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Research paper

Preliminary assessment of alginic acid as a factor buffering triethanolamine interacting with artificial skin sebum

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Abstract

Triethanolamine is an ethanolamine used as counter-ion for fatty acid soaps. Interaction between triethanolamine and free fatty acids is suggested to be useful for cleansing sebaceous follicles in acne prevention. This study describes the preliminary assessment of alginic acid as a factor buffering triethanolamine interacting with stearic acid – a compound of artificial skin sebum. Penetration of triethanolamine into artificial sebum, induced by the above mentioned interaction, was measured using a specific optical method. The values of the penetration depth amounted to 0.07–5.74 mm. pH values were measured. The value of pH is reduced from 10.06 for pure 1.49% (w/w) aqueous triethanolamine solution to 6.61 with the increase of the alginic acid to triethanolamine ratio in the preparations. The data of this in vitro research will support further study on other anionic polymers as factors buffering ethanolamines penetrating artificial skin sebum.

Keywords: Acne; Alginic acid; Anionic polymers; Ethanolamines; Fatty acids; Sebum; Triethanolamine

1. Introduction

Acne vulgaris is one of the most common skin conditions encountered in dermatology. Acne is a multifactorial disease affecting the pilosebaceous follicles. Known pathogenic factors of acne include high levels of skin sebum with free fatty acids, high counts of an anaerobic diphtheroid, *Propionibacterium acnes*, and abnormal keratinization in the sebaceous follicle.

Topical treatment consists of skin washes, antimicrobial agents, antibiotics and retinoids. The most frenetic washing, a process that is virtually complete in about 30 s, removes only surface lipids. The lipid in follicular reservoirs, considered as pathogenic, is not affected.

Antimicrobial agents such as benzoyl peroxide or antibiotics such as erythromycin and clindamycin reduce the population of *P. acnes* in sebaceous follicles. Retinoids are highly effective comedolytic agents, normalizing follicular keratinization. The above mentioned topically administered drugs possess adverse effects such as skin irritation, erythrema, dryness and peeling. In the case of antibiotics, the development of resistance to *P. acnes* is possible. Orally administered agents are associated with more significant and diverse effect profiles [1–3].

The authors suggest utilizing a spontaneous in situ reaction between triethanolamine and sebum fatty acids for deep sebaceous follicle cleansing in patients threatened with acne flares. In the course of the reaction a fatty acid soap with triethanolamine as the counter-ion is produced. The soap stabilizes O/W type emulsion, and binding water loosens the structure of the settled sebum. Thus, triethanolamine administration should lead to skin sebum removal. In addition *P. acnes* responsible for inflammation in acne is expected to be removed. This would provide preventive action in the disease. Considering the high pH values of pure triethanolamine solutions it was necessary to reduce the pH of the solution expected to react with the skin sebum. Thus, alginic acid, a natural anionic polymer, was used for buffering the triethanolamine solutions.

The aim of the study was to compare the triethanolamine penetration depth into artificial skin sebum from different triethanolamine preparations buffered with alginic acid.

2. Materials and methods

2.1. Materials

Triethanolamine (2,2′,2″-Nitrilotrisethanol, POCH Gli-

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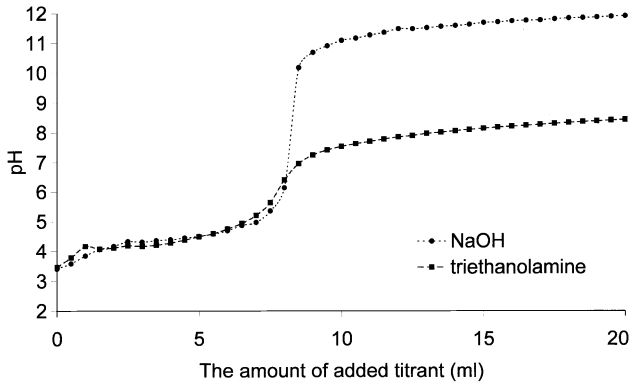

Fig. 1. Alginic acid titration using 0.01 M NaOH solution and 0.01 M triethanolamine solution.

wice, Poland), squalene (Sigma-Aldrich, Poznan, Poland), sodium hydroxide (POCH Gliwice, Poland), cholesterol (Sigma-Aldrich, Poznan, Poland) and stearic acid (POCH Gliwice, Poland) of analytical grade were used. Alginic acid (Sigma-Aldrich, Poznan, Poland) and lanolin (Cefarm Wroclaw, Poland) conformed to The European Pharmacopoeia 2000 standards. Animal triglycerides according to Pharmacopoea Polonica V [4] were purchased from Cefarm Wroclaw, Poland. The water used was de-ionized and purified by distillation.

2.2. Methods

2.2.1. Determining alginic acid molar-equivalent for a strong base and determining its dissociation rate

The titration endpoint was determined by potentiometric titration of alginic acid, using 0.1 M sodium hydroxide solution as titrant [5]. The alginic acid molar-equivalent binding 1 mol of a strong base was calculated. The titration curve was also used to determine the dissociation rate of alginic acid [6]. Potentiometric titration of alginic acid with 0.1 M triethanolamine solution was also performed to determine the pH of the titration endpoint.

2.2.2. Measurements of pH

pH measurements were taken using a pH 302 potentiometer (Hanna Instruments, USA) with a combined electrode ESAgP-301-W (Eurosensor, Poland).

2.2.3. Dynamic viscosity measurements

Viscosity measurements for alginic acid gels were performed with a rotational viscometer (Rheotest-2, Germany) at 37 °C, 1 h after setting the gel. Cylinder 'N' of 3.22 constant (z) was used, and the shearing rate (D) from 0 s^{-1} to 1312 s^{-2} of the dynamic viscosity (n) was examined. Shearing strength (T) and dynamic viscosity (n) were determined using the following equations:

$$T = za \ [\mathrm{dyn/cm^2}] \quad (1)$$

$$n = (T/D) \times 100 \ [\mathrm{cPs}] \quad (2)$$

where a is the measurement instrument indication on a conventional scale.

2.2.4. The measurement of triethanolamine penetration depth into artificial sebum

According to various authors, skin sebum consists mainly of fatty acids, triglycerides and waxes. Squalene, cholesterol and its esters are also present in skin sebum. Depending on the skin region there is also a variable amount of free fatty acids in the sebum [7–13]. Based on the above data a model artificial skin sebum was prepared, consisting of pork lard [4] as triglycerides of sebum (33%), stearic acid as free fatty acids in sebum (24%), lanolin as waxes (22%), squalene (12%) and cholesterol (4%). The components of artificial skin sebum were melted in a water bath, stirred and left to solidify.

Artificial skin sebum was placed in microbiological test tubes of 4 mm in diameter. The height of the sebum column was 2 cm. The exact height of the sebum column was measured with a 35 mm projector Diapol Automat (Poland) [14]. A 2 cm layer of triethanolamine, or triethanolamine buffered with an alginic acid layer, was placed on the artificial skin sebum. Under the influence of triethanolamine, artificial sebum brightened up. The brightened layer of sebum was washed out with water. Next, the remaining test tube layer of sebum was measured with a projector. Average penetration depths were calculated on the basis of the difference in sebum column height before and after the reaction between sebum and triethanolamine. Penetration depths were determined after 24, 48 and 72 h of incubation at 37 °C.

Table 1
The composition and characteristics of the examined preparations of triethanolamine with alginic acid

Gel no.	TEA (mg)	AA (mg)	Water (g)	Non-neutralized TEA concentration (%)	TEA/AA ratio expressed in molar-equivalents	pH	Dynamic viscosity measured at 243 s^{-1} shearing rate (cPs)
1	1492	2358	100	0	1:1	6.61	91.5
2	1492	1179	100	0.75	2:1	7.79	12.9
3	1492	589	100	1.12	4:1	8.44	5.1
4	1492	295	100	1.31	8:1	8.87	1.2

TEA, triethanolamine; AA, alginic acid.

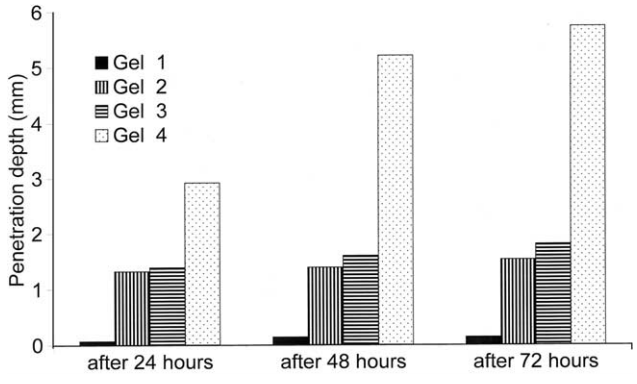

Fig. 2. Triethanolamine penetration depth from alginic acid gels into the artificial skin sebum after 24, 48 and 72 h of incubation at 37 °C.

3. Results and discussion

The molar-equivalent of alginic acid binding 1 mol of sodium hydroxide was determined to be 235.76 g. The dissociation rate constant determined in the above given conditions had a value of 4.39. The titration endpoint for alginic acid using triethanolamine as titrant was at pH 6.67 (Fig. 1).

Based on the determined gram-equivalents, four alginic acid gels were prepared with triethanolamine. The molar ratio of triethanolamine to alginic acid in the gels varied from 1:1 to 8:1 and the percentage of non-neutralized triethanolamine in the solutions was from 0% (w/w) to 1.31% (w/w) (Table 1).

The pH and viscosity of the examined gels are presented in Table 1. Among the examined preparations only gel 1 showed slightly acidic pH and gel 2 did not exceed pH 8, whereas the pure 1.49% (w/w) aqueous solution of non-neutralized triethanolamine acquired a pH of 10.06. A basic reaction was determined for the next two preparations, i.e. for gel 3 (pH 8.44) and gel 4 (pH 8.87).

All preparations when applied on artificial skin sebum brightened it up. The brightened layer was easily washed out with water. Penetration depths are presented in Fig. 2 and the results are summarized in Table 2.

The triethanolamine penetration depth into artificial sebum from examined preparations was differentiated and increased in the course of time. The obtained relation is approximately the function: $y = bx^a$, where x is penetration time, y is penetration depth, and a and b are proportionality coefficients. This resulted from the gradual increase of the distance that triethanolamine reached in deeper layers of artificial skin sebum.

The penetration depth increased also due to the concentration increase of non-neutralized triethanolamine in gel. These relations are presented in Table 2. The increase was not a linear one.

Following the increase of non-neutralized triethanolamine contents from 0% (w/w) in gel 1 to 0.75% (w/w) in gel 2, the penetration depth also increased rapidly, and in gel 1 was 0.07 mm after 24 h, 0.14 mm after 48 h, and 0.16 mm after 72 h. In gel 2 it was 1.32, 1.39 and 1.53 mm, respectively. This could be due to the difference in viscosity between the gels, and to the fact that in gel 1 triethanolamine was completely neutralized with alginic acid whereas in gel 2 it was only half-neutralized.

The further increase of non-neutralized triethanolamine contents up to 1.12% (w/w) in gel 3 accelerated the process of triethanolamine penetration into artificial sebum only up to 1.39, 1.60 and 1.81 mm after 24, 48 and 72 h, respectively (Fig. 2). This can be explained by the relatively low difference in dynamic viscosity between gel 2 and gel 3 and a minimal difference in non-neutralized triethanolamine percentage between both gels (Table 1).

Investigating increased non-neutralized triethanolamine contents of 1.31% (w/w) in the preparation, a considerable increase in the triethanolamine penetration depth into artificial sebum was observed. The obtained penetration depth was 2.92, 5.21 and 5.74 mm in respective time periods (Fig. 2, Table 2). The significantly enhanced penetration depth can be explained in terms of the considerably exceeded neutralization point of alginic acid with triethanolamine. Even a small excess of triethanolamine in gel 4 compared to gel 3 resulted in weaker alginic acid buffering activity and triethanolamine penetration into artificial sebum was deeper. As an additional factor, the decreased viscosity in gel 4 could influence the increase in the penetration depth.

The data of these in vitro investigations will support further study on other ethanolamines proposed as factors penetrating artificial skin sebum. On the other hand, because of possible skin irritation by preparations containing ethanolamines [15], the in vivo study should be performed after physical and chemical characterization of the inter-

Table 2
The influence of non-neutralized triethanolamine excess in alginic acid gels on the penetration depth of this ethanolamine into artificial skin sebum

Gel no.	% of non-neutralized amine	Penetration depth (mm) (± SD)		
		24 h	48 h	72 h
1	0	0.07 ± 0.034	0.14 ± 0.023	0.14 ± 0.024
2	0.75	1.32 ± 0.051	1.39 ± 0.050	1.53 ± 0.041
3	1.12	1.39 ± 0.039	1.60 ± 0.053	1.81 ± 0.042
4	1.31	2.92 ± 0.032	5.21 ± 0.041	5.74 ± 0.054

Gel numbers are in accordance with Table 1. SD, standard deviation.

action between artificial skin sebum and triethanolamine preparations.

4. Conclusions

Triethanolamine, completely or partially neutralized by using alginic acid, penetrates into artificial skin sebum from the formed gels and creates a mixture easily dispersed in water. The penetration depth into artificial skin sebum can be controlled providing proper gel composition. Triethanolamine from alginic acid gels of a high molar ratio ethanolamine/polymer penetrates deeply showing high pH up to 8.87. At a low molar ratio of triethanolamine/alginic acid penetration depth values are minimized due to the decreased pH of 6.61. The triethanolamine penetration depth from investigated preparations into artificial skin sebum depends also on viscosity and occurs from gels near to neutral pH. The in vivo studies should be performed to confirm the possibilities of the buffered triethanolamine application on human skin.

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