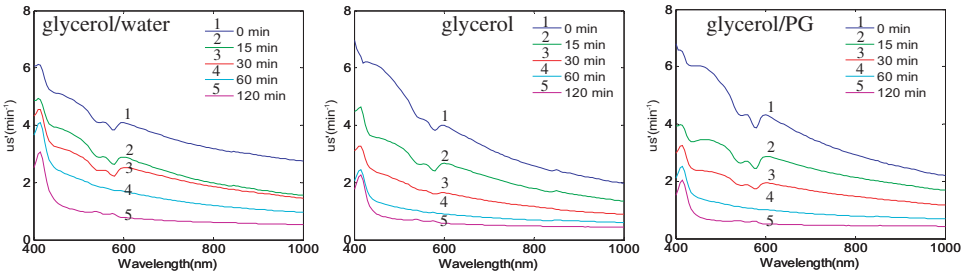
In the wavelength range of 400 to 1000 nm, the change in absorption coefficient is very slight for all of the skin samples during the optical clearing process. Here we only pay attention to the changes in reduced scattering coefficient (μ_s') of skin. Figure 3 shows the change in μ_s' (1/mm) at different treatment time intervals for all agents used.

Figure 3 shows that μ_s' decreased significantly in the whole wave band after all the agents treatment except for the control group, and with the increasing treatment time, scattering of skin became smaller and smaller. Nevertheless, the amount of decrease in scattering coefficient varies for different agents. There also exists difference in the initial value for different skin sample; thereby statistical analysis is still needed to better evaluate the effect of different agents.

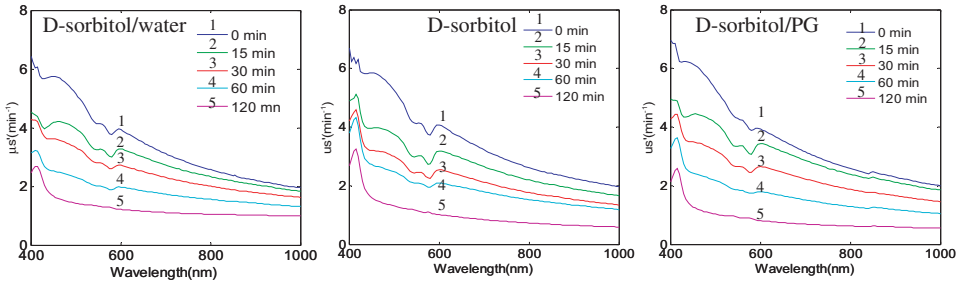
3.3. *Statistical analysis of optical properties at typical wavelength*

In order to better compare the optical clearing effect of different agents quantitatively, we analyzed the relative changes of $\Delta\mu_s'$ at 630 nm after 60 min and 120 min treatment of agents. Figure 4 presents the mean and the deviation of relative change of μ_s' at 630 nm. Since the relative changes of μ_s' at 120 min were all larger than those at 60 min, we only analyzed data of the three groups at 120 min. Further, statistical analysis was done for the values of several samples.

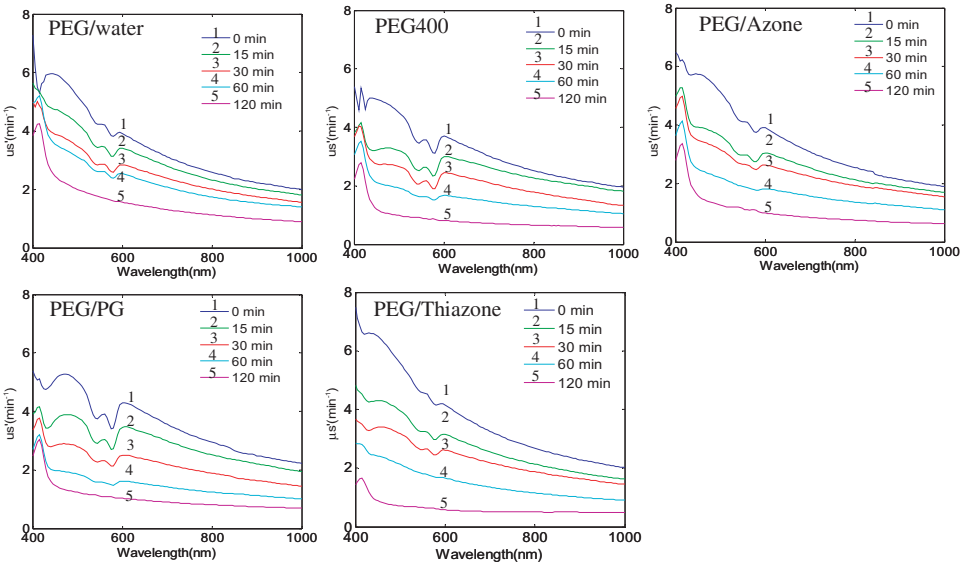
Figure 4 clearly demonstrates the enhancement of skin optical clearing by addition of PEs into OCAs. Figure 4(a) was the control group which showed the reduction of μ_s' caused by saline and pure PG. Saline application induced only minor reduction while PG induced more pronounced reduction. However, reduction induced by PG was smaller than the other three pure OCAs, which reflected suboptimal clearing effect. Hence, PG is not a good OCA as the other three alcohols. In Fig. 4(b), application of glycerol/PG resulted in more pronounced reduction of μ_s', and compared



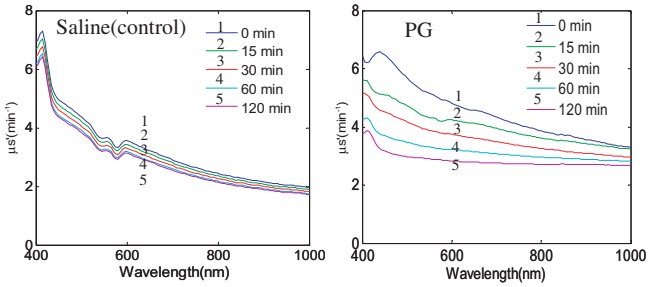
(a)



(b)

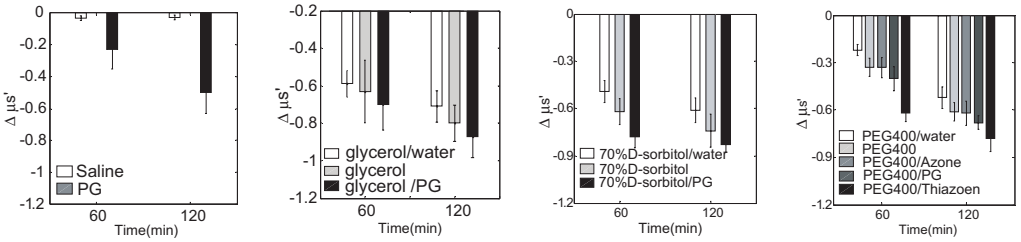


(c)



(d)

Fig. 3. Typical changes of calculated μ_s' (1/mm) spectra from measured reflectance and transmittance spectra with IAD program. (a) Glycerol group, (b) D-sorbitol group, (c) PEG400 group, and (d) control. The curves were obtained from the time intervals of 0, 15, 30, 60, and 120 min from top to bottom.



(a) (b) (c) (d)

Fig. 4. The relative changes of μ_s' at 630 nm after treatment with different agents. (a) Control group including saline and PG, (b) glycerol group, (c) D-sorbitol group, and (d) PEG group.

to glycerol and glycerol/water the reduction was enhanced by 7% and 16%, respectively. The differences are significant between glycerol/PG and glycerol ($p < 0.05$); between glycerol/PG and glycerol/water ($p < 0.05$). In Fig. 4(c), application of 70% D-sorbitol/PG also resulted in more pronounced reduction of μ_s', and compared to 70% D-sorbitol and 70% D-sorbitol/water, the reduction was enhanced by 9% and 22%, respectively. The differences are significant between 70% D-sorbitol/PG and 70% D-sorbitol ($p < 0.05$); between 70% D-sorbitol/PG and 70% D-sorbitol/water ($p < 0.01$).

Figure 4(d) demonstrates that the differences exist not only for agents with PE and water, but also for agents with different PEs. Compared to pure PEG400, application of PEG/Azone, PEG/PG and PEG/Thiazone enhanced the reduction of μ_s' by 2%, 8%, and 17%, respectively. Statistical analysis shows that the differences are significant between PEG/PG and PEG400 ($p < 0.05$) and between PEG/Thiazone and PEG400 ($p < 0.01$). However, there is no significant difference between PEG/Azone and PEG400 ($p > 0.05$).

4. Discussion

Integrated analysis of the three groups of agents, which include pure OCA, OCA/water and OCA/PG or other PEs, shows that after the addition of water into OCAs, the optical clearing effect they could induce are as poor as OCAs. This is in accordance with previous results reported, that is the higher the concentration of OCA, the better the clearing effect.[24,25]

In pure OCAs, after the same treatment time, glycerol induced the largest reduction in scattering of skin, i.e., the most effective optical clearing, while PG induced the least effect. This is due to the fact that the optical clearing effect of skin induced by alcohols is related to the number of hydroxyl groups according to our previous study, and the more the hydroxyl groups, the better the optical clearing effect.[14] Unfortunately, 70% D-sorbitol is a hexahydric alcohol, but its clearing effect is not as good as glycerol. Probably, because D-sorbitol's saturated concentration is only 70%. In our study, 70% D-sorbitol almost induced the same clearing effect as 95% glycerol. Hence, we could conclude that the optical clearing effect caused by 70% D-sorbitol was better than that by 70% glycerol.

Compared to pure OCAs, mixtures with PE induced better skin optical clearing effect. If we further compare the efficacy of different OCAs with PG (penetration enhancer), after the same treatment time, the decrease of reflectance and the increase of transmittance caused by different agents varies with the descending order of glycerol/PG, 70% D-sorbitol/PG and PEG400/PG, which is the same as pure OCAs. Previous investigation showed that the optical clearing effect induced by PG itself was worse than that by the three OCAs.[14] However, the efficacy was improved after the mixture of OCA with PG topically applied on skin. This proves that PG actually enhances the permeability of OCAs into skin, and has the effect of a penetration enhancer.

The penetration enhancing effect of PG was also compared to Thiazone and Azone. Our experiments show that mixtures of PEG400 with PG, Azone or Thiazone, vary in the effectiveness for the enhancement of optical clearing effect, and the effectiveness descends in the order of Thiazone, PG, and Azone. This indicates that differences exist in the enhancement of OCAs' permeability by different PEs. The reason may be due to that different PEs have different mechanisms. The solvation of keratin within the SC by competition with water for the hydrogen bond binding sites and the intercalation in

the polar head groups of the lipid bilayers by propylene glycol are postulated as mechanisms for the penetration enhancing effects of propylene glycol.[15] Whereas for Thiazone and Azone, a possible mechanism would be that the fluidity of the hydrophobic stratum corneum regions is increased and the permeation resistance of the horny layer against drug substances is reduced.[26] Although the penetration enhancing effect of PG is suboptimal to Thiazone, PG can be used more widely as it is a co-solvent and can be mixed with various OCAs.

According to our results, no significant difference exists between PEG400/Azone and pure PEG400 in the reduction of μ_s', which is different from the results in Xu's work.[13] This is because in Xu's work, the improvement of optical clearing effect was evaluated only through the change of reflectance and transmittance, but not the change of μ_s'. However, during experiments, thickness could not be guaranteed to be the same for all the skin samples, hence, μ_s' can better evaluate the change of skin optical clearing than transmittance or reflectance.

Although our work led to an improvement in the research about the effect of PG as a PE to enhance skin optical clearing and compared the effects of different PEs, further studies are needed to uncover the detailed mechanism of PE enhancing OCAs' permeability, and to find useful chemical penetration enhancing methods.

5. Conclusion

In this study, we investigated the influence on *in vitro* skin optical clearing effect caused by addition of PG into different OCAs with a concentration (5%) through direct observation and spectrometric measurement. The results indicated that OCA/PG-induced decrease of μ_s' (630 nm) was larger than that of pure OCA or OCA/water, and made the skin more transparent. However, the efficacy of optical clearing of OCA/PG also depends on the OCA itself. When different PEs were mixed with PEG400, the optical clearing effect varied. The penetration enhancing ability of PG was demonstrated to be much better compared to Azone, and suboptimal relative to Thiazone.

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References

1. V. V. Tuchin, I. L. Maksimova, D. A. Zimnyakov, I. L. Kon, A. H. Mavlutov, A. A. Mishin, "Light propagation in tissues with controlled optical properties," *J. Biomed. Opt.* **2**, 401–417 (1997).
2. V. V. Tuchin, "Optical immersion as a new tool to control optical properties of tissues and blood," *Laser Phys.* **15**, 1109–1136 (2005).
3. G. Vargas, J. K. Barton, A. J. Welch, "Use of hyperosmotic chemical agent to improve the laser treatment of cutaneous vascular lesions," *J. Biomed. Opt.* **13**(2), 021114 (2008).
4. B. Choi, L. Tsu, E. Chen, T. S. Ishak, S. M. Iskandar, S. Chess, J. S. Nelson, "Determination of chemical agent optical clearing potential using *in vitro* human skin," *Lasers Surg. Med.* **36**, 72–75 (2005).
5. G. Vargas, K. F. Chan, S. L. Thomsen, A. J. Welch, "Use of osmotically active agents to alter optical properties of tissue: Effects on the detected fluorescence signal measured through skin," *Lasers Surg. Med.* **29**, 213–220 (2001).
6. M. H. Khan, B. Choi, S. Chess, K. M. Kelly, J. McCullough, J. S. Nelson, "Optical clearing of *in vivo* human skin: Implications for light-based diagnostic imaging and therapeutics," *Lasers Surg. Med.* **34**, 83–85 (2004).
7. O. Stumpp, B. Chen, A. J. Welch, "Using sandpaper for noninvasive transepidermal optical skin clearing agent delivery," *J. Biomed. Opt.* **11**(4), 041118 (2006).
8. V. V. Tuchin, G. B. Altshuler, A. A. Gavrilova, A. B. Pravdin, D. Tabatadze, J. Childs, I. V. Yaroslavsky, "Optical clearing of skin using flashlamp-induced enhancement of epidermal permeability," *Lasers Surg. Med.* **8**, 824–836 (2006).
9. J. Yoon, T. Son, E. Choi, B. Choi, J. S. Nelson, B. Jung, "Enhancement of optical skin clearing efficacy using a microneedle roller," *J. Biomed. Opt.* **13**(2), 021103 (2008).
10. J. Hadgraft, "Modulation of the barrier function of the skin," *Skin Pharmacol. Appl. Skin. Physiol.*, **14**(suppl 1), 72–81 (2001).
11. H. Trommer, R. H. H. Neubert, "Overcoming the stratum corneum: The modulation of skin penetration," *Skin Pharmacol. Physiol.* **19**, 106–121 (2006).
12. J. Jiang, R. K. Wang, "Comparing the synergistic effects of oleic acid and dimethyl sulfoxide as vehicles for optical clearing of skin tissue *in vitro*," *Phys. Med. Biol.* **49**, 5283–5294 (2004).

13. X. Xu, Q. Zhu, "Evaluation of skin optical clearing enhancement with Azone as a penetration enhancer," *Opt. Commun.* **279**, 223–228 (2007).
14. Z. Mao, D. Zhu, Y. Hu, X. Wen, Z. Han, "Influence of alcohols on the optical clearing effect of skin *in vitro*," *J. Biomed. Opt.* **13**(2), 021104 (2008).
15. A. C. Williams, B. W. Barry, "Penetration enhancers," *Adv. Drug Deliv. Rev.* **56**, 603–618 (2004).
16. A. P. Funke, R. Schiller, H. W. Motzkus, C. Gunther, R. H. Muller, R. Lipp, "Transdermal delivery of highly lipophilic drugs: *In vitro* fluxes of antiestrogens, permeation enhancers, and solvents from liquid formulations," *Pharm. Res.* **19**, 661–668 (2002).
17. J. W. Wiechers, R. A. de Zeeuw, "Transdermal drug delivery: Efficacy and potential applications of the penetration enhancer Azone," *Drug Des. Deliv.* **6**, 87 (1990).
18. Z. Mao, Y. Hu, Y. Zheng, W. Lu, Q. Luo, D. Zhu, "Experimental study on influence of Thiazone on optical clearing of piglet skin *in vitro*," *Acta Optical Sinica* **3**, (2007).
19. J. Jiang, W. Chen, R. K. Wang, K. Xu, "Availability of Thiazone as an enhancer for optical clearing of skin tissue *in vitro*," in *Optics in Tissue Engineering and Regenerative Medicine II*, S. J. Kirkpatrick, R. K. Wang, Eds., *Proc. SPIE*, **6858**, 68580N (2008).
20. H. Li, Z. Ye, "Developments on synthesis of a new skin penetration enhancer: Thiazone," *Jiangsu Chemical Industry* **28**, 15–18 (2006).
21. J. Wang, Z. Zhi, S. Zeng, Q. Luo, D. Zhu, "Accessing the structure and function information of deep skin blood vessels with noninvasive optical method," *Proc. SPIE*, **7176**, 717626 (2009).
22. S. Prahl, G. Van, A.J. Welch, "Determining the optical properties of turbid media by using the inverse adding doubling method," *Appl. Opt.* **32**, 559–568 (1993).
23. S. Prahl, "Inverse adding-doubling for optical property measurements," http://omlc.ogi.edu (2007).
24. Z. Mao, Z. Han, X. Wen, "Influence of glycerol with different concentration on skin optical clearing and morphological changes *in vivo*," *Proc. SPIE*, **7278**, 72781T (2008).
25. G. Vargas, E. K. Chan, J. K. Barton, "Use of an agent to reduce scattering in skin," *Laser Surg. Med.* **24**, 133–141 (1999).
26. J. Kalbitz, R. Neubert, W. Wohlrab, "Modulation of drug penetration in the skin," *Pharmazie* **51**, 619–637 (1996).