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Studies of water-in-oil (w/o) emulsion stabilized with amino acids or their salts

YOSHIMARU KUMANO, SHIN NAKAMURA,
SADAAKI TAHARA, and SABURO OHTA *Shiseido Laboratories, 1050, Nippacho, Kohokuku, Yokohama, Japan.*

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Synopsis

WATER-IN-OIL (W/O) EMULSIONS STABILIZED by using gels formed between surfactants and aqueous solutions of AMINO ACIDS were studied. The gel can only be obtained with a fluid surfactant which has lipophilic properties and a specific orderly lamellar structure and amino acids or their salts which are readily soluble in water.

By dispersing these gels into the oil phase and then adding the water phase, extremely stable w/o emulsions with wide ranges of water content were obtained. This type of emulsification was termed the "gel-emulsification method" by the authors. When this new technology was applied to the preparation of cosmetics, products with outstanding characteristics were obtained.

The function of the amino acids in the emulsification were investigated by using physico-chemical methods such as X-ray analysis, nuclear magnetic resonance (NMR), heat of solution, electron microscopy (EM), and measurement of the water content solubilized in the surfactant phase. It may be concluded that the amino acids are effective in forming a tight surface atmosphere around the water particles and in preventing coalescence of water particles by strong hydration effect of the amino acids, thus stabilizing the w/o emulsion.

INTRODUCTION

Generally, w/o emulsions are said to be much more advantageous to human skin than an oil-in-water (o/w) emulsion. Gattefosse et al. (1,2) described the mechanism of application of w/o emulsions to the skin as follows.

The continuous fatty layer, in which minute droplets of water are distributed, is in contact with the epidermis and facilitates adhesion. After the water evaporates, the residual fatty phase of the emulsion on the skin is elastic and resistive, protecting the deeper layers of skin from dehydration and exaggerated hydration. Furthermore, other scientists (3) have also dealt on the properties of w/o emulsions of spreading well onto stratum corneum and aid in the prevention of chemical and natural attacks thereon, retarding moisture loss, which in turn helps to maintain flexibility.

Clar (4) has recently published results on skin impedance measurement that, in spite of the variation in the moisture of the atmosphere, when the w/o cream is applied, the moisture of the skin is preserved for some time.

However, oil-in-water (o/w) type emulsions have better consumer acceptance than the w/o type emulsion, despite the various benefits of the latter to the skin. This can be attributed to the difficulties of maintaining the stability of the w/o emulsion as well as the inferior feel during application. Generally, w/o emulsions are prepared by increasing the ratio as well as the viscosity of the outer phase (oil) in order to improve stability. This results in a product with a transparent and glaring appearance and with a greasy and oily feeling, which will not readily gain consumers' acceptance.

It is of great interest for cosmetic scientists to try to eliminate such defects from w/o emulsion (for example, the excessive addition of water caused separation (60 to 70 per cent)). The addition of oil-soluble polyvalent metallic soaps increased the stability of the w/o emulsions to some extent, but hardly altered the application defects.

From the above facts, the authors carried out a series of experiments to obtain w/o emulsions, which were designed to hold wide ranges of water ratio, a nongreasy feel, and still have good stability. As a result, it was possible to develop a new emulsification method, which the authors termed as the "gel-emulsification method."

The main points of this method are described as follows: By mixing an aqueous solution of amino acids or their salts with lipophilic surfactants having specific requirements in their chemical structure, a kind of gel, consisting of the surfactant in the continuous phase and an aqueous solution of amino acids or their salts in the dispersed phase, could be formed. In the following emulsification step, the gel was dispersed into the oil phase, and then the water phase was added into the mixture and emulsified. It was possible to obtain a stable w/o emulsion and/or cream having excellent characteristics with a wide range of water content.

The major characteristics of the creams obtained by this method were their excellent affinity and nongreasy feel to the skin which has never been achieved before with a w/o emulsion. Moreover, the surfactants such as the monoglycerides used in the creams prepared by this gel-emulsification method are highly safe materials found widely in nature and lipids. Furthermore, the amino acids used in this investigation are also found to be in the natural moisturizing factor (NMF) of the skin and safe enough to be used as food fortifiers for human nutritional purposes.

From this viewpoint, the creams obtained by the new method have great advantages over existing formulations, since they have been prepared from ingredients which have been proven to be physiologically safe for human beings to use.

The research findings will be discussed in 3 parts. First, it will be necessary to clarify the necessary requirements in the relationship between surfactants and amino acids in order to form the gels (which is characteristic in the new technology). Secondly, the details of the gel emulsification method, in which the gel is dispersed into the oil phase and water is added, will be discussed together with its characteristics. Finally, the examples of the practical application of the new technology to actual cosmetic formulations and their characteristics will also be explained. In addition, the similarity existing between the phenomena obtained in connection with amino acids and surfactants, and the spontaneous emulsifying phenomena on the skin will also be discussed to some extent.

EXPERIMENTAL

GEL FORMATION OBTAINED BETWEEN SURFACTANTS AND AMINO ACIDS

Materials: Most of the surfactants used in this study were commercially available. For example, Sunsoft® O-30B* (glycerol monooleate) was used as a standard surfactant for many of the experiments. Whenever necessary, those synthesized in the usual way or those fractionated by molecular distillation were used. The amino acids and their salts were of special reagent grade. Other reagents used were also of the same grade. Distilled water was used throughout the study.

METHOD

a. *Gel Formation:* Surfactants and amino acids or their salts, which were possible to form the gels, were classified by the following simple method. The surfactant was added to an aqueous solution of the amino acid or its salt at room temperature and stirred with a laboratory mixer. A gel was formed as shown in Fig. 1.

All of the gels obtained by this method were observed as to their electrical conductivity and their stability in hot water. Figure 2 illustrates this property and only those without electrical conductivity and insoluble in water were selected for further study.

b. *Other Measurements:* In order to investigate the various functions of the gels, the chemical structure of the surfactants, the structure and properties of the gels obtained, and the effects of amino acids, etc., were examined by the following methods:

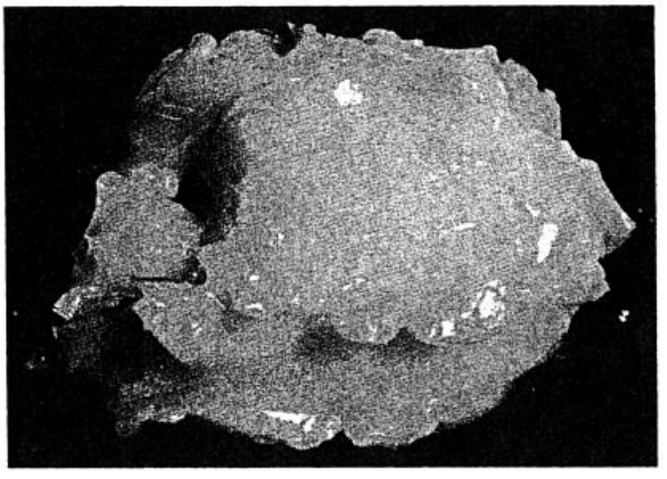


Figure 1. Example of gels prepared between surfactants and aqueous solution of amino acids

*Taiyo Kagaku Co., Ltd., 62 Akahori, Yokkaichi, Mie, Japan.

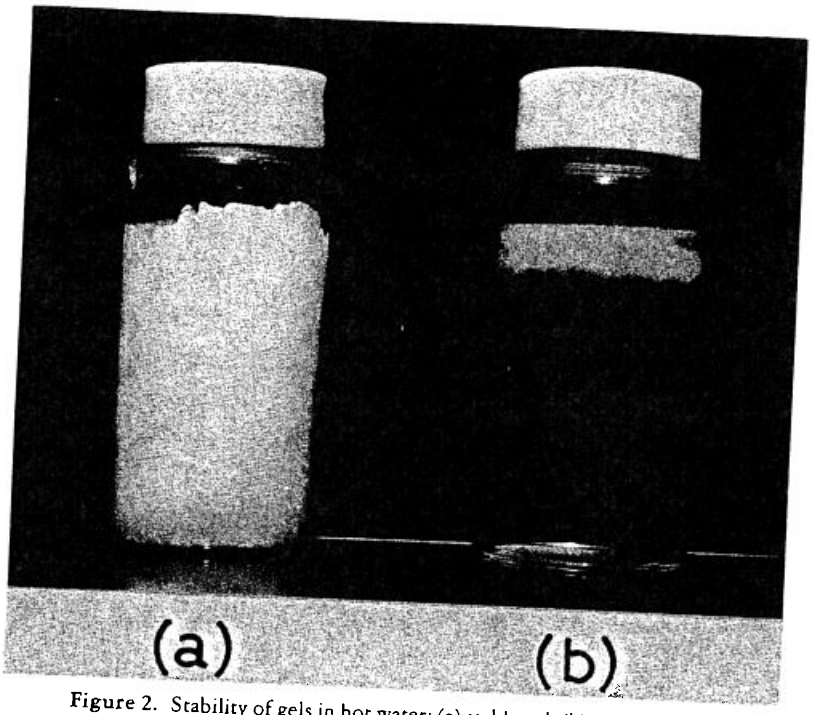


Figure 2. Stability of gels in hot water: (a) stable gel; (b) unstable gel

(1) *X-Ray Analysis:* Two types of X-ray diffractometers, Rotaflex* and JRX-12VA† were used to elucidate the structures of the surfactants and gels by means of small angle scanning and camera method at 25°C; Target; Cu.

(2) *Differential Thermal Analysis (DTA):* The gel sealed in aluminum cell was measured at 0 to 70°C, raising the temperature 2.5°C/min by a scanning type DSC.*

(3) *NMR:* Variation of chemical shift with proton in water of the aqueous solution to which various solutes were added, was measured by Hitachi‡ R-20 type NMR at 34°C.

(4) *Phase Inversion Temperature (PIT):* Influence of the addition of amino acids on the PIT was measured with Squalane—Beeswax—POE(6) oleyl alcohol ether 5 per cent (w/w)—water system (volume ratio = 0.6).

(5) *Heat of Solution:* A sealed ampule containing about 2 g of the surfactant, Sunsoft O-30B, was broken in 50 g aqueous solution of amino acid having various concentrations. Evolution of heat on mixing (cal/g) was measured by a twin type microcalorimeter** at 35°C.

(6) *Water Content Migration to the Surfactant Phase:* The amount of water migrating into the surfactant (Sunsoft O-30B) through an interface of the surfactant and an

*Rigaku Denki 8-2, Kandasurugadai, Chiyodaku, Tokyo, Japan.
†Japan Electron Optics Laboratory, 1418, Nakagami, Akishimashi, Tokyo, Japan.
‡2 Shibanishikubo, Minatoku, Tokyo, Japan.
**Applied Electric Laboratory, 4-672 Sekimachi, Nerimaku, Tokyo, Japan.

aqueous solution of the amino acids was measured by a MK-A type Karl Fischer apparatus* at 35°C; measuring point: 1 cm above the interface (constant).

(7) *Optical Microscopy:* Emulsion particles were observed by means of phase-contrast and polarizing microscopies; magnification; 400×.

(8) *Electron Microscopy:* Specimens were exposed to the saturated osmium tetroxide atmosphere for 72 h at 4°C, then dehydrated with ethanol and finally embedded in epoxy resin Epon 812. The specimens were sectioned at 500 Å thickness by a LKB III type ultramicrotome equipped with a diamond knife and observed under the Hitachi HU-12A EM.

(c). *Inorganic–Organic Property Balance (IOB):* The authors make reference to this concept in order to correlate the results of the experiment. Fujita (5) proposed the idea of the inorganic–organic property as a tool for predicting the various properties of organic substances. From the physical properties, such as boiling point, refractive index, etc., he gave an empirically specific number to each inorganic and organic property which corresponded to each functional group. Those surfactants capable of forming gels with an aqueous solution of amino acids or their salts are shown in Table I. Table II

Table I
Classification of the Surfactants Applicable to Gel Formation

Surfactant			Appearance	X-Ray Diffraction	Spacing (Å)		
Trade Name	Common Name	RT	IOB	Pattern	d_1	d_2	d_2/d_1
Sunsoft O-30B[a]	Glycerol monooleate	L	0.39	C	33.8	70.7	2.09
Arlacel 186[b]	Glycerol monooleate	L	0.47	C	33.8	73.9	2.18
G-EIS	Glycerol monoisostearate	L	0.42	C	33.8	70.7	2.09
POEM O-72-D[c]	Diglycerol dioleate	L	0.66	C	32.3	67.7	2.10
DIG-EIS[d]	Diglycerol diisostearate	L	0.47	C	33.0	67.7	2.05
PE-EIS	Pentaerythritol diisostearate	L	0.53	C	31.5	67.7	2.15
Arlacel 83[b]	Sorbitan sesquioleate	L	0.63	C	34.3	73.9	2.15
Emalex[e] EG2854-ol	POE (2.4) sorbitol tetraoleate	L	0.49	C	33.8	70.7	2.09

L: Liquid; S: Solid, C: Clear; I: Indistinct; N: No peak.
[a]Taiyo Kagaku Co., Ltd. (62 Akahori, Yokkaichi, Mie, Japan).
[b]Kao Atlas Co., Ltd. (1-1 Kayaba, Nihonbashi, Chuoku, Tokyo, Japan).
[c]Riken Vitamin Oil Co., Ltd. (3-8-10 Nishikanda, Chiyodaku, Tokyo, Japan).
[d]Matsumoto Trading Co., Ltd. (3-1 Nihonbashihoncho, Chuoku, Tokyo, Japan).
[e]5 Nihon Emulsion Co., Ltd. (5-32-7 Minami, Koenji, Suginamiku, Tokyo, Japan).
[f]Surfactants synthesized by the authors.

*Kyoto Denki, 68 Kisyoinshinden, Minamiku, Kyoto, Japan.

Table II
Classification of the Surfactant Lacking the Function of Gel Formation

Surfactant			Appearance		Spacing (Å)		
Trade Name	Common Name	RT	IOB	X-Ray Diffraction Pattern	d_1	d_2	d_2/d_1
G-di-nC$_8$[f]	Glycerol dioctanoate	L	0.58	I	24.2	(−)	(−)
G-tri-nC$_8$[f]	Glycerol trioctanoate	L	0.33	I	28.0	(−)	(−)
G-mono-brC$_8$[f]	Glycerol mono 2-ethylhexanoate	L	1.24	I	28.5	(−)	(−)
G-di-brC$_8$[f]	Glycerol di-2-ethylhexanoate	L	0.61	N	—	—	—
G-tri-brC$_8$[f]	Glycerol tri-2-ethylhexanoate	L	0.35	N	—	—	—
DIG-MO[d]	Diglycerol monooleate	L	0.80	I	38.2	86.6	2.27
DIG-TRO[d]	Diglycerol trioleate	L	0.26	I	29.3	(−)	(−)
DIG-TEO[d]	Diglycerol tetraoleate	L	0.17	I	27.7	(−)	(−)
TENOS®[a]	Glycerol monostearate	S	0.64	C	58.7	(−)	(−)
SPAN® 85[b]	Sorbitan trioleate	L	0.31	C	29.6	59.6	2.01
Nikkol®[g] GM-18IS	Batylalcohol monoisostearate	S	0.23	C	28.0	44.5	1.59
Hostaphat®[h] KO-300	Trioleyl phosphate	L	0.23	I	32.2	(−)	(−)
Emalex®[e] EG-O	Ethyleneglycol monooleate	L	0.40	I	30.5	(−)	(−)
EG-OPG-O[e]	Propyleneglycol monooleate	L	0.38	I	27.4	(−)	(−)
EG-O 300 dio[e]	Propyleneglycol 300 dioleate	L	0.54	I	(−)	(−)	(−)
EG-O 503[e]	POE(3)oleylethel	L	0.57	N	—	—	—
Nikkol®[g] MYO-2	POE(2)oleate	L	0.53	N	—	—	—
POEM®[c] O-105	POE(5)glycerol monooleate	L	1.03	N	—	—	—

[a]Taiyo Kagaku Co., Ltd. (62 Akahori, Yokkaichi, Mie, Japan).
[b]Kao Atlas Co., Ltd. (1-1 Kayaba, Nihonbashi, Chuoku, Tokyo, Japan).
[c]Riken Vitamin Oil Co., Ltd. (3-8-10 Nishikanda, Chiyodaku, Tokyo, Japan).
[d]Matsumoto Trading Co., Ltd. (3-1 Nihonbashihoncho, Chuoku, Tokyo, Japan).
[e]5 Nihon Emulsion Co., Ltd. (5-32-7 Minami, Koenji, Suginamiku, Tokyo, Japan).
[f]Nippon Fine Chemical Co., Ltd. (4-4-26 Honzanminami, Higashinodaku, Kobe, Japan).
[g]Nikko Chemicals Co., Ltd. (1-4-8 Bakurocho, Nihonbashi, Chuoku, Tokyo, Japan).
[h]Hoechst Dyestuffs & Chemicals Co., Ltd. (Frankfurt, Germany).

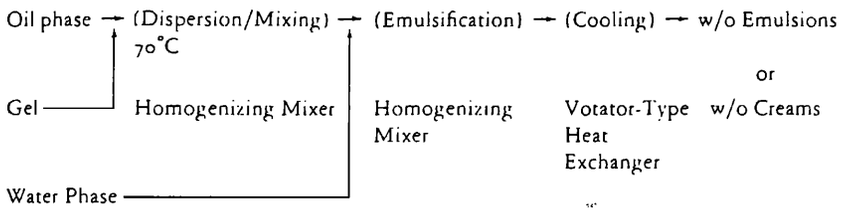
indicates those surfactants which did not produce a suitable gel. The various physical properties of the gel which were evaluated are shown in Figs. 3 to 17 and Tables III to IV.

GEL EMULSIFICATION METHOD

Materials: A standard gel was formulated with Sunsoft O-30B and an aqueous solution of monosodium L-glutamate monohydrate. Other surfactants used are shown in Table I. Squalane (special reagent grade) was used as the oil phase. For the formulation of the creams, materials readily available commercially were used.

METHOD

When the various premade gels were dispersed in the oil phase and emulsified by adding the water phase, excellent w/o emulsions were obtained. The emulsification method may be schematically shown as follows:



In order to compare the properties of these w/o emulsions and/or creams with those of the gels initially used, the following measurements were carried out.

a. *Hardness:* Hardness of the creams was measured at 25°C using a Curd Tension Meter.* The diameter of the needle was 8 mm and the load was 200 g.

b. *Viscosity:* Viscosity of the samples was measured using a B-type viscometer (at 30°C) and a Ferranti–Shirley cone and plate viscometer† (at 25°C, upper viscosity at the maximum rpm of 100 and a sweep time of 10 sec using M-cone).

c. *Emulsion Particles:* These were determined in a manner previously described.

d. *Stability:* The stability of the gels and w/o emulsions (or creams) stored for a month at 0°C, 25°C, 37°C was observed. These results are shown in Tables V and VI and Figs. 18 to 20.

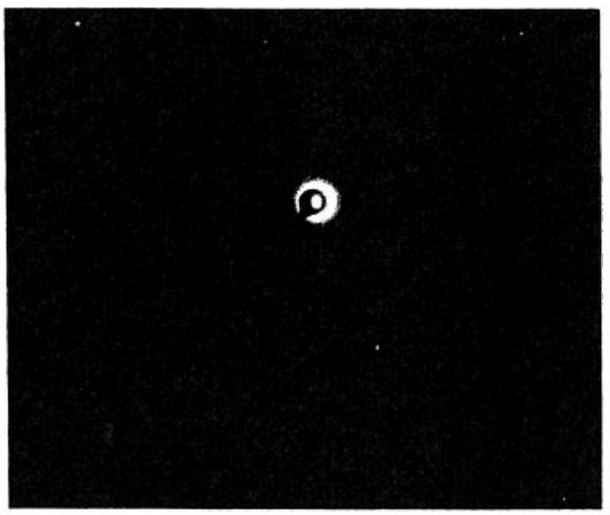


Figure 3. Typical small-angle diffraction pattern of surfactant, Sunsoft O-30B

*Iio Denki, 2-27 Yoyogi, Shibuyaku, Tokyo, Japan.
†Ferranti Ltd., Maston, Manchester 10.

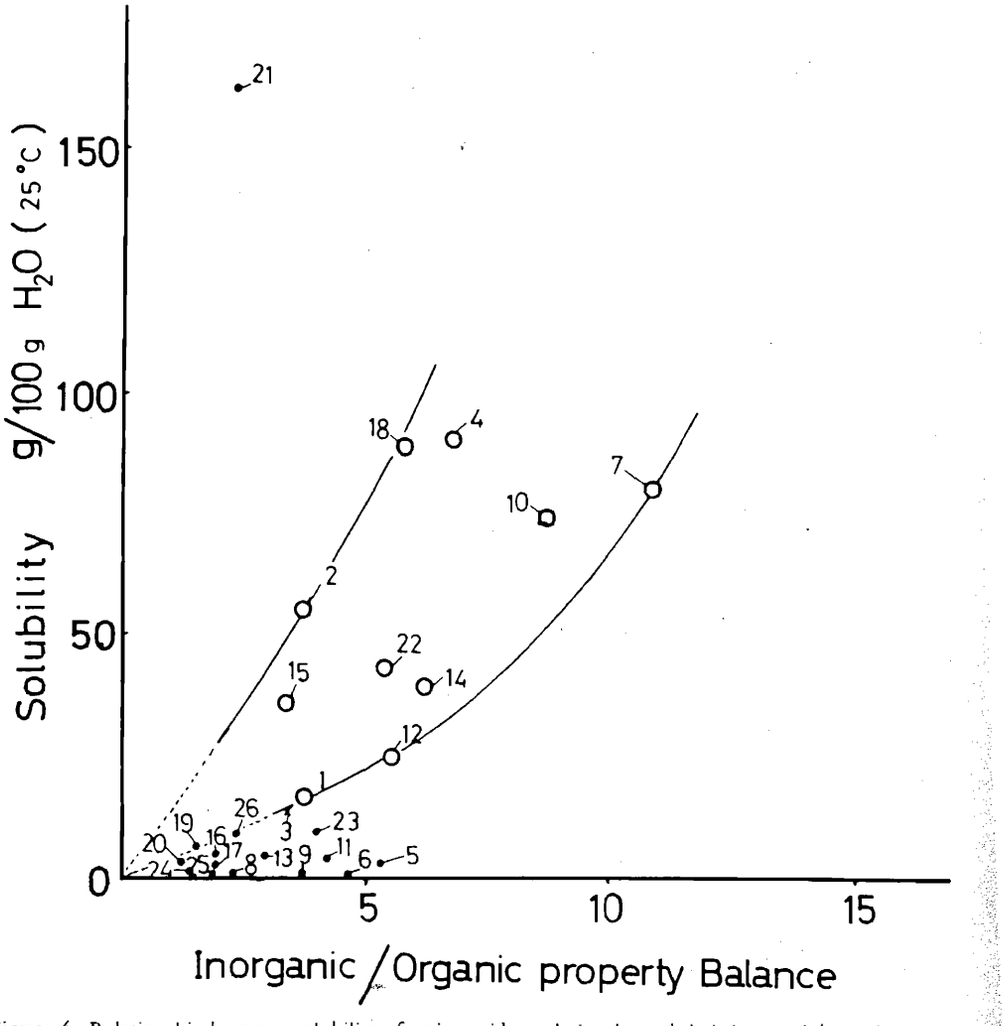


Figure 4. Relationship between solubility of amino acids or their salts and their inorganic/organic property balance

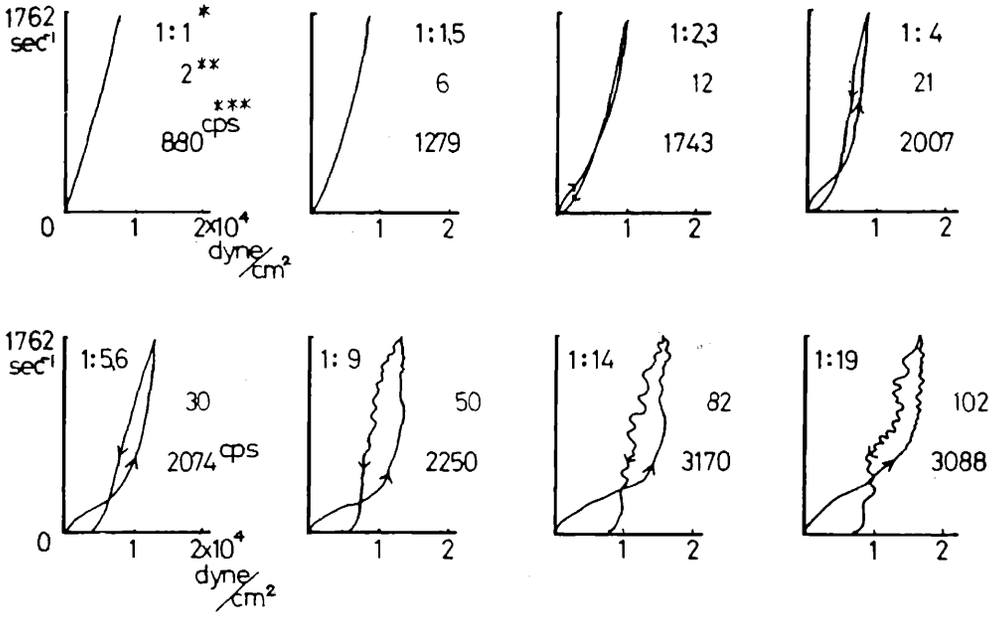


Figure 5. Rheological properties of gels obtained by combination of Sunsoft O-30B and 10 per cent aqueous solution of glycine: (*) Mixing ratio, (**) hardness (by curd tension meter), and (***) upper viscosity (by Ferranti-Shirley viscometer)

DISCUSSION OF RESULTS

The gels cannot be formed using any combination of surfactants and amino acids. To make the gelation possible, certain requirements in both surfactants and amino acids must not be neglected. Among the many surfactants tested, those capable of forming gels with aqueous solution of amino acids or their salts have been shown in Table I. It became evident from the results of the experiments, that the requirement common to the surfactants capable of forming gels were as follows.

1. Fatty acid partial esters of polyhydric alcohol having at least 3 hydroxyl groups in 1 molecule;
2. inorganic-organic property balance (IOB) of the molecules were within the range of about 0.4 to 0.7;
3. the carbon number of the esterified fatty acids was within 16 to 18; and
4. must be liquid at room temperature.

As is evident from Table II, no gels were formed with the surfactants which do not meet the above requirements, such as glycol esters, low fatty acid glycerides, surfactants that are solid at room temperature, higher alcohol ethers, higher fatty acid esters, and ethylene oxide adducts.

Polyhydric alcohols, which can be the main core of the gel forming surfactants were known from other experiments to include glycerin, diglycerin, trimethyl ethane, trimethylol propane, pentaerythritol, sorbitan, and sorbitol. On the other hand, the fatty acid part of the surfactants includes, for example, oleic acid, isostearic acid, ricinolic

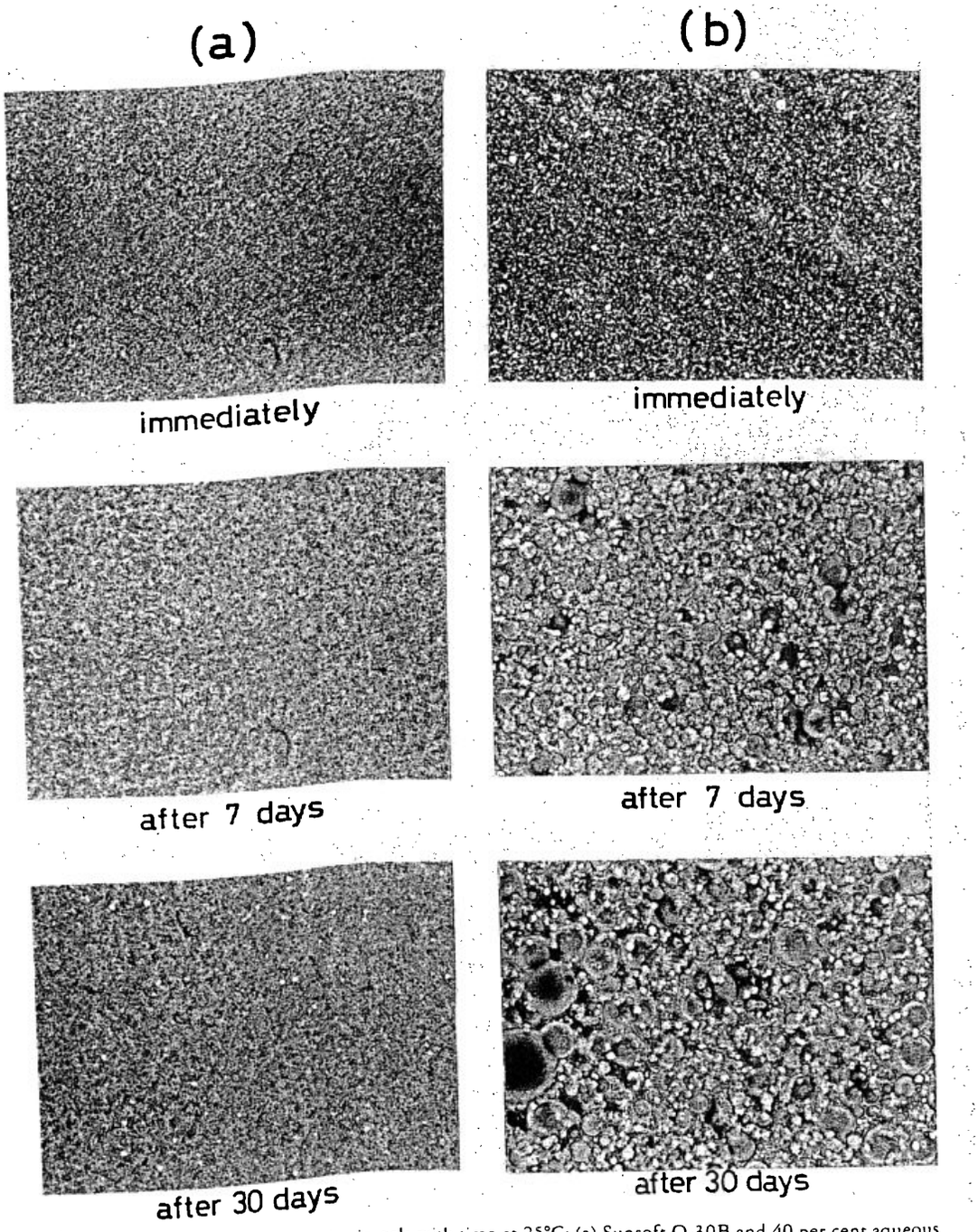


Figure 6. Change of water particles in gels with time at 25°C: (a) Sunsoft O-30B and 40 per cent aqueous solution of monosodium L-glutamate monohydrate system; (b) Sunsoft O-30B and water system

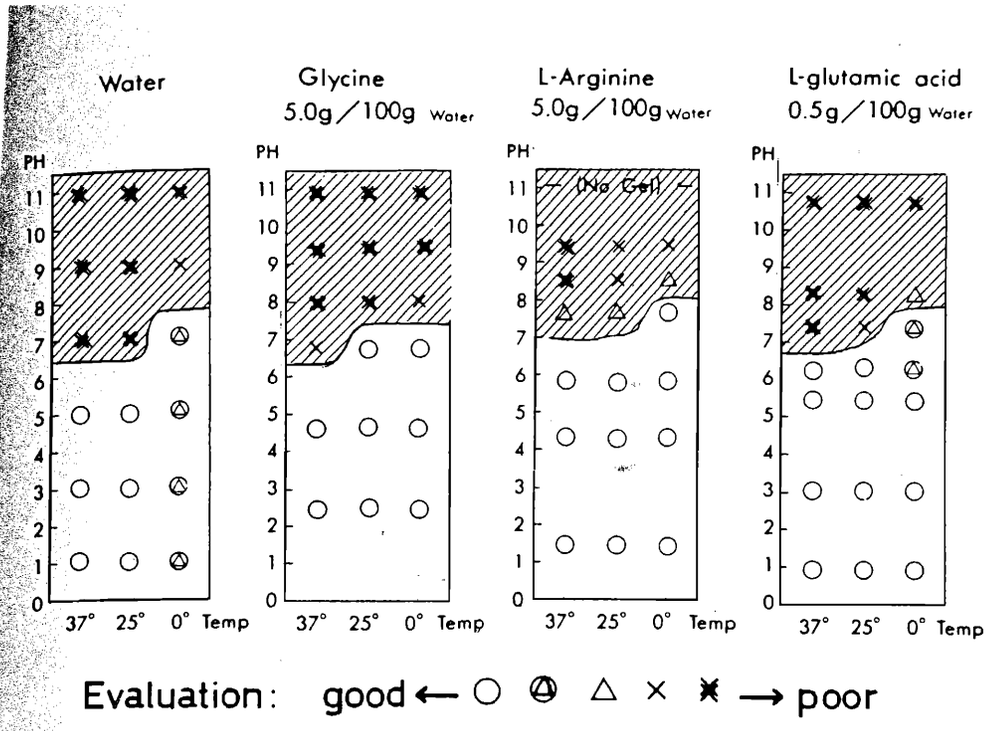


Figure 7. Effect of pH on stability of gels

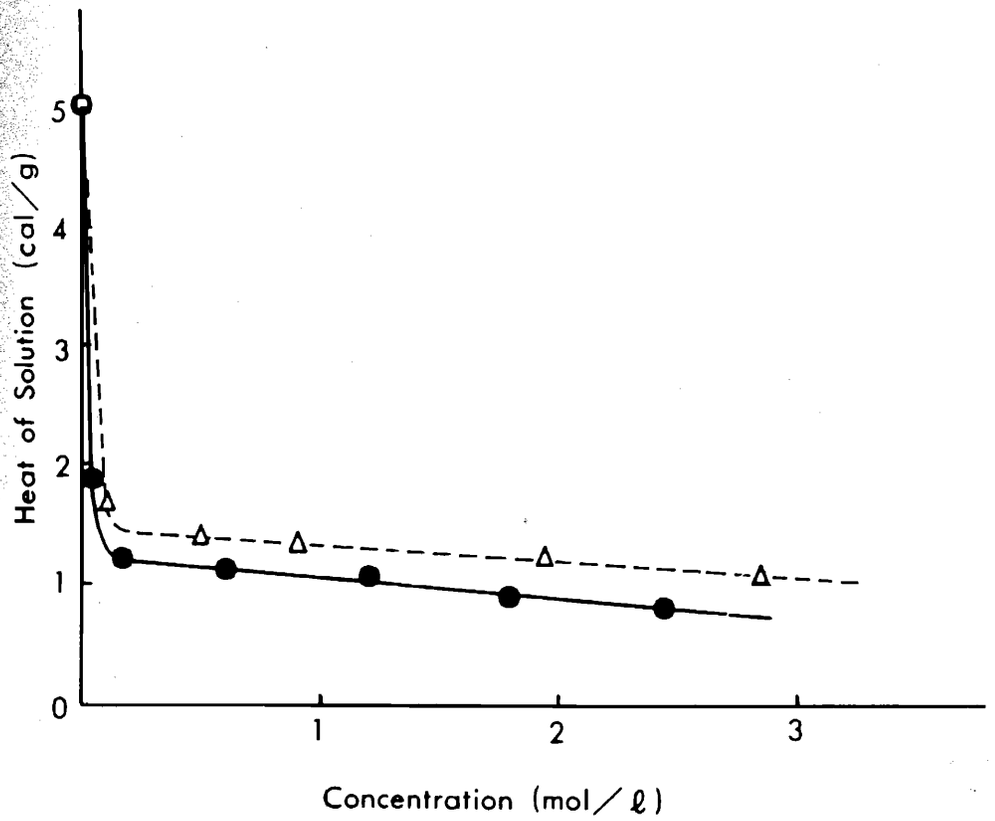


Figure 8. Heat of solution of surfactant to aqueous solution of amino acid or salt with various concentrations: (○) water, (△) L-serine, (●) Monosodium L-glutamate monohydrate

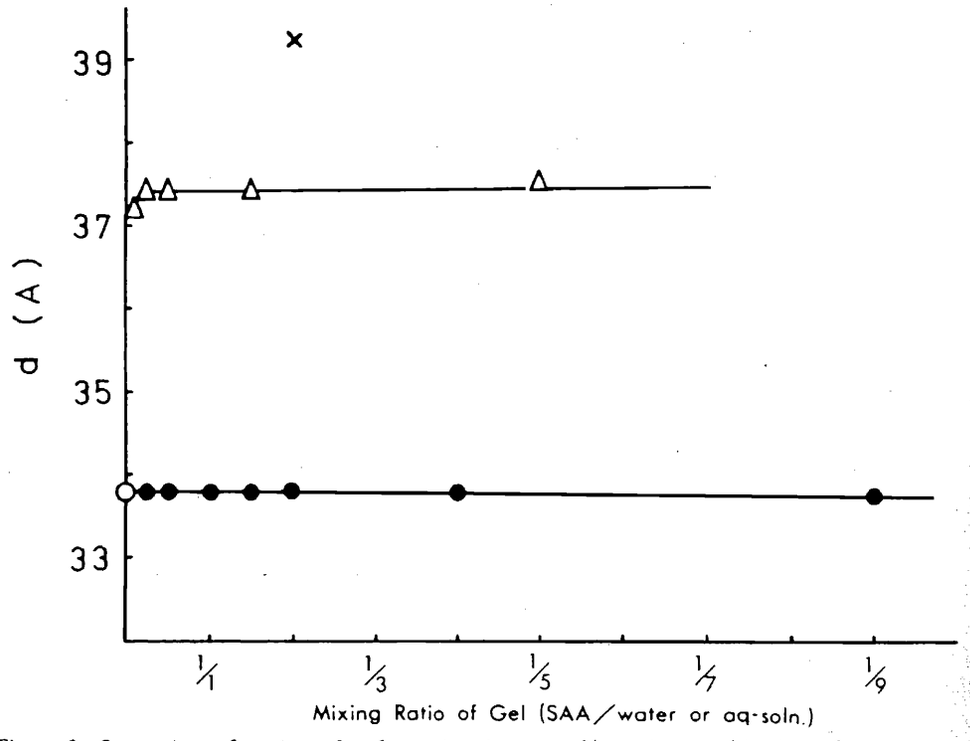


Figure 9. Comparison of spacings of surfactant in gels prepared by various combinations of water, monosodium L-glutamate monohydrate, and urea: (○) sunsoft O-30B itself, (△) water, (●) 40 per cent aqueous solution of monosodium L-glutamate monohydrate (×) 20 per cent aqueous solution of urea

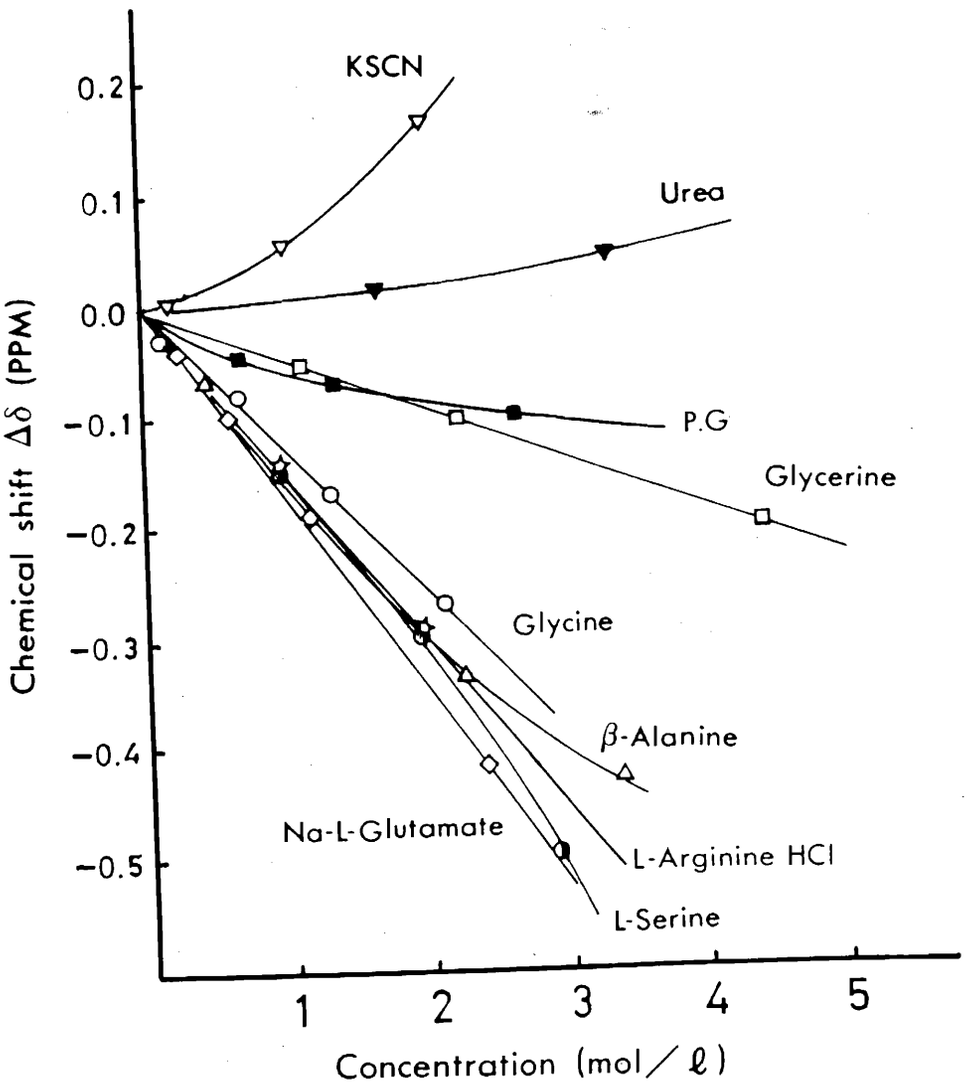


Figure 10. Chemical shift in proton of water for various aqueous solutions by nuclear magnetic resonance (NMR)

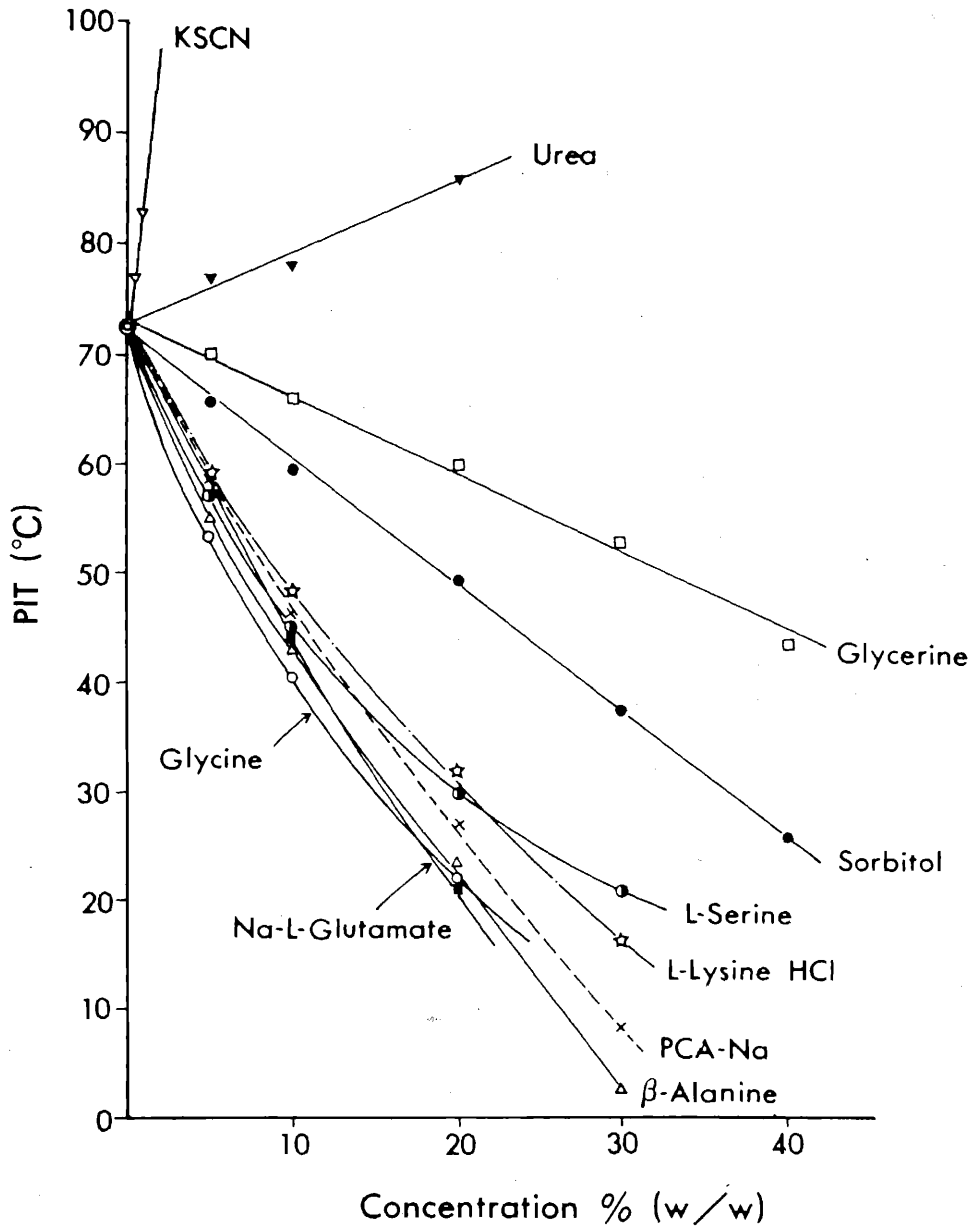


Figure 11. Effect of addition of amino acids or their salts to phase inversion temperature (PIT)

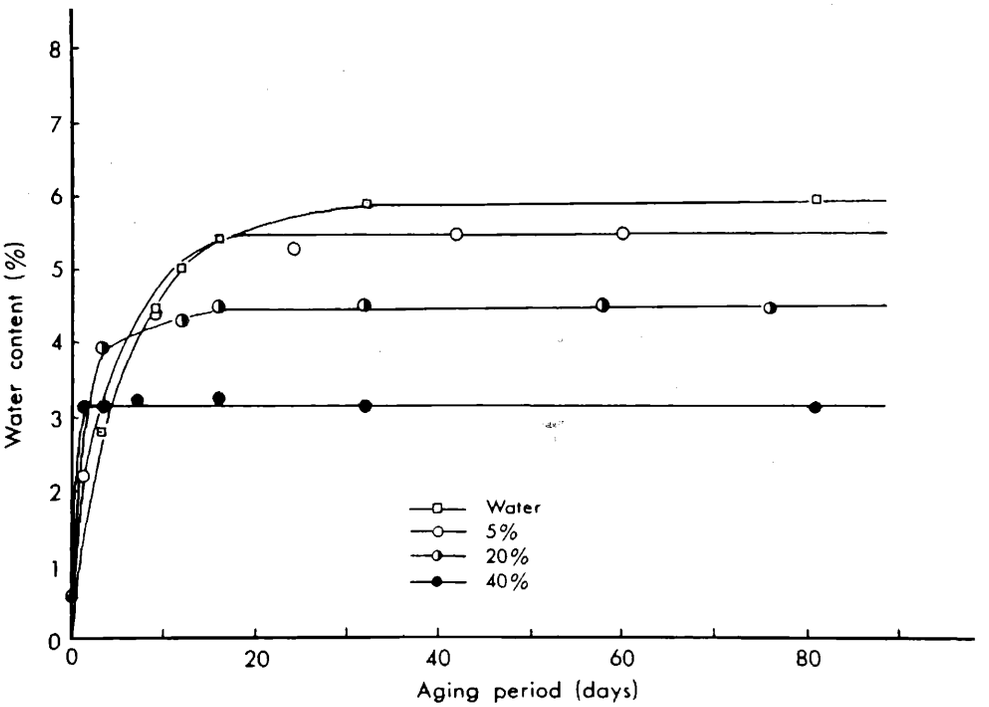


Figure 12. Change of water content migrating from water phase into surfactant phase (Sunsoft O-30B) with varied concentration of monosodium L-glutamate monohydrate with time

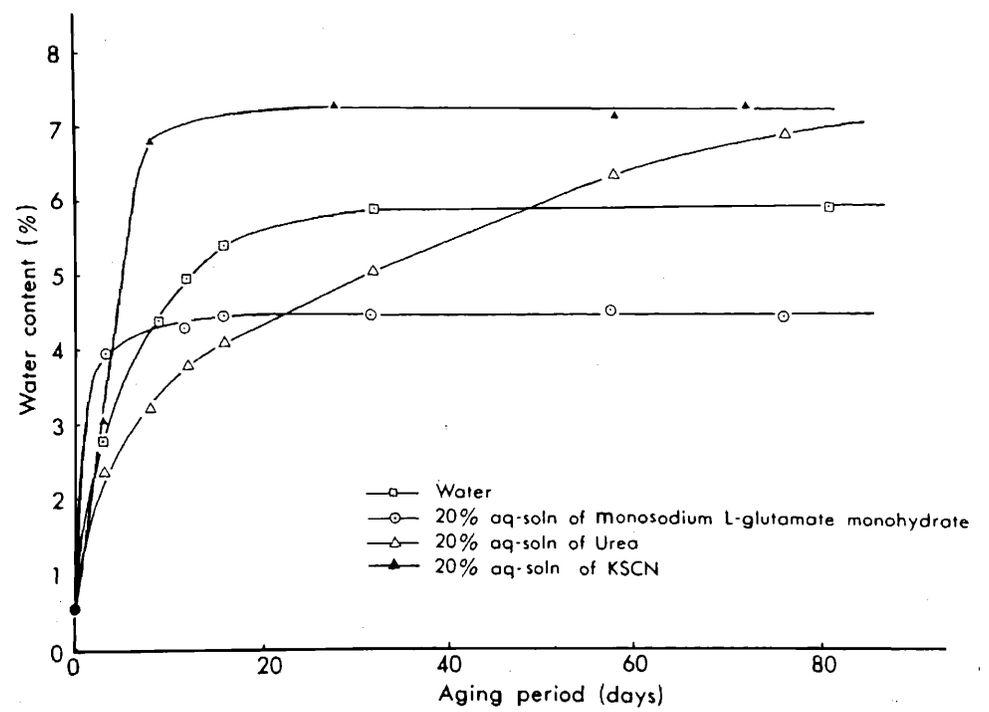


Figure 13. Change of water content, migrating from water phase into surfactant phase (Sunsoft O-30B) with varied solute with time

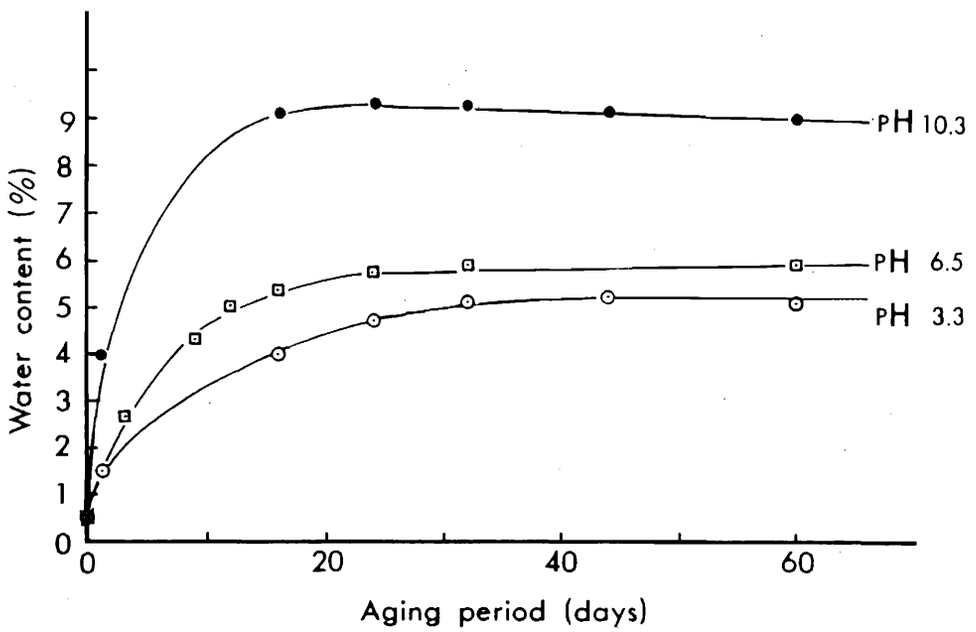


Figure 14. Change of water content, migrating from water phase into surfactant phase, with varied pH with time

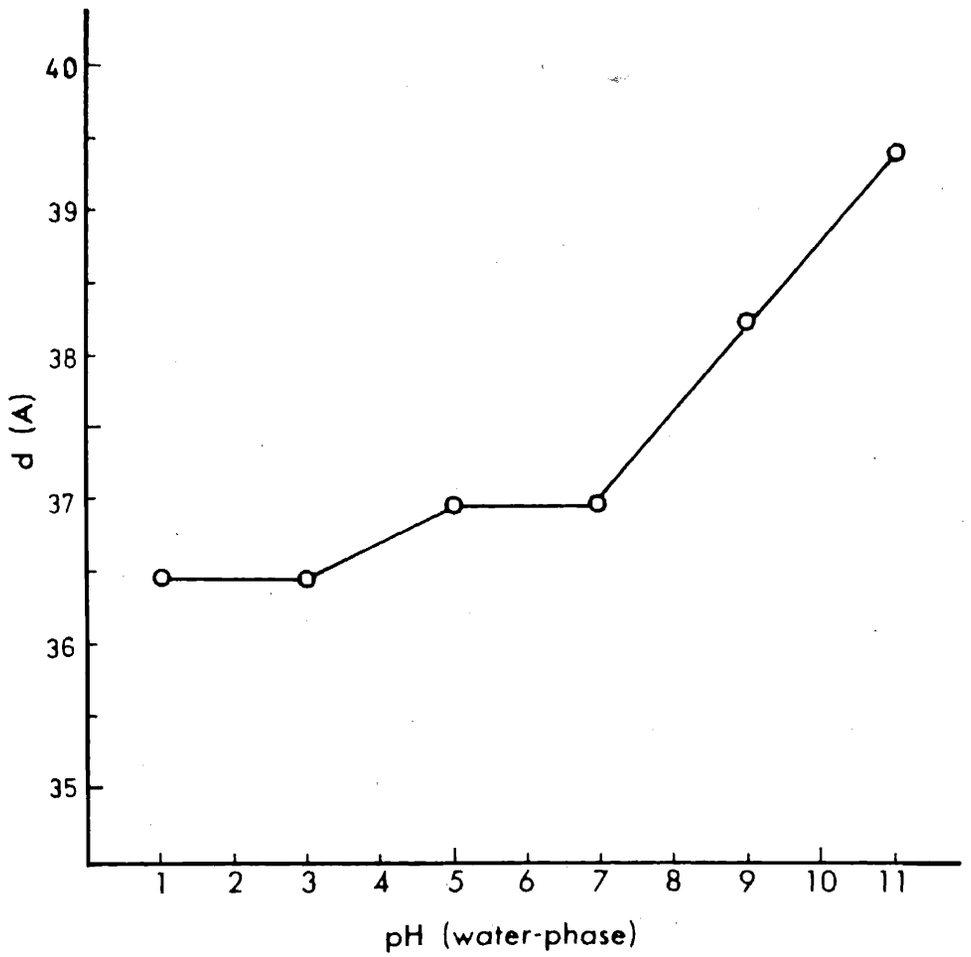


Figure 15. Effect of pH on spacings of surfactant in gel prepared between Sunsoft O-30B and water. Mixing ratio = 1:4

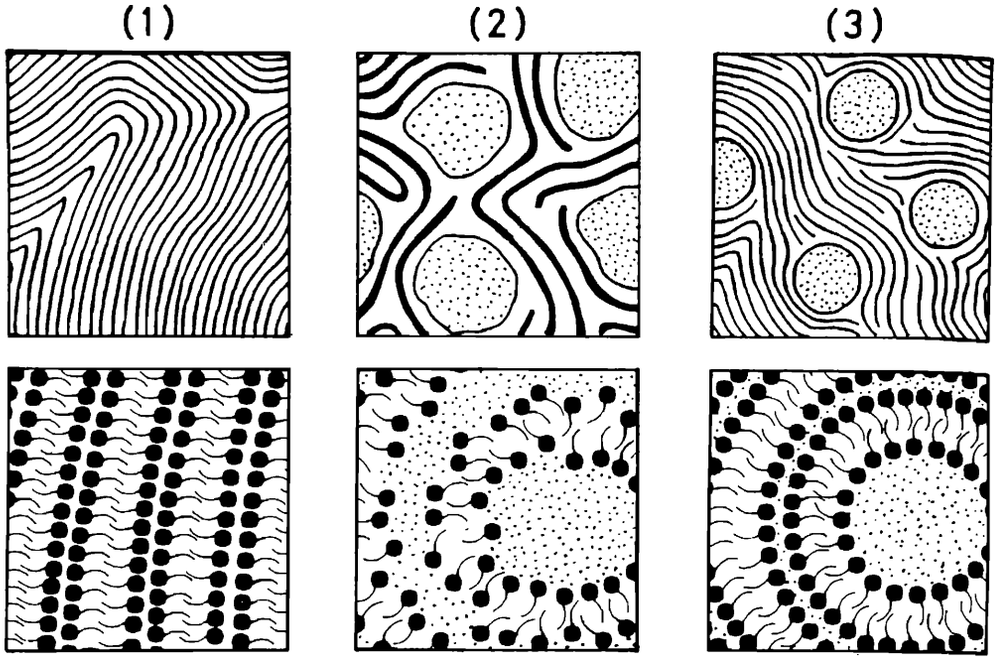


Figure 16. Structural model of surfactant and gel: (1) surfactant, (2) gel prepared between surfactant and water; (3) gel prepared between surfactant and aqueous solution of amino acid

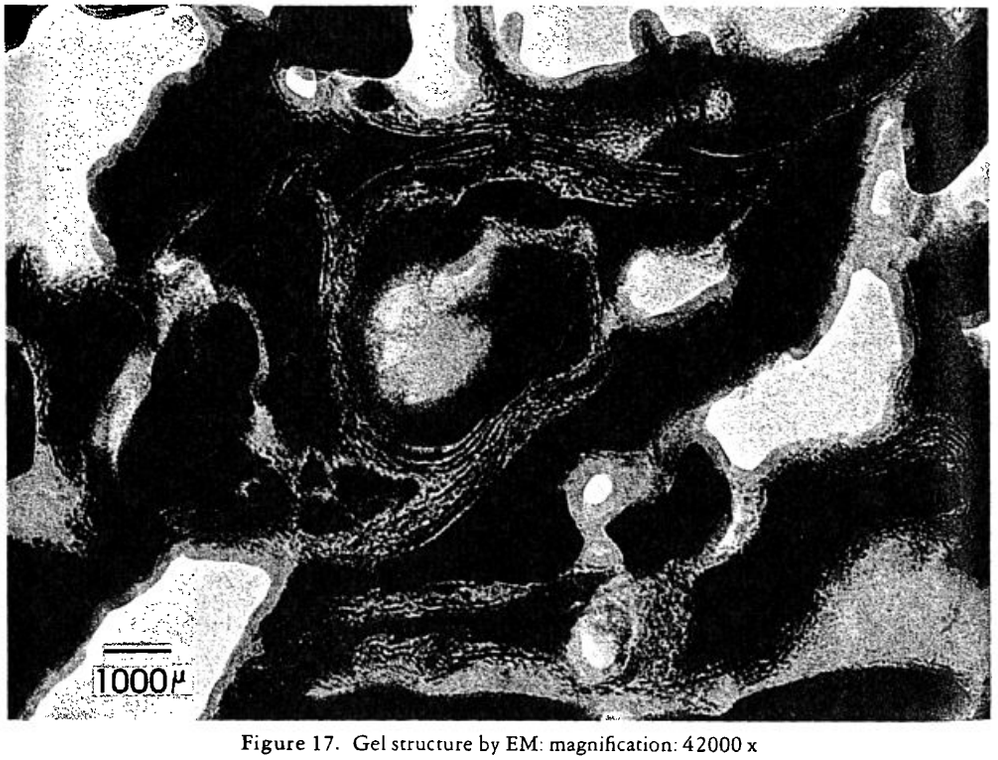


Figure 17. Gel structure by EM: magnification: 42000 x

Table III
Physical Properties of Amino Acids and their Salts

Number	L-Amino Acid and a Salt thereof	Solubility (6) $g/100_g H_2O$[25°C]	ΣN Inorganic	ΣN Organic	Gel Formation
1	L-Alanine	16.51	220	60	○
2	β-Alanine	55.50	220	60	○
3	L-Arginine	14.6 (20°C)	410	120	X
4	L-Arginine HCL	90.0	810	120	○
5	L-Asparagine H_2O	3.00	420	80	●
6	L-Aspartic acid	0.50	370	80	●
7	L-Asparate Na-H_2O	80.1	870	80	○
8	L-Cystine	0.011	240	100	●
9	L-Glutamic acid	0.84	370	100	●
10	L-Glutamate Na-H_2O	74.22	870	80	○
11	L-Glutamine	4.25	420	100	●
12	Glycine	24.99	220	40	○
13	L-Histidine	4.29	352	120	●
14	L-Histidine HCL-H_2O	39.0 (24°C)	752	120	○
15	Hydroxy L-proline	36.11	330	100	○
16	L-Isoleucine	4.12	220	110	●
17	L-Leucine	2.19	220	110	●
18	L-Lysine HCL	89.0	690	120	○
19	L-Methionine	5.92	230	140	●
20	L-Phenylalanine	2.97	231	180	●
21	L-Proline	162.3	230	100	●
22	L-Serine	43.0	320	60	○
23	L-Threonine	10.0	320	80	●
24	L-Tryptophan	1.14	321	220	●
25	L-Tyrosine	0.045	341	180	●
26	L-Valine	8.85	220	90	●

Evaluation: (○) good; (●) poor; (X) impossible.

Table IV
The Relationship of Water Content and Spacing to the Stability of Gel and W/O Cream

Test Items Gel's Aqueous Solution	Water Content (per cent)	Spacing (Å)	Stability Gel 1 day	Gel 7 days	Gel 30 days	W/O Cream 1 day	W/O Cream 7 days	W/O Cream 30 days
Water	5.93	37.6	△	x	X	△	△	x
5 per cent monosodium L-glutamate H_2O	5.49	37.1	○	◎	x	○	○	○
20 per cent monosodium L-glutamate H_2O	4.49	34.9	○	○	○	○	○	○
40 per cent monosodium L-glutamate H_2O	3.12	33.8	○	○	○	○	○	○
20 per cent potassium thiocyanate	7.32	40.4	x	X	X	△	x	X
20 per cent urea	6.91	39.2	X	X	X	X	X	X

Evaluation: good←○ ◎ △ x X⟶Poor

Table V
The Properties of the Creams Obtained from the Gels Prepared by the Addition of an Amino acid, and without an Amino acid

	Physical Properties of Cream						Stability of Cream					
	Viscosity		Hardness			Emulsion	After 1 Day			After 30 Days		
Number	70°C	30°C	0°C	25°C	37°C	Particles	0°C	25°C	37°C	0°C	25°C	37°C
	cps											
(1)[a]	18000	94400	42	11	6	(See Fig. 18(a))	○	○	○	○	○	○
(2)[b]	13900	101600	53	15	7		○	○	○	○	○	○
(3)[c]	8500	36000	12	3	1	(See Fig. 18(b))	△	◎	△	x	△	X
(4)[d]	5750	38400	15	4	1		◎	◎	△	△	△	x

Evaluation: good⟵○ ◎ △ x X⟶poor.
[a](1) 40 per cent aqueous solution of monosodium L-glutamate monohydrate~Sunsoft® O-30B.
[b](2) 40 per cent aqueous solution of monosodium L-glutamate monohydrate~POEM® O-72-D.
[c](3) Water~Sunsoft® O-30B.
[d](4) Water~POEM® O-72-D.

Table VI
The Relationship of the X-Ray Diffraction Patterns of the Surfactants to their Gel-Forming Function and the Properties of W/O Emulsions using the Gels

Surfactant			W/O Emulsion's	
Name	X-Ray Diffraction Pattern	Gel-Formation	Stability (RT)	Viscosity (30°C)
Sunsoft® O-30B[a]	Clear	○	○	94400[Hz]
DIG-EIS[b]	Clear	○	○	112800
Emalex® EG-O[c]	Indistinct	x	X (two-phase separations)	—
Emalex® 300dio[c]	Indistinct	x	x	1750
Emalex® 503[c]	No peak	x	x	750
Nikkol® MIO-2[d]	No peak	x	X (two-phase separations)	—

[a]Taiyo Kagaku Co., Ltd. (62 Akahori, Yokkaichi, Mie, Japan).
[b]Matsumoto Trading Co., Ltd. (3-1 Nihonbashihoncho, Chuoku, Tokyo, Japan).
[c]5 Nihon Emulsion Co., Ltd. (5-32-7 Minami, Koenji, Suginamiku, Tokyo, Japan).
[d]Nikko Chemicals Co., Ltd. (1-4-8 Bakurocho, Nihonbashi, Chuoku, Tokyo, Japan).

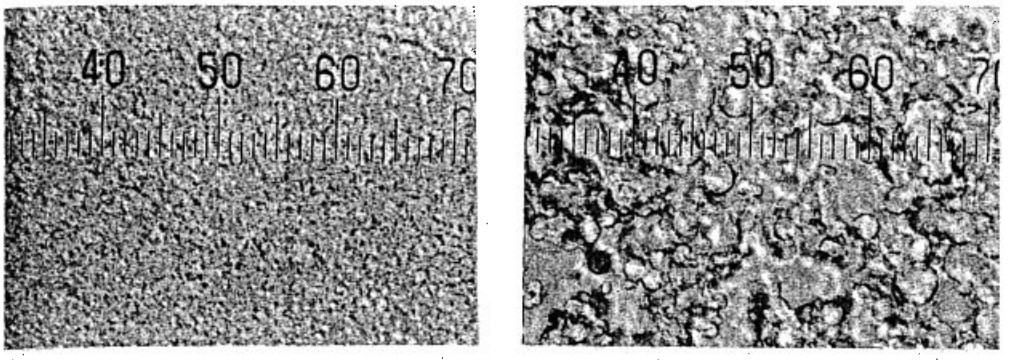


Figure 18. Comparison of emulsion particles of creams: division, 2.5μm

acid, linolic acid, and isohexadecanoic acid. These surfactants are supplied in the form of a mixture of partial esters. For example, when those with 3 hydroxyl groups were used, a mixture comprised mainly of monoester was preferred, and when those with 4 hydroxyl groups were used, a mixture comprising of diester gave the better results. On the other hand, even within the above mentioned range, those in which the hydroxyl groups were completely esterified, failed to form gels. Although, those surfactants which formed gels did not contain water, all of them gave 2 clearly discernible diffraction lines in the small angle region as seen in Fig. 3. As is seen in Table I, the spacings of the surfactants appeared at approximately 33 and 70 Å, and the ratio was about 1:2. From these facts, it was considered that, although, they were liquids, these surfactants possessed very orderly lamellar structures by themselves. On the other hand, almost all of those surfactants lacking the function of gel formation did not have a clear structure, as is shown in Table II. The X-ray diffraction patterns were indistinct or completely lacking even though they were clear; the spacings were either too wide or too narrow. Such results of X-ray analysis were identified as having a close correlation to gel formation.

As a result, it can be concluded that, in order to form gels, the corresponding surfactants are required to have at least a high orderly lamellar structure.

Contrary to the restriction of the surfactants used, almost all of the amino acids and their salts formed gels. As shown in Table III, nearly all of the monoamino-monocarboxylic acids (neutral amino acid) with an isoelectric point in the weak acidic range, such as monosodium salts of monoamino-dicarboxylic acid (acidic amino acid) and monohydrochlorides of diamino-monocarboxylic acid (basic amino acid) were capable of forming gels. Those readily soluble in water were very effective, such as glycine, L-alanine, B-alanine, hydroxy L-proline, L-serine, monosodium L-glutamate monohydrate, monopotassium L-glutamate monohydrate, monosodium L-aspartate monohydrate, monopotassium L-aspartate dihydrate, L-lysine monohydrochloride L-arginine monohydrochloride, and L-histidine monohydrochloride. The D- and DL stereoisomers also gave good gel formations.

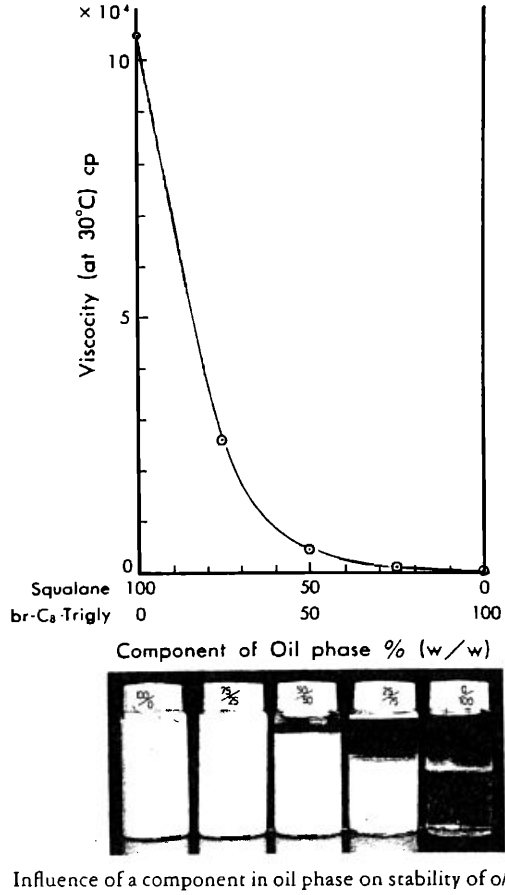


Figure 19. Influence of a component in oil phase on stability of o/w emulsions

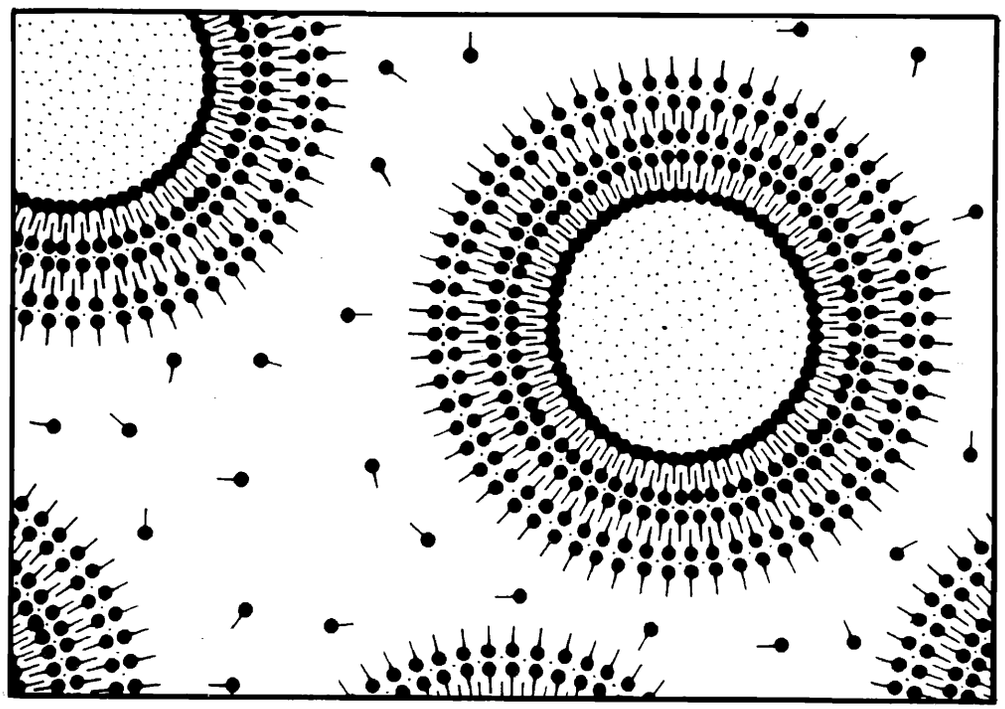


Figure 20. Model of emulsion obtained by gel-emulsification method

The fact that in the case of acidic amino acids and basic amino acids, the best gels were obtained only when the monosodium salts or monohydrochlorides, respectively, was considered to be closely related to the fact that the molecules were in the highest possible polyvalent state (III) among the 4 in the aqueous solution as is shown below.

$$\underset{\text{(I)}}{\overset{CO_2H}{H_3N^+-Glu-CO_2H}} \rightleftharpoons \underset{\text{(II)}}{\overset{CO_2H}{H_3N-Glu-COO}} \rightleftharpoons \underset{\text{(III)}}{\overset{COO^-}{H_3N-Glu-COO}} \rightleftharpoons \underset{\text{(IV)}}{\overset{COO^-}{H_2N-Glu-COO^-}}$$

$$pK_a' \quad 2.2 \qquad 3.2 \qquad 4.3 \qquad 9.7$$

In the case of neutral amino acids, the isoelectric range was also found to be the most suitable for the formation of the gels.

When one note the relationship between the solubility (6) and IOB as is shown in Fig. 4, it becomes apparent that the higher the solubilities in water the better the results obtained. The IOB is within the range of about 4 to 10. With acylated amino acids and their higher alcohol esters, there were no gel formations.

Various gels were obtained by a variety of combinations between amino acids and surfactants, such as their kinds and mixing ratio. The higher the concentration of amino acid, and it's mixing ratio to the surfactant and shearing stress the more stable was the gel obtained. In this case, the hardness and viscoelasticity of the gels increased and the gels became transparent. On the contrary, when the concentration and mixing ratio became lower, the gel appeared milky white and fluid. Figure 5 shows the rheological properties of the gels obtained between Sunsoft O-30B and a 10 per cent aqueous so-

lution of glycine. From the rheological properties, the previously mentioned results can also be explained. In this case, a maximum of 19 times the amount of glycine aqueous solution by weight to the surfactant was contained as the inner phase. When a 40 per cent aqueous solution of monosodium L-glutamate monohydrate was used, a maximum of 46 times was obtained. A gel in the metastable state lasting only for several hours was obtained in the absence of an amino acid as in the case of a system containing only surfactant and water. The water particles in the gel soon coalesced and the gel finally separated into 2 phases. The changes of water particles with time in these two cases are compared in Fig. 6.

It can be seen that the gels obtained by the addition of amino acids were very stable with no coalescence of droplets. The effect of pH on the gels was also examined. Figure 7 shows the stability of the gels obtained from buffer solution and that with a fixed amount of glycine (neutral amino acid, pI equals 5.97) L-arginine (basic amino acid, pI equals 11.15), L-glutamic acid (acidic amino acid, pI equals 3.22). It was apparent from the diagrams, that in all of cases, the gels were not stable in a higher pH range, but stable at the lower pH, i.e., the acid side. Gelation was poor in the range where the pH was extremely low and became better on the weakly acid side at 4.0 to 6.5. Stability also depended on the storage temperature. As the temperature increased, the stability shifted to the lower pH range.

As will be described later, the hydration power of the amino acids is considered to relate to the formation of stable gel, and it may be determined depending upon the ionic state of the amino acids.

From the fact that the aqueous solution of the amino acids and the above mentioned surfactants are combined to form gels having very high viscosity and showing no coalescence of particles of the aqueous solution, it is presumed that some specific interaction exists between the amino acid and surfactant at the interface. In order to confirm the possible existence of this interaction, first, the structure of the gel was investigated by the measurement of the heat of solution, X-ray analysis, DTA, and microscopy.

Figure 8 shows the results of the heat of solution at 35°C when the surfactant, Sunsoft O-30B, was mixed and dissolved into an aqueous solution of the amino acids. In the case of water only, exothermic heat of about 5 cal/g was measured. Should it be assumed that a new complex is formed by the addition of amino acid, an increase of exothermic heat higher than in the case of water will be expected and the curve should go upward.

However, as shown in the figure, in both the case of L-serine and monosodium L-glutamate monohydrate, the heat of solution decreased rapidly even with a minor increase of concentration. This indicates that the solubility of surfactant to amino acid solution decreases more than that to water by the possible reduction of the interaction between amino acid and surfactant and also confirms the fact that there are no new complex formations taking place.

Subsequently, as a result of X-ray analysis of the gels, which is the most common method, two diffraction lines were found in the small angle. It corresponded completely with the X-ray diffraction pattern of the surfactant itself as is previously described and was finally confirmed as that of the lamellar structure of the surfactants *per se*. However, it could not be confirmed that the formation of a new structure having new spacings between amino acid and surfactant was formed. The same results were

obtained by DTA and optical microscopic observations. From Fig. 6, it can be said that the gel itself is considered to be the same emulsion system as an ordinary w/o emulsion in which the particles of aqueous solution are dispersed in the lipophilic surfactant phase. It can be readily understood, as is shown in Fig. 5, that fluidity, high viscosity, and hardness of the gel are due to the increasing volume ratio and interfacial area. In the gels obtained by the addition of aqueous solution of amino acids, the interfacial films are very strong and stable so that the particles do not coalesce even though the inner phase is increased. These phenomena owe much to the functions of the amino acid. From these facts, the authors studied the influence of the amino acids on the change of the lamellar structure possessed in common with the surfactants used.

Figure 9 indicates the comparison between the spacings of stable gels with aqueous solutions of amino acid and those of unstable gels with water only and urea. Sunsoft O-30B, which was used as the surfactant in the experiments, had spacings of 33.8 Å. In the case of an aqueous solution of amino acid, even though the mixing ratio of the gel was altered, the original spacing of the surfactant did not change. However, in the case of water, it increased to 37.6 Å. Such change had been determined in either case at an early stage, regardless of the increased mixing ratio. It may be considered that the amino acid results in weakening the interaction between the water and the surfactant; that is, the amino acid decreases the solubility of the surfactant in the water phase and also prevents the solubilization of water into the hydrophilic parts of the lamellar structure, therefore, it seems to prevent the change in the original structure of the surfactant. It was found that urea further increased the spacing than in the case of water. Urea formed a gel with difficulty and though formed, was immediately destroyed and inverted to the o/w type.

The explanation of the results of NMR measurement are shown in Fig. 10 and shows how the amino acids or their salts interact with water. A negative sign Δd indicates a shift of proton in water to the lower magnetic field, and a positive sign means a shift to the higher magnetic field. The amino acids allows the proton in water to shift to the lower magnetic field. The gradient was sharp, hence, the effect was much greater even in lower concentration. On the contrary, potassium thiocyanate (KSCN) and urea shifted the proton to the higher magnetic field. In other words, these facts suggest a change in the physical properties of water. An amino acid, which shifts the proton to the lower magnetic field, indicates a condition in which the affinity of water to the amino acid is very strong and results in a decrease of free water. Therefore, these amino acids decreased the solubility of surfactant, which previously existed in water or prevented the solubilization of water in the hydrophilic part of the surfactant. These concepts are supported by the data obtained in the heat of solution shown in Fig. 8.

Furthermore, since the amino acid will expel the surfactant from the water phase, it becomes evident that a w/o type emulsion is the easier one to obtain. It is presumed that there is a decrease in the PIT with the increase concentration of the amino acids. This hypothesis was confirmed by the results of the PIT as is shown in Fig. 11. The amino acids decreased the PIT, and conversely, KSCN and urea increased the PIT. Such a trend in the PIT corresponded perfectly with the results of the NMR analysis. From this view point, it can be readily understood how the amino acid changes the physical properties of water and decreases the interaction with the surfactant. In order to investigate how much water causes a change of structure when solubilized in the hydrophilic part of the surfactant, the authors compared the changes in the amount of water migrat-

ing into the surfactant phase with that of the spacings. Figs. 12 and 13 show the changes in the water content caused by its migration into the surfactant phase with a lapse in time.

In the case of water only, the migration to the surfactant phase was as much as 6 per cent. In case of monosodium L-glutamate monohydrate, the amount of migration decreased with an increase in concentration. This corresponds well with the decreasing trend of affinity between the surfactant and water. The water content became constant rapidly and did not increase over a prolonged period.

The relationship of this water content to the spacings and the stability of the gels is indicated in Table IV. As is evident from this Table, with the increase of water migrating to the surfactant, the spacing becomes much wider. The spacing obtained with 40 per cent aqueous solution of monosodium L-glutamate monohydrate was exactly the same as that of the surfactant itself (Sunsoft O-30B). No changes in the structure of the surfactant was observed when the solubilized water was less than 3 per cent. The larger the variation of the spacings, the poorer the gel formation became.

The effect of pH on the stability of the gel can also be explained by the variation of water content and the spacings as shown in Figs. 14 and 15. As the pH of water increased, the water solubilized in the surfactant increased, and at the same time the spacings also increased rapidly.

From the above results and discussions, it is possible to draw the structural model indicating the mechanism for the stabilization of the gels by the amino acids as is shown in Fig. 16. The upper models indicate the overall view, and the lower models show a magnified view. The surfactant, though it is in the liquid state, has a lamellar structure as seen in view (1) of Fig. 16. In the case of the aqueous solution in the absence of amino acids, a large amount of water is solubilized in the hydrophilic part of the surfactant having an orderly form and its original structure becomes disordered and loosened due to the widening of its spacings. Under such conditions, water particles coalesce easily, which indicates instability. The same explanation can be made in the case of KSCN and urea, which change the structure of the surfactant. This is shown in view (2) of Fig. 16. View (3) of Fig. 16 indicates the case when amino acid is added. Since the amount of water to be solubilized in the hydrophilic part of the surfactant is limited and does not induce any change of structure, a concentrated and tight interfacial atmosphere is established around the particles. As a result, coalescence of the particles hardly occurs; thus, maintaining stability. As an important fact to support such a structural model, we succeeded in taking the photograph of the gel by EM (as is seen in Fig. 17). The sample shown was the gel obtained with Sunsoft O-30B and a 40 per cent aqueous solution of monosodium L-glutamate monohydrate mixed in the ratio of 1:4. Apparently, the water particles surrounded by the surfactant phase in the lamellar structure can be seen.

GEL-EMULSIFICATION METHOD

The relationship between the stability of the gels and that of w/o emulsions made by using the gels has also been noted. Hardness of the gels was influenced by the shear stress given at the time of preparation. When the hardness of the gels was changed, the

hardness of the w/o cream using such gels also changed depending on the former change.

As was previously described, the gel obtained by Sunsoft O-30B and the amino acid was stable; however, it is necessary to observe the changes in the cream when an unstable gel is used. Table V and Fig. 18 indicate the results obtained when an unstable gel, such as obtained between Sunsoft O-30B and water in the absence of an amino acid, was used. In comparing these results with the case where stable gels were used in the preparation of creams, remarkable differences were recognized as to viscosity, hardness, stability, and size of the emulsion particles. Table VI indicates the relationship of the X-ray diffraction patterns of the surfactants to its function in gel-formation and the properties of the w/o emulsions using these gels. As is evident from this Table, it is readily understood that the surfactants having clear X-ray diffraction patterns can readily form gels and the creams obtained were stable while those having indistinct X-Ray diffraction patterns did not form gels and produced unstable creams.

These facts can also be confirmed by other studies. As was mentioned previously, the amino acid functions to prevent the expansion of the spacings of the surfactant when an aqueous solution of amino acid is added to the surfactant phase. It was also found that amino acids possessing this property produced a good stable gel. Furthermore, the relationship between these properties and the stability of w/o emulsions were also studied. The results are shown in Table VII. There is a correlation between the stability of the gels and the stability of w/o emulsions; namely, the better the stability of the gels, the better the w/o emulsion obtained. Furthermore, the stability of the gels, which have been studied on the standard sample by using squalane as the main constituent of the oil, must be changed with the polarity of the oil. Figure 19 shows the influence of a component in the oil phase on the stability of the w/o emulsion. When squalane only was used as the oil phase, the destruction of the gel was not observed and the resulting w/o emulsion had a high viscosity of greater than 100,000 cps, but as the mixing ratio of glycerol tri-2-ethylhexanoate became higher, the viscosity dropped rapidly and finally, no emulsification occurred which was accompanied by a total destruction of the gel in the oil phase. In a nonpolar oil, such as squalane, there was no structural change of the surfactant; however, in a polar oil, such as glycerol tri-2-ethylhexanoate, the structure disappeared completely. Therefore, as the polar components in the oil phase increased, the emulsion became increasingly unstable. Thus, in the gel-emulsification method, it is advisable to use nonpolar oils and waxes in the oil phase, such as, squalane, liquid paraffin, and microcrystalline wax, etc. These facts have been proven to be identical in practical formulations of cosmetic creams as well as in the simple systems studied.

In the gel-emulsification method, the drawings of the emulsion when the oil is added can be assumed to be as is shown in Fig. 20. In the case of nonpolar oils, the water particles surrounded by several layers of surfactant disperse in the oil phase. On the contrary, in the case of polar oils, it may be assumed that the orientation of the surfactant is loosened and its adsorption at the interface are hindered, resulting in an unstable emulsion.

The above results may be summarized as follows. By initially forming a stable gel and maintaining conditions to maintain gel stability, a stable w/o emulsion having high vis-

Table VII
Example of w/o Type Emollient Cream

Squalane	25.0 parts
Ceresine	3.0
Beeswax	1.5
Lanolin	0.5
Petrolatum	6.0
w/o gel	20.0
Propylene glycol	5.0
Water	39.0
Perfume	proper amount
Antiseptics	proper amount

cosity and uniform particles may be obtained. On the other hand, when unstable gels are used (or under conditions inducive to gel destruction), unstable w/o emulsions and creams result. In other words, there is a correlation existing between the properties of the gel used and the stability of the final emulsion obtained.

APPLICATION TO COSMETICS

As mentioned above, the authors elucidated the requirements for surfactants that formed stable gel and the function of the amino acid contributing to the stability of the emulsions. It was noted therein that the characteristics of the gel used played an important role, and that the better the gel used in the formulation, the better the emulsions or creams produced were. We will discuss some of the advantages obtained by the application of this emulsification technique in the preparation of cosmetic base together with the characteristics of the finished products. Several creams were prepared by the gel-emulsification method and used as a base for cosmetics.

From the measured values and stability of the prepared creams by this method, we noted many advantages with this emulsifying method and products. For example, any type of cream with required values may be readily selected by referring to the component ratio, and a stable cream may be obtained by adding a definite quantity of the gel to cover the wide ranges of volume ratio. Even those at the range of extremely biased volume ratio where another surfactant such as soaps are used to adjust HLB for stabilizing the system can also be used.

Furthermore, from the evaluation of the application tests, we were able to know the suitable formulation ranges for 3 types of creams such as the cleansing, massage, and emollient types, respectively. It was especially interesting to note that suitable formulations for emollient creams were concentrated in the ranges, wherein there was a fairly large content of water contrary to the former 2 creams. By using these new techniques and know-how in the formulation of cosmetics, the authors were able to prepare various types of bases for cosmetics. An example of the emollient cream is shown in Table VII. This method may be applied not only to cosmetic creams, but also to stick type emulsion products with extremely low water content and also to different pharmaceutical preparations. The characteristics of the cosmetic bases prepared by using this method were their ready adaptation to the skin, their excellent spreadability and moisturizing effect with hardly any greasy feel and glaring appearance, which were

common to conventional w/o type cosmetics. In addition, the products were highly safe for skin application. These many advantageous characteristics of the creams may be attributed to the phenomena suggesting the role of NMF. The stability of these products were excellent and had long shelf life, even at elevated temperature together with less dependability of hardness on aging and temperature.

It is well known that the NMF content in stratum corneum is high and has water absorbing and water holding properties. It is said that the chapping of the epidermis in the winter and the dryness of the aged skin are due to insufficient secretion of sebum and NMF. In view of this fact, it is quite natural and reasonable to supply the deficiency of NMF with cosmetics. NMF is said to be found mostly at the boundary below the stratum corneum and is found also in stratum granulosum and sweat. Sixteen free amino acids have been identified such as serine with more than 30 per cent, glutamic acid, proline, etc. (7). It has been proven that mono-, di- and triglycerides of fatty acids also exist in sebum. Sebum flows readily and spreads on the surface of skin after reaching the hair follicles and is then mixed with sweat and eventually forms a skin surface lipid film which is a w/o type emulsion. It is easily inverted to o/w type emulsion under the condition when the ratio of sweat becomes high. But, this is reversible and the type emulsion is determined depending upon the physiological condition of the skin surface (w/o$\rightleftharpoons$o/w) (8).

We can find an interesting similarity between the natural emulsification phenomena on the skin surface and that of the gel-emulsification method developed by the authors. As mentioned before, in our emulsification method, monoglyceride, and aqueous solution of amino acid which correspond to sebum and NMF, respectively, were mixed and a w/o type emulsion was formed. On the contrary, urea and uric acid, which are found in sweat broke down such w/o emulsions and inverted to that of the o/w type. Furthermore, when the gel was kept in water, it expanded remarkably. This property is similar to semipermeability of the lipid film. As the amino acids in the gels retard moisture loss and maintain moisture by their strong hydration effect, it is considered that cosmetic bases utilizing amino acids play an important role in maintaining the flexibility and elasticity of the skin. The effect of products formulated with amino acids on the skin are now under investigation by the authors from the view point of viscoelasticity of stratum corneum and percutaneous absorption of amino acids using radioisotope.

CONCLUSION

The w/o emulsions stabilized with amino acids or their salts were investigated. The following may be concluded.

1. Stable w/o type emulsions which have the capacity to contain wide ranges of water ratio, were obtained by using the gels prepared in a given combination of the amino acids and their salts with lipophilic surfactants having high orderly lamellar structures.
2. The authors applied this technology to the formulation of cosmetics such as creams, stick type cosmetics, foundations, etc.
3. The outstanding characteristics of the products obtained by using this method are their refreshing, smoothing, ready adaptation to the skin and moisturizing effects

with hardly any greasy feel and glaring appearance which are common to w/o type emulsion.

4. It is important that such surfactants to be used in these w/o emulsions have lamellar structures.
5. Amino acids are very effective in forming lamellar structure and contribute to preserve the tight interfacial atmosphere concentrating the surfactant at the interface. As a result, coalescence of water particles is prevented and stabilization of the w/o emulsion is achieved.
6. The stabilization mechanism of the w/o emulsions using the new method was elucidated through surface chemical investigation synthetically by means of X-Ray analysis, heat of solution, NMR, electron microscopy, etc.

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