Chem. Pharm. Bull.
30(8)3024—3027(1982)

Dispersion State of Protein-stabilized Magnetic Emulsions

YASUNORI MORIMOTO,*,[a] MASAYUKI AKIMOTO,[a] and YOSHINORI YOTSUMOTO[b]

Faculty of Pharmaceutical Sciences, Josai University,[a] 1-1 Keyakidai, Sakado, Saitama, 350-02, Japan and Central Laboratory, Taiho Industries Co., Ltd.,[b] 9 Kirihara-cho, Fujisawa, Kanagawa, 252, Japan

(Received February 26, 1982)

Two types of oil-in-water emulsions consisting of oleic acid- or ethyl oleate-based magnetic fluid as the dispersed phase in water were prepared with casein as the emulsifier, and their characteristics relating to the emulsion stability were investigated.

The mean droplet diameter of ethyl oleate- or oleic acid-based magnetic emulsion was 5.0 or 6.4 μm, respectively. Visual observation of sedimentation volume revealed that ethyl oleate-based emulsion was more stable than oleic acid-based emulsion, and this observation was in accord with the experimental results of droplet number of each emulsion. Concerning the rheological properties, oleic acid- or ethyl oleate-based magnetic fluids showed non-Newtonian flow, but the emulsions prepared from both fluids showed Newtonian flow.

These results indicate that ethyl oleate-based magnetic emulsion may be superior to oleic acid-based magnetic emulsion as a magnetic drug-carrying material.

Keywords——oleic acid; ethyl oleate; magnetic fluid; magnetic emulsion; sedimentation volume; droplet number; rheological properties; magnetic drug-carrying material

Colloid suspensions of magnetic particles, which have been referred to as ferromagnetic fluids or ferrofluids, are well known[1] and have been proposed for medical application.[2] Widder *et al.*[3] suggested that magnetic microspheres might be utilized as a drug carrier for site-specific drug delivery. We also reported the utility of ferrofluid-containing albumin microspheres as a drug carrier which might localize chemotherapeutic agents in the target sites.[4,5] Similarly, magnetic emulsions might be used as supports for drugs which could be brought to the target site with the aid of a magnetic field.[6] However, there are many unsolved problems in connection with the stability of magnetic emulsions, because of the inherent problem of stability.

In this paper, we examined the stability of magnetic emulsions prepared with two types of ferrofluids and casein, which was chosen as a natural emulsifier.

Experimental

Materials——Oleic acid and ethyl oleate were obtained from Wako Pure Chemicals Industries., Ltd. Casein prepared by the method of Hammersten was obtained from Wako Pure Chemicals Industries, Ltd. Synthetic magnetites, Fe_3O_4 fine particles, were obtained from Taiho Industries Co., Ltd. All other chemicals were reagent grade products.

Preparation of Magnetic Fluids——Two types of oil-based magnetic fluid were prepared by the modified method of Shimoiizaka.[7] Oleic acid-based magnetic fluid and ethyl oleate-based magnetic fluid contained 30.0% (w/w) magnetite in oleic acid and 22.3% (w/w) magnetite in ethyl oleate, respectively.

Preparation of Magnetic Emulsions——The dispersed phase was oleic acid-based magnetic fluid or ethyl oleate-based magnetic fluid, and the continuous phase was 1% (w/v) casein solution (pH 8.0).

The two phases were mixed and the mixture was agitated using the Ultra Turrax® (Janke and Kunkel Co., Ltd., Germany) at 10000 rpm for 5 min. The emulsion always contained 20% of dispersed phase.

In all cases, emulsification was carried out in a water bath maintained at 37°C. After emulsification, a sample was immediately taken to evaluate the emulsion stability.

Determination of Emulsion Stability——Emulsion stability was evaluated by visual observation of the sedimentation volume.[8] Ten ml of the emulsion sample were poured into 10 ml glass-stoppered graduated cylinders. The sedimentation volumes were measured as a function of time at 37°C.

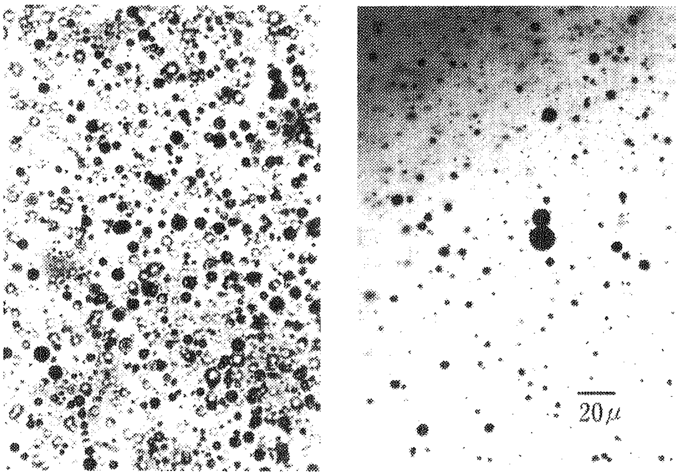
Measurement of the Number and Size of Droplets——The stability of the emulsion was examined in terms of the number and size of droplets by the use of a Coulter Counter (Model ZB instrument, Coulter Electronics Co., Ltd., Florida, U.S.A.). In the present experiments, a 50 μm aperture diameter orifice was used with the counter, and 0.9% NaCl in water was used as the electrolyte.[9,10]

Measurement of Viscosity——Rheological properties were evaluated using a viscometer (Rotovisco RV 100/CV 100 type, Haake Co., Ltd., Germany) at shear rates from 0 to 180 s^{-1}.

Measurement of Density——Densities were measured with a digital density meter (Anton Paar Co., Ltd., Germany) at 37°C.

Results and Discussion

Fig. 1 shows microphotographs of oleic acid-based magnetic emulsion and ethyl oleate-based magnetic emulsion immediately after preparation. Both emulsion types show broad size distribution patterns. The approximate average particle sizes of oleic acid- and ethyl oleate-based magnetic emulsions were 6.4 and 5.0 μm, respectively.



a b

Fig. 1. Microphotographs of Magnetic Emulsions

(a) ethyl oleate-based magnetic emulsions,
(b) oleic acid-based magnetic emulsions.

Fig. 2 shows the visually estimated phase separation processes of both emulsions. For presentation of the sedimentation volume, the parameter F, *i.e.*, the ratio of the volume of sediment at time t, V_t, to the original volume of the emulsion, V_o, was used.

$$F = V_t / V_o$$

As is clear in Fig. 2, oleic acid-based magnetic emulsion separated fairly rapidly into two phases, *i.e.* cloudy supernatant and sediment, from the top. On the other hand, the separation of ethyl oleate-based magnetic emulsion proceeded slowly, and the emulsion showed three distinct zones: a zone of cloudy supernatant at the top, a zone of concentrated emulsion in the middle, and a zone of sediment at the bottom of the cylinder. The system showed extremely slow settling after the initial sediment formation.

The number of droplets, which was taken as one of the quantitative criteria of emulsion stability, was measured with the Coulter counter.[9] The change in the number of droplets with standing time of the magnetic emulsion is shown in Fig. 3. The number of droplets was found to be influenced by the type of base oil used. The number of droplets for the magnetic emulsion prepared with ethyl oleate was greater than that for the magnetic emulsion prepared with oleic acid. Therefore, the, the magnetic emulsion containing ethyl oleate was more stable than that containing oleic acid.

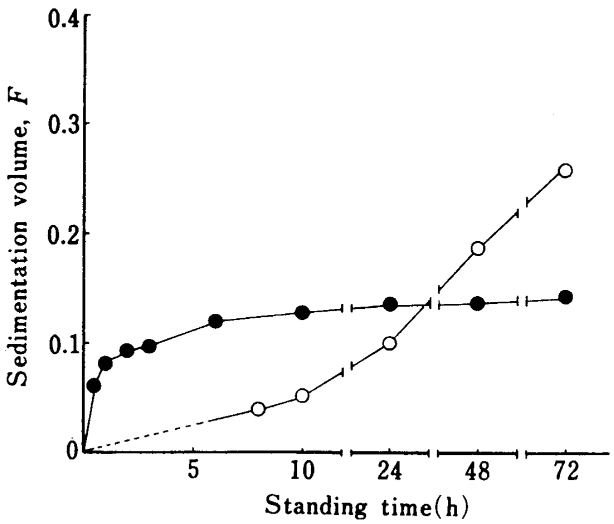


Fig. 2. Plot of Sedimentation Volume *versus* Standing Time for Magnetic Emulsions

(○) ethyl oleate-based magnetic emulsion,
(●) oleic acid-based magnetic emulsion.
Results are expressed as the mean values of five experiments.

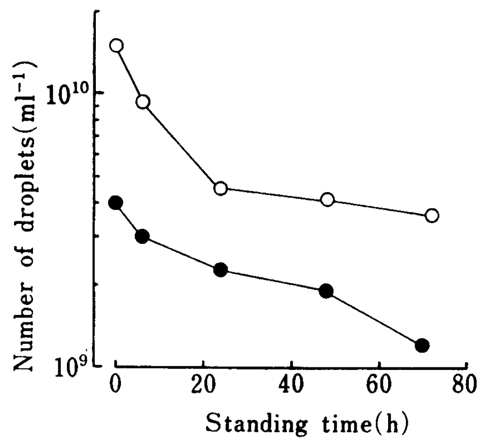


Fig. 3. Change of the Number of Droplets with Standing Time for Magnetic Emulsions

(○) ethyl oleate-based magnetic emulsion,
(●) oleic acid-based magnetic emulsion.
Results are expressed as the mean values of three experiments.

Generally, the stability of emulsions is proportional to the size of dispersed droplets and the difference of density between the oil and water phase.[11,12]

The density of the magnetic fluids based on an oleic acid or ethyl oleate is about 1.047 or 1.036 g/ml, respectively. The density of the continuous phase and that of the dispersed phase should preferably be equal to each other.

Fig. 4 shows flow curves of both emulsions and both magnetic fluids. The results for oleic acid and ethyl oleate are illustrated for comparison. As is clear from Fig. 4, both magnetic fluids exhidited non-Newtonian plastic flow. Oleic acid-based magnetic fluid was 8 times

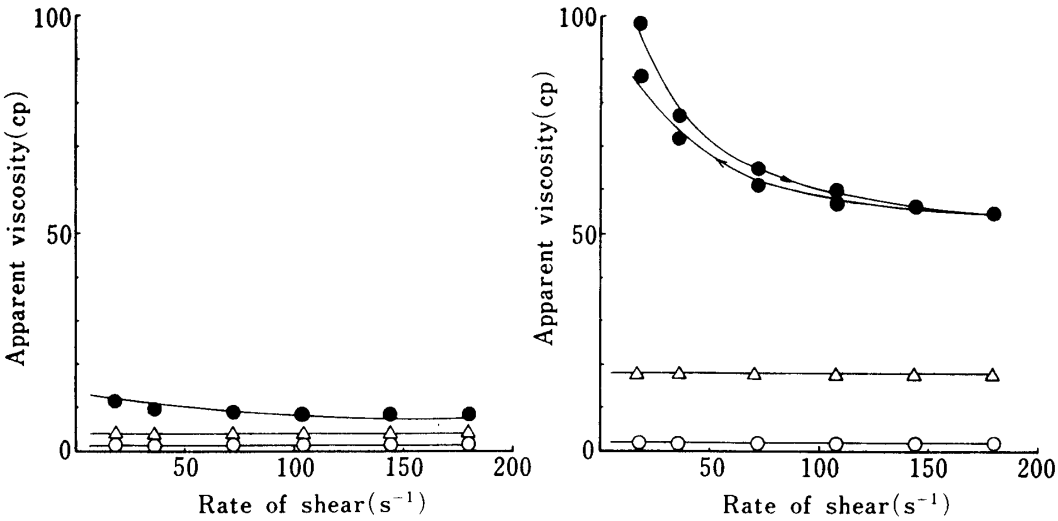


Fig. 4. Rheograms of Various Oily Vehicles

(a) (○) ethyl olrate-based magnetic emulsion,
(●) ethyl oleate-based magnetic fluid,
(△) ethyl oleate.
(b) (○) oleic acid-based magnetic emulsion,
(●) oleic acid-based magnetic fluid,
(△) oleic acid.
Results are expressed as the mean values of three experiments.

viscous than the ethyl oleate-based magnetic fluid, and the viscosity of ethyl oleate-based magnetic fluid was 9.0 cp and that of oleic acid-based magnetic fluid was 76.5 cp at a shear rate of 36 s^{-1}. On the other hand, both emulsions exhibited Newtonian flow. Similarly, oleic acid and ethyl oleate also showed simple Newtonian flow. Since the o/w emulsions are composed of a relatively low volume fraction of dispersed phase, they might exhibit Newtonian flow behavior.[13] These results in Fig.4 are in agreement with the theoretical prediction that Newtonian flow in a range of low shear rates can be obtained with the fluids whose dispersed concentration is low, such as below 0.2 volume fraction.[14] On comparing the two emulsion types, there was only a small difference in emulsion viscosity, irrespective of the viscosity of the oily phase emulsified. Generally, the higher the viscosity is, the more stable an emulsion is.[15] However, the emulsion stability obtained by varying the type of magnetic fluid did not appear to be related to the viscosity of the base oil emulsified.[16]

Emulsions of ethyl oleate-based magnetic fluid were obtained which are extremely stable over relatively long periods of times. Such a system, which has a high sedimentation volume, has a cloudy supernate, and is easily redispersible by simple shaking. However, the oleic acid-based magnetic emulsions rapidly settled and formed an initial sediment which was difficult to redisperse. Flocculated systems have high final sedimentation volume, which is defined as F_∞, whereas deflocculated systems settle toa com pact sediment and have low F_∞ values.[8] Generally, systems with low F_∞ values are difficult to redisperse.

The use of magnetic particles has created a great deal of interest in clinical medicine[17] and biomedical research.[18] The magnetic emulsion presented in this report has many potential clinical applications in a wide variety of disesase states. Work is now in progress on the magnetic responsiveness of the emulsions *in vivo* and *in vitro*.

References and Notes

1) K. Kaiser and G. Miskolzy, *J. Appl. Phys.*, **41**, 1064 (1970).
2) K. Mosbach and U. Schroder, *FEBS Lett.*, **102**, 112 (1979).
3) K. Widder, A. Senyei, and D. Scarpelli, *Proc. Soc. Exp. Biol. Med.*, **58**, 141 (1981).
4) Y. Morimoto, K. Sugibayashi, M. Okumura, and Y. Kato, *J. Pharm. Dyn.*, 3, 264 (1980); Y. Morimoto, M. Okumura, K. Sugibayashi, and Y. Kato, *ibid.*, **4**, 624 (1981).
5) K. Sugibayashi, M. Okumura, and Y. Morimoto, *Biomaterials*, 3, 181 (1982).
6) M. Akimoto and Y. Morimoto, *J. Pharm. Dyn.*, **5**, s–15 (1982).
7) J. Shimoiizaka, K. Nakatsuka, R. Chubachi, and Y. Sato, *Nippon Kagaku Kaishi*, **1976**, 6.
8) I.W. Kellaway and N.M. Najib, *Inter. J. Pharm.*, **9**, 59 (1981).
9) A. Takamura, T. Minowa, S. Noro, and T. Kubo, *Chem. Pharm. Bull.*, **27**, 2971 (1979).
10) E.L. Rowe, *J. Pharm. Sci.*, **54**, 260 (1965).
11) S. Noro, A. Takamura, and M. Koishi, *Chem. Pharm. Bull.*, **27**, 309 (1979).
12) L.T. Lomankiw, N.Y. Briachift, U.S. Patent, 3981844 (1975).
13) P. Shermand(ed.), "Emulsion Science," Academic Press, London, 1969.
14) Y. Kita, S. Matsumoto, and D. Yonezawa, *J. Colloid Interface Sci.*, **62**, 87 (1977).
15) J.C. Acton and R.L. Saffle, *J. Food Sci.*, **35**, 852 (1970).
16) J.C. Acton and R.L. Saffle, *J. Food Sci.*, **36**, 1118 (1971).
17) L. Goldman and R. Dreffer, *Arch. Derm. Res.*, **257**, 227 (1976).
18) N. Lynn, *Clin. Chim. Acta*, **69**, 387 (1976); S. Margel, S. Zisblatt, and A. Rembaum, *J. Immunol. Methods*, **28**, 341 (1979).