Preliminary Evaluation of Interactions between Selected Alcoholamines and Model Skin Sebum Components

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Received December 13, 2005; accepted May 14, 2006

The aim was to evaluate the interaction between selected alcoholamines and components of artificial skin sebum. The rate and depth of penetration into the lipophilic bead imitating pilosebaceous unit lumen was applied for alcoholamine penetration activity assay. The activity differentiation of 0.5% aqueous alcoholamine solutions with a potential cleansing effect on the pilosebaceous unit was performed. The depth of aminomethylpropanol penetration increased from 0.080 mm after 15 min to 3.049 mm after 72 h. The depth of aminomethylpropendiol penetration increased with time from 0.148 to 4.064, respectively, of diisopropanolamine from 0.481 to 4.626, triethanolamine from 0.236 to 4.342, triisopropanolamine from 0.275 to 2.392 and trometamol from 0.338 to 4.580. The products of alcoholamines reaction with the model skin sebum are easily dispersed in water. The rate of alcoholamines reaction with the model skin sebum depends on the alcoholamine, being the highest in the case of diisopropanolamine, decreasing to minimum for triisopropanolamine. Selected alcoholamines would be applied in *ex vivo* and *in vivo* research.

Key words alcoholamine; stearic acid; model skin sebum; pilosebaceous unit

Agents used topically in the treatment and prophylaxis of acne may bring significant benefits, however in many cases recurrences are not uncommon. For this reason new active substances and new drug forms offering better control of *Propionibacterium acnes*, management of keratosis of the sebaceous follicles and more effective removal of lipophilic bead are developed. Some authors suggest incorporating the active substance into liposomes or microspheres consisting of lactic acid and glycolic acid copolymer, whichever may facilitate its penetration into the pilosebaceous unit.[1—3)]

One of the suggested approaches to the management and prophylaxis of acne involves binding of free fatty acids to triethanoloamine in the form of soap.[4,5)] In aqueous environment, in the presence of a free electron pair at the nitrogen atom, an alcoholamine molecule, combining with a water molecule produces an alcoholamine ion—a counterion for fatty acids[6)] present in human sebum. The ions together with fatty acids form so-called alcoholamine soaps, well tolerated by the skin; 50% solution of the soap does not produce any skin lesions, which is important if they are to be used as anion soap constituents.[7)] Alcoholamines, by diffusing to the pilosebaceous units and forming amine soaps may change the lipophilic sebum into hydrophilic bead. Due to the presence of hydroxide groups, alcoholamine soaps bind significant amounts of water, inducing volume increase of sebum bead, which could facilitate the sebum removal from the pilosebaceous unit with simultaneous bacteria eradication. Binding of free fatty acids should decrease the irritating action of bacteria on the pilosebaceous unit, the epithelium, preventing its excessive cornification. Possible irritating effect of alcoholamines on the sebaceous epithelium may be suppressed by binding alcoholamines to, for example, anionic polymers. Selected alcoholamines could be applied as components of pharmaceutical or cosmetic products to prevent or treat acne.

The aim of the paper was to evaluate the interaction of selected alcoholamines with components of artificial skin sebum, *i.e.*, the penetration depth and the penetration rate into the space simulating lipophilic interior of pilosebaceous unit. The height of the reaction products and the height increase rate were also considered.

Experimental

Materials Alcoholamines characterized by the lack of or relatively mild irritating effect on the skin, long-term use in pharmaceutical and cosmetic products, legal regulations concerning allowable concentrations, and structural diversity were selected for the research. The following alcoholamines were researched: 2-amino-2-methyl-1,3-propanediol (AMPD) (Lancaster, England), 2-amino-2-methyl-1-propanol (AMP) (Lancaster, England), tris-(2-hydroxyethyl)-amine (triethanolamine, TEA) (Sigma-Aldrich, Poland), 1,1-iminodi-2-propanol (diisopropanolamine, DIPA) (Sigma-Aldrich, Poland), tris-(2-hydroxypropyl)-amine (triisopropanolamine, TIPA) (Sigma-Aldrich, Poland), and 2-amino-2-hydroxymethyl-1,3-propanediol (trometamine, trometamol, TRIS) (Sigma-Aldrich, Poland). The DIPA, the only representative of alcoholamines with a secondary amine group is authorized to be used in mixtures. Preliminary comparative studies of alcoholamine solutions were performed using 0.5% w/w solutions, which corresponds to 1/20 (AMP)–1/5 (TEA) concentration permitted in products designed for dermal application.

Other materials included triglyceride mix, stearic acid, lanolin, squalene, and cholesterol, all of analytical grade, supplied by Sigma-Aldrich. Demineralized, bidistilled water was used.

Preparation of Alcoholamines 0.5% w/w Aqueous Solutions Solutions of the following alcoholamines: TEA, TRIS, AMP, AMPD, DIPA and TIPA were prepared.

Conductivity and pH Measurement Conductivity and pH were assessed using pH 302 apparatus (Hanna Instruments, U.S.A.). For pH measurements, a combined electrode of OSH 10-10 type (Metron, Poland, U.S.A.) was applied. The measurements of conductivity were performed with electrolyte-resistance sensor EPS-2ZM (Eurosensor, Poland) with the sensor constant $c = 0.85\ \mathrm{cm}^{-1}$.

Preparation of Artificial Skin Sebum, Measurements of Penetration Depth, and Height of the Reaction Products Biological models currently used to investigate phenomena occurring in the pilosebaceous unit are quite complex.[8—10)] They enable determination of numerous biochemical factors and functions of the unit. In the proposed model, the focus was on differentiating the activity of pure alcoholamines solutions with a potential cleansing effect on the lipid bead of the unit. The differentiation of interaction of selected alcoholamines with the components of the model sebum can be evaluated in terms of the penetration depth and penetration rate into the space imitating lipophilic interior of the pilosebaceous unit. Also, the height of the reaction products and the height increase rate were considered (Fig. 1). Details concerning the assessment were presented in the previous report.

Calculation of Alcoholamines Dissociation Constant Alcoholamines dissociation constants were determined by titration method according to Inczedy. Dissociation constants of the investigated alcoholamines (K_A) were

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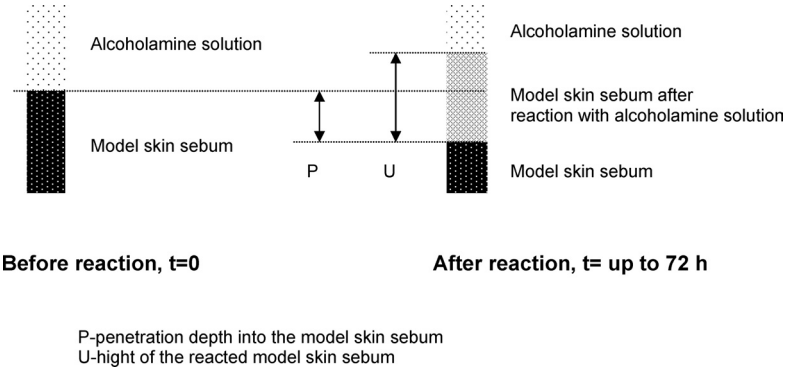

Fig. 1. Measurement of Penetration Depth into the Model Skin Sebum, and Height of the Reacted Sebum

Table 1. pH, Conductivity, Dissociation Rate and Molecular Weight of Selected Alcoholamines and Their Percentage in the Delivered Samples

Sample	pH	S.D.	Conductivity [mS]	S.D.	Dissociation rate	S.D.	Molecular weight [g/mol]
AMP	11.01	0.09	0.476	0.005	3.18×10^{-5}	0.02×10^{-5}	89.14
AMPD	10.42	0.24	0.166	0.003	4.78×10^{-6}	0.09×10^{-6}	105.14
DIPA	10.32	0.11	0.15	0.002	7.12×10^{-6}	0.11×10^{-6}	133.19
TEA	10.07	0.16	0.083	0.001	5.22×10^{-7}	0.07×10^{-7}	149.19
TIPA	9.48	0.19	0.071	0.001	7.31×10^{-7}	0.09×10^{-7}	191.27
TRIS	9.85	0.10	0.083	0.001	1.08×10^{-6}	0.02×10^{-6}	121.14

S.D., standard deviation.

determined using data from five-times-repeated potentiometric titration of weighed portion of respective alcoholamines with 0.1 mol/l hydrochloric acid as titrant. The following formula was used:

$$\log K_A = \{\log\{(aC_A - [H^+] + [OH^-])/[(1-a)C_A + [H^+] - [OH^-]]\} + pH\} - 14 \quad (1)$$

with titration fraction from 0.2 to 0.8, where: C_A, total alcoholamine concentration; a, titration fraction.

Determination of Rate of Process between Model Skin Sebum and Alcoholamines Penetration rates were determined to compare the penetration rates of various amines into the model sebum. For this reason, an assumption was made that the depth of triethanolamine penetration into the model sebum from its non-neutralized reference solution of 1.5% w/w concentration (0.1 mol/l) after 72 h corresponds to 100%. The solution, analyzed in previous research, was assumed as reference solution. Penetration rates were calculated from the general kinetic formula for first order processes:

$$P = P_0 \cdot e^{-K_p \cdot t} \quad (2)$$

where P_0, depth of triethanolamine penetration into the model sebum from 1.5% solution after 72 h; P, depth of alcoholamine penetration into the model sebum at individual time intervals; t, time; K_p, penetration rate.

The same method was applied to determine the height increase of the reaction products column. The height of the products column arose in the presence of triethanolamine at the reference 1.5% w/w concentration was assumed as 100%. The following equation was used to determine respective rates:

$$W = W_0 \cdot e^{-K_s \cdot t} \quad (3)$$

where W_0, height of the reaction products column after 72 h reaction in presence of 1.5% w/w triethanolamine solution; W, height of the reaction products column in the presence of researched alcoholamines at individual time intervals; t, time, K_s, rate of height increase of reaction products.

Description of the processes of penetration and height increase of the reaction products column enables evaluation of reactivity of individual alcoholamine preparations with the components of artificial skin sebum.

Calculation of the Ratio: the Penetration Depth/the Height of Reaction Products Column To compare the activity of investigated alcoholamine preparations, the ratio between the reaction products column height (S) and depth of penetration (P) were determined according to the following formula:

$$R = S/P \quad (4)$$

where R, ratio the penetration depth/the height of reaction products column; S, maximum height of model sebum and alcoholamine reaction products column after 72 h; P, maximum depth of penetration of alcoholamine into the model sebum after 72 h.

Statistical Analysis Data obtained from alcoholamines and model sebum reactions were analyzed by means of variance analysis (ANOVA), applying the simple classification. Respective means, variances, standard deviations, and root square deviations sum were calculated. Intra- and intergroup variance analysis was performed by dividing the sum of root square deviations by respective degrees of freedom. To determine whether the differences between groups reach statistical significance, the ratio of variations between groups to intragroup variations was calculated and compared with respective values of F distribution in Tables for 0.05 level of significance. The correlation analysis of variables with the characteristics of linear function was performed by calculating linear regression with the use of Pearson's correlation index and determination coefficient at 0.05 significance level. The calculations were performed using Statistica 6.0 software.

Results

Conductivity pH and of 0.5% Aqueous Solution of Alcoholamines The mean pH of the investigated 0.5% alcoholamines solutions, shown in Table 1, ranged from 9.48 in case of TIPA solution to 11.01 for AMP solution. The pH of alcoholamines is not only determined by the nitrogen atom order, but also depends on the electrochemical character of radicals attached to the nitrogen atom. Conductivity ranged from 0.071 mS for TIPA solution to 0.476 mS for AMP solution and was mainly affected by the molecular weight.

Alcoholamine Dissociation Rates The dissociation rates for respective alcoholamines ranged from 4.23×10^{-7} for TEA to 3.75×10^{-5} for AMP (Table 1). Assuming the properties of alcoholamines as alkaline (according to Broensted), their dissociation rates in aqueous solutions can be determined. Dissociation rates reflect the electrolyte potency and provide information about the rate of its disintegration into ions. Dissociation rates were determined by means of

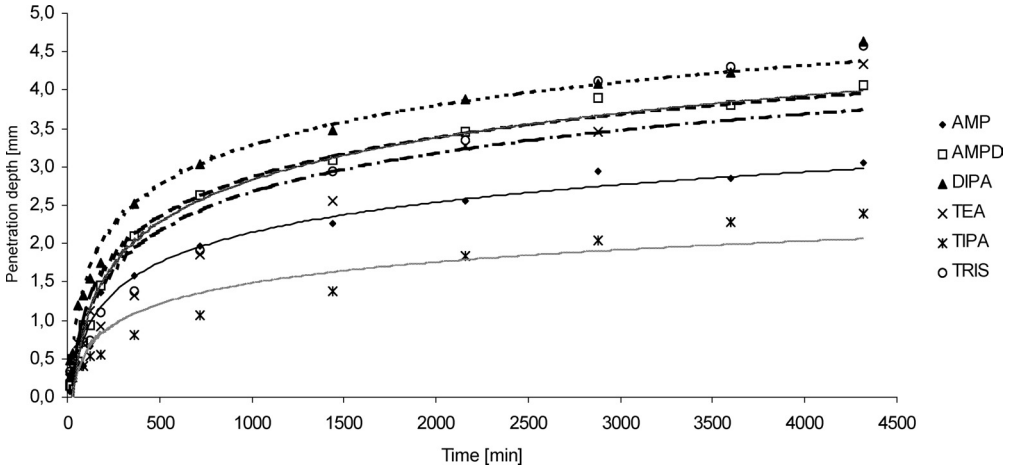

Fig. 2. Penetration Depths of the Alcoholamines 0.5% Aqueous Solutions into the Artificial Skin Sebum

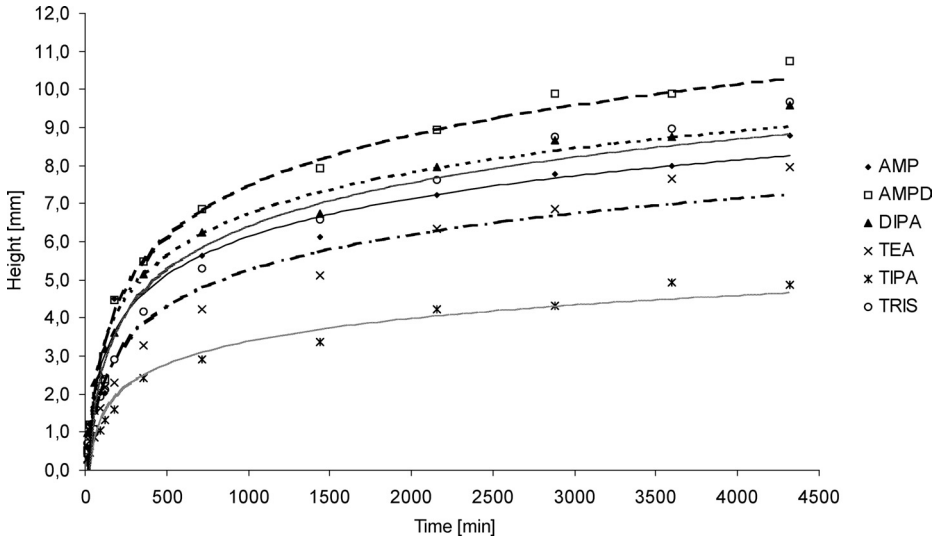

Fig. 3. Height of the Column of the Reacted Artificial Sebum

potentiometric titration method and the calculations were performed according to Indeczy's formula. The dissociation rates enable differentiation of alcoholamines taking into account their dissociation into ions that should affect the intensity of alcoholamine reaction with model skin sebum.

Interaction of Selected Alcoholamines with Artificial Skin Sebum Artificial skin sebum reacted with 0.5% alcoholamine solutions. As shown in Fig. 2, the depth of AMP penetration increased with time from 0.080 mm after 15 min to 3.049 mm after 72 h. The depth of AMPD penetration increased with time from 0.148 to 4.064 respectively, of DIPA from 0.481 to 4.626, TEA from 0.236 to 4.342, TIPA from 0.275 to 2.392, and TRIS from 0.338 to 4.580. The highest penetration depth was observed in case of DIPA, whereas TIPA and AMP solutions revealed low penetration into the model sebum. The observed relationship between the penetration depth and time shows the nonlinear character of the process.

Figure 3 illustrates changes in the height of the reaction products column in relation to time. The height of the column increased with time in a nonlinear mode and the assessed values for AMP were from 0.621 mm after 15 min to 8.788 mm after 72 h. For AMPD they were 0.521 and 10.734 respectively, for DIPA from 0.979 to 9.568, for TEA from 0.280 to 7.967, for TIPA from 0.303 to 4.877, and for TRIS from 0.717 to 9.663. The height of the reaction products column was highest when the solution contained AMPD, and the lowest, as in the case of the penetration depth, when the solution contained TIPA.

Graphic presentations of the penetration rates into sebum and of the products height increase rates suggest that the course of the process approaches the shape of an exponential function (Figs. 4, 5).

On a semilogarithmic graph, Fig. 4 presents the depths of penetration conversed according to Eq. 2, whereas Fig. 5 presents the height values of the products of reaction column conversed using Eq. 3. Considering the slope of the straight lines, the reaction rate is much higher in the first stage than in the second. Pearson's correlation coefficients for the respective linear equations were from about 0.88 to about 0.99. Penetration rates were calculated from the slope of respective lines and are presented in Table 2. In the first stage they ranged from $3.24\times10^{-5}\,\mathrm{min}^{-1}$ for TIPA to $1.40\times10^{-4}\,\mathrm{min}^{-1}$ for DIPA, whereas in the second stage they ranged from $6.44\times10^{-6}\,\mathrm{min}^{-1}$ for AMP to $1.48\times10^{-5}\,\mathrm{min}^{-1}$ for TRIS. The height increase rates are also presented in Table 2. They

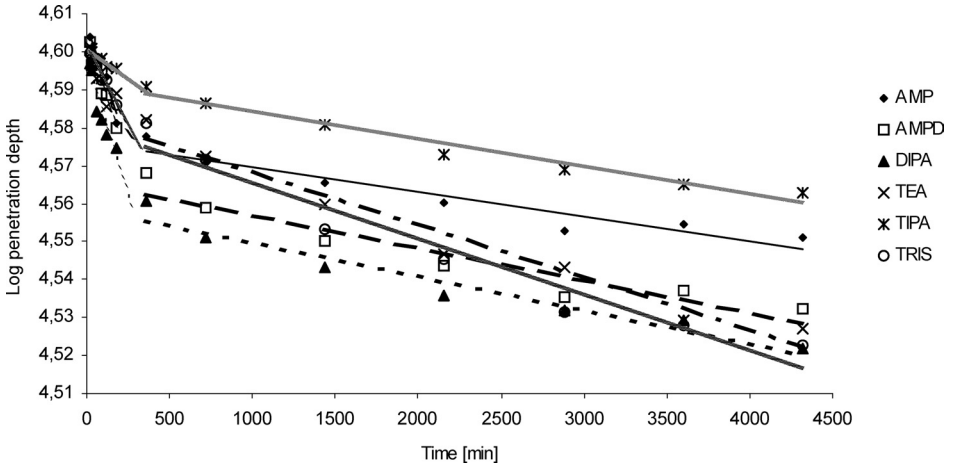

Fig. 4. Penetration Rates of the Alcoholamines 0.5% Aqueous Solutions into the Artificial Skin Sebum, a Semilogarithmic Graph

Table 2. Interaction Rates between Alcoholamines and Artificial Skin Sebum, and the Respective Ratio *R* Values

Substance	K_p in I stage [min^{-1}]	S.D.	K_p in II stage [min^{-1}]	S.D.	K_s in I stage [min^{-1}]	S.D.	K_s in II stage [min^{-1}]	S.D.	*R* in the range between 3 to 72 h	S.D.
AMP	1.23×10^{-4}	3.86×10^{-6}	6.44×10^{-6}	1.85×10^{-7}	1.81×10^{-4}	3.91×10^{-6}	8.91×10^{-6}	1.43×10^{-7}	2.82	0.11
AMPD	1.29×10^{-4}	7.82×10^{-7}	8.55×10^{-6}	1.38×10^{-7}	1.97×10^{-4}	3.63×10^{-6}	1.15×10^{-5}	1.31×10^{-7}	2.60	0.03
DIPA	1.40×10^{-4}	3.03×10^{-6}	8.91×10^{-6}	1.96×10^{-7}	1.50×10^{-4}	2.33×10^{-6}	9.68×10^{-6}	1.11×10^{-7}	2.05	0.05
TEA	8.47×10^{-5}	4.61×10^{-6}	1.39×10^{-5}	1.44×10^{-7}	1.04×10^{-4}	2.30×10^{-6}	1.07×10^{-5}	1.02×10^{-7}	2.04	0.25
TIPA	3.24×10^{-5}	3.84×10^{-6}	7.25×10^{-6}	1.15×10^{-7}	6.98×10^{-5}	2.40×10^{-6}	5.73×10^{-6}	1.18×10^{-7}	2.39	0.35
TRIS	7.92×10^{-5}	2.68×10^{-6}	1.48×10^{-5}	1.28×10^{-7}	1.09×10^{-4}	2.18×10^{-6}	1.25×10^{-5}	8.51×10^{-8}	2.38	0.37

K_p, alcoholamine penetration rate; K_s, height increase rate; *R*, ratio height of the products of reaction column/penetration depth.

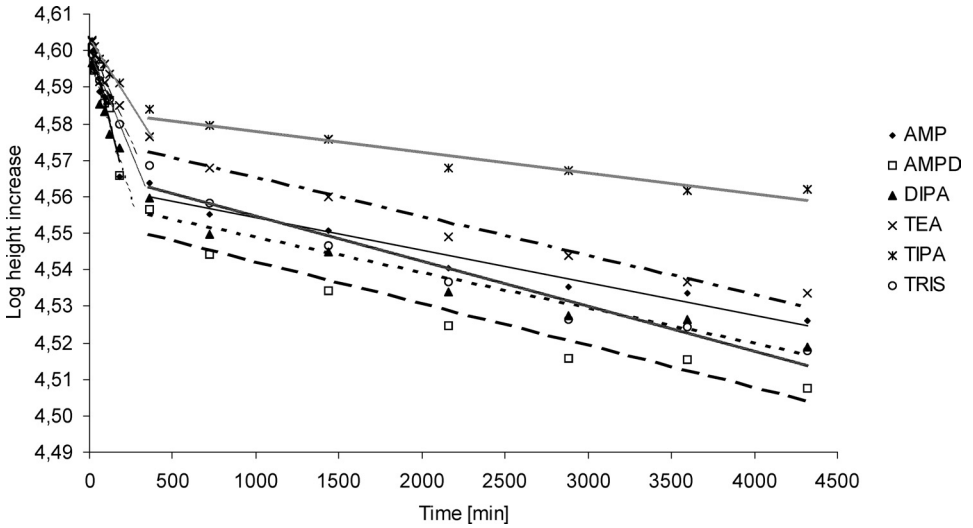

Fig. 5. The Rate of Reacted Sebum Height Increase, a Semilogarithmic Graph

ranged from $6.98\times10^{-5}\,min^{-1}$ for TIPA to $1.97\times10^{-4}\,min^{-1}$ for AMPD in the first stage, whereas in the second stage they were from $5.73\times10^{-6}\,min^{-1}$ for TIPA to $1.25\times10^{-5}\,min^{-1}$ for TRIS.

The ratio of the height of the products of reaction column to the depth of alcoholamines penetration into the artificial skin sebum is an interesting observation. As shown by data presented in Fig. 6, the ratio is differentiated between individual alcoholamines.

Mean ratio of the height of the products of reaction column to alcoholamine penetration depth was from 8.27 after 15 min to 2.88 after 72 h for AMP and from 3.66 to 2.64 for AMPD, from 2.04 to 2.07 for DIPA, from 1.19 to 1.84 for TEA, from 1.94 to 2.04 for TIPA, and from 2.13 to 2.11 for TRIS respectively. A significant differentiation of the products of reaction column and alcoholamine penetration rate ratio, which was from 1.09 to 8.27, was observed in the time from 15 min to 180 min. Next, the values of the ratio stabilized for all alcoholamine solutions at levels of about 2.00 to about 3.00.

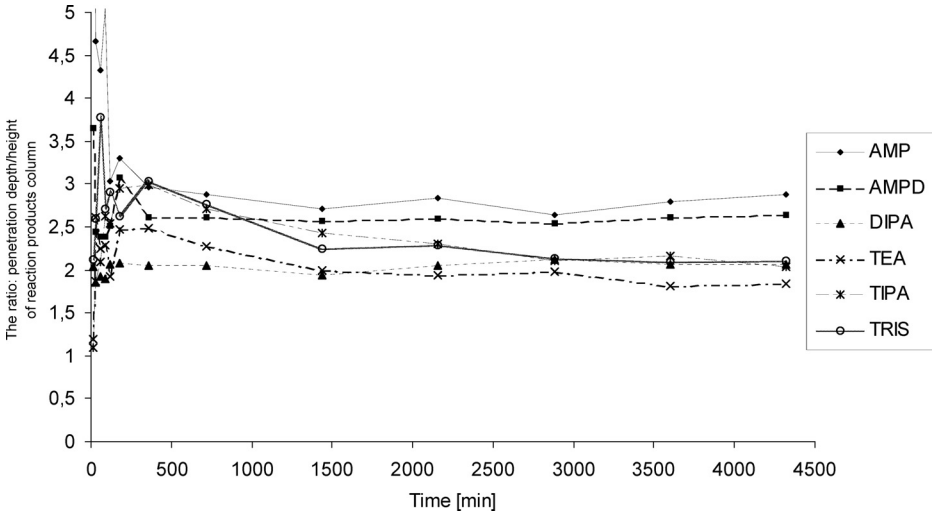

Fig. 6. Time Dependent Changes of the Ratio: Penetration Depth/Height of Reaction Products Column

Discussion

Numerous changes, both in the lipophilic layer of the artificial skin sebum as well as in the aqueous solution over the sebum were observed during the reaction of alcoholamines and artificial skin sebum (Fig. 1). Alcoholamine reacts initially only with the superficial layer of the model sebum. The reaction occurs rapidly, as there is no diffusion barrier that would prevent the diffusion of alcoholamine into the artificial sebum. With time, a column of the reaction products forms on the water/lipid border of the model sebum, which contains alcoholamine stearate and other constituents of the model sebum. The reacted layer of sebum becomes less dense, but thicker. Penetration of alcoholamines and molecules of water into the model sebum influence this. It results in bulging and softening of the reaction products. Thus, the process of diffusion of alcoholamine into deeper layers of the stearic acid contained in the model sebum decreases. As the thickness of the diffused layer of sebum increases, the rate of penetration as well as the height of the reaction products column in time unit decreases. Obtained amine soaps, as a good emulsifier,[11] facilitate spontaneous dispersion of the remaining compounds of the model sebum into the aqueous stage, which is manifested as turbidity of the solution over the reacted sebum.

Characteristic of DIPA Out of all the investigated alcoholamines, DIPA revealed the highest activity on the artificial sebum in the first stage of the reaction from 15 min to 3 h. Although its molar concentration in 0.5% solution is about 0.038 mol/l, which is a middle value considering other amines, the rate of penetration of this alcoholamine into the model sebum in the first stage is the highest among all the investigated compounds. This fact may be associated with the presence of a secondary nitrogen atom in the DIPA molecule; therefore, the reaction with stearic acid occurs with high intensity. As a result of amine depletion in the first stage of the reaction, the second stage is much slower. The rate of the swelling process of the reacted lipophilic bead is intermediate in relation to the remaining amines both in the first 3 h as well as in the next 72 h. The total depth of DIPA penetration after 72 h is 40 mm. The ratio between the height of the products of reaction column and the depth of DIPA penetration is about 2, and this is the lowest value observed for the investigated amines. However, the height of the swelled reaction products is twice as much as the depth of penetration of the amine.

The penetration rate of the remaining alcoholamines increases with the increase of molar concentration. Several groups may be distinguished: alcoholamines with a low molecular weight, *i.e.* AMP and AMPD, compounds with alcoholamines with intermediate molecular weight, such as TEA and TRIS, and the low-reactive TIPA.

AMP and AMPD Characteristics Penetration rate of AMP and AMPD is similar to that of DIPA. AMP and AMPD are compounds with relatively low molecular weight, high pH, and conductivity of 0.5% solution. As in the case of DIPA, the amines rapidly deplete in the first stage of the reaction, thus the penetration into lipophilic bead in the second stage is much slower. Swelling of the reacted lipophilic bead is most rapid in the presence of AMP and AMPD in the first and second stage of the reaction. Comparing the molecular weight, conductivity, pH and dissociation constant of the investigated amines, it can be assumed that the high rate of reaction is associated with high thermodynamic activity of amine ions in the amine solutions. This fact is confirmed by both high values of dissociation constants as well as of conductivity in 0.5% solutions. The depth of AMP penetration is about 24 mm, and AMPD penetration is about 39 mm. These amines reveal the most favorable ratio of the column height of swelled products to the depth of penetration into the model sebum, which is 2.82 and 2.60 for AMP and AMPD respectively. The reacted components of the model sebum are intensively elevated over the surface of sebum and the elevation is threefold higher than the depth of the amine penetration. Moreover, in the first stage the column height of the products of AMP reaction with the components of the model sebum is eightfold higher than the depth of penetration of the amine into the sebum. This phenomenon may play a significant role in primary preliminary cleansing of the pilosebaceous unit in severe oily acne skin.

TEA and TRIS Characteristics The effect of TEA and TRIS, amines with the same number of hydroxyl groups and different molecular weights, on the penetration rate in the

first stage is similar for both amines and intermediate in relation to others. However, the penetration rate into the model sebum in the second stage remains at a high level, higher than in case of the remaining alcoholamines and not much different than in the first stage. The lipophilic bead swelling rates influenced by TEA and TRIS did not differ much between the first and the second stage and reached intermediary level. Total depth of penetration was 29 mm and 30 mm respectively, and the ratio between the height of the column of reaction products and penetration depth was 2.04 and 2.38 respectively. Thus, the amines are characterized by a prolonged action on the components of the model sebum, both in the first as well as in the second stage of the reaction. The reaction products are elevated above the primary level of sebum by up to 30 mm. Considering safety, TEA and TRIS are the most utilized alcoholamines in drug delivery studies.[12,13]

TIPA Characteristic The penetration rate of TIPA from its 0.5% solution in the first stage of the reaction is the slowest in relation to other alcoholamines; however, in the second stage it reaches values similar to those of DIPA, AMP, and AMPD. Swelling of the reaction products column occurs at a slow rate in both stages. TIPA penetrates the model sebum at a depth of only 14 mm, and the column of the reaction products reaches about 32.5 mm. This is the only alcoholamine solution that over a layer of the model sebum remained clear throughout the whole investigation period. Comparing with DIPA, an alcoholamine different from TIPA in only one radical, the penetration decreases dramatically. There are two main factors influencing decreased diffusion of TIPA into the model sebum layer. Structural obstacles as well as high molecular weight exert an inhibitory effect on the diffusion of triisopropanolamine ion in water. According to Nugent *et al.*, the three asymmetric centers in alcoholamine molecule, for example in TIPA, may provide a highly asymmetric environment in the coordination sphere of the transition metal and provide the corresponding trialkanolamine complexes.[14] Actually, the TIPA penetrates the model sebum up to 14 mm, whereas the DIPA up to almost three folds deeper. This suggests binding of the TIPA in tight complexes with the model skin sebum components.

The selection of alcoholamine for further *in vivo* studies is determined both by the physicochemical properties of the investigated chemical compounds as well as by safety of their use. Among compounds that have been authorized for use in humans, the reaction of DIPA with sebum components is the fastest among all alcoholamines. However its use has been authorized only in cosmetic products in a mixture with other amines, and in some cases contact dermatitis was reported.[15] TRIS and TEA, different forms of which have been authorized for use in medicine and cosmetology, are characterized by interesting properties. Their reaction with the components of model sebum occurs at a moderate rate, and the ratio of the height of swollen reaction products to the penetration depth suggests a beneficial cleansing effect on the pilosebaceous unit.

The volume of the reaction products of alcoholamines with artificial skin sebum is higher than the volume of the original sebum. The reaction products of the alcoholamines with the model skin sebum undergo dispersion in water, which facilitates their washing off the space imitating a pilosebaceous unit. The reaction of alcoholamines with the model sebum runs in two stages, the first stage being of significantly higher rate than the second one. The rate of alcoholamines reaction with the model skin sebum depends on the alcoholamine structure, reaction being the highest in the case of DIPA, decreasing to a minimum in the presence of TIPA. Selected alcoholamines will be applied in *ex vivo* and *in vivo* research.

Acknowledgments The work was supported by grant P05F 022 from Polish Ministry of Science (PL). The authors would like to thank to Prof. Eugeniusz Baran from Department of Dermatology of Wroclaw Medical University (PL) for dermatological consultations.

References and Notes

1) Biju S. S., Ahuja A., Khar R. K., *J. Pharm. Sci.*, **94**, 240—245 (2005).
2) Mordon S., Sumian C., Devoisselle J. M., *Lasers Surg. Med.*, **33**, 119—125 (2003).
3) Meidan V. M., Bonner M. C., Michniak B. B., *Int. J. Pharm.*, **306**, 1—14 (2005).
4) Musial W., Kubis A. A., *Eur. J. Pharm. Biopharm.*, **55**, 237—240 (2003).
5) Musial W., Kubis A. A., *Polim Med.*, **34**, 17—30 (2004).
6) Patil G. S., Matthews R. H., Cornwell D. G., *J. Lipid Res.*, **17**, 197—202 (1976).
7) Smith M. B., *J. Am. Optom. Assoc.*, **51**, 502—504 (1980).
8) Burkhart C. N., Burkhart C. G., *Int. J. Dermatol.*, **42**, 925—927 (2003).
9) Downie M. M., Sanders D. A., Kealey T., *Br. J. Dermatol.*, **147**, 869—878 (2002).
10) Nishimuta K., Yito Y., *Arch. Dermatol. Res.*, **294**, 544—551 (2003).
11) Gutierrez X., Silva F., Morles A., Pazos D., Rivas H., *Petrol. Sci. Techn.*, **21**, 1219—1240 (2003).
12) Gwak H. S., Choi J. S., Choi H. K., *Int. J. Pharm.*, **297**, 156—161 (2005).
13) Roy S. D., Manoukian E., *J. Pharm. Sci.*, **84**, 1190—1196 (1995).
14) Nugent W. A., Licini G., Bonchio M., Bortolini O., Finn M. G., McCleland B. W., *Pure Appl. Chem.*, **70**, 1041—1046 (1998).
15) Umebayashi Y., *J. Dermatol.*, **32**, 145—146 (2005).