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Original Article

Effect of sample Concentration on the Characterization of Liposomes using Dynamic light Scattering Technique

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ABSTRACT

Objective: The objective of the study was to assess the effect of liposomal concentration on the characterization of liposome using dynamic light scattering technique. **Method:** Liposome of a water soluble active, Niacinamide, was taken for the study. Phospholipid combination (INCI name lecithin and Propane diol) was used to form liposomes of Niacinamide. Concentrated liposomal suspension was prepared using various ratios of phospholipid. The concentrated liposomal suspension was diluted five times using distilled water. Concentrated liposomal suspension and diluted suspension were characterized for particle size, PDI, conductivity and Zeta potential. Their surface morphology was studied with SEM images. **Results:** The data on conductivity, PDI, zeta potential and particle size were significantly different between concentrated and diluted liposomal samples. The use of various phospholipid ratios appears to significantly affect PDI, zeta potential and particle size in concentrated sample. The data on diluted samples indicated that the phosholipid level has no significant effect on these parameters. **Conclusion:** In conclusion, characterizing liposomes using techniques which involves dynamic light scattering, that is widely used, liposome concentration during measurement is important to get reliable results.

Key words: Liposomal dilution, Niacinamide, Polydispersity index, Particle size.

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INTRODUCTION

Liposomes are concentric bi-layered vesicles in which aqueous volume is entirely enclosed by a lipid layer composed of natural or synthetic phospholipids. Various amphipathic molecules have been used to prepare the liposomes, and the method of preparation can be tailored to control their size and morphology. Drug molecules can either be encapsulated in the aqueous space or intercalated into the lipid bilayer; the exact location of a drug in the liposome will depend upon its physicochemical characteristics and the composition of the lipids.[1,2]

Cholesterol may improve the bilayer characteristics of liposomes by increasing the microviscosity of the bilayer, reducing the permeability of the membrane to water-soluble molecules, stabilizing the membrane and increasing the rigidity of the vesicle. Liposomes have been widely used in improving the dermal penetration of hydrophilic drugs. Sino C *et al*[3] have reviewed the value of lipid vesicles in localizing drugs within the skin at the site of action. El Magharab *et al*[4] have reviewed the use of liposomal vesicles in permeation enhancement studies. Ada Pola[5] reviewed the role of liposomes in cosmetics.

Various parameters including particle size, poly dispersity index (PDI) and zeta potential should be monitored in order to assess the liposomes quality and obtaining quantitative measures that allow comparison between different batches and different types of liposomes. *Laouinil et al*[6] have reviewed various techniques used in the characterization of liposomes. The average size and size distribution are assessed by various techniques like microscopy, field flow fractionation, and static or dynamic light scattering.

Dynamic light scattering (DLS) also known as photon correlation spectroscopy (PCS), is extensively used in liposome size distribution analysis. DLS measures the time-dependent fluctuation of light scattered from particles experiencing Brownian motion. DLS is also used to assess zeta potential. Various literatures have demonstrated the characterization techniques of liposomes; however, no details have been published concerning the effect of dilution of the liposome sample.

Niacinamide (Pyridine-3-Carboxamide) is colorless crystal or a white crystalline powder, with faint and characteristic odor. It is freely soluble in water and in ethanol. Niacinamide is used for preventing vitamin-B3 deficiency, pellagra. Niacinamide reported to have skin benefits for the treatment of skin pigmentation, acne, wrinkles. Niacinamide, an active ingredient widely used at 1% to 3% levels, in cosmetics and cosmeceuticals for skin benefits. The dermal penetration of Niacinamide is poor. Liposomal delivery system can enhance dermal delivery of niacinamide and therefore for the present study, liposomes with niacinamide were formulated. Benjamin[7] had filed a patent idea on "Therapeutic Treatment using niacin for skin disorders". Niacin and/or Niacinamide are incorporated with liposomes in therapeutic compositions for topical application to prevent or alleviate the conditions and symptoms of skin aging and other histological disorders. In particular, niacin and/or niacinamides are combined with liposomes to increase the penetration of the niacin and/or niacinamides into the deep epidermis" This is a patent idea and no technical substantiation had been submitted.

In the present study liposomal suspension was formulated using a phospholipid and active niacinamide. Pro-Lipo™ Neo is a ready-to-use mixture of selected phospholipids already organized in lamellar bilayers by their solubilization in an appropriate medium. This pro-liposome's structure requires only the addition of a water phase to spontaneously form, at room temperature, an alcohol free suspension of multilamellar liposomes. This type of liposomes is small enough to present high cutaneous absorption and release the entrapped ingredients while membranes merge with skin one by one.[8]

In the present study, liposomes of niacinamide was formulated using various levels of the phospholipids and data of concentrated and diluted samples of liposomes were characterized and compared.

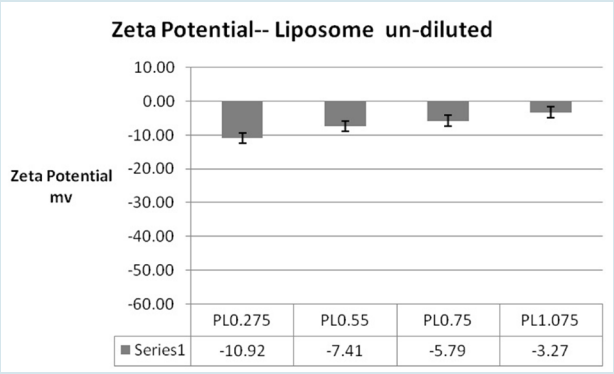

Figure 1: Effect of Lipid Concentration on zeta potential in undiluted samples.

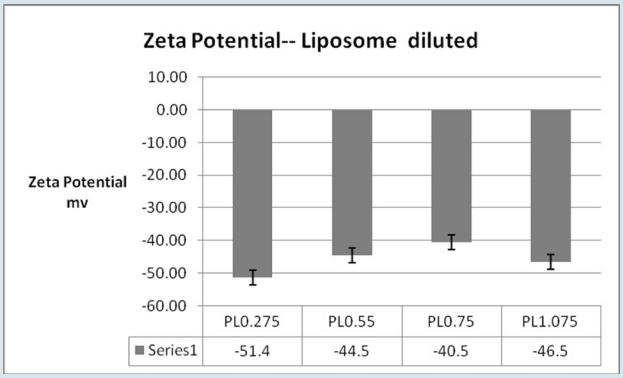

Figure 2: Effect of lipid concentration on zeta potential in diluted samples.

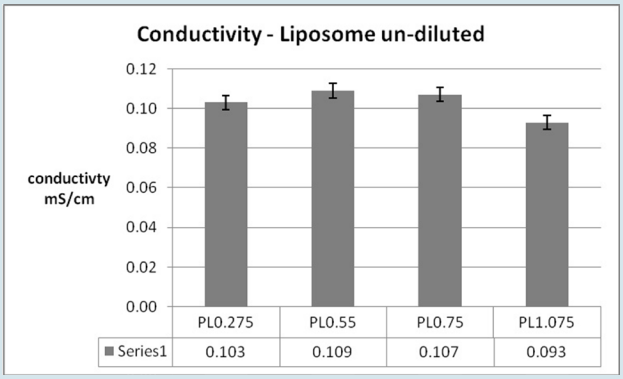

Figure 3: Effect of lipid concentration on conductivity in undiluted samples.

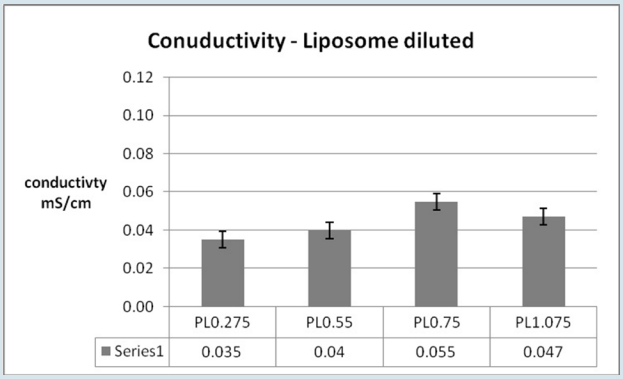

Figure 4: Effect of lipid concentration on conductivity in diluted samples.

MATERIAL AND METHODS

Materials

Pro-Lipo™ Neo (Lecithin and propane diol) was provided by Generex Pharmacist Pvt. Ltd. Mumbai, India/Lucas Meyer Cosmetics, France. Niacinamide was provided as a gift sample by Veer Chemie and Aromatics Pvt. Ltd., Hyderabad, India.

Preparation of Lecithin and propane-diol based liposomal suspension

Niacinamide liposomal suspension was prepared by dispersing solution of niacinamide in distilled water with the phospholipid Pro-Lipo™ Neo. The dispersion was achieved by homogenization at 5000 rpm for 5 min using homogenizer polytron PT 1600 E. Liposome suspension was made with 1 part of niacinamide, 2.5 parts of water and lipid at four different levels 0.275, 0.5, 0.75 1.075. Prepared liposomal suspension was stored in an airtight container under refrigerated conditions for further use.

Characterization of liposomes

The prepared, undiluted and diluted (1:5), liposomal suspension were characterized for Particle size, Zeta Potential, PDI and Conductivity using Malvern Zetasizer (Nano ZS90, Malvern instruments.). Surface morphology was studied using Joel-5400.

RESULTS AND DISCUSSION

Zeta Potential

The Zeta potential of undiluted and diluted samples was significantly different. The zeta potential of undiluted sample was found to be significantly influenced by phospholipid levels used (Figure 1). With the increase of lipid levels from 0.275 to 1.075 the zeta potential increased from -10.92 to -3.27. Whereas, in diluted samples as lipid level was increased from 0.275 to 1.075, zeta potential increased from -51.4 to -40, (Figure 2) but the variation in the case of undiluted samples was not statistically significant. It was observed that the zeta potential increased by 10 times on dilution of the sample.

Conductivity

The conductivity of undiluted and diluted samples was significantly different. However, phospholipid levels had no significant effect on conductivity in both undiluted and diluted samples (Figure 3, 4). There was about 0.5 fold decrease in conductivity in diluted samples compared to undiluted samples.

Particle size

The particle size of undiluted and diluted samples was significantly different. The particle sizes of undiluted sample was found to be signifi-

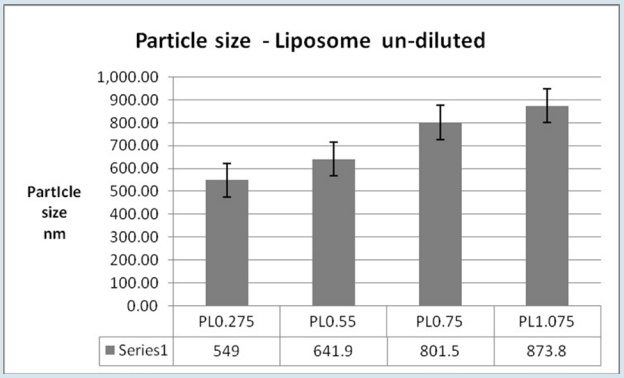

Figure 5: Effect of lipid concentration on particle size in undiluted samples.

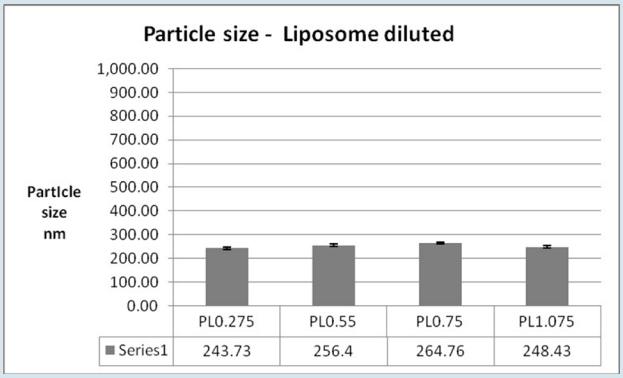

Figure 6: Effect of lipid concentration on particle size in diluted samples.

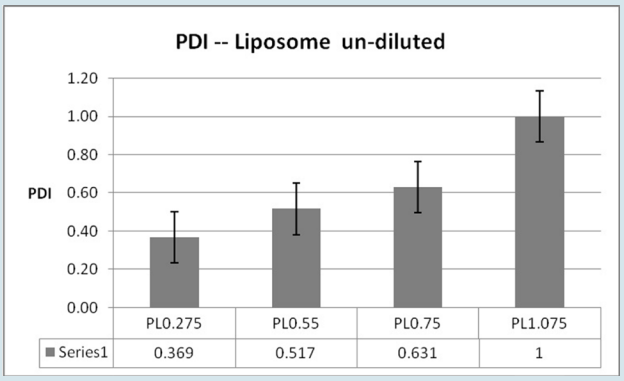

Figure 7: Effect of lipid concentration on PDI in undiluted samples.

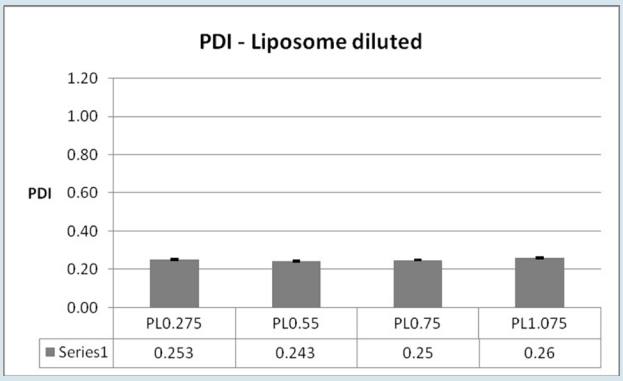

Figure 8: Effect of Lipid Concentration on PDI in diluted samples.

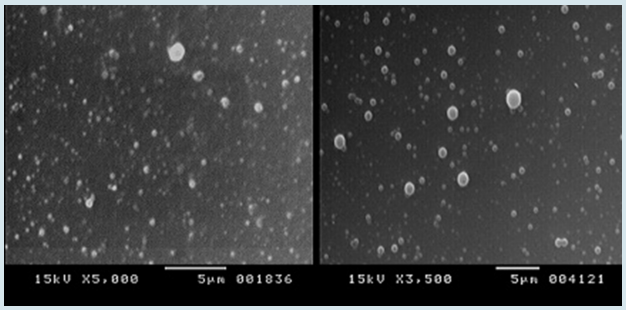

Figure 9: SEM images of A) Undiluted and B) diluted samples.

cantly influenced by the amount of lipid used and ranged from 650 nm to 950 nm with the increase of phospholipid levels from 0.275 to 1.075 (Figure 5). In diluted sample the particle size ranged from 243 nm to 264 nm. Phospholipid levels had no significant effect on particle size of diluted samples (Figure 6).

PDI

The PDI of undiluted sample was found to be significantly increased by 2.5 folds with the increase in lipid levels from 0.275 to 1.075 (Figure 7). The PDI ranged from 0.4 to 1 in undiluted samples. PDI of diluted samples were found to be in a range of 0.25 to 0.27 (Figure 8) and were not significantly influenced by phospholipid levels.

In all the above data it is clearly seen that the liposome in two different concentrations shows significantly different results.

Surface Morphology by SEM

Even though the particles size results of undiluted and diluted sample differ, the particle size and shape of both the samples were found to be similar when observed under SEM microscope (Figure 9). However results obtained through the instrument were significantly different.

DISCUSSION

The results can be explained through the principle of DLS technique. DLS measures Brownian motion and relates to the size of the particles. Normally DLS is concerned with the measurement of particles suspended within a liquid, the speed at which the particles are diffusing due to Brownian motion. This is done by measuring the rate at which the intensity of the scattered light fluctuates when detected using a suitable optical arrangement. A typical light scattering system comprises of six

main components laser, sample cell, detector, attenuator, correlator, and a computer.

Laser provides light source to illuminate the sample contained in the cell. For dilute concentration, most of the laser bean passes through the sample, but some is scattered by the particles within the sample at all angles. A detector is used to measure the scattered light. The intensity of the scattered light must be within the specific range for the detector to successfully measure it. If too much light is detected, then the detector will become saturated. To overcome this, an attenuator is used to reduce the intensity of the laser source and hence reduce the intensity of scattering. For samples that do not scatter much light, such as very small particles or samples of low concentration, the amount of scattered light must be increased. For samples that scatter more light, such as large particles or samples at higher concentration, the intensity of scattered light must be decreased. The scattering intensity signal from the detector is passed to a digital processing board called a correlator. The correlator compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying. This correlator information is then passed to a computer where the data is analyzed to derive size information.

In zeta potential measurements, electric field is applied across the cell. Any particles moving through the measurement volume will cause intensity of light detected to fluctuate with a frequency dependant on the particle speed and digitally processed to obtain the electrophoretic mobility and thus arrive at zeta potential data.

Malvern in their technical note[9,10] has discussed on the sample requirements to obtain reliable results. The optical properties, particle size and PDI are important factors affecting the zeta potential data. The samples have to be optically clear.

Technical literature from nano Composix,[11] explains that in a highly conductive sample, the movement of conductive ions can lead to electrode polarization and degradation and therefore can lead to inconsistent Zeta potential values.

In our study SEM pictures of both diluted and concentrated samples were similar. However, they showed different characterization parameter. The concentration of the sample that had altered conductivity, and optical properties, were important contributors to get different results. The concentrated samples in the experiment scatter more light that gave inconsistent particle size data with high PDI. The diluted samples scatter light within the detection limits and therefore the results were consistent.

It is also seen in the diluted samples conductivity decreased significantly that can be an important factor in getting consistent Zeta potential values. Conventional equipments for measuring particle size using Dynamic light scattering has been upgraded regularly. The non-invasive back scatter (NIBS) technology, of Malvern[12] not only increases the concentration limits at which DLS can be successfully applied, but also increases the sensitivity of the technique.

CONCLUSION

The dilution of the sample had altered conductivity and optical properties of the samples. The un-diluted samples in the experiment scatter more light that gave inconsistent particle size data with high PDI. The diluted samples scatter light within the detection limits and therefore the results were consistent. In diluted samples conductivity decreased significantly that can be a factor in getting consistent zeta potential values. This study re-emphasizes the importance of sample preparation in characterizing liposomes using techniques which involves dynamic light scattering. Sample concentration during measurement is important to get reliable results. Sample preparation and standardization is important in characterizing and to compare the properties of various batches of liposomal formulation.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

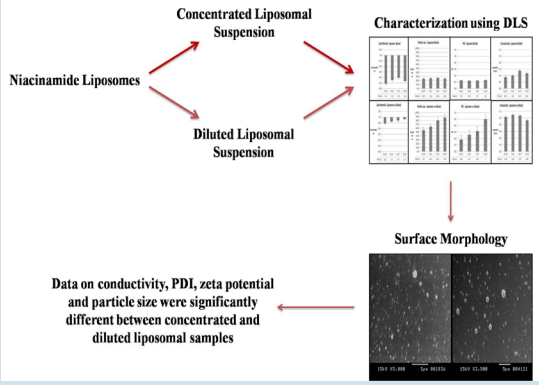
ABBREVIATIONS USED

PDI: Polydispersity index; **DLS:** Dynamic light scattering; **PCS:** Photon correlation spectroscopy; **SEM:** Scanning electron microscopy; **NIBS:** Non-invasive back scatter technology.

REFERENCES

1. Gregoriadis G. The carrier potential of liposomes in biology and medicine. N Engl J Med. 1976;295(13):704-10.
2. Fendlerv JH, Romero A. Liposomes as drug carriers. Life Sci. 1977; 20(7):1109-20.
3. Sinico C, Fadda AM. Vesicular carriers for dermal drug delivery. Expert Opin Drug Deliv. 2009;6(8):813-25.
4. El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: from drug delivery to model membranes. Eur J Pharm Sci. 2008;34(4):203-22.
5. Siram K, Chellan VR, Natarajan T, Krishnamoorthy B, Mohamed EHR, Karanam V, *et al*. Solid lipid nanoparticles of diethylcarbamazine citrate for enhanced delivery to the lymphatics: *in vitro* and *in vivo* evaluation. Expert Opin Drug Deliv. 2014; 11(9):1351-65.
6. Ada P. Penetration of Cosmetics into and through the Stratum Corneum. Cosmetics and Toiletries. 2013;128(2):92-6.
7. Johnson B. Therapeutic Treatment Using Niacin for Skin Disorders. United States patent application 2009/0246234 A1. October 1, 2009.
8. Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: State of the Art A. J Colloid Sci Biotechnol. 2012;1(2):147-68.
9. Malvern, DLS technical note, mrk 656-01, Dynamic light scattering: an introduction in 30 min.
10. Malvern. Zetasizer nano technical note, mrk 669-01, concentration limits for zeta potential measurements in the zetasizer nano.
11. Nano Composix. Zeta Potential Analysis Of Nanoparticles, September 2012, v1.1
12. Kaszuba M, Connah M, Mattison K. High concentration particle size measurements using dynamic light scattering, LabPlus international, September 2004.

PICTORIAL ABSTRACT



SUMMARY

- Liposomal suspension was formulated using a phospholipid and active niacinamide.
- Data of concentrated and diluted samples of liposomes were characterized and compared.
- The dilution of the sample had altered conductivity, zeta potential and optical properties of the sample.
- This study showed that sample concentration during measurement is important to get reliable results.

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