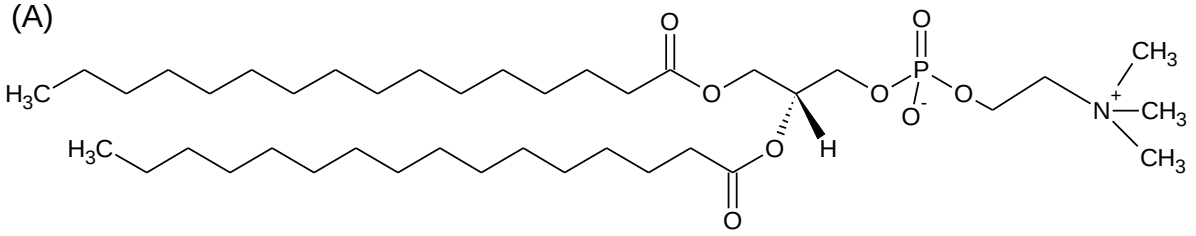
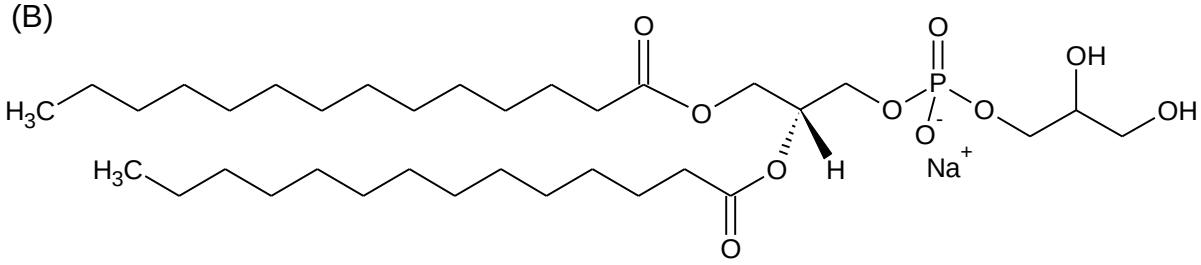
Supplementary Material

Structures of phospholipids, cyclodextrin and drug used for liposome preparation (A)1, 2-dipalmitoyl-sn-glycero-3-Phosphocholine, (DPPC) (B) 1, 2-dimyristoyl-sn-glycero-3-(Phospho-rac-(1-glycerol), (DMPG) (C) 2-Hydroxypropyl-γ-cyclodextrin (D) Curcumin - 1, 7-Bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione.

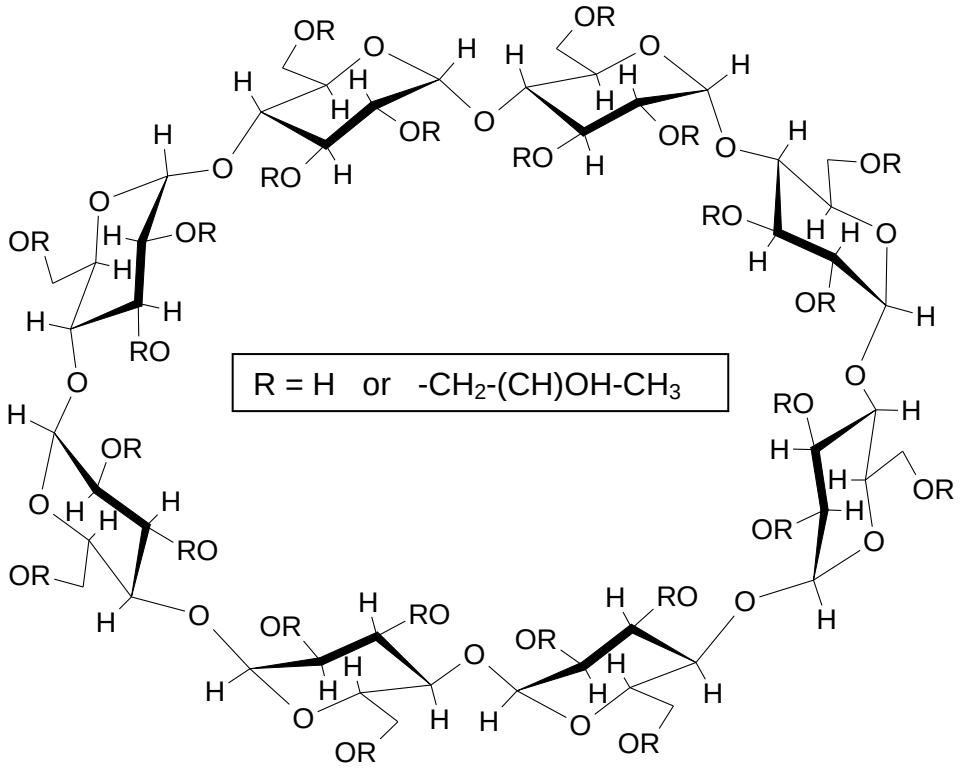
(A)



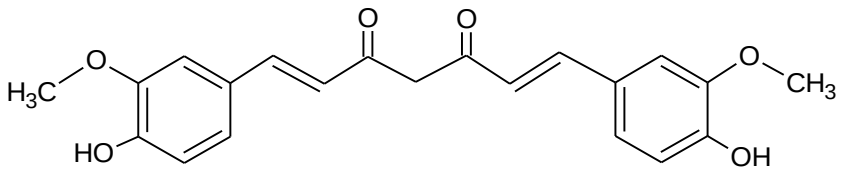
(B)



(C)



(D)



Analysis of DLS and cryo-TEM data in the present study

Table 1. Size distribution of liposomal nanoparticles using DLS and Cryo-TEM in present study.

Formulation	DLS Data			Cryo-TEM Data		
	Diameter (nm)	Half Width (SD)	SE	Diameter (nm)	SD	SE
Conventional curcumin liposomes	104.7	23.1	0.9	71	20	1.38
HPγCD-curcumin liposomes	98.2	30.1	0.7	67	16	2.35

$$Standard\ Deviation = SD = \ \sigma = \sqrt{\frac{\sum_{i=1}^{i=N}(Y_i - \mu)^2}{N}}$$

$$Standard\ Error\ of\ the\ Mean = SE = \frac{Standard\ Deviation}{\sqrt{N}}$$

N – Number of data points

Yi – individual value

μ - Population mean

DLS measures the effective diffusion coefficients of colloidal particles and relates the diffusivity to the particle diameter through the Stokes Einstein equation.

$$D = \frac{k_B T}{6\pi\eta r}$$

D - Diffusion coefficient, k_B – Boltzmann's constant, T- Absolute temperature, η - viscosity, r – radius of the spherical particle

DLS offers the intensity average based diameter of the particles measured, related to the diffusion coefficient by Stokes Einstein relation for hydrodynamic radius derived from the intensity autocorrelation function (Bern et al. 1976), expanded in terms of cumulants (Koppel 1972). The size distribution provided by analysis of the measurements is merely a qualitative description of the population distribution in the sample, since it is largely model dependent. Moreover, if the solution to be measured contains aggregates even in the smallest percent, or a smaller population of large particles, these dominate the scattering signal; hence one has to be cautious in interpreting data. This is clearly seen in an example of HPγCD-curcumin liposomes below, where multi-size distribution (MSD) is shown based on particle number and by intensity. A small number of particles mean particles having small population as shown by arrow in Figure 1A. A small number of particles in HPγCD-curcumin liposomes have a high scattering signal/intensity (shown in Figure 1B by arrow), with a significant effect on the averaged particle size.

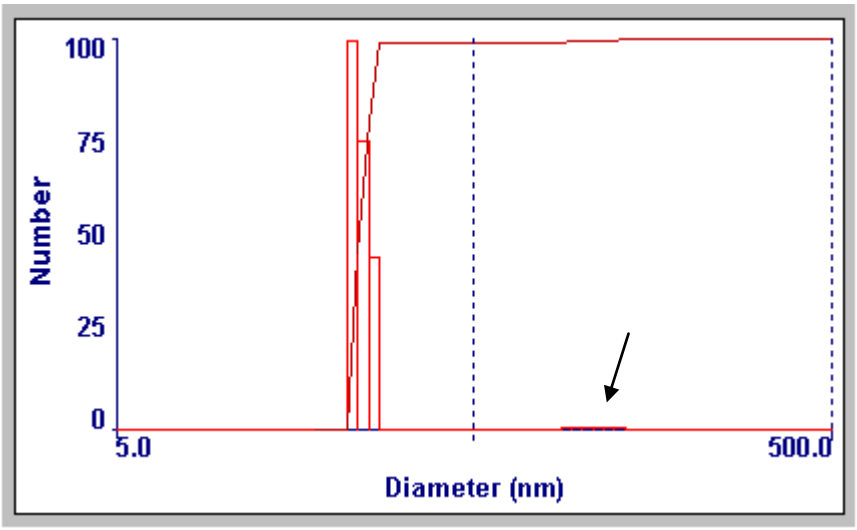


Figure 1A. MSD data for HPγCD-curcumin liposomes based on particle number

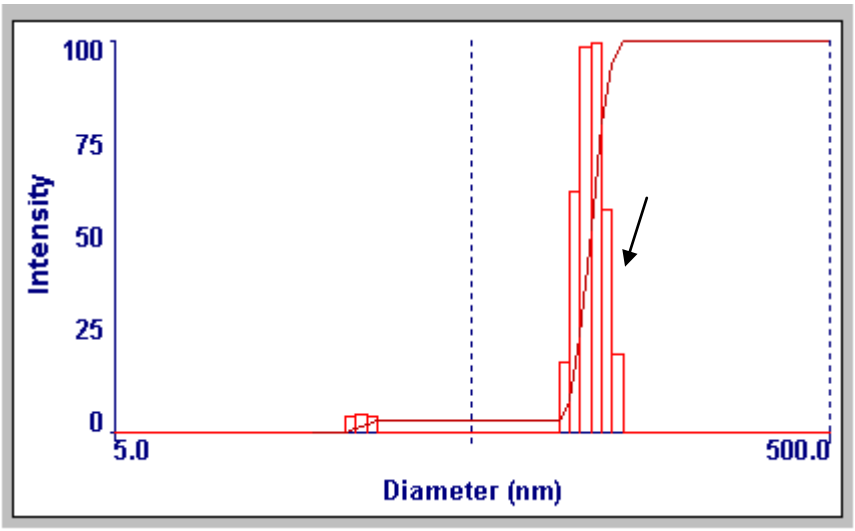


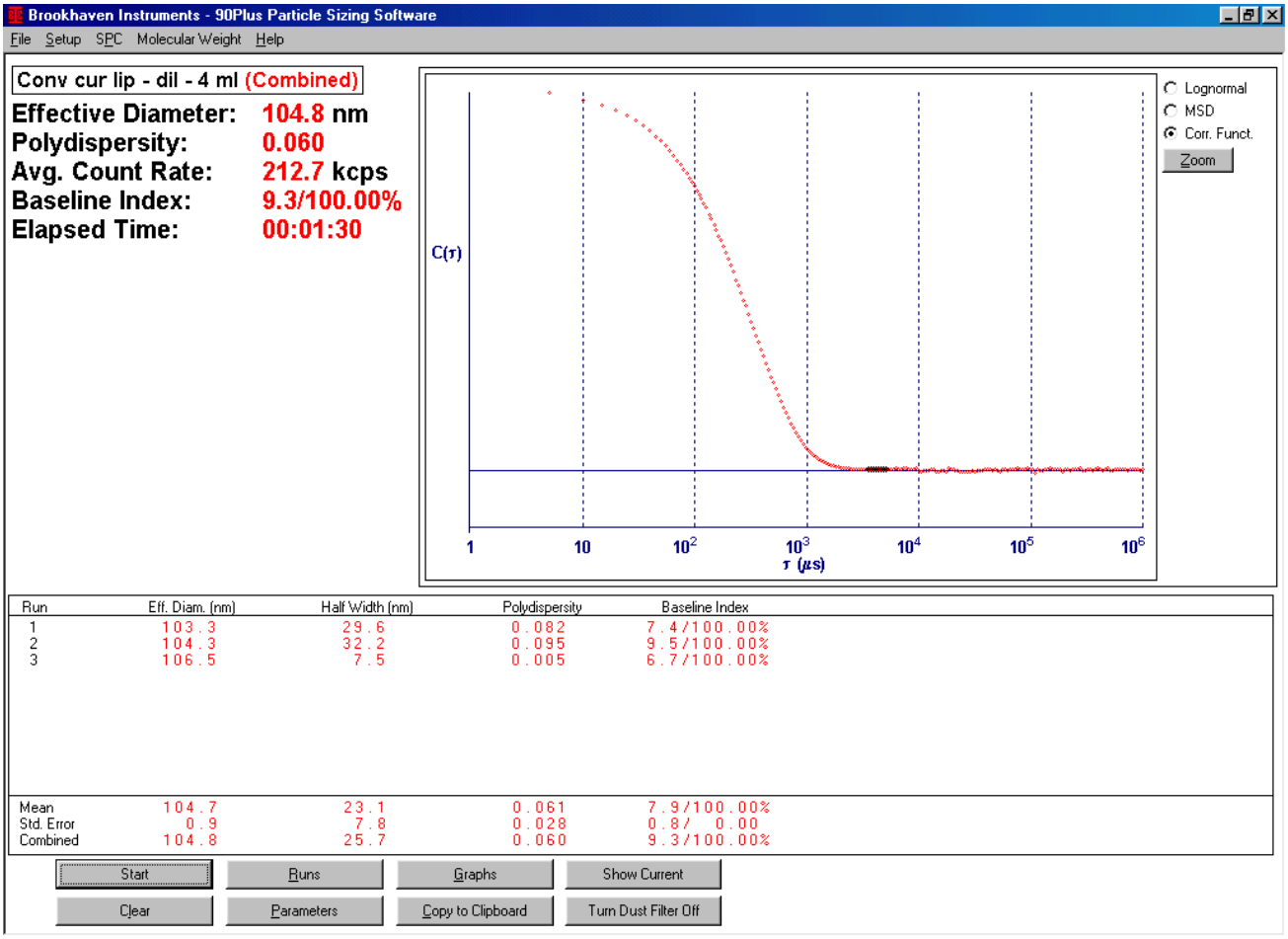
Figure 1B. MSD data for HPγCD-curcumin liposomes based on intensity

The effective diameter from DLS is an average diameter weighted by the intensity of light scattered by each particle. This intensity weighting is not the same as the population or number weighting used in a single particle counter such as in electron microscopy. We are extremely confident in the Cryo-TEM results on our system because it is a direct method. It has to be done carefully to avoid artifacts, but when done carefully Cryo-TEM can give information that reveals

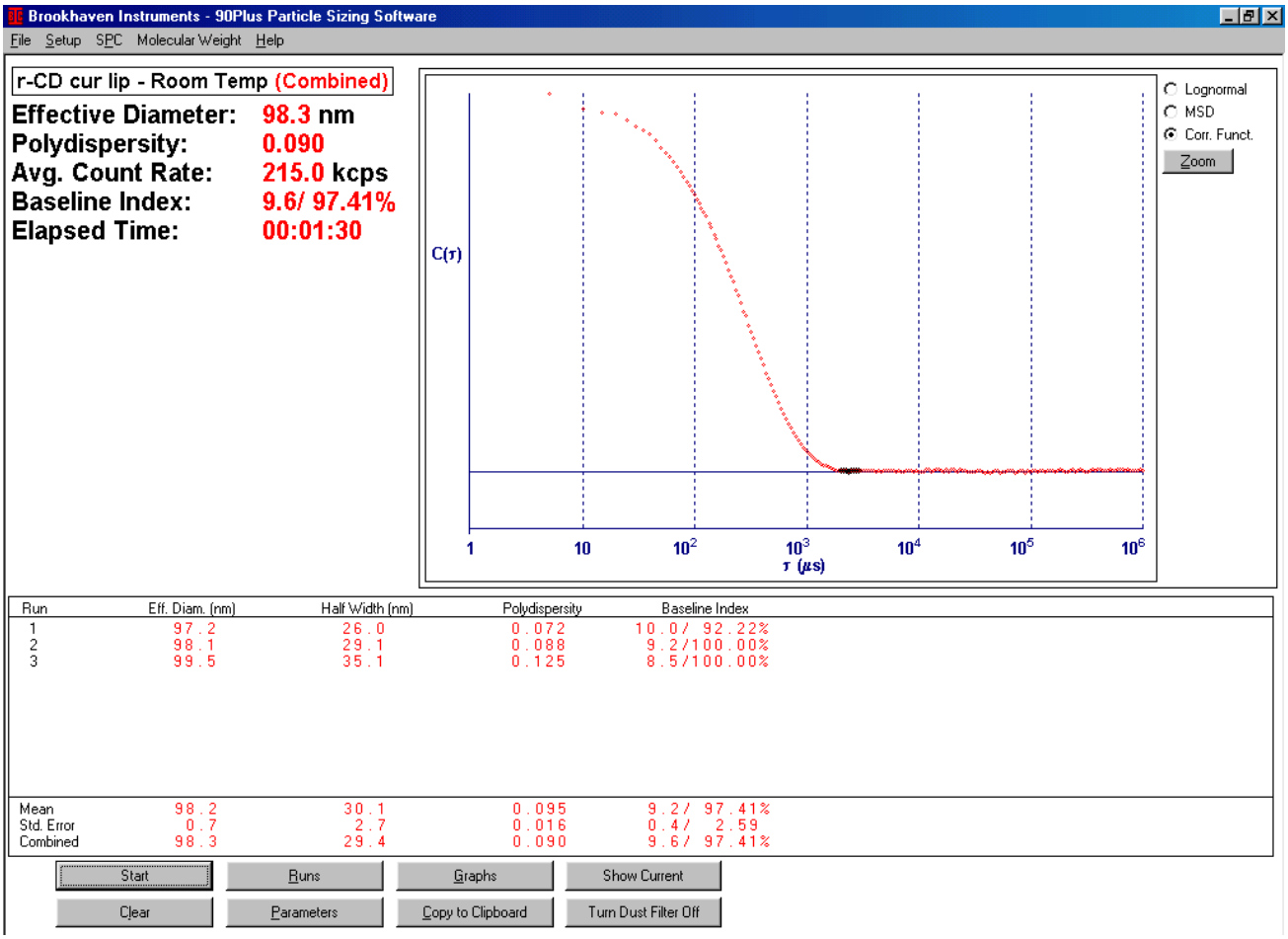
not just the size and size distribution of liposomes but also shape effects due to incorporation of other components in the liposome bilayer. In recent work from the co-authors laboratory (Tan et al. 2009), we have shown for example the formation of highly tubular liposomes upon ceramide incorporation into the bilayer.

Additional data showing effective mean diameter and standard error

Conventional Curcumin Liposomes



HPγCD-Curcumin liposomes



The conflicting information provided by DLS and cryo-TEM has also been mentioned in the recent literature (Crawford et al. 2011, Holzer et al. 2009).

References:

- Berne, B. and Pecora R. Dynamic Light Scattering, John Wiley & Sons, N.Y. 1976

- Koppel, DE. *J. Chem. Phys.* (1972) *11*, 4814-4823.
- Tan G, Xu P, He J, Lawson L, McPherson G and John V. Highly aspherical silica nanoshells by templating tubular liposomes. *Soft Matter* (2009), 5, 3006-3009.
- Crawford R, Dogdas B, Keough E, Haas RM, Wepukhulu W, Krotzer S, Burke PA, Sepp-Lorenzino L, Bagchi A, Howell BJ. Analysis of lipid nanoparticles by Cryo-EM for characterizing siRNA delivery vehicles. *Int J Pharm* (2011), 403(1-2), 237-244.
- Holzer M, Barnert S, Momm J, Schubert R. Preparative size exclusion chromatography combined with detergent removal as a versatile tool to prepare unilamellar and spherical liposomes of highly uniform size distribution. *J Chromatography A* (2009), 1216, 5838-5848.