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RESEARCH PAPER

Characterization of a Nanoparticulate Drug Delivery System Using Scanning Ion Occlusion Sensing

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ABSTRACT

Purpose To explore the application of scanning ion occlusion sensing (SIOS) as a novel technology for characterization of nanoparticles.

Methods Liposomes were employed as model nanoparticles. The size distribution of the liposomes was measured by both SIOS and dynamic light scattering (DLS). Particle number concentration was determined based on particle translocation rate. The ability of SIOS and DLS to resolve bimodal samples was evaluated by measuring a mixture of 217 and 355 nm standard nanoparticles. Opsonization of liposomes by plasma was also studied using SIOS.

Results SIOS was shown to measure the size of different liposomes with higher sensitivity than DLS and it requires a smaller sample volume than DLS. With appropriate calibration, SIOS could be used to determine particle number concentrations. In comparison, SIOS analysis of the mixture showed accurate resolution of the population as a bimodal distribution over a wide range of number ratios of the particles. SIOS could detect plasma opsonization of liposomes by demonstrating a increase in particle size and also changes in the particle translocation rate.

Conclusion SIOS is a useful technology for nanoparticle characterization. It shows some advantages over DLS and is clearly a useful tool for the study of nanoparticle drug delivery systems.

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INTRODUCTION

The rapidly expanding application of nanoparticles for drug delivery requires more diversified techniques for the characterization of particle size, surface charge, number-concentration and shape. There are various techniques available for characterizing nanoparticles including dynamic light scattering (DLS) (1), electronic microscopy (EM) (2), size exclusion chromatography (3), gel electrophoresis (4) ultrasound spectroscopy (5) and nanoparticle tracking analysis (6). However, no single technique meets all the requirements of characterization of different nanoparticulate drug delivery systems.

Scanning ion occlusion spectroscopy (SIOS) is a recently developed approach for particle analysis. SIOS utilizes the well established Coulter principle (7) for particle analysis, i.e. particles are individually analyzed as they traverse a pore. SIOS provides a method for analysis of particles from microns in size down to approximately 60 nm. One of the unique features of SIOS is that it employs a tunable pore. This pore is formed centrally within an elastomeric polyurethane cruciform (8). The cruciform is placed within the fluid cell of the qNano instrument. The four arms of the cruciform can be mechanically stretched and/or relaxed in the XY axis, allowing for nanoscale adjustment of the centrally located pore. A fluid cell attached to the qNano instrument encompasses the central portion of the cruciform allowing containment of an electrolyte solution above and below the pore. Ag/AgCl electrodes in the upper and lower portions of the fluid cell are used to apply a potential difference across the pore. When an electrolytic fluid bridge is

established across the pore, the ionic current across the pore can be measured by the qNano electronics. By stretching or relaxing the cruciform, the pore size can be adjusted and optimized for the particles that are being analyzed. Charged nanoparticles migrate in the electric field and may traverse the pore by electrophoresis and electro-osmosis. As particles traverse the pore, a resistive current pulse can be detected; these pulses are called blockage events. Particles can also be driven through the pore under pressure by using a variable pressure module (VPM) to provide pressure or vacuum (Fig. 1). This allows detection of weakly charged or neutral particles and offers detection at lower particle concentrations.

Theoretical models have been developed to describe the translocation of nanoparticles through the pore under electrophoresis (9) or VPM conditions (10). The mean blockade magnitude has been found to scale linearly with mean particle volume for spherical particles (11) enabling particle size measurement using SIOS. Recent applications of this technique include the detection and controlled gating of DNA molecules (8) and the characterization of DNA-coated nanoparticles (12) and adenovirus (11). In this paper we explore the use of SIOS for the characterization of pharmaceutical nanoparticles.

Unilamellar liposomes were used as model nanoparticles as they have been extensively studied and there are now several liposomal products available for clinical use (13). Pores were calibrated using synthetic carboxylated polystyrene nanoparticles. Liposomes and polystyrene nanoparticles were characterized by both SIOS and DLS in order to compare their advantages and disadvantages. Currently, DLS is the technique most commonly used for liposome characterization. The liposomes size distribution, the liposome particle concentration (liposome number concentration) and also the interaction between liposomes and plasma proteins were measured by SIOS.

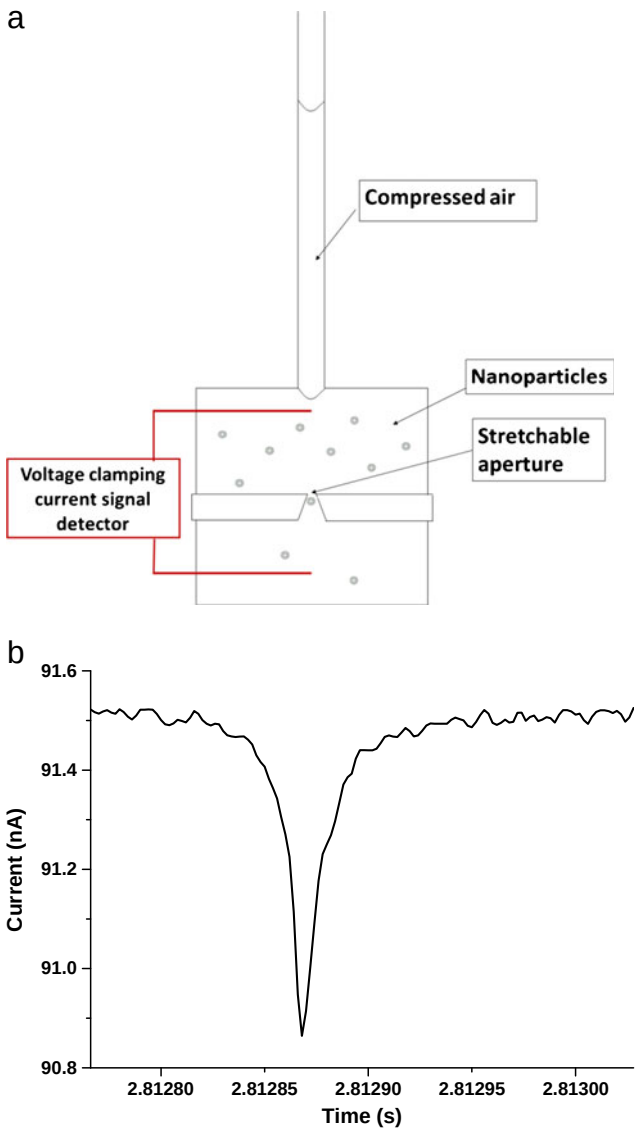

Fig. 1 Diagram of SIOS using a variable pressure module (**a**) and a representative blockage event (**b**).

MATERIALS AND METHODS

Materials

Soy phosphatidylcholine (SPC) was donated by Lipoid (GmbH, Ludwigshafen, Germany). Ringer's buffer (10 mM D-glucose; 0.23 mM $MgCl_2$; 0.45 mM KCl; 120 mM NaCl; 0.70 mM Na_2HPO_4; 1.5 mM NaH_2PO_4) was purchased from Sigma-Aldrich (USA). Nucleopore Track-Etch membranes (50, 100 and 200 nm pore size) were purchased from Whatman (UK). Heparinized rat blood was obtained from the Hercus-Taieri Resource Unit, University of Otago. Carboxylated standard nanoparticles with nominated diameter of 100 nm (CPS100), 118 nm (CPS118), 217 nm (CPS217) and 355 nm (CPS355) containing $5e^{12}$, $5e^{12}$, $5e^{12}$ and $5e^{10}$ particles/ml respectively were bought from Bangs Labs (USA).

Methods

Liposome Preparation

SPC liposomes were prepared by the thin film hydration method and extruded to obtain large unilamellar vesicles (LUVs) with a narrow size distribution. Briefly, SPC (100 mg) was dissolved in 5 ml chloroform-methanol (3:1, v/v) in a round-bottomed flask. The organic solvent was removed by rotary evaporation (Rotavapor R110, BÜCHI) at 35°C and the residue was kept under vacuum overnight to remove traces of organic solvent. The lipid film was then

Table I Optimized Experimental Conditions for Studying 400lip, 200lip and 100lip

Liposomes	Cruciform	Applied XY stretch on the cruciform (mm)	Voltage (V)	Pressure (kPa)	Calibration standards
400lip	NP200	3.6	+0.5	1.0	CPS355
200lip	NP200	2.9	+0.5	1.0	CPS217
100lip	NP100	2.5	+0.5	1.0	CPS100

hydrated with 5 ml Ringer's buffer in the presence of 0.5 g glass beads at room temperature for 30 min. After hydration, the liposomal suspension was sonicated (bath sonicator, RK100H, Bandelin Electronic, Germany) for 1 min and extruded through 400, 200 and 100 nm Nucleopore Track-Etch membranes using a Lipex Extruder at room temperature (Northern Lipids, Canada) to produce liposomes of different sizes. These liposomes were designated 400lip, 200lip and 100lip.

Particle Measurement by SIOS

Particle characterization by SIOS has been fully described (8–10,12,14). SIOS analysis was performed using the Izon qNano instrument with the NP100-NP400 apertures supplied. After positioning of the cruciform on the instrument, the electrolyte (Ringer's solution) was added into the lower cell (75 µl) and upper cell (40 µl) compartments respectively. The four arms of the cruciform were mechanically stretched

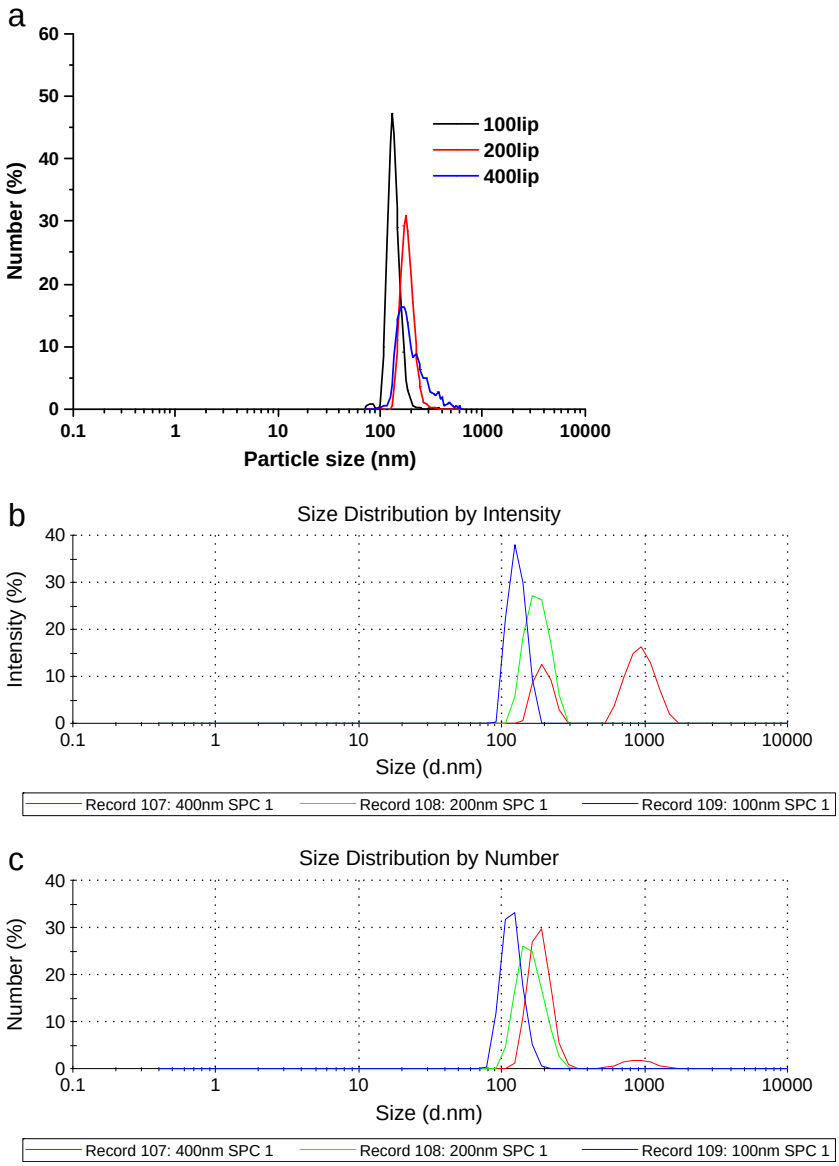

Fig. 2 Size distribution of liposomes extruded through different pore-sized membranes. (**a**) measured by SIOS; (**b**) distribution by intensity measured by DLS; (**c**) distribution by number measured by DLS.

Table II Size of Liposomes Extruded Through Different Pore-Sized Membranes

liposomes	DLS			SIOS		
	Z-average (nm)	Peak (nm)	PDI	Mean (nm)	Media (nm)	Mode (nm)
100lip	126	128	0.007	142	138	124
200lip	175	179	0.058	191	187	182
400lip	418	950/196[a]	0.438	234	204	159

[a]Two peaks of bimodal distribution in DLS result

in the XY axis to +3 mm and the pore was allowed to wet with electrolyte. The establishment of a stable baseline of ionic current indicated that an electrolytic fluid bridge was established across the pore. The apertures were tuned by adjustment of XY deformation to optimize the resolution of each liposome preparation. Apertures were calibrated with carboxylated polystyrene nanoparticles (CPS100, CPS217 and CPS355).

For the liposome experiment, 40 μl of diluted liposome suspension was added to the upper fluid cell compartment and the lower cell contained Ringers solution (75 μl). The experimental conditions such as cruciform stretch (pore size adjustment) and applied voltage were adjusted to optimize the resolution of each liposome preparation (Table I). A minimum of 500 translocation events for each sample were recorded for statistical purposes.

Determination of Particle Number Concentration Using SIOS

The 100lip suspension (0.05 mg/ml phospholipids) was further diluted 2.5, 5.0, 7.5 and 10 times using Ringer's buffer. The standard particle CPS100 was diluted to 9×10^8 particles/ml using Ringer's buffer. The translocation rate (translocation events per min) of the diluted CPS100, the diluted 100lip and the four further diluted samples were measured using SIOS under 1.0 kPa pressure until a minimum 500 translocation events were recorded.

Study of Multimodal Samples Using SIOS and DLS

Two standard particle suspensions (CPS217 and CPS355) were diluted to 0.5×10^9/ml respectively using Ringer's buffer. Bimodal samples were prepared by mixing the two particle suspension in 5:95; 20:80; 50:50; 80:20; 95:5 volume ratios. The particle size distributions of the individual particles samples (CPS217 and CPS355) and the mixtures were determined using SIOS. A trimodal sample was prepared by mixing CPS118, CPS217 and CPS355 at a 1:1:1 ratio. For this analysis the cruciform (NP200) had an applied XY stretch of 4.9 mm, the voltage was 0.3 V and the pressure 1 kPa. The samples were also analyzed by DLS.

Liposome/Plasma Interaction

Heparinized whole rat blood was centrifuged at 2000 g for 15 min; the plasma was collected and filtered through a 0.22 μm membrane. An aliquot (1 μl) of filtered rat plasma was added to 5 μl of 100lip, the sample was incubated for 30 min at room temperature and then diluted to 1000 μl with Ringers solution for SIOS analysis. Control samples of 100lip were incubated with 1 μl of Ringers solution instead of plasma and after 30 min diluted to 1000 μl in Ringers Solution for SIOS analysis.

Particle Size Measurement by DLS

The Z-average size of the liposomes was determined in Ringer's buffer at 25°C by DLS using a Zetasizer (Nano ZS, Malvern Instrument). The values of viscosity and refractive index of the dispersion medium were taken as 1.02 cP and 1.330, respectively.

Statistical Method

Data for liposomes size increases due to opsonization were analyzed by independent-sample t-test using SPSS Statistics 19 (IBM, US).

Table III Precision of Size Measurement by DLS and SIOS (n=4)

	DLS			SIOS		
	Z-average	Peak	PDI	Mean	Medium	Mode
average	106	112	0.072	112	109	106
Standard Deviation	2.8	2.7	0.024	1.2	1.0	1.2
CV (%)	2.7	2.4	14	1.1	1.0	1.1

RESULTS AND DISCUSSION

Particle Size Measurement

A liposome preparation was extruded through 400, 200 and 100 μm filters to generate three samples for analysis (400lip, 200lip and 100lip). These samples were measured by both DLS and SIOS. Particle size distributions of each sample are shown in Fig. 2a, b and c. As expected the liposome populations obtained after extrusion differ in size and the smaller liposomes have a tighter size distribution. Particle size distributions measured by SIOS showed a single peak for 100lip and 200lip (Fig. 2a) which is consistent with the results of the DLS measurements for the two samples (Figs. 2b and c). Moreover, the mean size of 100lip and 200lip measured by SIOS and the Z-average size of the two samples measured by DLS are similar (Table II). Under SIOS analysis, the size distribution of 400lip shows a major peak with multiple smaller higher sized peaks. It was found that the size of 400lip is around 100~250 nm which is smaller than the pore size of the extrusion membrane. This phenomenon has been reported previously (15) and it has been demonstrated that the size of extruded liposomes depends not only on the pore-size of extrusion membrane but also extrusion pressure (16). With DLS analysis, the 400lip sample has a bimodal distribution and a polydisperse index (PDI) (Table II) higher than 0.3, indicating the sample is not monodisperse. The Z-average size of the 400lip sample is unreliable as it was determined based on the assumption that the sample is monodisperse. Unlike DLS where the distribution can be adversely affected by the presence of a relatively low number of larger particles, SIOS builds its size histogram by accumulating data from many individual blockade events.

The measurement precision of the two techniques was also compared by repeated measurement (n=4) of a 100lip batch (Table III) which is a different batch from that used in Table II and Fig. 2 and although the CV for SIOS was smaller than that for DLS, the difference was not significant (p>0.05).

The concentration of liposomes used in the above studies was 0.5 mg/ml phospholipid. In order to compare the sensitivity of the two methods, serially diluted samples were measured by both DLS and SIOS. Starting with a liposome with phospholipid concentration of 0.5 mg/ml that was detectable with both methods, it was found that DLS could accurately still measure this sample when diluted 2-fold but not after a 5-fold dilution (Table IV). In comparison, SIOS could measure the same sample diluted 100-fold. Theoretically SIOS could measure the sample at an even higher dilution by extending the run time to collect enough data for statistical purposes.

Table IV Size of Liposomes Measured by Both DLS and SIOS at Different Concentrations

Dilution factor of liposomes (0.5 mg/ml phospholipid)	Z-average size (DLS), nm	Mean (SIOS), nm
undiluted	106	112
2	108	N.D.
5	x	N.D.
10	x	108
25	x	109
50	x	108
75	x	109
100	x	108

N.D.: Not determined

x: No result obtained as DLS software detected that particle concentration was too low

Based on these results for size and size distribution measurements, it is apparent that SIOS is a useful technique to measure liposomes with some superior features to DLS. SIOS collects information of individual particles whereas DLS measures the particle population. SIOS provides information about size (mean, medium and mode) whereas DLS gives the Z-average size. The Z-average size measurement relies on the assumption that the sample is monodisperse. With DLS, a small number of large particles can lead to a skewed distribution. SIOS has previously been shown to provide accurate size measurement compared to electron microscopy (11). SIOS is more sensitive than DLS and the sample volume required for SIOS analysis is significantly less than that required for DLS.

An advantage of DLS is that it does not require calibration (17). In comparison SIOS requires calibration of the aperture with reference particles under the identical conditions to those used to measure an unknown. If the aperture

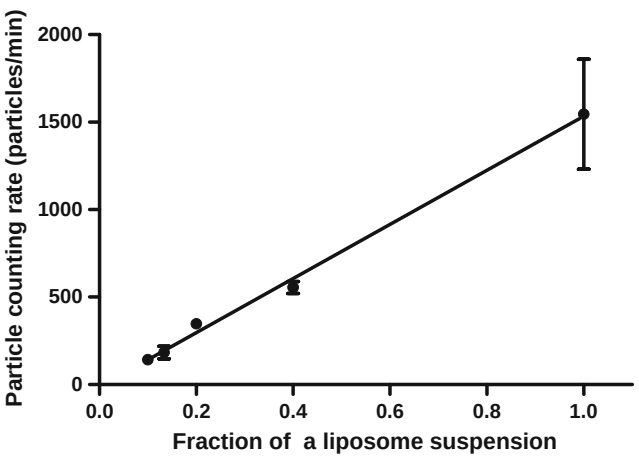

Fig. 3 Measured particle concentration as a function of fraction of a liposome suspension (data are linear with r>0.98, points are mean ± SD, n=3).

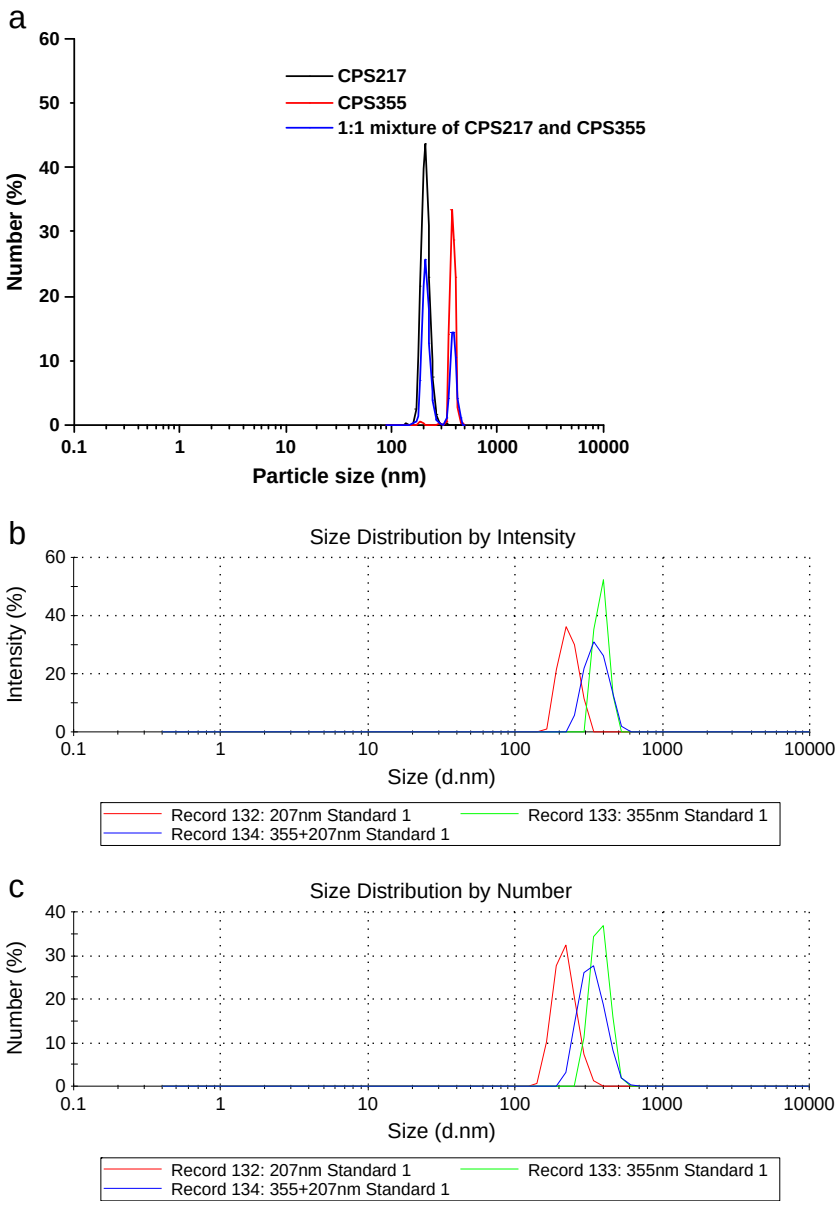

Fig. 4 Size distributions of CPS217 and CPS355 nm and a mixture of the two nanoparticles with a number ratio of 1:1 measured by (**a**) SIOS and (**b** and **c**) by DLS.

size, the voltage, the electrolytic conditions, or the applied pressure is altered, the aperture should be recalibrated with reference particles. Although SIOS can detect particles with sizes from approximately 60 nm to 10 μm (18), different format apertures are required to span such a wide distribution. It is also difficult to integrate the results of measurements conducted using different apertures under varying experimental condition.

Particle Number Concentration Measurement

The number of liposomes present in a sample (number concentration) is important information which DLS is not able to provide. The normal approach for calculating liposome number concentration, (15,16) relies on several limiting assumptions such as spherical geometry and monodisperse distribution (19). SIOS can determine particle number concentration, based on the fact that particle counting rate is proportional to particle number concentration (18). Figure 3 shows a linear relationship ($r>0.98$) between the dilution factor and particle counting rate. From this, the relative concentrations of particles in different samples can be determined by comparing their counting rates. In order to obtain an absolute particle concentration, calibration with standard nanoparticles of known size and concentration is required and the count rate must be independent of the physical properties of the particles.

The effect of the properties of nanoparticles, particularly their surface charge on the translocation frequency or particle counting rate can be minimized by using the pressure

Table V Size of Carboxylated Polystyrene Nanoparticles Measured by SIOS and DLS

Standard sample	DLS			SIOS		
Number ratio of CPS217 /CPS355	Z-average (nm)	Peak (nm)	PDI	Mean (nm) CPS217/CPS355 (nm/nm)	Medium (nm)	Mode (nm)
100:0	227	232	0.022	219	219	211
5:95	N.D.	N.D.	N.D.	212/346	213/346	215/345
20:80	N.D.	N.D.	N.D.	216/343	217/343	215/341
50:50	392	360	0.20	215/348	215/342	208/340
80:20	N.D.	N.D.	N.D.	217/343	217/342	217/341
95:5	N.D.	N.D.	N.D.	220/348	221/348	216/355
0:100	385	385	0.047	340	340	338

N.D.: Not determined

mode (10). It has been shown (10) that the ratio of pressure-driven nanoparticle flux to electrophoretic-driven nanoparticle flux is:

$$\frac{J_{pressue}}{J_{electrophoretic}} \frac{3\pi a' a_0^2 \Delta P}{4qV_0}$$

where α' is the hydrodynamic radius, α_0 is the radius of the nanopore, ΔP is the pressure difference applied across the nanopore, q is the effective charge on the nanoparticle and V_0 is the voltage applied across the nanopore. Based on this equation, pressure is approximately 5 fold more significant than electrophoresis to drive the translocation of 100 nm carboxylated polystyrene nanoparticles (zeta potential: −50 mV) at a pressure of 1.0 kPa. For liposomes, the effect of pressure is even more significant as liposomes have a slightly larger radius (α') and much lower effective charge (q) compared to carboxylated polystyrene nanoparticles. Thus the absolute particle number concentration of liposomes can be calculated with reference to the counting rate of the calibration sample.

Multimodal Sample

The particle size distributions of two standards (CPS217 and CPS355) and their 50:50 mixture are shown in Fig. 4a (SIOS) and 4b (DLS). SIOS could resolve the mixture clearly, showing a bimodal distribution whereas DLS could not resolve the mixture but showed a unimodal distribution. The values for particle size are shown in Table V. The results of SIOS for the mixture are very consistent with the results when the two particle size distributions were measured separately. Thus SIOS has higher resolution than DLS for samples with bimodal distributions and can accurately measure particle size and size distribution of individual populations in a mixture.

DLS measures particle size based on the intensity of light scattered by particles and assumes samples are monodisperse and spherical. This limits its application with polydispersed samples. It has been shown that DLS can only resolve two populations of particles with a diameter ratio greater than 2.71 (20); this is twice the size ratio of the two

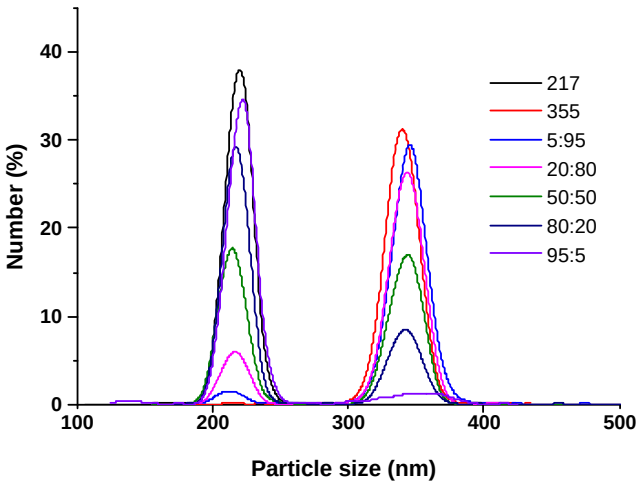

Fig. 5 Size distribution of mixtures of CPS217 and CPS355 with number ratios of 5:95; 20:80; 50:50; 82:20 and 95:5.

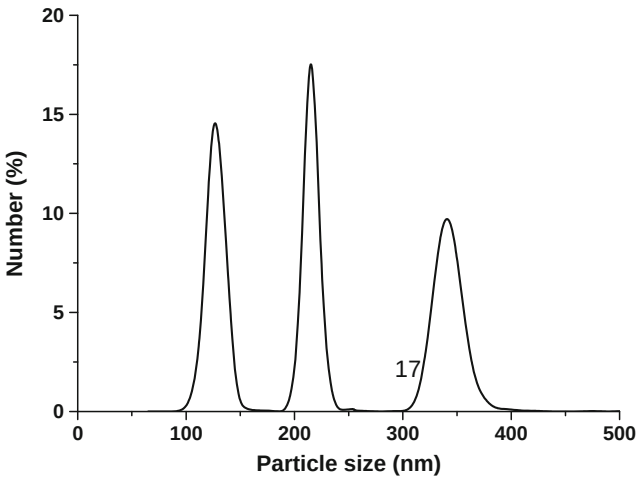

Fig. 6 Size distribution of a mixture of CPS118, CPS217 and CPS355 with particle number ratio of 1:1:1.

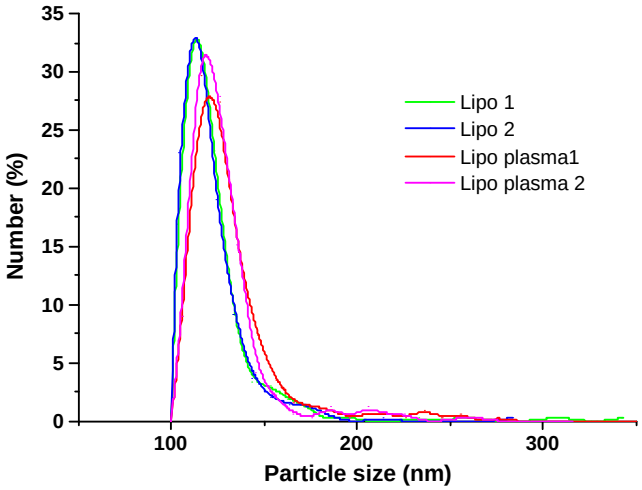

Fig. 7 Size distributions of liposomes in the presence (Lipo plasma 1&2) and absence (Lipo 1&2) of rat plasma.

standards used here (355/217=1.37). Thus, it is not surprising that DLS could not accurately measure the size distribution of the mixture. DLS measures the properties of whole populations of particles whereas SIOS measures a large number of individual particles. In order to obtain a good comparison, a mixture with 1:1 particle number ratio was used. The results show that the counting rates of the CPS217 and the CPS355 were 357 and 350 particles/min respectively. Therefore, SIOS not only accurately measures particle size of a polydisperse sample but also can be used to determine the ratio of particle concentrations of the two particle populations. It has been shown that the ability of DLS to resolve bimodal samples is not only limited by size ratio but also by number ratio of the two subpopulations (21). Either a low or high number ratio will lead to failure in resolving the 2 subpopulations. However the accuracy of SIOS was adversely affected across the full range of number ratios (5:95–95:5) of CPS217 and CPS355 particles (Fig. 5 and Table V). In addition, Fig. 6 shows SIOS successfully resolved a trimodal sample (a mixture of CPS118, CPS217 and CPS355 with number ratio of 1:1:1). SIOS has demonstrated a superior resolution compared with single angle DLS. Multiple-angle DLS would be required to improve the discrimination by DLS. The ability to resolve more complex mixes will depend on the polydispersities of the populations of particles and on the differences between the population modes. Success will depend not just on the technical aspects of SIOS but on the statistical treatment of the data.

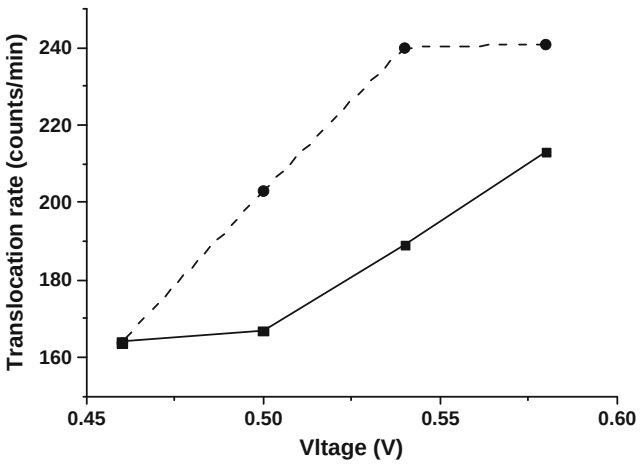

Fig. 8 Translocation rate of liposomes in the presence (red) and absence (black) of rat plasma under electrophoresis conditions with different voltages.

Liposome/Plasma Interaction

The interaction of nanoparticles with plasma proteins is believed to influence their *in vivo* behavior and determine the fate of nanoparticles (22). Understanding the interaction between nanoparticles and plasma protein is a fundamental requirement for developing a nanoparticulate drug delivery system. Therefore SIOS was used to investigate the size change of liposomes in plasma. Pure plasma analyzed by SIOS produced blockade events; these may be derived from microvesicles or large subcellular complexes in the plasma. The presence of these background events prevented the analysis of liposomes in pure plasma. However, pre-incubated liposomes with a small aliquot of plasma followed by dilution of the sample into Ringer's buffer could be analyzed. At this dilution, the contribution of plasma components to blockade rate was negligible. After incubation with plasma, the size of liposomes significantly ($P<0.05$) increased by 10 nm (Fig. 7). These results suggest that plasma proteins are binding to the liposomes. Furthermore, the translocation rates of liposomes incubated with rat plasma were higher than those of liposomes without rat plasma when using SIOS without application of pressure (Fig. 8). This result supports the proposal that the liposomes are rendered more negatively charged due to plasma protein binding to the liposome.

The nanopore is made of polyurethane which is considered to be inert. Several different nanoparticles including polystyrene nanoparticles (10,18), silica nanoparticles (10), DNA-coated nanoparticles (12), Baculovirus occlusion bodies (18), marine cyanobacterium Prochlorococcus (18), adenovirus (11), exosomes (not published results) and now liposomes (this paper) have been studied using SIOS without any incompatibility problem. However, the compatibility should be studied case by case.

CONCLUSION

SIOS is able to measure particle size and size distribution of nanoparticles with good accuracy, precision and sensitivity. It demonstrates excellent ability to resolve bimodal sample

across a wide range of number ratios and detect minor changes in the nanoparticle size. SIOS is a useful complementary technique to DLS for nanoparticle characterization.

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REFERENCES

1. Bootz A, Vogel V, Schubert D, Kreuter J. Comparison of scanning electron microscopy, dynamic light scattering and analytical ultracentrifugation for the sizing of poly(butyl cyanoacrylate) nanoparticles. Eur J Pharm Biopharm. 2004;57:369–75.
2. Krauel K, Girvan L, Hook S, Rades T. Characterisation of colloidal drug delivery systems from the naked eye to Cryo-FESEM. Micron. 2007;38:796–803.
3. Husain A, Hamielec AE, Vlachopoulos J. Particle size analysis using size exclusion chromatography. Size exclusion chromatography (GPC). Am Chem Soc. 1980;138:47–75.
4. Xu X, Caswell KK, Tucker E, Kabisatpathy S, Brodhacker KL, Scrivens WA. Size and shape separation of gold nanoparticles with preparative gel electrophoresis. J Chromatogr A. 2007;1167:35–41.
5. Liu L. Application of ultrasound spectroscopy for nanoparticle sizing in high concentration suspensions: A factor analysis on the effects of concentration and frequency. Chem Eng Sci. 2009;64:5036–42.
6. Filipe V, Hawe A, Jiskoot W. Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. Pharm Res. 2010;27:796–810.
7. Henriquez RR, Ito T, Sun L, Crooks RM. The resurgence of Coulter counting for analyzing nanoscale objects. Analyst. 2004;129:478–82.
8. Sowerby SJ, Broom MF, Petersen GB. Dynamically resizable nanometre-scale apertures for molecular sensing. Sensors Actuators B Chem. 2007;123:325–30.
9. Willmott GR, Moore PW. Reversible mechanical actuation of elastomeric nanopores. Nanotechnology. 2008;19:475504.
10. Willmott GR, Vogel R, Yu SSC, Groenewegen LG, Roberts GS, Kozak D, *et al.* Use of tunable nanopore blockade rates to investigate colloidal dispersions. J Phys.: Condens Matter. 2010;22:454116.
11. Vogel R, Willmott G, Kozak D, Roberts GS, Anderson W, Groenewegen L, *et al.* Quantitative sizing of nano/microparticles with a tunable elastomeric pore sensor. Anal Chem. 2011; 83:3499–3506.
12. Roberts GS, Kozak D, Anderson W, Broom MF, Vogel R, Trau M. Tunable nano/micropores for particle detection and discrimination: Scanning ion occlusion spectroscopy. Small. 2010; 6:2653–8.
13. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discovery. 2005;4:145–60.
14. Kozak D, Anderson W, Vogel R, Trau M. Advances in resistive pulse sensors: devices bridging the void between molecular and microscopic detection. Nano Today. 2011;6:531–45.
15. Mayer LD, Hope MJ, Cullis PR. Vesicles of variable sizes produced by a rapid extrusion procedure. BBA - Biomembranes. 1986;858:161–8.
16. Hunter DG, Frisken BJ. Effect of extrusion pressure and lipid properties on the size and polydispersity of lipid vesicles. Biophys J. 1998;74:2996–3002.
17. Shekunov BY, Chattopadhyay P, Tong HHY, Chow AHL. Particle size analysis in pharmaceutics: principles, methods and applications. Pharm Res. 2007;24:203–27.
18. Roberts GS, Yu S, Zeng Q, Chan LCL, Anderson W, Colby AH, *et al.* Tunable pores for measuring concentrations of synthetic and biological nanoparticle dispersions. Biosens Bioelectron. 2012;31:17–25.
19. Epstein H, Afergan E, Moise T, Richter Y, Rudich Y, Golomb G. Number-concentration of nanoparticles in liposomal and polymeric multiparticulate preparations: empirical and calculation methods. Biomaterials. 2006;27:651–9.
20. Tscharnuter W. Photon correlation spectroscopy in particle sizing. In: Meyers RA, editor. Encyclopedia of analytical chemistry. Chichester: John Wiley & Sons Ltd; 2000. p. 5469–85.
21. Frantzen CB, Ingebrigtsen L, Skar M, Brandl M. Assessing the accuracy of routine photon correlation spectroscopy analysis of heterogeneous size distributions. AAPS PharmSciTech. 2003; 4:62–70.
22. Karmali PP, Simberg D. Interactions of nanoparticles with plasma proteins: Implication on clearance and toxicity of drug delivery systems. Expert Opinion on Drug Delivery. 2011;8:343–57.