

# Viscosity Change after Dilution with Solutions of Water-Oil-Water Emulsions and Solute Permeability Through the Oil Layer

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**Abstract** □ A water-in-oil-in-water (W/O/W) multiple phase emulsion was prepared by a two-step emulsification procedure. The oil phase consisted of paraffin oil and sorbitan monooleate. The inner aqueous phase and the outer aqueous phase were 0.5% glucose solution and 3% polysorbate 80 solution, respectively. Viscosity measurements were carried out on the W/O/W emulsion after diluting it with a number of solutions. A given sequence for the solutes that would increase the emulsion viscosity after dilution was determined. This sequence was identical with that obtained with the solutes in an independent permeability experiment using a planar membrane composed of sorbitan monooleate alone. As a result, it was suggested that solutes as well as water can permeate the oil layer of vesicles of the emulsion to change the vesicle volume, thereby causing a change in the emulsion viscosity.

**Keyphrases** □ Emulsions—water-oil-water, viscosity change after dilution with solutions, permeability through the oil layer □ Permeability—solutes, through oil layer of water-oil-water emulsions □ Viscosity—changes, water-oil-water emulsions after dilution with solutions

Recently, a great deal of work has been done on preparation methods and applications of water-in-oil-in-water type multiple phase emulsions (denoted hereafter as W/O/W emulsions). Matsumoto *et al.* (1–3) reported a systematic procedure for the preparation of W/O/W emulsions. It was shown that an anticancer agent is localized at the cancer site for prolonged periods when administered in the form of a W/O/W emulsion, with the drug dissolved in inner aqueous phase (4–8). The application of W/O/W emulsions in foods (9) and cosmetics (10) also has been described.

In the application of W/O/W emulsions, it is necessary to estimate their stability experimentally. Viscosity has been claimed as a convenient method to determine stability (11). Although the viscosity of W/O/W emulsions is affected by a number of factors, the most important is the osmotic pressure gradient between the inner aqueous

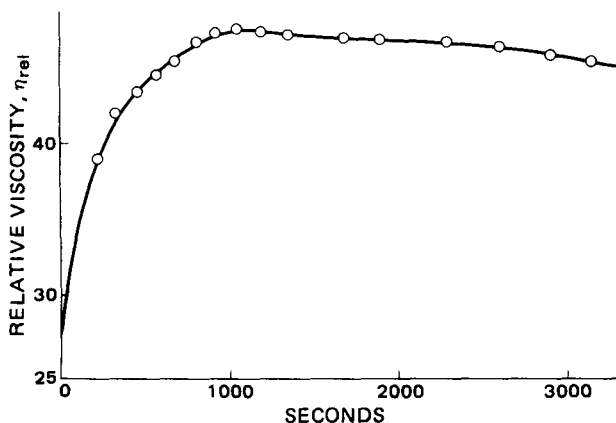


Figure 1—Viscosity change of W/O/W emulsion after dilution with water.

phase and the outer aqueous phase. This may produce changes in the volume of the inner aqueous phase due to the migration of water and/or solutes through the oil layer. In fact, the initial viscosity rise of some W/O/W emulsions on aging was explained by assuming the permeation of water containing a solute through the oil layer, from the outer aqueous phase to the inner aqueous phase due to the osmotic pressure gradient between the two phases (12). The present study will try to show that the viscosity change of W/O/W emulsions after dilution with solutions is interpreted more reasonably by assuming that solutes as well as water can permeate the oil layer.

## EXPERIMENTAL

**Preparation of W/O/W Emulsions**—The W/O/W emulsion was prepared by a two-step emulsification procedure according to Matsumoto *et al.* (1). Sorbitan monooleate<sup>1</sup> (8 g) and liquid paraffin<sup>2</sup> (10 g) were mixed thoroughly in a 100-ml beaker with a pin-mixer<sup>3</sup>. The stirring bar of the mixer was two six-pin blades which were fixed in a pile with a stainless steel bar. The rotation speed was maintained at 196 rpm by a constant speed stirrer<sup>4</sup>. As the mixture was being stirred, 30 ml of 0.5% (w/v) aqueous glucose solution was added slowly into the beaker at a constant rate. Addition of the glucose solution was controlled at a 3 ml/min rate by a pump<sup>5</sup>. The W/O emulsion formed was then stirred for 10 more min. It took 100 min for the first emulsification.

Thirty-five grams of the newly prepared W/O emulsion was dispersed into 35 ml of 3% aqueous solution of polysorbate 80<sup>6</sup> using a homogenizer. The blade was a part of another homogenizer<sup>7</sup>, and consisted of a fixed outer blade and an inner rotatable blade. The viscous W/O emulsion was torn and dispersed when passed through the narrow gap between the

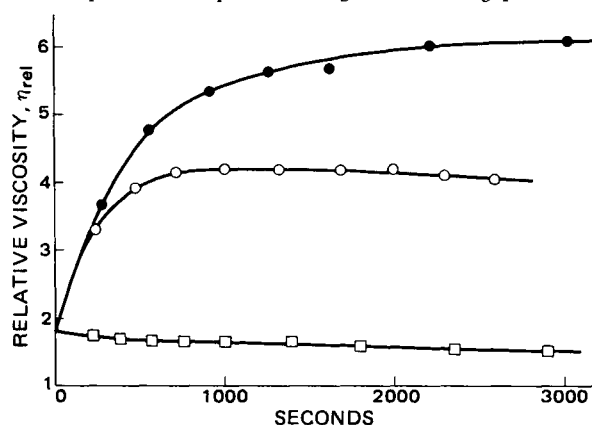
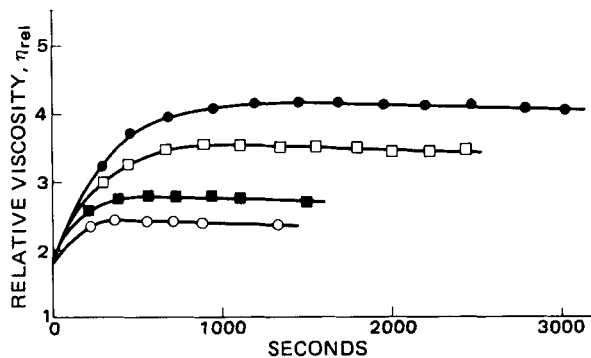


Figure 2—Viscosity changes of W/O/W emulsion after dilution with aqueous potassium chloride solutions. Key: ●, water; ○, 10<sup>-3</sup> M; and □, 10<sup>-2</sup> M.

- 1 Tokyo Kasei Co., Tokyo.
- 2 Kokusan Chemical Works, Tokyo.
- 3 National Mfg. Ltd., Lincoln, Neb.
- 4 Tokyo Rikakikai Co., Tokyo.
- 5 Kuramochi Kagaku Kikai Mfg. Ltd., Tokyo.
- 6 Nikko Chemicals Co., Tokyo.
- 7 Polytron PCU-2, Kinematica Co., Basel, Switzerland.



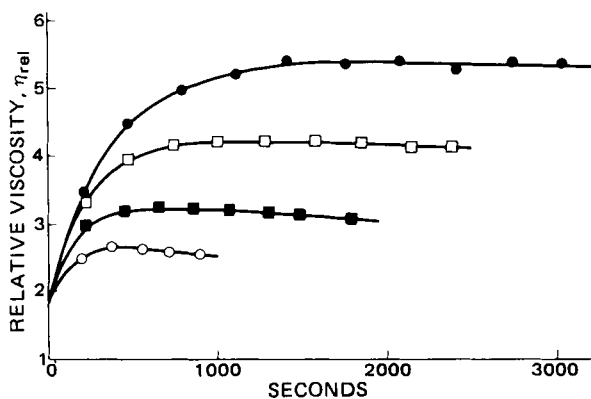
**Figure 3**—Viscosity changes of W/O/W emulsion after dilution with aqueous sucrose solutions. Key: ●,  $1 \times 10^{-3}$  M; □,  $2 \times 10^{-3}$  M; ■,  $4 \times 10^{-3}$  M; and ○,  $6 \times 10^{-3}$  M.

blades. The blade was connected to the constant speed stirrer used in the first emulsification since slow rotation was necessary. The rotation speed was set at 324 rpm and it took ~10 min to complete the second emulsification. The W/O/W emulsion prepared was denoted Sample A.

**Viscometry of W/O/W Emulsion**—The viscosity of W/O/W emulsions was measured with a capillary viscometer<sup>8</sup> in a constant temperature water bath maintained at  $25 \pm 0.1^\circ$ . The time course was started at the time of Sample A dilution with water or an aqueous solution. The measurement time was taken as the average of the start and finish time of each liquid addition. All diluents were kept at  $25^\circ$  beforehand. As the ratio of diluent volume to Sample A weight was not constant for all runs, it is noted where necessary.

**Planar Membrane Experiment**—To determine if solutes can migrate through the oil layer, the following planar membrane experiment was done using an apparatus consisting of two acrylic chambers separated by a millipore filter<sup>9</sup> to which sorbitan monooleate was applied. Sorbitan monooleate acts not only as a W/O emulsifying agent, but as water solubilizer in oil. Liquid paraffin was omitted because it made the applied liquid easy to detach from the millipore filter. The millipore filter was compatible with the surfactant without swelling or dissolving. Its thickness was measured as  $150 \mu\text{m}$ . The diameter of the diaphragm was 2.5 cm, and therefore the membrane area was  $4.91 \text{ cm}^2$ . Water and a solution (200 ml each), were placed in the two cells, respectively, and the whole apparatus was immersed in a thermostated water bath ( $30^\circ$ ). Since the cells were clear and transparent after each run, sorbitan monooleate was probably not removed from the millipore filter.

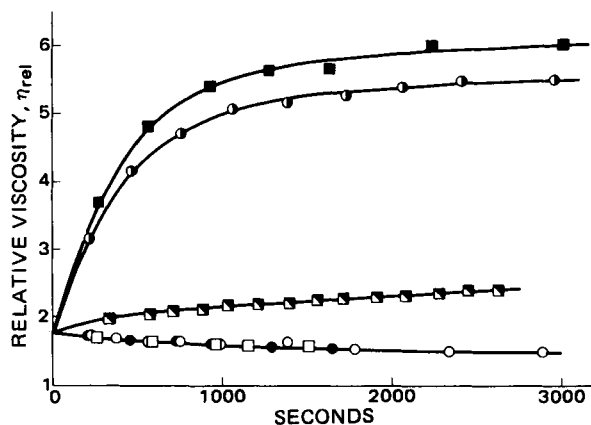
Fluxes for electrolytes were measured with a conductance meter equipped with platinum electrodes immersed in the water in one of the cells. In the conductometry, it was necessary to keep room temperature at  $19 \pm 1^\circ$  throughout the measurement to obtain reproducible data. Measurements were taken every 30 min. Conventional colorimetric methods were employed for determining the fluxes of nonelectrolytes. Every hour, 3 ml of sample was withdrawn and then an equal volume of water previously kept at  $30^\circ$  was added to the cell to prevent hydrostatic pressure differences from being created.



**Figure 4**—Viscosity changes of W/O/W emulsion after dilution with aqueous glucose solutions. Key: ●,  $1 \times 10^{-3}$  M; □,  $2 \times 10^{-3}$  M; ■,  $4 \times 10^{-3}$  M; and ○,  $6 \times 10^{-3}$  M.

<sup>8</sup> Ostwald type.

<sup>9</sup> Type GSWP 02500.



**Figure 5**—Viscosity changes of W/O/W emulsion after dilution with a variety of aqueous solutions. Key: ■, water; ○,  $2 \times 10^{-2}$  M urea; ●,  $10^{-2}$  M KSCN; □,  $10^{-2}$  M KCl; ●,  $2 \times 10^{-2}$  M glucose; and □,  $2/3 \times 10^{-2}$  M  $\text{CaCl}_2$ .

## RESULTS AND DISCUSSION

Figure 1 shows the viscosity change with time for a W/O/W emulsion after dilution with water, in which the ratio of Sample A weight to diluent volume was 1:1.

The viscosity increased with time to a maximum and then decreased monotonically. A similar viscosity change was obtained even with the original emulsion. Matsumoto and Kohda (2) also reported this type of viscosity change for their W/O/W emulsions, the inner and outer aqueous phases of which were glucose solution and water, respectively. This viscosity change was ascribed to the volume change of vesicles in the W/O/W emulsions (2). To deal with this phenomenon quantitatively, an appropriate equation which correlates the relative viscosity to the volume fraction of vesicles should be known. However, it is impossible to determine an equation that would be appropriate, since the volume fraction changes on aging from the time of preparation and also changes on dilution. Hence, the volume change of vesicles in W/O/W emulsions can only be estimated qualitatively by means of viscometry, light scattering measurement, photomicroscopy, etc. For example, vesicle swelling after dilution of the original sample was confirmed by photomicroscopy. Similar observations were reported for a water/*n*-heptane/water emulsion (13).

Figures 2–4 show viscosity changes caused by dilution with aqueous potassium chloride, sucrose, and glucose solutions of various concentrations, respectively. In these cases the ratio of Sample A weight to diluent volume was 1:4.

The rate of viscosity change decreases with increasing concentration of solute in the diluent, and the viscosity falls even below its initial value when the emulsion is diluted with  $10^{-2}$  M potassium chloride. These findings may be explained by the osmotic pressure difference between the inner aqueous phase and the outer aqueous phase as suggested previously (12, 14). The oil layer of vesicles in the W/O/W emulsion has a permeability to water, and the amount and direction of water movement depend on the osmotic pressure gradient. Figure 5 shows the viscosity change produced by dilution with a variety of aqueous solutions which have identical  $\pi$  values in the equation:

$$\pi = zcRT \quad (\text{Eq. 1})$$

where  $\pi$  is osmotic pressure,  $c$  is the concentration of solute,  $R$  and  $T$  are the usual gas constants, and  $z$  is an integer which is 1 for nonelectrolytes, 2 for uni-univalent electrolytes, and 3 for calcium chloride. The ratio of Sample A weight to diluent volume is 1:4. The equation gives the osmotic pressure of the outer aqueous phase when the oil layer has ideal semi-permeability to the solute.

Urea and potassium thiocyanate increase viscosity, while potassium chloride, glucose, and calcium chloride decrease it. This suggests that solutes which produce a greater increase in viscosity contribute to a smaller degree to the osmotic pressure of the outer phase. In other words, these latter solutes can more or less permeate the oil layer. Therefore, the permeability sequence for the solutes is: urea > potassium thiocyanate > potassium chloride > glucose > calcium chloride.

Tanaka and coworkers<sup>10</sup> found a dependency of W/O/W emulsion

<sup>10</sup> Personal communication.

**Table I—Permeability Coefficients Evaluated from Planar Membrane Experiments**

Solute	Permeability Coefficient (30°), cm/sec × 10 <sup>9</sup>
Urea	440
Potassium thiocyanate	21.9
Cesium chloride	3.76
Rubidium chloride	2.84
Potassium chloride	2.37
Sodium chloride	2.19
Lithium chloride	1.77

viscosity at the time of preparation on the species of solute enclosed in the inner aqueous phase. For example, potassium chloride shows a much higher viscosity than potassium thiocyanate. Although it was suggested<sup>10</sup> that potassium thiocyanate prevents formation of W/O/W to give a lower viscosity, this can also be explained in terms of the permeability difference between the two salts. Since potassium thiocyanate, which is more permeable than potassium chloride, would contribute to the osmotic pressure of the inner aqueous phase less than potassium chloride, there would be less movement of water from the outer to the inner aqueous phase, thus giving a lower viscosity.

Table I lists the permeability coefficients calculated for some solutes from the fluxes determined in the planar membrane experiment. The sequence of permeability coefficient values in the table is identical with that obtained in the viscometry of the W/O/W emulsion. Furthermore, the absolute values of the permeability coefficient for sodium chloride and urea are in good agreement with those obtained with phospholipid bilayer membranes (15, 16). The sequence for alkali chlorides is also identical with that obtained with the lipid bilayers (15).

It would be reasonable to conclude that solutes can permeate the oil layer of vesicles in the W/O/W emulsion, probably due to a large total surface area of the vesicles, even though there is a considerable difference in composition between the oil layer and the planar membrane. Water would act as the carrier of solutes across the oil layer, presumably in the solubilized form.

Matsumoto *et al.* (1) proposed to measure the yield of W/O/W emulsion by a dialysis method. That is, when the emulsion containing a solute in the inner aqueous phase is dialyzed against distilled water, the concentration of the solute in the water should correspond to the yield. However, if the solute can permeate the oil layer of vesicles in the emulsion, there is no way to distinguish the solute diffused from the inner

aqueous phase from the solute that leaked out as a result of vesicle destruction. The dialysis method cannot give correct yield. Moreover, if a high solute concentration in the water and a low emulsion viscosity are found (as in the case where a highly permeable solute is used) it could be incorrectly concluded that the solute has prevented the formation of a W/O/W emulsion.

## REFERENCES

- (1) S. Matsumoto, Y. Kita, and D. Yonezawa, *J. Colloid Interface Sci.*, **57**, 353 (1976).
- (2) S. Matsumoto, M. Kohda, and S. Murata, *ibid.*, **62**, 149 (1977).
- (3) Y. Kita, S. Matsumoto, and D. Yonezawa, *J. Chem. Soc. Jpn.*, **1977**, 748.
- (4) C. J. Benoy, L. A. Elson, and R. Schneider, *Br. J. Pharmacol.*, **45**, 135 (1972).
- (5) T. Takahashi, M. Mizuno, Y. Fujita, S. Ueda, B. Nishizawa, and S. Majima, *Gann*, **64**, 345 (1978).
- (6) T. Takahashi, U. Satoshi, K. Kono, and S. Majima, *Cancer*, **38**, 1507 (1976).
- (7) T. Takahashi, K. Kono, and T. Yamaguchi, *J. Exp. Med.*, **123**, 235 (1977).
- (8) M. Hashida, M. Egawa, S. Muranishi, and H. Sezaki, *J. Pharmacokinet. Biopharm.*, **5**, 225 (1977).
- (9) S. Matsumoto, Y. Ueda, Y. Kita, and D. Yonezawa, *Agr. Biol. Chem.*, **42**, 739 (1978).
- (10) H. Fukuda and M. Tanaka, Japanese pat. 134,029 (1977).
- (11) Y. Kita, S. Matsumoto, and D. Yonezawa, *J. Colloid Interface Sci.*, **62**, 87 (1977).
- (12) S. Matsumoto and M. Kohda, *ibid.*, **73**, 13 (1980).
- (13) S. Matsumoto, T. Inoue, M. Kohda, and K. Ikura, *ibid.*, **77**, 555 (1980).
- (14) Y. Kita, S. Matsumoto, and D. Yonezawa, *J. Chem. Soc. Jpn.*, **1978**, 11.
- (15) V. K. Miyamoto and T. E. Thompson, *J. Colloid Interface Sci.*, **25**, 16 (1967).
- (16) R. E. Wood, F. P. Wirth, Jr., and H. E. Morgan, *Biochim. Biophys.* **163**, 171 (1968).

## ACKNOWLEDGMENTS

The authors express their thanks to Dr. Minako Tanaka of the Lion Co., Tokyo, for her valuable discussion.

## Ion Chromatographic Determination of the Principal Inorganic Ion in Bulk Antibiotic Salts

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Received May 13, 1981, from Pfizer Inc., Quality Control Division, Groton, CT 06340.  
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Accepted for publication July 28, 1981.

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**Abstract** □ Two ion-chromatographic procedures were developed for determining the principal inorganic ions in bulk antibiotic salts. The anion system enables sulfate determination in sulfate salts of polymyxin, neomycin, streptomycin, and dihydrostreptomycin, with typical analysis times of 8 min/sample and a relative precision of ±2% (95% confidence interval). The cation system is applicable to sodium or potassium analysis in the respective penicillin salts in 9–16 min with a relative precision of

±2.5%. These methods offer speed, specificity, and easy sample preparation compared with traditional assays.

**Keyphrases** □ Ion chromatography—determination of inorganic ion in bulk antibiotic salts □ Antibiotics—determination of inorganic ion in salts using ion chromatography □ Inorganic ion—determination in salts of various antibiotics by ion chromatography

Bulk antibiotic salts can be characterized by the determination of their principal components for the purposes of quality assurance. An accurate assay is required for both

the pharmacologically active organic ion and the inorganic counterion. For the organic moiety, many specific chromatographic assays (1) are applicable to bulks. However,