

1 **Technical Note: Evaluation of the NanoAerosol Generator by**
2 **Kanomax FMT Inc. in Aerosolization of Size Standard**
3 **Nanoparticle and Protein**

4
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9
10 **Abstract**

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12 A new nebulizer called NanoAerosol Generator (NAG) by Kanomax FMT, Inc. was evaluated
13 experimentally in their performance in aerosolizing sub-100 nm particles and sub-10 nm protein
14 molecules which cannot be aerosolized with conventional compressed-air jet nebulizers due to
15 the interference by a number of residue particles. For the challenge particles, 29 nm-polystyrene
16 latex (PSL) particles and bovine serum albumin (BSA) protein molecules were used as sub-100
17 nm and sub-10 nm test materials, respectively. For both particles, the aerosolization by the NAG
18 was successful with a clear separation between the peak of the challenge particle and the peak of
19 residual particles in the size distribution spectra. The size distributions of aerosolized 29 nm-PSL
20 particles were compared between the NAG and an electrospray aerosol generator (EAG) with
21 liquid samples of the same particle concentration. The EAG showed clearer separation of the PSL
22 peak from the residual peak, but the peak height was taller for the NAG by a factor of 6 than for
23 the EAG, which indicated the generation of airborne particles from a liquid suspension/solution
24 of the same concentration was more efficient for the NAG than for the EAG.

25
26 **Keywords:** Nebulizer; Electrospray, Polystyrene latex, Bovine serum albumin
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29 INTRODUCTION

30

31 Generation of aerosols by spraying a suspension of solid particles or a solution of non-volatile
32 materials is an essential technique in aerosol and particle studies, and various types of sprayers
33 are used in the laboratory for this purpose (Hinds, 1999; Chen *et al.*, 2011). In our laboratory
34 where aerosol instruments are regularly tested and calibrated, for example, size standard
35 polystyrene latex (PSL) particles are aerosolized by the spray method. Conventional compressed-
36 air jet nebulizers (or atomizers) and electrosprays are most common sprayers. The conventional
37 nebulizer can aerosolize particles in the size range from about 50 nm to a few μm . The
38 electrospray is good at 50-100 nm and even smaller sizes. The difference in the lower size limit
39 between these techniques comes from the difference in the initial size of the droplets formed by
40 the two sprayers. That is, the initial droplet size of the nebulizer is greater than that of the
41 electrospray, which results in larger residual particles. The size of the residual particles from the
42 nebulizer is often as large as 50 nm and overlaps with the particles of 50 nm or smaller. For
43 example, the number weighted size distribution of residue particles when deionized water was
44 sprayed with a conventional nebulizer, TSI model 3076 Constant Output Atomizer, had the mode
45 diameter of 21.5 nm and extended to about 50 nm (Stabile *et al.*, 2013). For this reason,
46 aerosolization of sub-50 nm particles is usually done with the electrospray. However, the
47 electrospray technique may not be applicable for some types of liquids.

48 Recently, Kanomax FMT Inc. introduced a new nebulizer, i.e., the NanoAerosol Generator
49 (NAG) model 3250. Similar to other nebulizers, the NAG has an impactor after the exit of the
50 nebulizing nozzle to remove large droplets that are formed by spraying. For the NAG, the
51 impactor is located closer to the nozzle, compared with conventional nebulizers, to reduce the
52 cut-off size of the impaction. The initial mode diameter of the droplets generated by the NAG is
53 550 nm (Kanomax FMT, Inc., 2017), which is greater than the droplet sizes of not only the
54 electro spray but also the conventional nebulizer, based on the information provided by the
55 manufacturers. For example, the initial droplet size of the TSI model 3480 Electro spray Aerosol
56 Generator (EAG) and that of the TSI model 3076 Constant Output Atomizer (a conventional
57 nebulizer) are 150 nm (TSI Inc., 2012) and 350 nm for water (TSI Inc., 2005), respectively.
58 Nevertheless, the manufacture of the NAG claims that the NAG "produces significantly smaller
59 droplets minimizing the influence of non-volatile residue" (Kanomax FMT, Inc., 2017) when
60 compared with conventional nebulizers. To verify the claim by the manufacturer, we performed
61 experimental evaluations of the performance of the NAG in aerosolizing particles that we often
62 use in our laboratory, namely size standard PSL nanoparticles and proteins, and investigated
63 whether the NAG can aerosolize those sub-50 nm particles without burying them in residual
64 impurity particles.

65
66 **EXPERIMENTAL**

67

68 A schematic of the experimental setup is given in Fig. 1. The setup was simple: An aerosol
69 generator was connected with a tube to an instrument for the measurement of size distribution.
70 The NAG was operated with the sample and nebulizer gas pressures set at 18 psi (0.12 MPa) and
71 35 psi (0.24 MPa), respectively. The evaporation temperature was set at 60 °C. The dilution gas
72 mode was set passive, i.e., the excess air was bled through the vent in the NAG.

73 The size distribution of the aerosols from the NAG was measured with a scanning mobility
74 particle sizer spectrometer (SMPS) by TSI Inc. (Shoreview, Minnesota, U.S.A.) with a bipolar
75 charge conditioner with Am-241 (3 MBq) in a stainless steel housing by Tsukasa Sokken Co.,
76 Ltd. (Tokyo, Japan). The SMPS consisted of a model 3085A Nano DMA, model 3776 Ultrafine
77 Condensation Particle Counter, and model 3082 controller platform, with TSI Aerosol Instrument
78 Manager ver. 10.1 software for measurement control and data acquisition. We did not use the pre-
79 cut impactor. The sheath air and aerosol flow rates of the DMA were set at 15 L min⁻¹ and 1.5 L
80 min⁻¹, respectively. The CPC was operated in high flow mode. The upscan time was 160 s. The
81 multiple charge and diffusion loss corrections were both turned off in the size distributions
82 presented in this paper, since these corrections were not essential in this study. The SMPS
83 covered the size range between 2.5 nm and 65 nm. The size distribution spectra shown in this
84 paper are the results of averaging of sequential measurements repeated for at least three times.
85 The tubing connection between the NAG outlet and the inlet of the bipolar charge conditioner of

86 the SMPS was made with a conductive silicone tube of the inner diameter of about 6 mm and the
87 length of about 50 cm. There was no diffusion dryer used after the NAG.

88 To compare directly the performance of the NAG with that of the electrospray, a unit of the
89 electrospray aerosol generator (EAG, TSI model 3480) was placed next to the NAG and the size
90 distribution measurement for the aerosol by the EAG was made with the same SMPS. The tubing
91 connection between the EAG and SMPS was identical in the length (i.e., 50 cm) except that there
92 was a branch for bleeding. The EAG was operated with clean air at the flow rate of 1.5 L min^{-1}
93 without carbon dioxide gas added, and with a capillary of $40 \mu\text{m}$ in inner diameter. An Am-241
94 (4 MBq) alpha-ray source was installed in the spray chamber for bipolar charge conditioning.

95 Two liquid samples were used in the evaluation of the NAG, namely, suspensions of PSL
96 nanoparticles and solutions of bovine serum albumin (BSA) protein. The PSL and BSA used in
97 this study were SC-0030-A by JSR Corp. (Tokyo, Japan) and A9418 by Sigma-Aldrich (Saint
98 Louis, Missouri, U.S.A.), respectively. For the PSL, the raw suspension liquid was diluted with
99 ultrapure water at three concentration levels. BSA was dissolved into ultrapure water at three
100 concentration levels. For the PSL suspension that was aerosolized by the EAG, ammonium
101 acetate was dissolved at the concentration of 2 mM to adjust the electrical conductivity of the
102 liquid. The sample dilution factor was determined by weighing both the liquid to be diluted and
103 the liquid after dilution with an analytical balance of 0.1 mg precision. The relative standard

104 uncertainty of the dilution factor by the above method was estimated to be approximately 10 %.
105 The ultrapure water used for the preparation of the above liquid samples had the nominal
106 resistivity and total organic contents of 18 MΩ cm and 3 ppb, respectively, and was processed
107 with a final filter of the pore size of 0.22 μm.

108 109 **RESULTS AND DISCUSSION**

111 The NAG was first evaluated with ultrapure water for the size of residual particles. The size
112 distribution obtained is shown in Fig. 2. Most of the residual particles were smaller than 6 nm,
113 and the mode diameter was 3.5 nm. The size of the residual particles was significantly smaller
114 than that by conventional nebulizers which is usually greater than 20 nm (Stabile *et al.*, 2013).

115 The second evaluation test was done with the 29 nm PSL. The size distributions are given in
116 Fig. 3. The left plot (Fig. 3(a)) shows the size distributions with the absolute scale in y-axis.
117 Suspensions of three dilution levels, i.e., the dilution factors of 10^3 , 10^4 , and 10^5 , were prepared
118 and used in the evaluation. The height of the PSL peak at about 30 nm decreased as the dilution
119 factor was increased. About the stability of the aerosol generation and the measurement
120 repeatability of the experiment, the total concentration for the PSL peak in the size range between
121 15 nm and 50 nm varied by 5 % or less in repeated measurements. This good stability and
122 repeatability were maintained throughout this study. The broad peak for residual particles on the
123 left became shorter and shifted to smaller sizes as the dilution factor was increased. The

124 separation between the two peaks was clear at all the three concentration levels, although the
125 bottom of the gap between the two peaks did not go to zero completely. In Fig. 3(b), the size
126 distribution functions were normalized so that the top of the PSL peaks match at unity for the
127 three traces. The PSL peak for the dilution factor of 10^3 had a slight shoulder around 40 nm on
128 the right, which indicated the formation of PSL doublets. Indeed, the mobility-equivalent
129 diameter of doublet particles of 29-nm spheres was calculated based on Cheng et al., (1988) and
130 found to be 35.1 nm and 39.7 nm when the particles are assumed to align parallel and
131 perpendicular to the direction of the particle motion, respectively. These apparent diameters are
132 comparable to the particle size that corresponds to the shoulder. Another possibility for the
133 formation of the shoulder is due to thick coating of the PSL particles with non-volatile residue
134 when the dilution factor was the lowest. From Fig. 3(b), the largest residual particles for the
135 dilution factor of 10^3 seemed to be about 20 nm. Based on this, we assumed that the maximum
136 volume of the non-volatile residue that coated the 29-nm PSL particles was equal to the volume
137 of 20 nm spherical particles and calculated the diameter of the coated PSL particles. The result
138 was 32 nm, which was too small to explain the formation of the shoulder at about 40 nm.
139 Therefore, we concluded that the shoulder was due to the formation of doublets, and for this PSL
140 with the NAG, the dilution factor should have been greater than 10^3 to avoid completely the
141 doublet formation. Except the formation of doublets, the NAG was successful in aerosolizing 29

142 nm-PSL particles without being significantly interfered by the residual peak in the distribution
143 spectra.

144 The third evaluation test was performed with BSA. The size distributions obtained for the three
145 concentration levels are shown in Fig. 4. At the highest concentration level of $100 \mu\text{g mL}^{-1}$ in Fig.
146 4(a), the monomer peak at 6.4 nm was clearly separated from other peaks. There was no peak for
147 residual particles. This was probably because all droplets formed in the NAG contained at least
148 one BSA molecule and the majority of the particles formed were BSA multimers. While the
149 monomer peak was present, the fraction of the monomers was not large. For the purpose of the
150 formation of airborne BSA monomers, the solution concentration was too high and most of BSA
151 put into the solution was wasted. At the second concentration level of $1 \mu\text{g mL}^{-1}$ in Fig. 4(b), the
152 monomer peak was the strongest, with some multimers still being present to the right. There was
153 no residual peak. The height of the monomer peak ($4.3 \times 10^6 \text{ cm}^{-3}$ in $dC_N/d\log d$), was greater than
154 that of the monomer peak at the concentration of $100 \mu\text{g mL}^{-1}$ ($2.2 \times 10^6 \text{ cm}^{-3}$). That is, between 1
155 and $100 \mu\text{g mL}^{-1}$, more BSA monomers were aerosolized with less BSA in the solution. At the
156 third, lowest concentration level of $0.01 \mu\text{g mL}^{-1}$ in Fig. 4(c), the most dominant peak was that
157 for the monomer. Most of the mulimer peaks seen at the higher concentration levels diminished,
158 and instead the signal from residual particles were visible in the size range between 2 nm and 6
159 nm in this spectrum. There was no interference of the residual peak signal to the monomer peak.

160 From these observations, the aerosolization of BSA monomers was most successful at the
161 solution concentrations of $0.01 \mu\text{g mL}^{-1}$ and $1 \mu\text{g mL}^{-1}$.

162 An additional measurement was made with the $1 \mu\text{g mL}^{-1}$ BSA solution to investigate the
163 concentration of the monomers after the classification by the DMA. The size classification by the
164 DMA in the SMPS was fixed at 6.4 nm and the concentration after the DMA was recorded with
165 the Ultrafine CPC. The concentration was about $2.5 \times 10^4 \text{ cm}^{-3}$, which would be sufficiently high
166 for many applications, such as instrument testing in sub-10 nm size range.

167 The fourth evaluation test was performed with the 29 nm-PSL particles again, and the size
168 distributions of the PSL particles aerosolized by the NAG and EAG were compared with each
169 other. For this test, both generators aerosolized PSL suspensions of the same dilution factor of
170 10^3 , while the suspension for the EAG had additional ammonium acetate of 2 mM as stated
171 earlier. The size distributions taken by the two generators are plotted together in Fig. 5. The gap
172 between the PSL and residual peaks was wider for the EAG. The mode diameter of the residual
173 peak for the EAG was about 4 nm while that for the NAG was about 7 nm, which indicated that
174 the initial droplet size of the NAG was greater than that of the EAG. The mean diameter of the
175 PSL peak calculated for the size range between 25 nm and 33 nm was 29.1 nm for both
176 generators. The above size range was determined so that the shoulder due to doublets in the size
177 distribution of the NAG was not included. The agreement of the mean diameter between the two

178 generators indicated that the residual coating did not affect the particle size when the PSL
179 particles were aerosolized with the NAG. The intensity of the PSL peak was higher for the NAG
180 by about a factor of 6 when compared with the peak height for the EAG. Since the particle
181 concentration in the liquid suspension was about the same (i.e., the raw PSL suspension liquid
182 was diluted by a factor of 10^3 for both generators), and since the relative uncertainties for the
183 dilution ($< 10\%$) and measurement repeatability ($< 5\%$) were small, the taller PSL peak for the
184 NAG in this side-by-side comparison indicated that the NAG was more efficient in aerosolization
185 of this PSL. It should be noted that, since the droplet size was smaller for the EAG, the particle
186 concentration of the liquid suspension could be increased to increase the PSL peak intensity until
187 the magnitude of doublet formation becomes unacceptable, while for the NAG the dilution factor
188 of 10^3 was already at the limit.

189 **CONCLUSION**

191
192 The NAG was evaluated with the PSL nanoparticles and protein molecules regarding its
193 capability of generating the particles and protein molecules without suffering from the
194 interference with residual particles and multimers in the size distribution. We found the NAG was
195 successfully able to aerosolize the 29 nm-PSL particles and BSA molecules of about 6.4 nm
196 without significant interference. Based on these results, we expect the NAG could be an

197 alternative of the electrospray for aerosolizing particles and molecules of sub-100 nm and even
198 sub-10 nm.

199 We would like to note that the consumption rate of the sample liquid was much faster for the
200 NAG than for the EAG. In their catalogs, for example, the sample liquid flow rates of the NAG
201 and EAG are $0.5 - 3.0 \text{ mL min}^{-1}$ and $50 - 100 \text{ nL min}^{-1}$ (TSI model 3480 EAG), respectively, and
202 these values are consistent with our experience.

203

204 **ACKNOWLEDGMENTS**

205

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209 his assistance in designing the experimental evaluation of this study.

210

211 **DISCLAIMER**

212

213 Publication of this paper does not imply recommendation or endorsement of any commercial
214 products by the National Institute of Advanced Industrial Science and Technology (AIST).

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Figure Captions

238 **Fig. 1.** Schematic of the experimental setup.

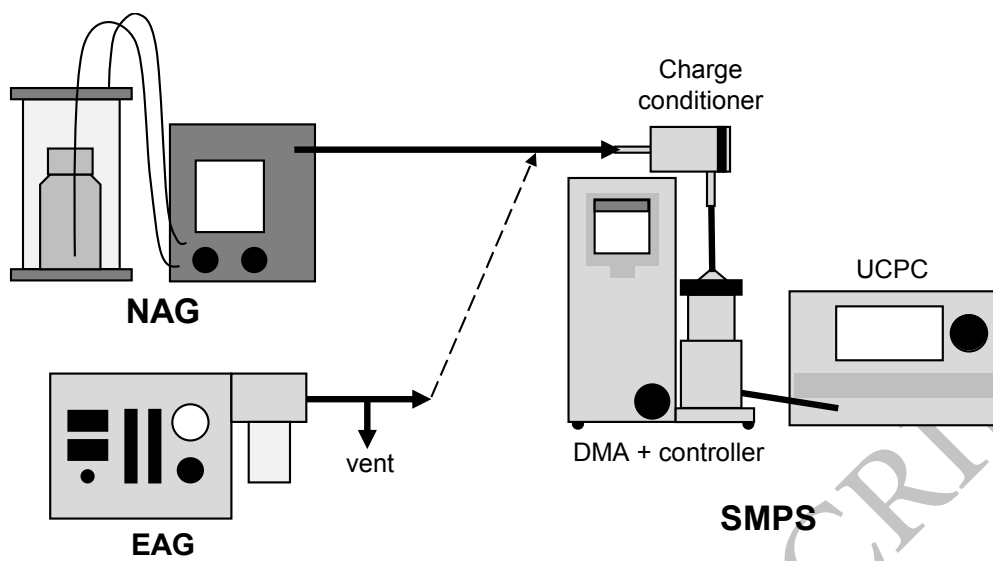
239 **Fig. 2.** Size distribution of the residual particles formed by nebulization of ultrapure water with
240 the NAG.

241 **Fig. 3.** Size distributions of 29 nm-PSL particles by nebulization with the NAG. The three
242 traces in each plot correspond to suspensions prepared at different dilution factors: (a) y-
243 axis in the absolute scale, and (b) y-axis in normalized scale so that the peak heights for
244 the PSL match at unity for the three traces.

245 **Fig. 4.** Size distributions of BSA protein molecules nebulized with the NAG at three
246 concentration levels: (a) $100 \mu\text{g mL}^{-1}$, (b) $1 \mu\text{g mL}^{-1}$, and (c) $0.01 \mu\text{g mL}^{-1}$. The down
247 arrow in (a) indicates the position of the monomer peak at about 6.4 nm.

248 **Fig. 5.** Comparison of the size distributions of 29 nm-PSL particles aerosolized by the NAG and
249 EAG with suspensions of about the same dilution factor of 10^3 .

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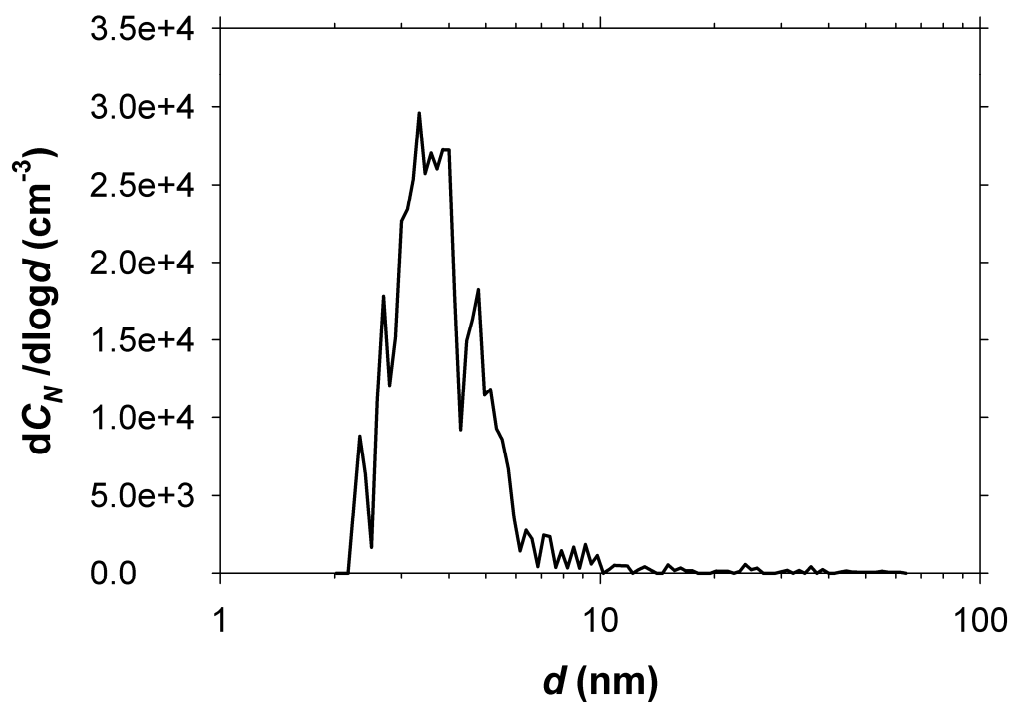
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Fig. 1

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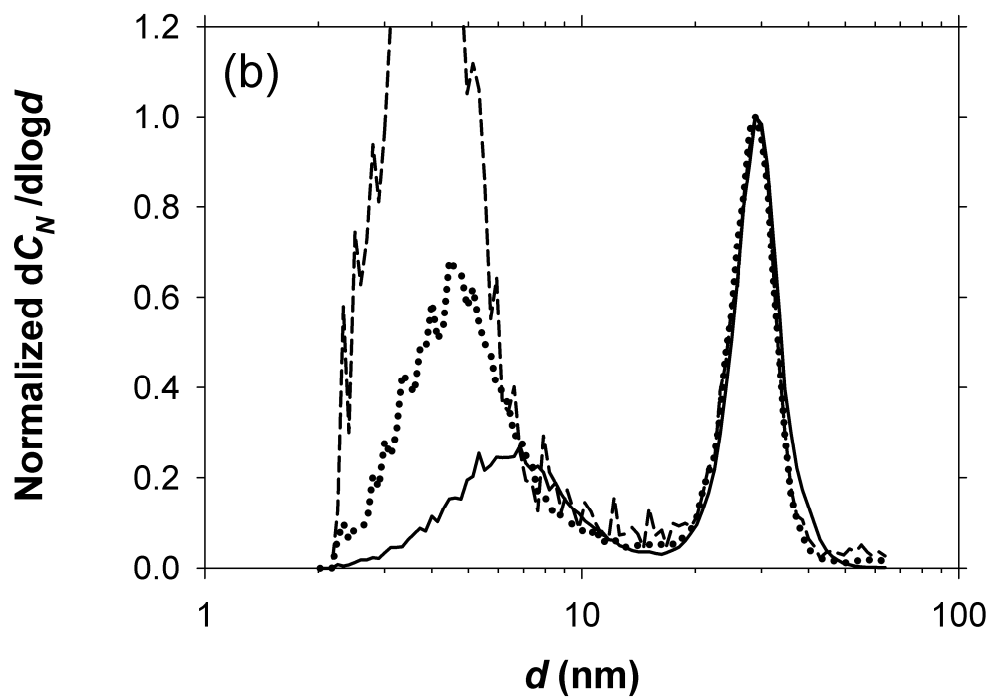
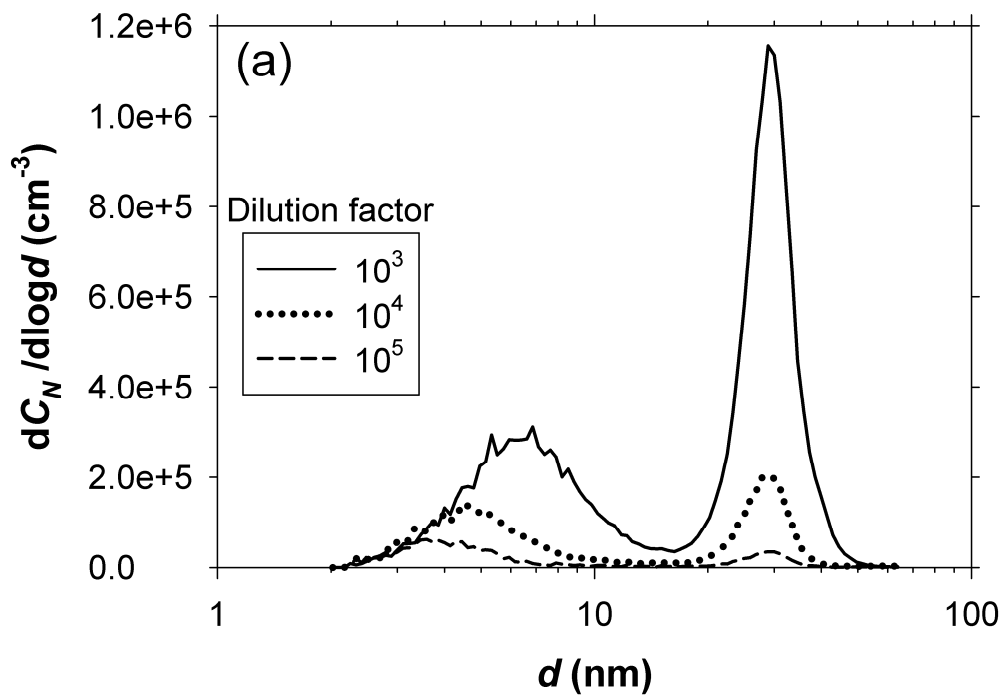
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Fig. 2

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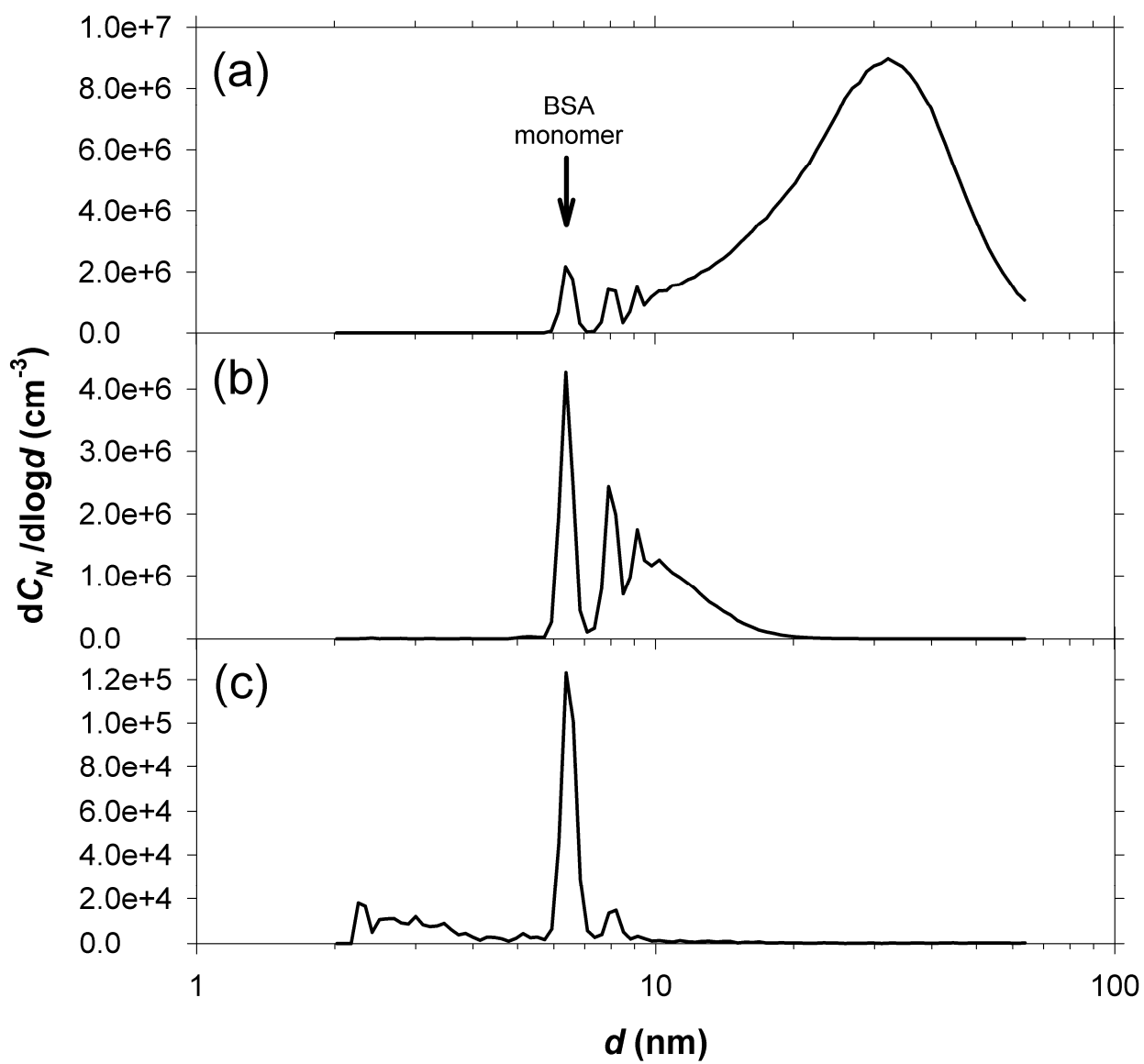
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Fig. 3



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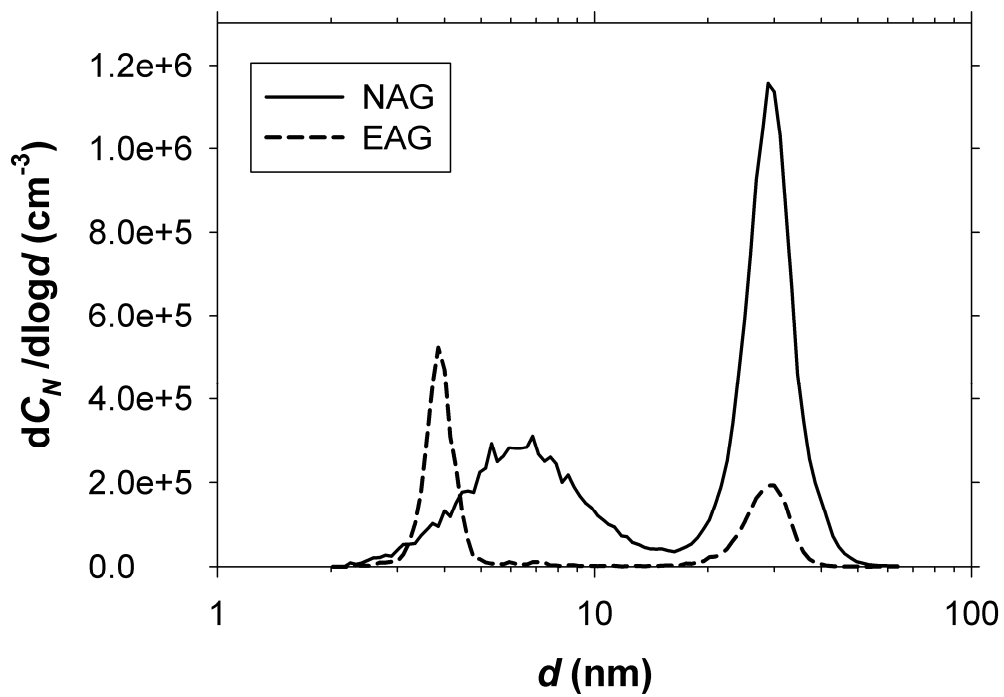
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Fig. 4



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Fig. 5

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