Near-Infrared (NIR) Spectroscopic Investigation of Novel Bionanoparticles by using MOEMS Spectrometer

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***Abstract*-Near-Infrared (NIR) spectroscopy is one of the vibrational spectroscopic techniques which has been utilized to characterize the structural information of biological samples by means of associated "molecular fingerprints". Recent advances in the optical technology have enabled us to utilize this method for the investigation of various properties of nano-sized biomaterials such as biopolymers or bionanoparticles. In this study, a compact, cost-effective, portable, field-deployable, dual-detector micro-mirror NIR spectrometer based on micro-opto-electro-mechanical systems (MOEMS) technology has been employed for the NIR spectroscopic investigations of a novel kind of biomaterials originating from self-forming synthesized lipids. This miniaturized spectrometer is capable to operate in the wide spectral range of 9000-4800 cm^{-1} (~1100-2100 nm) which is relevant to the vibrational bands corresponding to the biological samples. The NIR spectra of such biopolymers are dominated by overtones and combinations bands originating from C−H, O−H and C=O vibrational modes. Furthermore, various characteristics properties including Beer's law has been tested for different polar solvents and found that this law is valid for the studied biopolymers at the low concentration regime.**

***Index Terms*- Bionanoparticles, Lipids, Beer's law, MOEMS spectrometer, NIR spectroscopy.**

I. INTRODUCTION

Near-infrared (NIR) spectroscopic technique provides an appropriate means for the characterization of chemical structures and conformation of the materials. Although this technique has been widely applied in the identification of minerals and food [1-4], it has been less frequently used for the analysis of biological samples such as lipids and liposomes. However, substantial progress has been made in recent years in the applications of NIR spectroscopy to many other areas including but not limited to pharmacy, medicine, nutrition and cosmetic products [3]. It should be noted that NIR spectroscopy is also known as proton spectroscopy because this type of spectroscopy is most useful for measuring bonds involving hydrogen such as O−H, C−H, N−H etc. [4]. Thus, the technique appears most suitable for the identification of compounds having hydrated and hydroxyl groups. NIR spectroscopy can also be used to monitor the behaviour of lipid nanovesicles (liposomes) in aqueous suspensions with different temperatures and concentrations of lipids [5-7]. This technique has proven to be tremendously useful to study the interactions and stability of nanoparticles in an aqueous environment.

NIR spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the spectral region between approximately 12500 and 4000 cm^{-1} (0.8-2.5 μm). This spectral region contains absorption bands corresponding to overtones and combinations of fundamental vibrations. The anharmonicity in the potential function as described by Morse's potential is responsible for such overtones and combinations vibrational modes (Frank-Condon Principle) [8].

The conventional NIR spectrometers, mostly based on detector arrays, are mainly designed for laboratory use and are generally, too large, heavy, costly and delicate to handle for remote field applications [9, 10]. Hence, in the present study, a new type of NIR spectrometer has been developed and employed as an alternative which eliminates the various problems associated with traditional spectrometers. Such novel miniaturized spectrometer is based on new and optimized MOEMS technology and consists of a scanning micro-mirror and dual

detector. This mirror is combined with a diffraction grating and other imaging optical components [9, 10]. It periodically disperses the polychromatic radiation generated from infrared light source into its spectral components. The radiation is measured by dual detector system to cover broad spectral range. The radiation coupling is accomplished by using fiber optics. The dual-detector system offers an attractive alternative to much more expensive systems based on InGaAs-Arrays. Furthermore, a compact design provides the advantages of improved versatility, high sensitivity, fast and accurate measurement in a wide spectral region at low costs [9, 10].

In this work, we have employed a NIR spectrometer system based on MOEMS technology for a comprehensive analysis of bionanoparticles namely lipids and liposomes or nanovesicles trademarked as QuSomes. Liposomes or lipid vesicles are spherical, self-closed nano-structures composed of curved lipid bilayers. Their size ranges from about 20 nanometer (nm) to several hundreds nm. Such liposomes are made predominately from amphiphiles, a special class of surface-active molecules, which are characterized by having a hydrophilic (water-soluble) and hydrophobic (water-insoluble) group [11]. These structures have numerous unique physical properties, such as osmotic activity, permeability of their membrane(s) to different solutes, solubilising power, chemical composition and surface characteristics of the membranes etc. More recently, liposomes have also been evaluated as delivery vehicles for drugs, vitamins and cosmetic materials. Thus liposomes can be custom designed for almost any need by varying the lipid content, size, surface charge due to different methods of preparation. The formation of liposomes requires careful quality control methods for the encapsulation process and efficient production capacity. Moreover most of the known liposome suspensions are not completely thermodynamically stable. Therefore, thermodynamically more stable liposome systems have to be investigated to avoid some of these current problems [12, 13]. Recently, BioZone Laboratories Inc. (Pittsburg, CA), introduced new patented revolutionary delivery technology such as QuSomes, as a lipid based delivery system that is more stable and cost effective, when compared to conventional technologies and are substantially more versatile for topical and oral delivery [14].

II. EXPERIMENTAL METHODS

A. Materials

The QuSomes samples have been provided for our experiments by BioZone Laboratories Inc. They are directly produced in an aqueous solution while competing traditional liposomal techniques require the use of expensive and environmentally toxic organic solvents. This new delivery approach adopts a lipid-based delivery system that is more cost effective than conventional technology and substantially more versatile. Those QuSomes spontaneously form liposomes upon addition of any polar solvents, without additional heating, cooling and agitation methods. Therefore, it is easier to encapsulate active ingredients. Such QuSomes formulations have been developed for an ample range of applications. Furthermore QuSomes can encapsulate both hydrophobic and hydrophilic ingredients and solubilize small molecules, peptides and proteins. Hence, QuSomes can be delivered in various routes including oral, topical, nasal, ophthalmic, injectable, transdermal, transmucosal and pulmonary delivery modes [15].

As a representative example, we have shown here NIR absorption spectra of sample lipid originating from 1,2-dimyristoyl-*rac*-glycerol-3-dodecaethylene glycol referred as GDM-12 hereafter. This is a synthetic lipid and the number after nomenclature indicates the number of C_2H_4O subunits in the PEG chain. As base polar solvents we have used Deuterium or heavy water (D_2O) and Phosphate Buffered Saline (PBS) in our experiments. To obtain the spectra with high signal to noise ratio, several aqueous solutions of nanovesicles were prepared using different concentration. All of the data have been recorded at room temperature.

One of the fundamental properties of the studied samples is the ability to form liposomes by virtue of having the proper packing parameters. These packing parameters are relative measures of a given lipid composition and depends on factors such as size relationships between the lipid head and

hydrocarbon chains, the electric charge, and the presence of stabilizers such as cholesterol [11]. In general, for such QuSomes type liposome compositions the packing parameters have the following values: P_a (packing parameter with respect to the surface) lies in the range of 0.84 to 0.88, whereas P_v (packing parameter with respect to the volume) lies between 0.88 and 0.93 [14].

B. MOEMS NIR Spectrometer

The NIR absorption spectra of pure QuSomes and its aqueous solution with D_2O and PBS have been recorded using our highly sensitive and compact spectrometer provided by the Fraunhofer Division and developed by Colour Control Farbmesstechnik GmbH, Germany. The schematic of such a spectrometer is illustrated in Fig. 1 and the picture of the assembled unit with light source and sample holder are demonstrated in Fig. 2. According to the schematic, near-infrared radiation generated by a quartz tungsten halogen lamp, is absorbed by a sample and enters a fiber optics. The sample is presented using a quartz cuvette with different cell pathlength as per experimental requirements. To acquire the NIR spectra of pure lipids and nanovesicles, cuvette with cell pathlength ranging from 1 mm to 10 mm was employed. The samples in the cuvette were mounted in front of infrared light source to obtain NIR spectra. The radiation enters the spectrometer through an entrance slit by means of fiber-optics connector. Then the light is reflected by a deflection mirror toward a spherical mirror. This mirror collimates the incident photons and illuminates the micro mirror. Finally, it is directed onto the diffraction grating, which divides it into different spectral components [9, 10].

According to the wavelength dependent reflection angle of the micro mirror the desired wavelength of diffracted light reaches the two exit slits. Behind the spatially separated exit slits two detectors convert the monochrome radiation acquired into electrical signals for further data processing [9, 10]. We have utilized the software IrSys developed by Colour Control Farbmesstechnik GmbH, Germany for initial data acquisition. Subsequent data processing such as base line correction, peak position identification and data fitting has been performed by software Origin 7.0 (OriginLab Corporation, Northampton, MA).

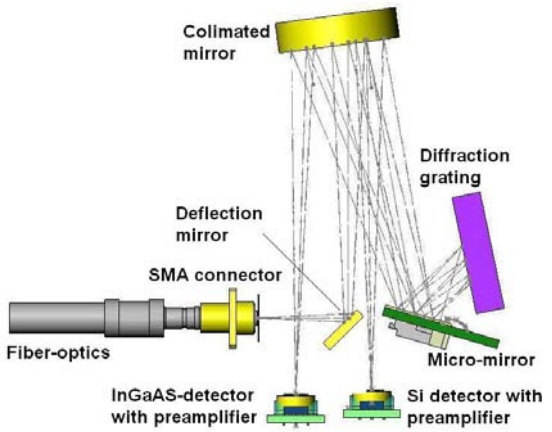

Fig.1. Schematic of dual-detector micro-mirror MOEMS NIR Spectrometer [9].

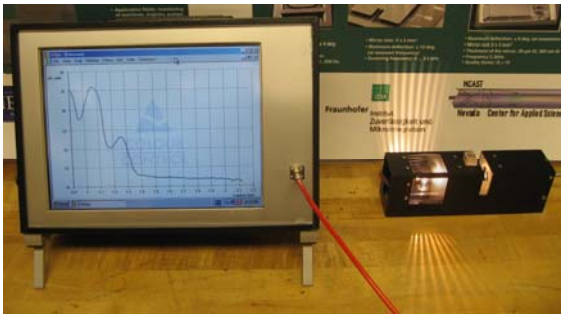

Fig.2. Picture of assembled prototype of MOEMS NIR Spectrometer showing light source, sample holder and fiber-optics connector.

III. EXPERIMENTAL RESULTS

A. NIR Spectra of Bionanoparticles

The collected NIR spectra originating from biological samples usually comprise molecular bands arising from overlapping absorptions structures, which mainly attribute to overtones and combinations of functional groups involving C-H, O-H and C=O molecular bonds [1]. These peaks correspond to the doubling or tripling of fundamental bands arising in the infrared and Raman region. In Fig. 3, we have exhibited a typical NIR spectrum of pure GDM-12 in the spectral range of 11100 to 4700 cm^{-1} corresponding to the wavelength range of 900-2120 nm. The main observed band structures for lipid GDM-12 are listed in Table 1 [16, 17].

Table 1: Band center and suggested assignments for NIR spectra of lipid GDM-12

Band Center (cm^{-1})	Suggested assignments
8278	2nd overtone C−H band
7018	1st overtone O−H band
5659	1st overtone C−H band
5196	2nd overtone C=O band
4878	Combinations O−H band

As observed in Fig. 3, for the QuSomes sample three distinct NIR spectral regions are identified, (a) the high wavenumber region between 6500 and 9000 cm^{-1} attributed to the first overtone of the hydroxyl stretching and second overtone of the C−H stretching mode; (b) the 5350−5900 cm^{-1} region attributed to first overtone of the C−H stretching mode; and (c) the 4800−5300 cm^{-1} region attributed to the combination O−H stretching and second overtone of the C=O stretching mode [1]. In particular, the first band at 8278 cm^{-1} (1208 nm) is due to the 2nd overtone of C−H group whereas the second dominant band located at 7018 cm^{-1} (1424 nm) may be interpreted as 1st overtone of O−H functional group. In addition, the other dominant peak at around 5659 cm^{-1} (1767 nm) can be identified as 1st overtone of C−H group. Furthermore, the peak positioned at 5196 cm^{-1} (1924 nm) is attributed to 2nd overtone of C=O band. Finally, the band occurring at 4878 cm^{-1} (2050 nm) may be ascribed to combinations O−H band [1].

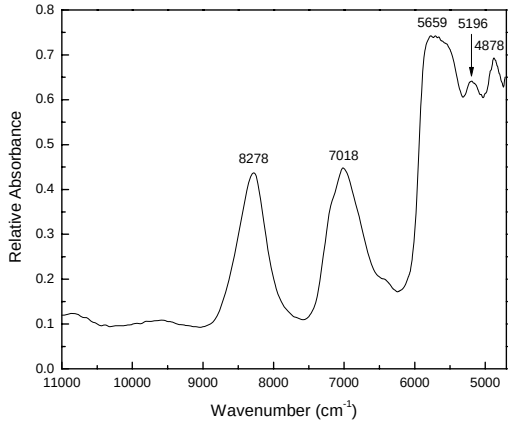

Fig.3. NIR absorption spectrum of pure QuSome sample (GDM-1) with prominent vibrational peak positions.

B. Beer-Lambert (Beer's) Law

Beer-Lambert Law, more frequently known as Beer's Law, states that the optical absorbance of a chromophore in a transparent solvent varies linearly with both the sample cell pathlength as well as the atoms or molecular concentration. In general, Beer's law provides the linear relationship between absorbance and concentration and pathlength of an absorbing species. If radiant power I_0 is directed at a sample solution, then the portion of the beam of radiation leaving the sample I is defined as,

$$I(\times) = I_0 e^{-\alpha \times} = I_0 e^{-\varepsilon c \times}$$

In above relation, α is εc where, ε is the molar absorptivity with units of L mol^{-1} cm^{-1} and c is the concentration of the compound in solution, expressed in mol L^{-1}. Similarly, $\times$ is the pathlength of the sample in cm corresponding to the pathlength of the cuvette in which the sample is contained. The expression $\alpha \times = \varepsilon c \times$ relates to absorbance depending on the total quantity of the absorbing compounds in the light path through the cuvette. However, it should be noted that this law is valid only for low concentration regime [7].

To validate one of the features of the Beer's law, we have studied the variation in the absorption pattern of lipid nanovesicles as a function of different light pathlength. The NIR spectra of nanovesicles for different pathlength are illustrated in Fig. 4. As shown in this figure, we have observed mainly two distinct peaks arising at around 7100 and 6000 cm^{-1} labeled as peak 1 and peak 2, respectively.

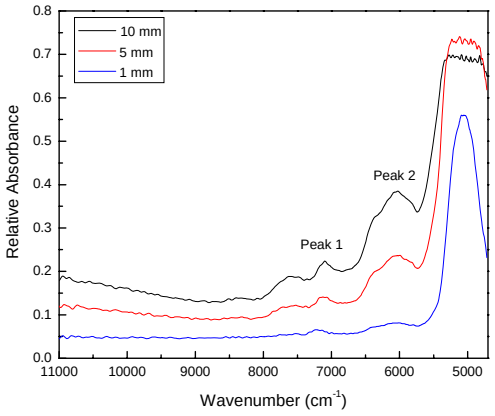

Fig.4. NIR spectra of nanovesicles originating from one of the studied lipid sample (GDM-12) for different light pathlength as indicated.

We have further investigated the effect of cell pathlength on the absorbance behavior of lipid nanovesicles in D_2O suspension (2%, v/v). For these experiments the cuvettes of different

light pathlength, i.e. 1, 5 and 10 mm have been used. Fig. 5 shows the plot of cell pathlength as a function of absorbance for lipid (GDM-12) nanovesicles. This plot provides further evidence that the absorbance increases linearly with increase in cell pathlength showing linear dependence i.e. Beer's law is valid. Moreover, we should note that this behavior is demonstrated by both of the prominent absorbance bands of lipid nanovesicles in D_2O suspension but showing different trends [6].

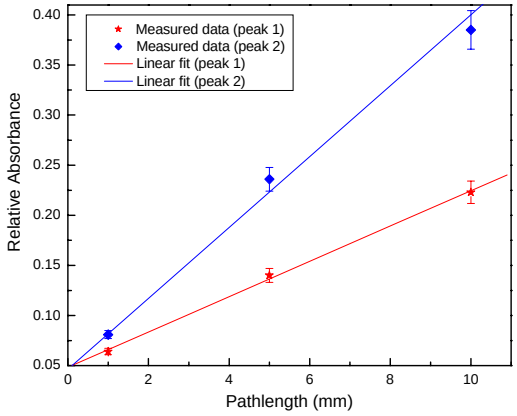

Fig.5. Relation of absorbance vs. pathlength of cuvette for QuSomes (GDM-12) in aqueous suspension of D_2O proving Beer's Law.

In addition, we have analyzed the behavior of peak intensity ratios as a function of cell pathlength. For this purpose, peak intensity ratios (peak 2/peak 1) have been calculated and plotted vs. cell pathlength as shown in Fig. 6. Once again, it also demonstrates the linear relationship providing the evidence for the validity of Beer's law.

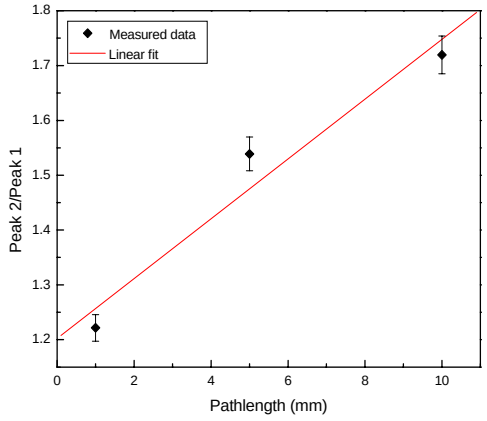

Fig.6. Relationship of intensity ratios of peak 2 and 1 (6000 cm^{-1}/7100 cm^{-1}) vs. cell pathlength of GDM-12 nanovesicles in aqueous suspension of D_2O validating the Beer's law.

In summary, our miniaturized MOEMS technology based NIR spectrometer has enabled us for the detailed band analysis of bionanoparticles. The dual detector systems allowed here to cover extensive spectral range which is significantly relevant in particular for the biological samples. The use of fiber-optics and integrated computerized data acquisition system improved the efficiency of the spectrometer further to save time as well as cost. The acquired data are easily reproducible and accurate with compared to the data obtained from other NIR spectrometer system.

Similarly, near-infrared spectroscopy is a technique, which has not been previously applied for comprehensive study of self-forming synthetic lipids and its nanovesicles in aqueous environment. Unlike other conventional lipids, these lipids have a PEG head group which is composed of hydroxyl units; they show strong O−H stretching vibration bands in addition to other vibrational modes. Raman and IR spectra of the lipids have been used to verify the NIR overtones band positions [12, 13, 18].

Furthermore, the technique of NIR spectroscopy to study QuSomes type lipids shows great potential for the understanding of the molecular structure and conformation. In particular, this technique can be employed to monitor the behavior of lipid nanovesicles (liposomes) with different concentrations and temperatures suspended in an aqueous environment. From this study, it has been proven that the NIR absorption technique is suitable for the band component analysis of the lipid samples in liquid form.

As a part of the comprehensive studies of such novel lipids using various vibrational spectroscopic techniques, we have also performed detailed NIR spectroscopic studies of absorption spectra of self-forming nanovesicles in PBS and D_2O suspensions. From the observed distinctive band structures we have confirmed that the nanovesicles in liquid are stable and not interacting strongly in this environment. It is also found that Beer's law is valid in the low concentration regime for both of the suspensions. However, from our earlier study it has been revealed that the nanovesicles appear more stable in D_2O than in PBS suspension [5].

IV. CONCLUSION

In this study, we have performed for the first time detailed NIR spectroscopic investigations of absorption spectra of self-forming lipids and nanovesicles by using MOEMS technology based compact, dual detector micro-mirror NIR spectrometer. From the observed prominent band structures, we have been able to confirm that the nanovesicles are stable and not interacting strongly in the aqueous setting. It is also shown by using different light pathlength that Beer's law is valid in the low concentration regime. Our spectroscopic results also indicate that specific band structures can be used as fingerprints for the sample identification and characterization. These data may be useful for the development of new type of lipid based drug and substance delivery systems.

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