



Factors affecting droplet size of sodium caseinate-stabilized O/W emulsions containing -carotene

著者	Kanafusa Sumiyo, Chu Boon-Seang, Nakajima Mitsutoshi
journal or publication title	European journal of lipid science and technology
volume	109
number	10
page range	1038-1041
year	2007-10
権利	(C) 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
URL	http://hdl.handle.net/2241/97928

doi: 10.1002/ejlt.200700100

論文内で示した連絡先は、下記のとおり変更しています。

2007年4月より

筑波大学大学院生命環境科学研究科

中嶋 光敏

〒305-8572 つくば市天王台 1-1-1

Tel 029-853-4703

e-mail mnaka@sakura.cc.tsukuba.ac.jp

**Factors affecting droplet size of sodium caseinate-stabilized O/W emulsions
containing β -carotene**

Sumiyo Kanafusa¹, Boon-Seang Chu² and Mitsutoshi Nakajima^{1,2*}

¹Graduate School of Life and Environmental Sciences,
University of Tsukuba,
Tennodai 1-1-1, Tsukuba,
Ibaraki 305-8572, Japan

²National Food Research Institute,
Kannondai 2-1-12, Tsukuba,
Ibaraki 305-8642, Japan

*To whom correspondence should be addressed:

Tel: +81 29 838 7997

Fax: +81 29 838 8122

E-mail: mnaka@affrc.go.jp

1 **Abstract**

2 It has been reported that solubilization, and therefore, bioavailability of water insoluble
3 bioactive compounds can be improved by incorporating the compounds in emulsions. This
4 work was initiated to prepare an oil-in-water (O/W) emulsion containing β -carotene by
5 microfluidization. The β -carotene was dissolved in triolein and microfluidized with an
6 aqueous phase containing sodium caseinate (SC) as the emulsifier. Microfluidization at 140MPa
7 resulted in O/W emulsions with a mean droplet diameter of *ca.*120nm which was further
8 confirmed with a transmission electron microscopy (TEM) analysis. The influences of SC
9 concentration and microfluidization parameters on the droplet size of the emulsions were
10 studied. The results showed that mean droplet diameter decreased significantly ($P<0.05$) from
11 310nm to 93nm with the increase in SC concentration from 0.1%wt to 2%wt. However,
12 further increase in SC concentration did not change the droplet diameter much, although the
13 polydispersity of the emulsions was slightly improved. The droplet diameter of the emulsions
14 was found to decrease from 200nm to 120nm with increasing microfluidization pressure, with
15 narrower droplet size distribution. Storage study showed that the emulsions were physically
16 stable for about two weeks at 4°C in dark. The results provide a better understanding on the
17 performance of SC in stabilizing the O/W emulsions.

18 **Keywords:** β -carotene, emulsion, high-pressure homogenization, microfluidization

19 **Introduction**

20 Bioactive compounds such as carotenoids, phytosterols, natural antioxidants and many others
21 have been receiving much attention from pharmaceuticals and food industries for many years.
22 Although the physiological functions of many bioactive compounds have not yet fully
23 understood, it is well recognized that these compounds contribute to the improvement of
24 public health. The effectiveness of bioactive compounds in preventing diseases depends on
25 preserving the bioavailability of the bioactive ingredients. However, many of the bioactive
26 compounds are almost water-insoluble and thus have a low uptake in the body. A strategy to
27 improve the bioavailability of these bioactive compounds involves the preparation of O/W
28 emulsions with droplet size in sub-micrometer range.

29 Preparation of an emulsion generally requires energy input *via* a homogenization process. The
30 energy must overcome Laplace pressure, which increases with decreasing emulsion droplet
31 size (McClements, 2004). High-pressure homogenization is extensively used in the food,
32 pharmaceuticals and biotechnology industries to mix, disperse, emulsify and process many
33 products (Floury *et al.* 2002). Microfluidization is a high-pressure homogenization technique
34 that is efficient for preparing O/W emulsions with fine droplet size. The interaction chamber
35 of the microfluidizer has a fixed geometry and is present in the form of a confined capillary
36 tube where disruption of the droplets occurs due to liquid-liquid, and above all, liquid-solid
37 shear forces (Perrier-Cornet *et al.*, 2005).

38 Milk proteins are good emulsifiers and hence are used as ingredients in a wide range of
39 formulated food emulsions. Caseinates are important milk proteins and have a number of
40 advantages over the whey proteins. SC has a better solubility in water and more thermally
41 stable compared to whey proteins, presumably because the relatively flexible casein
42 molecules do not undergo appreciable heat-induced conformational changes like the globular

43 whey proteins (Srinivasan *et al.*, 2002). SC contains a soluble mixture of surface-active
44 caseins that absorb rapidly at the oil-water interface during emulsification and stabilize the
45 dispersions by a combination of electrostatic repulsion and steric stabilization (Dickinson *et*
46 *al.*, 1998). SC is also highly effective at protecting emulsified oils from oxidation, owing to
47 their unique iron chelating property and the ability to produce thick interfacial layers around
48 the droplets (Hu *et al.*, 2003).

49 In this work, O/W emulsions containing β -carotene as a model of water-insoluble bioactive
50 compounds were prepared using a microfluidizer. The β -carotene was dissolved in triolein
51 and emulsified with the aqueous phase containing SC as the emulsifier. The main objectives
52 of this work were to study the performance of SC in stabilizing the emulsions and the effect of
53 microfluidization in the emulsions droplet size.

54 **Materials and methods**

55 ***Materials***

56 SC, β -carotene and triolein were purchased from Wako Pure Chemical Industries, Ltd., Osaka,
57 Japan. Deionized water purified by a Milli-Q Organex system (Millipore, Bedford,
58 Connecticut, USA) was used for preparing the aqueous phase. All other chemicals used were
59 of analytical grade.

60 ***Preparation of the β -carotene O/W emulsions***

61 Unless otherwise specified, β -carotene O/W emulsion was prepared by the following method.
62 SC (1%wt) was dissolved in 0.05M phosphate buffer pH7 (at 20°C), containing 0.02%wt
63 sodium azide. The aqueous solution was magnetically stirred for 1h before the organic phase
64 (0.1%wt β -carotene in triolein) was added. The ratio of organic phase to aqueous phase was
65 1:9 by weight. The pre-mix was homogenized using a conventional homogenizer (Polytron[®]

66 PT300, Kinematica AG, Lucerne, Switzerland) at 5000rpm for 5min to produce a coarse O/W
67 emulsion, immediately followed by microfluidization (Model M-110EHi Microfluidizer
68 Processor, Microfluidic™ Corporation, Newton, Maine, USA) for a single pass at 140MPa
69 (Tan and Nakajima, 2005).

70 The O/W emulsions containing β -carotene were prepared under various operating conditions
71 to study the effect of the experimental parameters on the droplet size of the emulsions. The
72 concentration of SC was varied from 0.1 to 10%wt to study the effect of the protein
73 concentration. Several other batches of emulsions were prepared under different
74 microfluidization pressures (20 to 160MPa). For storage stability study, emulsions prepared
75 with 1%wt SC and microfluidized at 140MPa were kept at 4°C in dark. Samples were
76 withdrawn for analysis at weekly interval. Each sample was analyzed for particle-size
77 distribution.

78 ***Particle-size analysis***

79 A laser-diffraction particle-size analyzer (LS 13320, Beckman Coulter, Inc., Florida, USA)
80 was used to measure the droplet size distribution of the emulsions. A refractive index of 1.47
81 for β -carotene in polar solution (water) and that of 1.33 for water were used to calculate the
82 droplet size. The reported droplet diameter was calculated from the average of at least three
83 measurements.

84 ***TEM analysis***

85 O/W emulsion prepared with 1%wt SC and 0.1%wt β -carotene, and microfluidized at
86 140MPa was selected for TEM analysis to observe the microstructure and droplet size
87 distribution of the emulsion. The sample was prepared using the freeze-fracture replica
88 method. The surface of the fractured sample was coated with a platinum layer followed by a

89 carbon layer in vacuum. The metal atoms were applied at 40° to the fractured surface to
90 produce a shadow effect. TEM images were then obtained using a JEOL-JEM 200CX TEM
91 (JEOL, Tokyo, Japan) working at an accelerating voltage of 80kV.

92 ***Statistical analysis***

93 Statistical analysis was performed on the data by a one-way analysis of variance using SAS
94 (SAS, 1989) software package release 6.1. The significant differences ($P<0.05$) between
95 means were further determined by Duncan's multiple-range test.

96 **Results and discussion**

97 Fig.1 shows the typical changes in droplet size of the O/W emulsion after the coarse
98 homogenization and microfluidization at 140MPa. Coarse homogenization by a conventional
99 rotor-stator homogenizer resulted in large droplets with a mean diameter of 1.7 μ m with CV
100 61%. The coarse homogenization involved the breakup and intermingling of bulk oil phase
101 and aqueous phase. Fairly large droplets were formed due to the low homogenization pressure.
102 The following microfluidization reduced the droplet size distribution into the range of 40 to
103 700nm with a mean value of 120nm (CV45%), owing to the fact that microfluidization
104 applied higher disruptive energy than the conventional rotor-stator homogenizer. The breakup
105 of the large droplets to smaller ones in the microfluidizer was initiated by a combination of
106 turbulence and laminar-shear stress, which increased the droplet-specific surface area up to
107 disruption. The protein rapidly adsorbed at the surface of the newly formed smaller droplets.

108 Fig.2 depicts the effect of SC concentration on the droplet size distribution of the emulsions
109 after microfluidization. At low SC concentration (0.1%wt), the emulsion exhibited a bimodal
110 droplet distribution, with a mean droplet size of 310nm and CV104%. Even the microfluidizer
111 was capable of producing smaller droplets, there were insufficient protein molecules to adsorb

112 onto the newly formed surface and formed a protective interfacial layer around the droplets
113 that prevented them from coalescing with their neighbors. Increasing SC concentration to
114 2%wt significantly ($P<0.05$) decreased the mean droplet diameter to 93nm, as well as the
115 polydispersity of the emulsion (CV35%). Higher protein concentration in the aqueous phase
116 provided better availability of the emulsifier to stabilize the droplets before they re-aggregated
117 and therefore narrowed the range of droplet size. However, there was no significant difference
118 ($P>0.05$) in the mean droplet size with further increase in SC concentration to 10%wt. There
119 were more SC molecules present than were required to cover the droplet surface formed
120 during microfluidization and the droplet size was independent of the protein concentration.
121 The results were in accordance with previous findings that there was a critical concentration
122 of SC at which the emulsifying capacity was the maximum (Carrera Sánchez and Rodríguez
123 Patino, 2005). Nevertheless, the polydispersity of the emulsions was improved slightly in
124 system with excess SC. The CV decreased from 35% to 27% with increasing SC
125 concentration from 2%wt to 10%wt.

126 The effect of microfluidization pressure on the droplet size distribution is shown in Fig.3. The
127 mean droplet size of the emulsions decreased significantly ($P<0.05$) from 200 to 120nm with
128 the increase in microfluidization pressure from 20 to 100MPa. Increasing the
129 microfluidization pressure or energy input caused the generation of more intense disruptive
130 forces when the fluid inside the homogenisation chamber collided with each other, resulting in
131 emulsion with smaller droplet size. The results agreed well with other works with several
132 high-pressure homogenizers (Trotta *et al.*, 2001; Tan and Nakajima, 2005). However, there
133 was a maximum limit of homogenization efficiency for the microfluidizer at which a
134 minimum emulsion droplet size could be achieved under a given condition. In this work, there
135 was not much change in mean droplet size when the microfluidization pressure was further
136 increased to 140MPa, although the droplet diameter showed was some tendency to increase at

137 160MPa.

138 Fig.4 shows the changes in droplet size distribution during storage at 4°C in dark. There was
139 no significant ($P>0.05$) change in mean droplet diameter after one week of storage, but the
140 mean droplet size increased gradually from 120nm in the first week to 201nm in the fourth
141 week with increasing polydispersity from CV 45% to 53%. Phase separation was observed
142 after two weeks of storage but the creaming layer disappeared after gentle shaking.
143 Flocculation, which occurred due to the inability of SC to prevent close approach of the
144 droplets, partly caused the phase separation (Dalglish, 1997). Other reason for the creaming
145 included the gravitational separation of the oil phase from the aqueous phase owing to the
146 large density difference between the two phases (McClements, 2004).

147 A representative TEM image of O/W emulsion containing β -carotene prepared with 1%wt SC
148 and homogenized at 140MPa is illustrated in Fig.5. The droplet size of the emulsion in the
149 image agreed well with the results from particle size analyser, although some smaller droplets
150 were also observed. The droplets had a well-defined boundary and fairly smooth surface
151 which was of characteristic of oil droplets.

152 One of the difficulties in application of emulsions as bioactive compound delivery systems is
153 the limitation in the choice of emulsifier. This work demonstrated the potential of SC as an
154 emulsifier for preparing the emulsions. The droplet size of the emulsions depended on the SC
155 concentration and microfluidization pressure. Due to the high solubility of β -carotene in
156 triolein, the use of triolein in preparation of the emulsions would allow more β -carotene to be
157 loaded in the system. Future works include the addition of a co-emulsifier into the system to
158 improve the storage stability of the emulsions, as well as the bioavailability study with *in vivo*
159 animal trials.

160 **Acknowledgements**

161 The authors gratefully acknowledge the financial support by the Ministry of Agriculture,
162 Forestry and Fisheries of Japan through the Nanotechnology Project for Utilization of the
163 Renewable Biomaterials. The authors thank Fumiko Yukuhiro and Reiko Nagata for their
164 contributions to this work.

165 **References**

166 Carrera Sánchez, C., J.M. Rodríguez Patino, Interfacial, foaming and emulsifying
167 characteristics of sodium caseinate as influenced by protein concentration in solution, *Food*
168 *Hydrocolloids* 19, 407-416 (2005).

169 Dalgleish, D.G., Adsorption of protein and the stability of emulsions, *Trends in Food Sci.*
170 *Technol.* 8, 1-6 (1997).

171 Dickinson, E., M.G. Semenova, A.S. Antipova, Salt stability of casein emulsions, *Food*
172 *Hydrocolloids* 12, 227-235 (1998).

173 Flourey, J., A. Desrumaux, M.A.V. Axelos, J. Legrand, Degradation of methylcellulose during
174 ultra-high pressure homogenisation, *Food Hydrocolloids* 16, 47-53 (2002).

175 Hu, M., D.J. McClements, E.A. Decker, Lipid oxidation in corn oil-in-water emulsions
176 stabilized by casein, whey protein isolate and soy protein isolate, *J. Agric. Food Chem.* 51,
177 1696-1700 (1995).

178 McClements, D.J., in *Food Emulsions: Principles, Practices and Techniques*, 2nd edn., CRC
179 Press, Boca Raton, Florida, 2004, pp.175-231; 269-339.

180 Perrier-Cornet, J.M., P. Marie, P. Gervais, Comparison of emulsification efficiency of
181 protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill
182 homogenisation, *J. Food Engineering* 66, 211-217 (2005).

183 SAS, in: *Statistical Analysis System User's Guide: Statistics*, Cary: SAS Institute Inc., pp.125-
184 154, 1989.

185 Srinivasan, M., H. Singh, P.A. Munro, Formation and stability of sodium caseinate emulsions:
186 influence of retorting (121°C for 15min) before or after emulsification, *Food Hydrocolloids*
187 16, 153-160 (2002).

188 Tan, C.P. & M. Nakajima, β -Carotene nanodispersions: preparation, characterization and
189 stability evaluation, *Food Chem.* 92, 661-671 (2005).

190 Trotta, M., M. Gallarate, F. Pattarino, S. Morel, Emulsions containing partially water-miscible
191 solvents for the preparation of drug nanodispersions, *J. Controlled Release* 76, 119-128
192 (2001).

List of figures

Fig.1: Changes in droplet diameter of O/W emulsion containing β -carotene after coarse homogenisation and microfluidization.

Fig.2: Effect of sodium caseinate concentration on the droplet size distribution of O/W emulsion containing β -carotene after microfluidization at 140MPa.

Fig.3: Effect of microfluidization pressure on the droplet size distribution of O/W emulsion containing β -carotene stabilized with 1%wt sodium caseinate.

Fig.4: Changes in droplet size distribution of O/W emulsion containing β -carotene during storage at 4°C for 4 weeks.

Fig.5: TEM image of a typical O/W emulsion containing β -carotene prepared with 1%wt sodium caseinate and microfluidized at 140MPa.

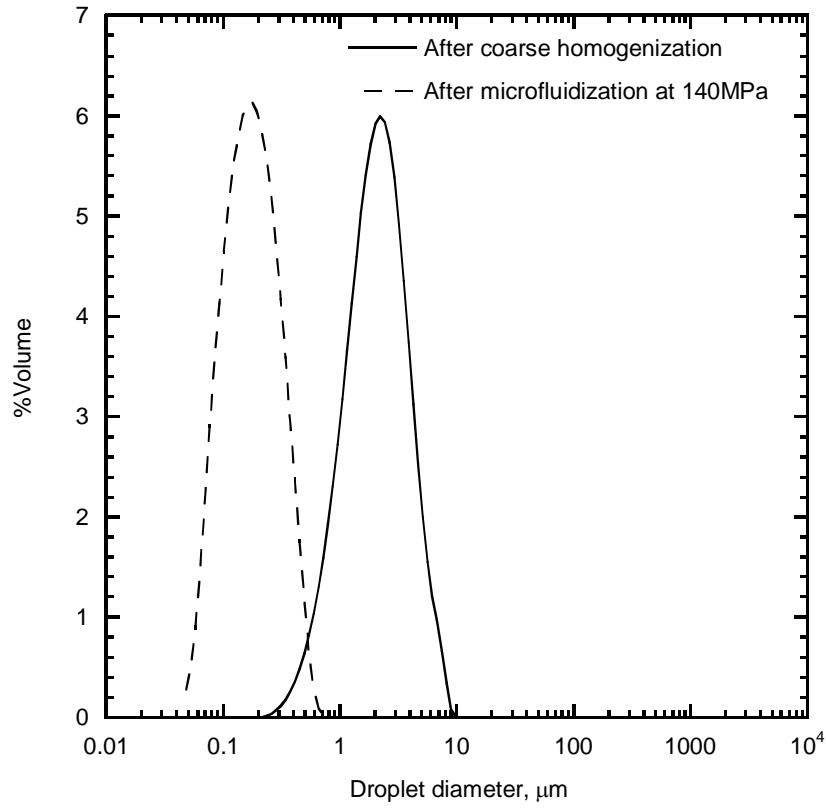


Fig.1

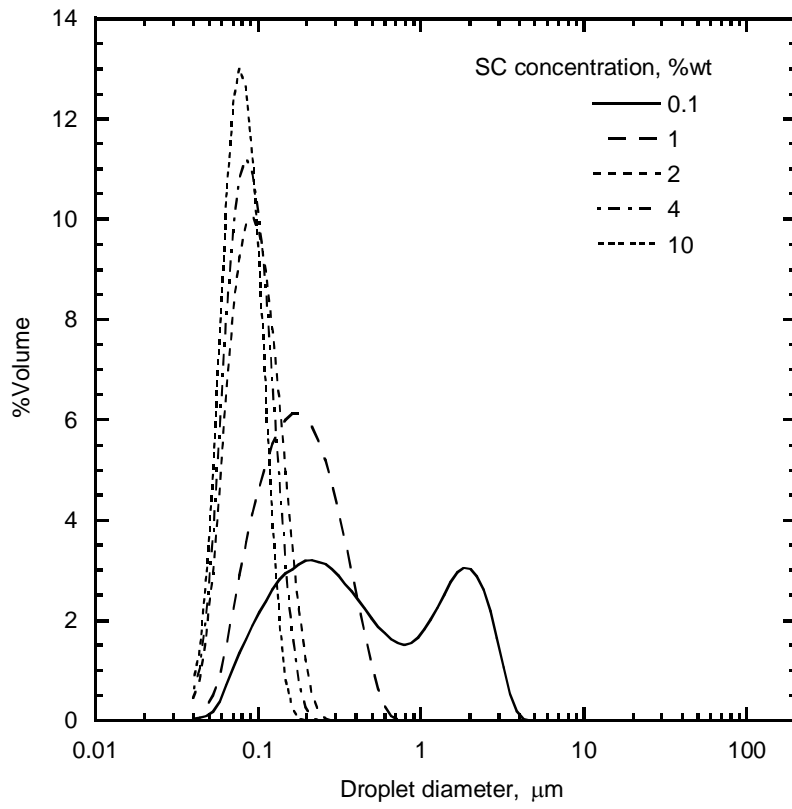


Fig.2

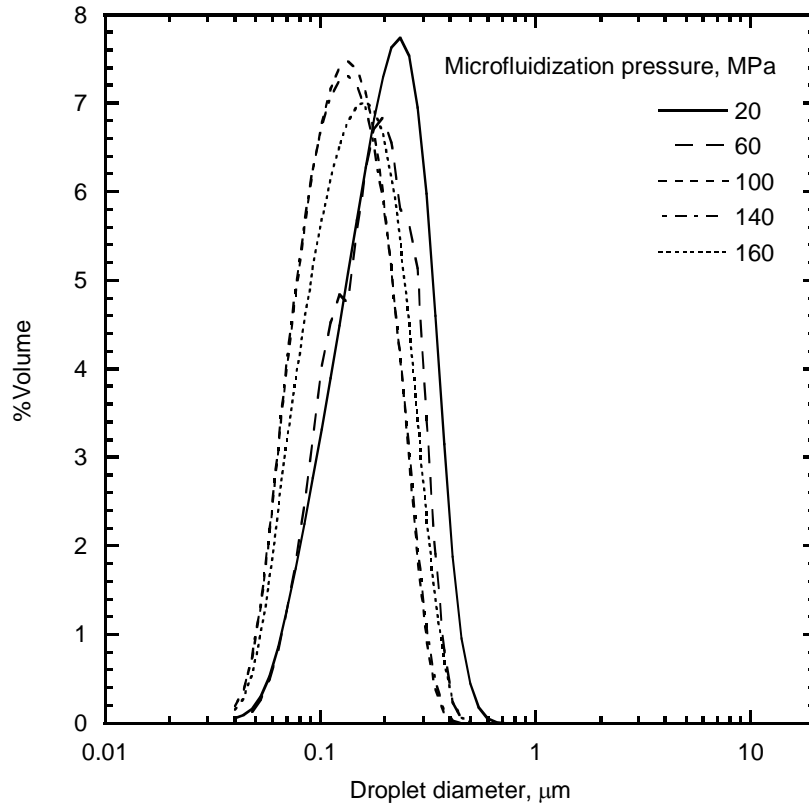


Fig.3

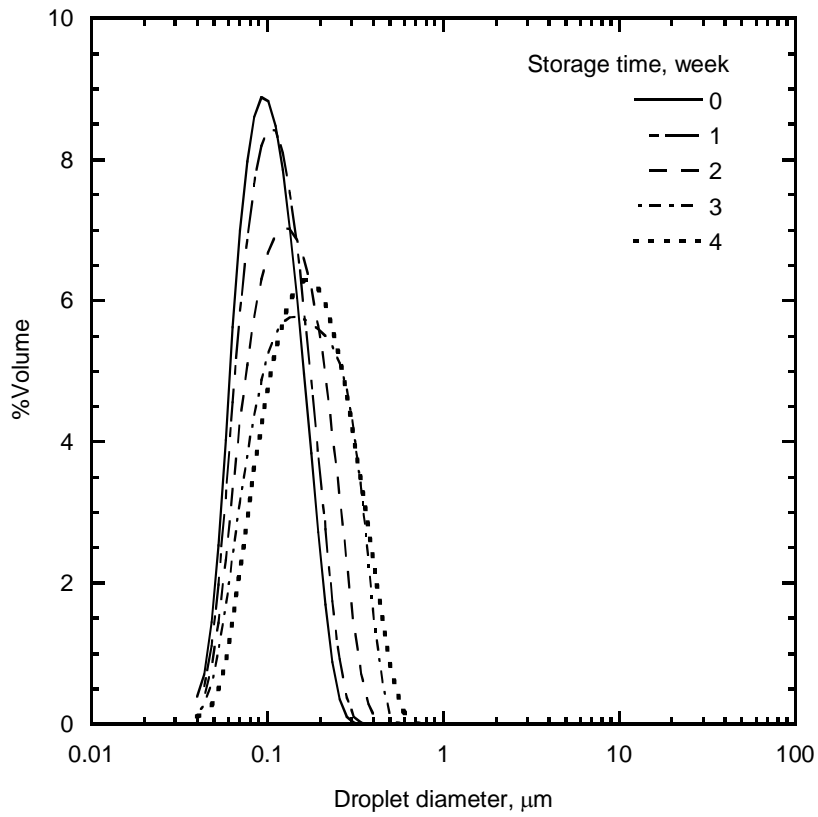


Fig.4

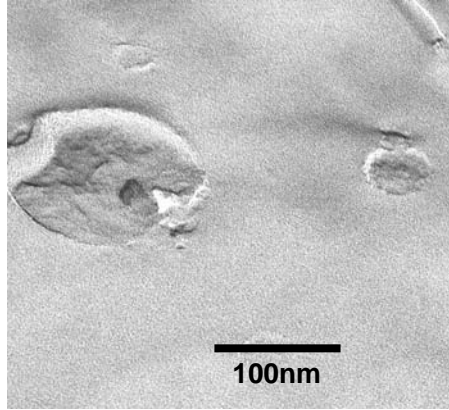


Fig.5