The use of surfactants to enhance the solubility and stability of the water-insoluble anticancer drug SN38 into liquid crystalline phase nanoparticles

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Graphical abstract

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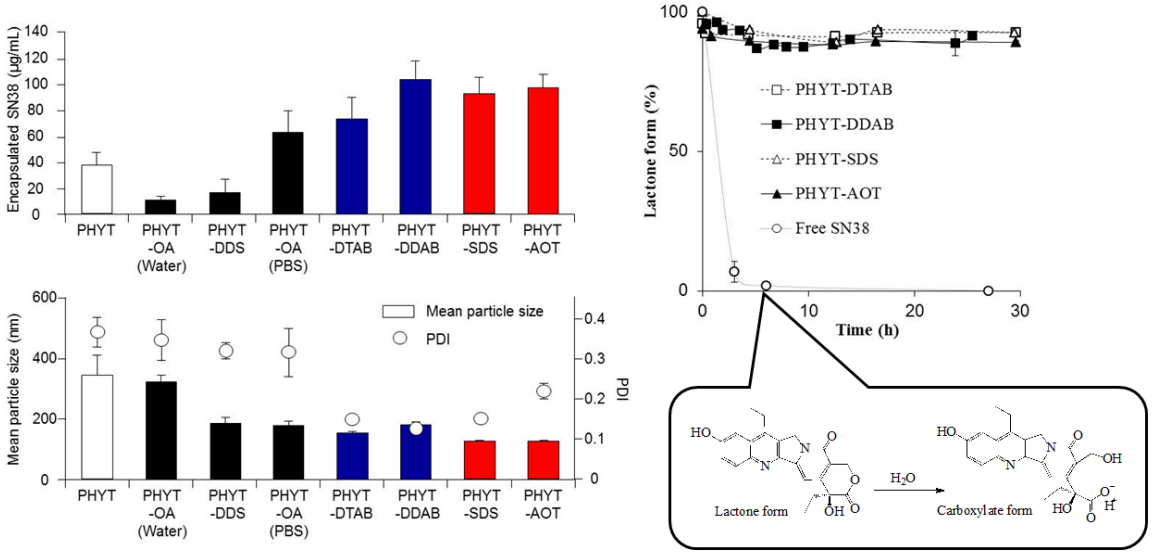

Abstract

Cubosomes were used to increase the aqueous solubility of the water insoluble anticancer drug SN38. The results showed that the use of a common cubosome formulation consisting of phytantriol (PHYT) as the matrix amphiphile (PHYT-cubosome) led to a 6-fold increase in the solubility of SN38. However, mean hydrodynamic diameter (D_H) and polydispersity index (PDI) of these PHYT-cubosome particles were 345±49 nm and 0.37±0.05, respectively, making them unsuitable for intravenous applications. Several additives were investigated to increase the solubility of SN38 and reduce the D_H and PDI values of the resulting particles. Charged additives such as didodecyldimethyl ammonium bromide (DDAB) and sodium dodecyl sulfate (SDS) led to improvements in the physiochemical properties of the cubosomes. Notably, the PHYT-DDAB and PHT-SDS cubosomes led to 15- and 14-fold increases in the aqueous solubility of SN38, respectively. Moreover, the SN38 loaded into the PHYT-DDAB and PHYT-SDS cubosomes was found to be highly stable, with very little hydrolysis to its inactive acid form. In summary, the addition of DDAB and SDS to PHYT-cubosome nanoparticle drug delivery systems not only led to considerable improvements in their physiochemical properties, but also enhanced the aqueous solubility of SN38 and increased its chemical stability.

Keywords: cubosomes; poorly water-soluble drug; SN38; hydrolysis; chemical stability

Solubility is one of the most important factors affecting the absorption and biodistribution properties of drugs. Poor aqueous solubility therefore represents a considerable limitation during the development of therapeutic agents for oral or intravenous administration, because water insoluble drugs struggle to achieve the concentrations required to elicit therapeutic efficacy. Recent developments in the field of nano-ordered drug delivery systems have allowed for the successful encapsulation of a wide variety of anticancer drugs, leading to considerable improvements in their targeting efficiency. These systems have also led to a dramatic reduction in the side effects of these drugs (Murakami et al., 2011) by increasing their solubility (Funakoshi et al., 2013; Barman et al., 2014).

Cubosomes are lyotropic liquid crystalline phase nanoparticles that can be prepared from amphiphiles, such as phytantriol (PHYT), which provide an inner structure composed of lipid bilayers arranged in a contorted pattern around adjacent water channels. This structure allows the lipid bilayer to act as a matrix making the particles like a sponges. The properties of these systems make them ideal candidates for the loading of highly hydrophobic drugs such as 7-ethyl-10-hydroxycamptothecin (SN38), which has a log*P* value of 3.37 (Thakur et al., 2010) (**Fig. 1**). SN38, which is formed as an active metabolite of irinotecan (Zhang et al., 2004), is a potent anticancer drug with an IC_{50} value of less than of 4 nM (Mariani et al., 2012). However, the clinical application of SN38 has been limited by its poor aqueous solubility (7 μg/mL) and poor stability, with its lactone ring being prone to hydrolysis under physiological conditions to give the corresponding carboxylate, which is inactive (Zhang et al., 2004). A recent successful attempt in increasing the solubility of SN38 was reported using cationic materials with mesoporous silica (Bala et al., 2016). Here, we envisioned that the use of additional excipients with either cationic or anionic hydrophilic head, that have similar structural characteristics to PHYT would allow us to increase the loading of SN38 into cubosome nanoparticles and overcome both of those problems.

Firstly, we tried to load SN38, which was gifted from Yakult (Tokyo, Japan), into a conventional cubosome formulation consisting of PHYT (Tokyo Kasei chemicals, Tokyo, Japan) and Pluronic F127® (F127, BASF, Tokyo, Japan), which was used as a steric stabilizer. SN38 was dissolved in a solution of 50 mg of PHYT and 5 mg of F127 at 60–70 °C. One thousand, seven hundred and fifty microliters of water was then added to the SN-38 solution, and the resulting mixture was probe sonicated in pulse mode for 2 min. Two hundred microliters of 10-times concentrated phosphate buffered saline (PBS) was added to the sonicated solution, and the resulting mixture was sonicated for 0.5 min at 25 °C. The final PBS solution contained NaCl, KCl, Na_2HPO_4 and KH_2PO_4 with the following concentrations, 137, 2.7, 10 and 1.8 mM, respectively. The mixture was centrifuged at 1000 ×*g* for 3 min at 25 °C to remove any unencapsulated drug agglomerates or metal debris. The supernatant was collected and vortexed for 10 min at 2500 rpm.

The concentration of SN38 in the PHYT cubosomes was measured using a Shimadzu LC-2010C HT® HPLC system with a mobile phase consisting of a 1:1 (v/v) mixture of acetonitrile and an aqueous solution of 25 mM Na_2HPO_4 at a pH of 7.4 (Xuan et al., 2006). The results revealed that the use of PHYT led to a 6-fold increase in the aqueous solubility of SN38 (39±8 µg/mL) (**Fig.2A**). The mean hydrodynamic diameter (D_H) and poly dispersity index (PDI) were determined by dynamic light scattering (Zetasizer Nano ZS®, Malvern, Worcestershire, UK). The results revealed that the suspension was polydispersed with PDI and D_H values of 0.37±0.05 and 345±49 nm, respectively (**Fig.2B**), indicating that this formulation would be unsuitable for intravenous application because of the potential for enhanced permeability and retention effects (Matsumura and Maeda, 1986).

To further increase the aqueous solubility of SN38 and improve the physiochemical properties of the particles, we investigated the effects of adding a variety of different additives to the initial SN38-PHYT mixture. A PHYT/additive molar ratio of (1:13) was used in these experiments (Muir et al.,

2012). Several additives were evaluated, including didodecyl sulfoxide (DDS), dodecyltrimethyl ammonium bromide (DTAB) and dioctyl sodium sulfosuccinate (AOT), which were obtained from Tokyo Kasei Chemicals (Tokyo, Japan). Oleic acid (OA) and didodecylammonium bromide (DDAB) were purchased from Sigma Aldrich (Tokyo, Japan), and sodium dodecyl sulfoxide (SDS) was obtained from Wako chemicals (Osaka, Japan).

Both PHYT-OA (water), prepared in deionized water, and PHYT-DDS were used as neutral additive-based formulations. It has been reported that OA exists in its un-ionized form when it is used as an additive in similar liquid crystalline systems. It was therefore envisioned that OA would exist as a charge-neutral species under salt-free conditions, with the ability to become anionic under more basic conditions through deprotonation (Salentinig et al., 2010). Disappointingly, however, both of these formulations led to negligible increases in the solubility of SN38, with PHYT-OA (water) and PHYT-DDS leading to 1.5-fold (11±3 μg/mL) and 3-fold (18±9 μg/mL) increases, respectively. Moreover, these formulations were polydispersed with PDI values of 0.42±0.05 and 0.33±0.02 for PHYT-OA (water) and PHYT-DDS, respectively. Based on the ionization of OA, we subsequently investigated the effect of the nature of the additive head on the properties of the system. Interestingly, we observed a 9-fold increase in the solubility of SN38 (62±16 μg/mL) in PHYT-OA formulation prepared in PBS, with no noticeable difference in polydispersity properties of the suspension, which gave a PDI value of 0.35±0.07. This result therefore highlighted the importance of having charged additives for the loading of SN38 into these particles. To further evaluate this hypothesis, we investigated several other charged additives.

The use of cationic additive-based formulations led a considerable increase in the solubility of SN38, as exemplified by PHYT-DTAB and PHYT-DDAB formulations, which resulted in 10-fold (74±17 μg/mL) and 15-fold (104±14 μg/mL) increases in the solubility, respectively. The resulting suspensions were monodispersed with mean particle sizes of less than 200 nm, making them suitable for future *in-vivo* applications. Furthermore, both PHYT-DTAB and PHYT-DDAB

formulations had PDI values of 0.15±0.01 and 0.13±0.02, respectively. Anionic additive-based formulations showed similar trends to their cationic counterparts in terms of their ability to enhance the solubility of SN38, with PHYT-SDS and PHYT-AOT formulations leading to 13-fold (93±13 µg/mL) and 14-fold (98±10 µg/mL) increases in the solubility. These formulations were also monodispersed with PDI values of 0.15±0.01 and 0.22±0.02 for the PHYT-SDS and PHYT-AOT particles, respectively. Furthermore, the mean particle sizes of the PHYT-SDS and PHYT-AOT particles were 125±2 and 123±11 nm, respectively. The charged additive-based systems showed higher SN38 loadings than the neutral systems, leading to higher aqueous solubility, regardless of the differences in the number of hydrophobic tails or the chemical structures of the tails. These results suggested that an interaction could have taken place in the lipid mixture prior to the addition of an aqueous solution or the sonication of the mixture. This interaction is supposedly manifested by the charged head of the surfactant electrostatically interacting with transient charges on the SN38 structure, such as the pyridine nitrogen or the phenol moiety, thereby facilitating the loading of the SN38 molecules into the lipid bilayer of the cubic phase. Based on the SN38 loading, PDI and mean particle size characteristics of these systems, we selected the PHYT-DTAB, PHYT-DDAB, PHYT-SDS and PHYT-AOT formulations for further studies.

To gain a better insight into these systems, we evaluated their inner structures using small angle X-ray scattering (SAXS) analysis (NANO-Viewer system, Rigaku, Tokyo, Japan). These data allowed us to determine the space groups of these formulations based on the reciprocal spacing ratios of their three peaks, as mentioned elsewhere (Nakano et al., 2002). The cell parameter a (Å) was calculated as follows: $a = \frac{\sqrt{h^2+k^2+l^2}}{Q*10}$

where Q is the norm of the scattering vector (nm^{-1}), and h, k and l are the Miller indices. Also, the channel size was calculated as mentioned elsewhere (Negrini et al., 2012) (**Table.1**). The details of all the formulations prepared in

the current study are shown in (**Fig.3A**). All of the formulations had the $Pn\bar{3}m$ cubic space group with peaks corresponding to miller indices of (110), (111), (200) and (211). The PHYT-SDS formulation had an extra set of peaks with reciprocals, indicating the presence of another space groups. Similar to the other formulations, the first of these groups was determined to be the cubic space group $Pn\bar{3}m$ with peaks corresponding to miller indices of (110), (111) and (211). The second set of peaks corresponded to miller indices of (110), (200) and (211). The absence a peak corresponding to (111) was attributed to the emergence of the $Im\bar{3}m$ cubic space group. SAXS data concluded that the particles in these formulations were cubosomes.

The second major issue concerning the use of SN38 is the susceptibility of its lactone ring to hydrolysis under physiological conditions (pH 7.4), diminishing the pharmacological activity of SN38 (Zhang et al., 2005). Under the chromatographic conditions described above, we observed two distinct peaks with retention times of 1.4 (carboxylate form) and 2.2 min (lactone form), for samples composed of a 2:1 (v/v) mixture of acetonitrile and PBS (pH 7.4). Free SN38 (i.e., SN38 not loaded into a cubosome formulation) was rapidly hydrolyzed under these conditions to give the inactive carboxylate form almost completely after 24 h. Furthermore, we only observed a small amount of lactone by HPLC analysis (6.7% by peak area) after 3 h, with less than 0.1% remaining after 24 h (**Fig. 3B**). However, SN38 showed much greater chemical stability when it was loaded into a variety of cubosome formulations, as exemplified by the PHYT-DTAB, PHYT-DDAB, PHYT-SDS and PHYT-AOT cubosomes, which gave peak areas of 92.8, 91.7, 92.8 and 89.5% for the active lactone form after 26 h, respectively. These results suggested that the lactone ring of SN38 was integrated into the cubic bilayer of the cubosome structure in these formulations, making it much less susceptible to being attacked by water molecules. This kind of interaction would most likely take place in the lipid mixture after the sonication and self-assembly of the lipid bilayer. These results therefore provide clear evidence that the loading of SN38 into cubosomes led to a considerable

196 increase in the chemical stability of this drug.

197 In conclusion, this study has demonstrated that the addition of charged
198 additives to PHYT-cubosomes loaded with the hydrophobic drug SN38 led to
199 considerable increases in the solubility of this system. Furthermore, the loading
200 of SN38 into these cubosome systems led to a dramatic increase in the
201 chemically stability of SN38 against hydrolysis at biological pH. The *in vivo*
202 evaluation of these systems in mouse models of human cancer would be of
203 considerable interest, since the cell membrane disrupting ability of PHYT- and
204 F127-based systems (Zhai et al., 2015) would show synergy effect with SN38 in
205 eliminating cancer cells.

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Table captions

Table 1. Inner structure data of chosen formulations. The properties of the inner structures were determined using small angle X-ray scattering

Formulation	Space group	Cell parameter, *a* (Å)	Channel diameter (Å)
PHYT	$Pn\bar{3}m$	65.20 ± 0.10	20.99 ±0.03
PHYT-DTAB	$Pn\bar{3}m$	78.84 ± 0.04	31.65 ±0.02
PHYT-DDAB	$Pn\bar{3}m$	74.80 ± 0.15	28.49 ±0.06
PHYT-SDS	$Im\bar{3}m$	104.97 ± 0.05	34.03 ±0.02
	$Pn\bar{3}m$	80.25 ± 0.16	32.76 ±0.07
PHYT-AOT	$Pn\bar{3}m$	70.90 ± 0.04	25.44 ±0.01

Figure captions

Figure 1. Chemical structures of the compounds used in this research.

Figure 2. Properties of the formulations prepared using phytantriol and several different additives. The solubility of SN38 in the different formulations was measured by HPLC (a). The D_H and PDI results for the prepared formulations were measured by DLS (b). Each point represents the mean ± S.D (n=3). Samples indicated with (*) showed statistically significant differences, p<0.05 in t-test, for both of the vertical axes. Samples indicated with (#) showed statistically significant differences, p<0.05 in t-test, for PDI only.

Figure 3. Inner structure information and stability of the lactone form of SN38. The inner structure data of chosen formulations with peaks showing corresponding miller indices and space group was obtained using SAXS (a). The hydrolysis of the lactone from of SN38 in PBS (pH 7.4) (b). The dotted line represents free SN38 without any cubosomes. The other lines represent SN38 encapsulated in cubosomes formulations with different additives, as indicated. Each point represents the mean ± S.D. (n=3). Schematic showing SN38 dispositioning within the lipid bilayer of one of the cubosomes formulations (PHYT-SN38) where it is protected against hydrolysis.

Fig. 1

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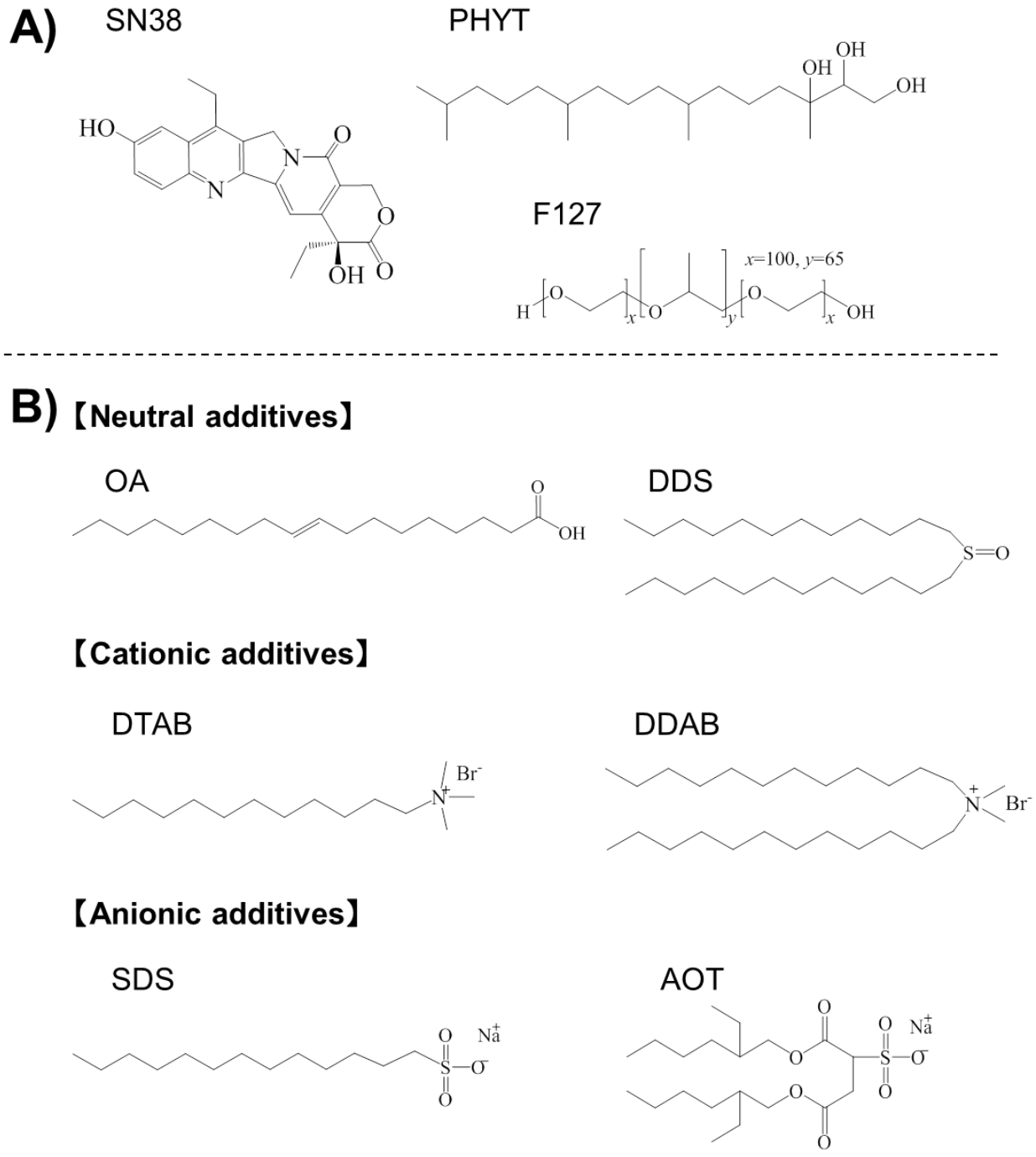

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Fig. 2 **Ranneh *et al***

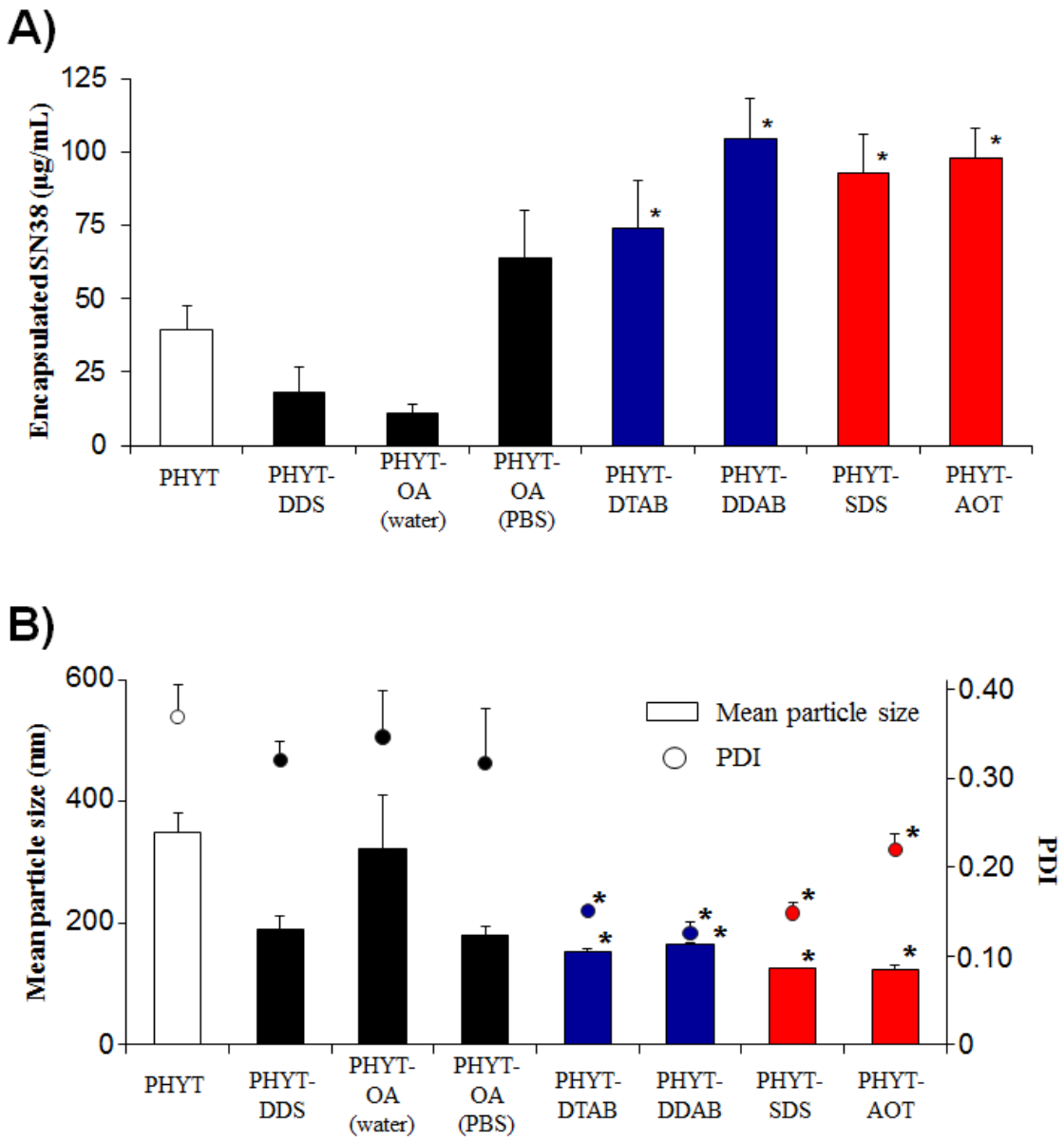

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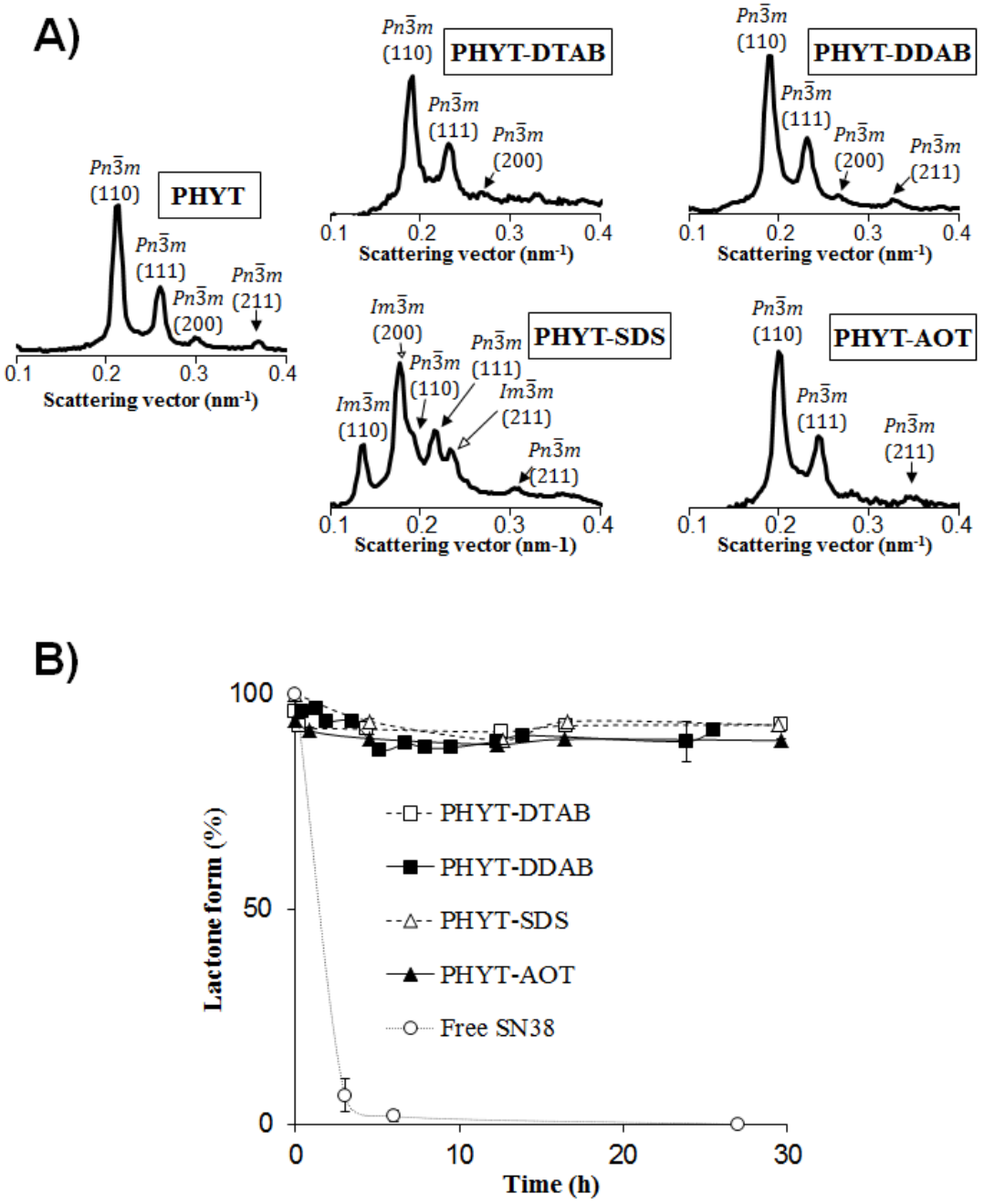
Fig. 3 **Ranneh *et al***



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