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RESEARCH PAPER

Preparation and characterization of fluorescent probe (carbon nano dot) encapsulated liposome

Tanaya Palit[1],Poulami Sarkar[2],Saronik Bose[3], Md.Zulqarnain[4] and Om Prakash Kumar[5]
[1]Department of Pharmaceutical science , Pitambara College Pharmacy , Ranchi, Jharkhand – 835217
[2]Department of Pharmaceutical Technology, Brainware University,Barasat, Kolkata–700125
[3]Department of studies in Biotechnology, University of Mysore, Mysuru , Karnataka – 570006
[4]Department of Pharmacy Practice, Jamia Hamdard University, New Delhi – 110062
[5]Department of Pharmacognosy, Pitambara College Pharmacy , Ranchi, Jharkhand – 835217

*Corresponding Author: **Saronik Bose**

ABSTRACT

Liposomes are colloidal vesicles, having a size range of 20nm – 5.0µm in diameter. They are generally made up of Phospholipids and cholesterol. Due to their biocompatibility, unique structure, and capability to get fused with biological membranes, they are extensively used for targeted drug delivery and gene delivery. Again, fluorescent probes like Carbon Dot (CD) can be used for bio-imaging so, CD encapsulated liposomes targeted against tumours can be used for bio-imaging of tumour tissue. Here to prepare and characterize carbon dot encapsulated liposomes by the Reverse Phase Evaporation method for large unilamellar vesicles (LUV). Nitrogen-dopedCDs were encapsulated by suitable modification of method. Size distribution was studied by the Dynamic Light Scattering (DLS) technique. The fluorescent property was evaluated by fluorescence spectroscopy. The carbon dot size was 14nm, whereas the average diameter of blank liposomes was 134nm.Upon encapsulating the carbon dots, the size of liposomes was increased to 323.5nm. Upon excitation at 280nm. The carbon dot-encapsulated liposomes emitted visible radiation at 543nm.These CDs liposome encapsulation efficiency is 50.4% and the quantum yield was 21.4%.After the Fourier-transform infrared spectroscopy (FTIR) process wave number 1653 was present in CDs liposome which means Carbon dot was present in the liposome, which also in Carbon dot.

Keywords: - *Carbon Dot, biocompatibility, fluorescent, reverse phase evaporation, Fourier-transform infrared spectroscopy*

INTRODUCTION

***CORRESPONDING AUTHOR**
Saronik Bose
Department of Studies in Biotechnology, University of Mysore, Mysuru , Karnataka – 570006
E.Mail: saronikbosebiotech@gmail.com
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The liposome is a small, spherical artificial vesicle made of natural phospholipids that are safe for consumption and cholesterol. Both molecules that are lipophilic and those that are water soluble are transported by liposomes. The interior watery compartments are filled with hydrophilic materials. Most lipophilic medications are confined inside

lipid bilayers [1-4].

Types of Liposomes are based on vesicle size, number of lamellae, and preparation method. The degree of drug encapsulation in the liposomes is influenced by the size and number of the bilayers, which is an important parameter in determining the circulation half-life of liposomes [1-3]. Based on the ability of liposome to the interact with the cell : Non – interactive sterically stabilized (longcirculating) liposome and high interactive cationic liposomes. Based on size and number of bilayers : multilamellar vesicles(MLV) , large unilamellar vesicles(LUV) and small unilamellar vesicles(SUV). Based on composition and mechanism of intracellular : conventional liposomes (CL), pH – sensitive liposomes, cationic business, immunoliposomes, long-circulating business(LCL) [1,2].

Drug targeting for a particular cell, tissue, receptor, and pH determination. Inactive: Unmodified liposomes collect in a particular tissue's reticuloendothelial system when not in use.Active:ligand (antibodies, enzymes, protein A, carbohydrates) modifies liposome surface [4-6]. Protection: Reduce negative side effects by changing the location of medication accumulation in the body. Effectiveness and toxicity in pharmacokinetic: -Modifies the biodistribution and absorbance. Deliver medication in the desired form. Protection: Reduce negative side effects by changing the location of medication accumulation in the body [7,8]. Affect the drug's release time and lengthen the release,period -prolong the action's duration and reduce administration. Subject to drug and liposome characteristics Environment, osmotic gradient, and liposome composition [8].

Material & Methods

Preparation and Characterization of Fluorescent Probe (Carbon Nano dots) encapsulated liposome: -

The whole process was done by the Reverse Phase Evaporation method (REV)

Phosphate buffer saline (PBS) (PH 7.4), for 40ml solution: -

Table 1. Amount of Buffer solution –

Material	Amount(gm)
Nacl	0.032
KCL	0.008
Na_2PO_4	0.562
KH_2PO_4	0.009

Phospholipid solution –

Table 2. Amount of phospholipid solution

Material	Amount
Phospholipid (Egg Yolk)	2.50gm
Cholesterol	0.030mg
Di ethyl ether	7-8 drops

Figure 1: Blank liposome

Figure 2: Carbon Nano dot encapsulated liposome

Methods

For Blank Liposome by Reverse Phase Evaporation method

Phase A (Phospholipid + Cholesterol + Di ethyl ether) was injected into Phase B (phosphate buffer saline) after ultra-sonication oil in emulsion form. After that these solutionswere heated at theRotary vacuum Evaporator at (81°C) theyturned to a gel-like consistency and then diluted with water and Large Unilamellar vesicles (LUV) formed [10].

Synthesis of Carbon Dot

In this study, the carbon Dot used for the liposomal formulation is our laboratory which can be reflected in D G Dastidaret al. [9].

For Carbon-dot encapsulated liposomes

1ml raw carbon dot diluted with doubled distilled water and volume up to 10ml. Then add the PBS buffer solution and the whole process is the same as blank liposomes. Then liposomes will produce. Here, we use two types of Carbon Nano dots size [1] 14nm and size [2] 0.9nm -1.4nm.

Schematic diagram of Preparation of liposomes

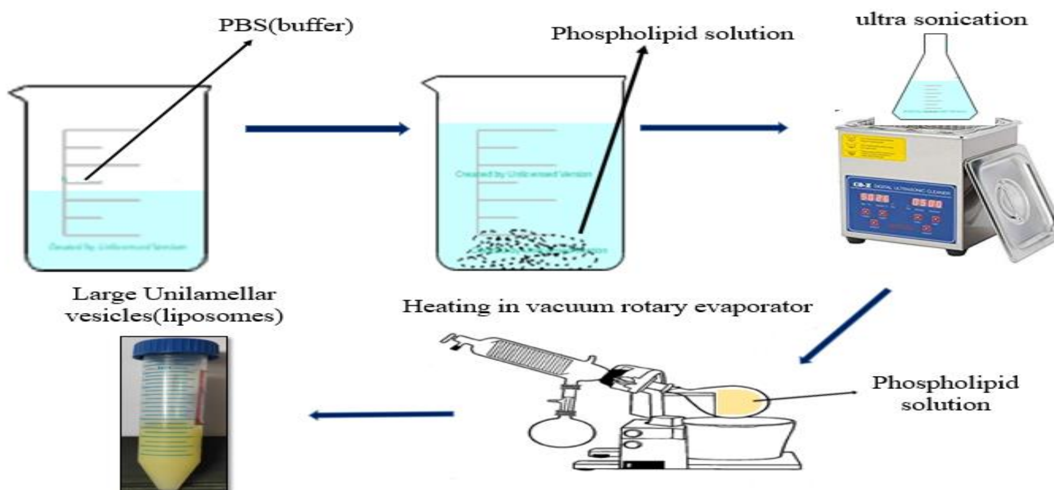


Figure 3: Preparation of liposomes

Optimization of variation Product

After all through the preliminary optimization experiment, we can determine that amount of phospholipid, amount of Cholesterol and Temperature affect the particle size, encapsulation efficiency and quantum yield of the product [11,12]. So we vary these all material. All variation is done by using encapsulated liposomes,

Table 3. Variation amount of all material

Run	Factor 1 Amount of Phospholipid (gm)	Factor 2 Amount of Cholesterol(mg)	Factor 3 Temperature ◦C
1	3.00	17.50	75.00
2	3.00	6.89	75.00
3	2.00	10.00	60.00
4	3.00	17.50	96.21
5	3.00	28.11	75.00

Study of CNDs Encapsulation within Liposome by Dialysis Membrane

Dialysis membrane kept in the hot water bath until it opens (time 1hr.) In 2ml falcon tubes 0.5ml encapsulated CNDs liposomes are taken then make volume up to 2 ml by PBS buffer. From this tube 1ml CNDs liposomes are taken and add 1ml PBS buffer into the dialysis membrane [13,14]. Kept these dialysis membranes in 50ml buffer solution and magnetically stirred for 1 hr.

These between products are used for DLS, FTIR and Unentrapped and entrapped products are used for Fluriometry spectroscopy and Encapsulation efficiency.

Schematic Diagram of Encapsulation Efficiency experiment –

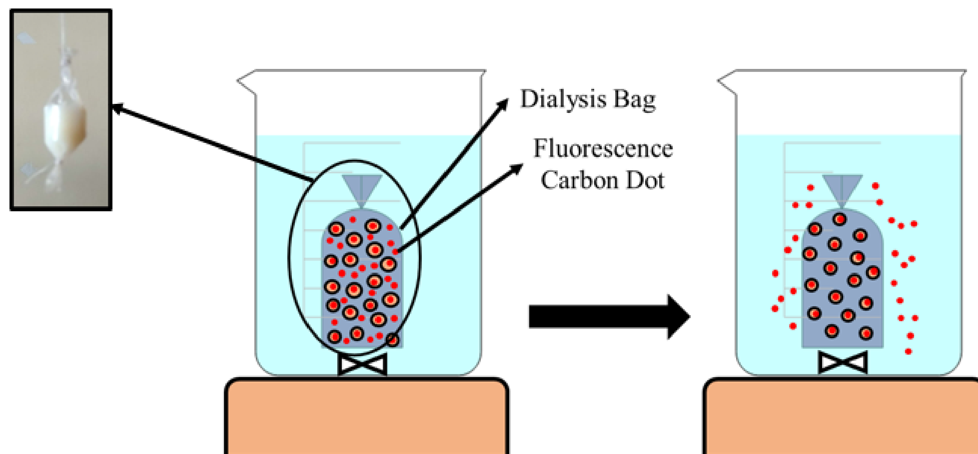


Figure 4: Encapsulation efficiency

Result and Discussion

Study of Particle Size Distribution with Zetasizer

Liposomes were diluted 100 times with PBS buffer 0.01ml sample is taken into the cuvette & placed into the machine. Encapsulated liposomes were diluted 500 and 5000 times with PBS buffer 0.01ml and 0.001ml.

Table 4. Size range of all Liposomes

Formulation	Size
Blank liposome (100times)	134nm
Encapsulated liposome [1] (500 times)	323.5nm
Encapsulated liposome [2] (5000 times)	423.8nm

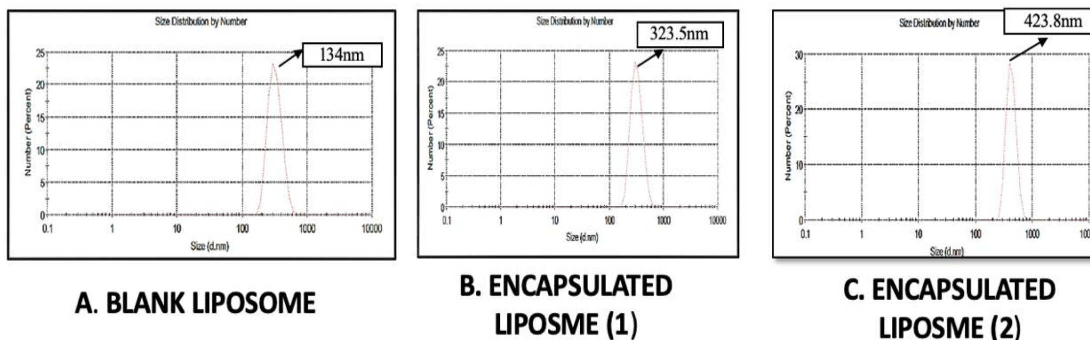


Figure 4: graph of Particle size distribution of Blank liposome, Encapsulated liposome (1) and Encapsulted liposome (2)

All variation formulation DLS size range chart is below (All formulation done by 5000times dilution.)These all are in-between dialysis membrane product.

Table 5: All variation formulation's liposomes size

Formulation Number	Size
1	245.0nm
2	216.3nm
3	263.0nm
4	227.8nm
5	296.4nm

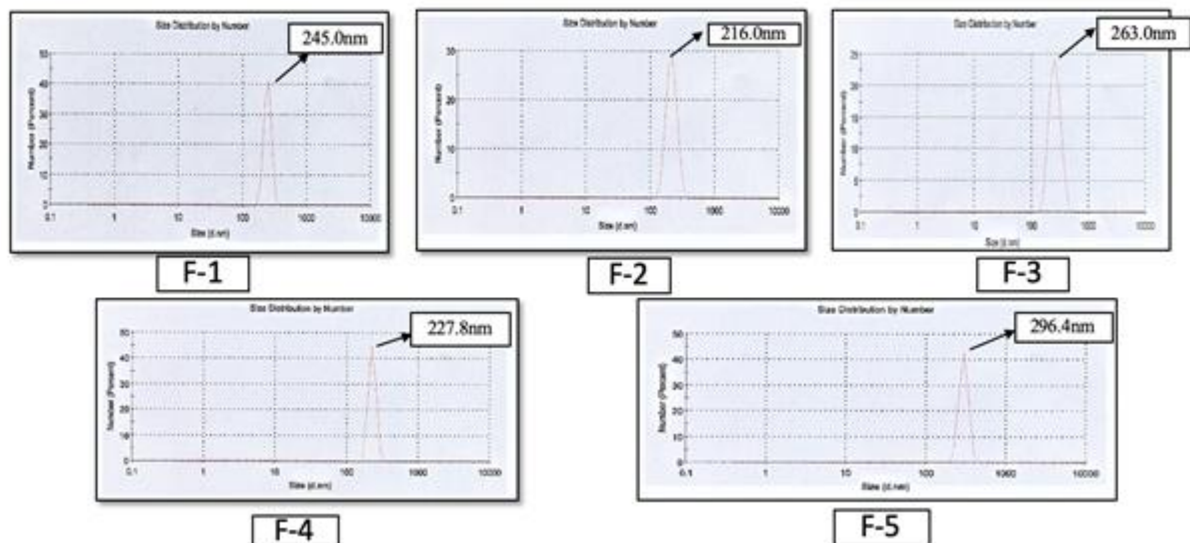


Figure 5: All variation formulation DLS size range graphs

Liposome size is 20nm - 5µm, here we made large unilamellar vesicles (LUV).Normally LUV size is 100nm – 100nm. So here blank liposome size we get 134nm and after carbon dot [1] (size -14nm) encapsulation size was 323.5nm and carbon dot [2] (Size – 0.9nm -1.4nm) encapsulated liposome size was 423.8nm. Here, two encapsulated liposomes were diluted 500 times and 5,000 times.

All five optimization products' sizes were 245.0nm, 216.3nm, and 263.nm, 227.8nm, and 296.4nm. These all products were encapsulated by Carbon dot [1] (Size-14) and all were done by 5000 times dilution.For timing of dilution liposomes size show less. But large unilamellar vesicles (LUV) were formed.

UV – Spectroscopic analysis

Sample preparation for encapsulation efficiency method

Encapsulated liposomes (1) and (2) were diluted 2500 and 5000 times with PBS buffer 0.01ml and 0.001ml. All optimization products were diluted 500times times.

This entrapment efficiency is calculated by the formula

% Entrapment Efficiency = 1 − Unentrapped drug content/Total drug content × 100

Encapsulated liposome (1)

Table 6: Excitation wavelength – 249 nm

Sample Name	Degree of Dilution	Value
Carbon Nano Dots	500	0.578nm
Encapsulated Carbon Nano dots	2500	0.538nm

Encapsulation Efficiency = [1-(0.05738*5/0.578)] *100% = 50.4%

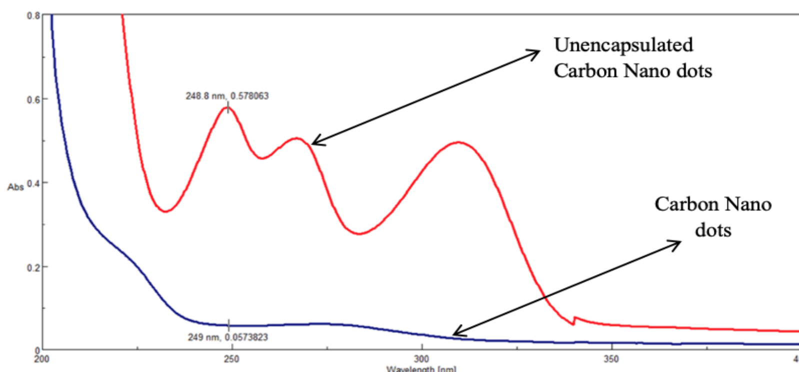


Figure 6: Overlay of Carbon Nano dots and Unencapsulated Carbon Nano dots UV spectra

Encapsulated liposome (2) –

Table 7: Excitation wavelength – 317 nm

Sample Name	Degree of Dilution	Value
Carbon Nano Dots	100	0.130nm
Unencapsulated Carbon Nano dots	1200	0.584nm

Encapsulation Efficiency = [1-(0.584*12/0.130)] *100% = 53.46%

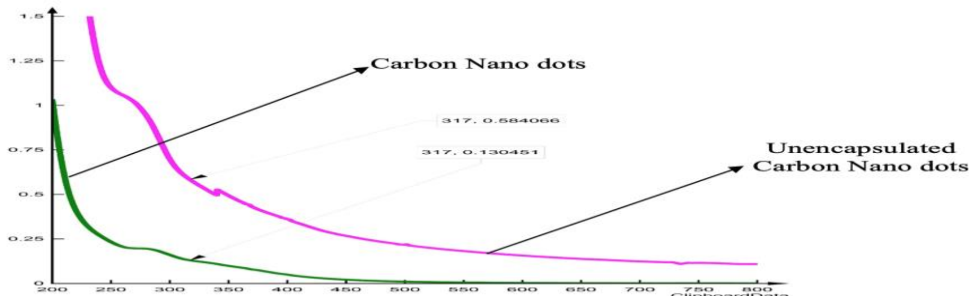
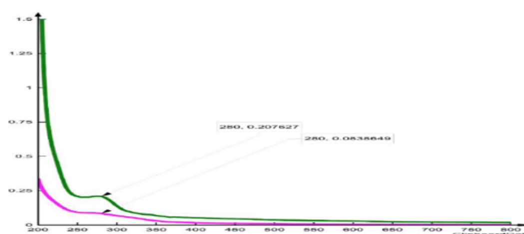


Figure 7: Overlay of Carbon Nano dots and Unencapsulated Carbon Nano dots

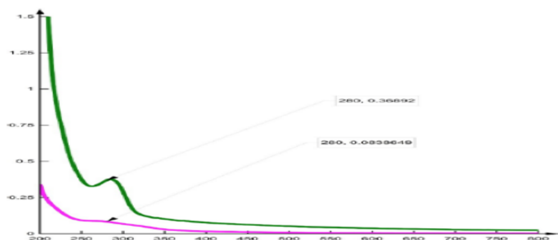
All optimization product results are given below,
Here carbon dot excitation wavelength – 280nm

Table 8: All formulation of Encapsulation Efficiency

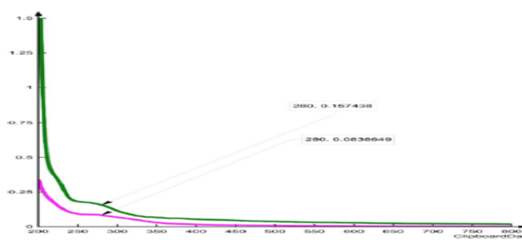
Sample Name	Degree if Dilution (Times)	Value (nm)	Encapsulation Efficiency
Carbon Name Dot	500	0.083	
Formulation 1	500	0.207	40.15%
Formulation 2	500	0.368	50.26%
Formulation 3	500	0.157	50.62%
Formulation 4	500	0.140	59.51%
Formulation 5	500	0.088	52%



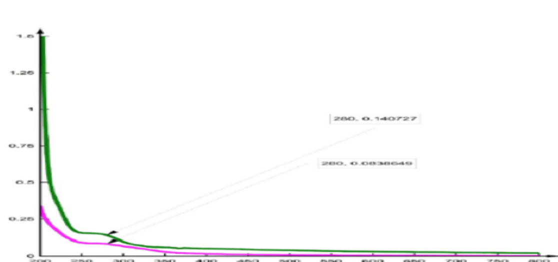
Formulation 1. Overlay of Carbon Nano dots and Unencapsulated Carbon Nano dots UV spectra



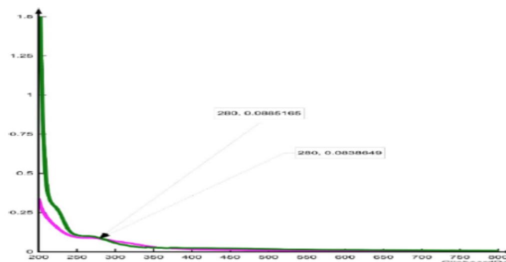
Formulation 2 .Overlay of Carbon Nano dots and Unencapsulated Carbon Nano dots UV spectra



Formulation 3. Overlay of Carbon Nano dots and Unencapsulated Carbon Nano dots UV spectra



Formulation 4. Overlay of Carbon Nano dots and Unencapsulated Carbon Nano dots UV spectra



Formulation 5. Overlay of Carbon Nano dots and Unencapsulated Carbon Nano dots UV spectra

Figure 8: Encapsulation efficiency of all formulation

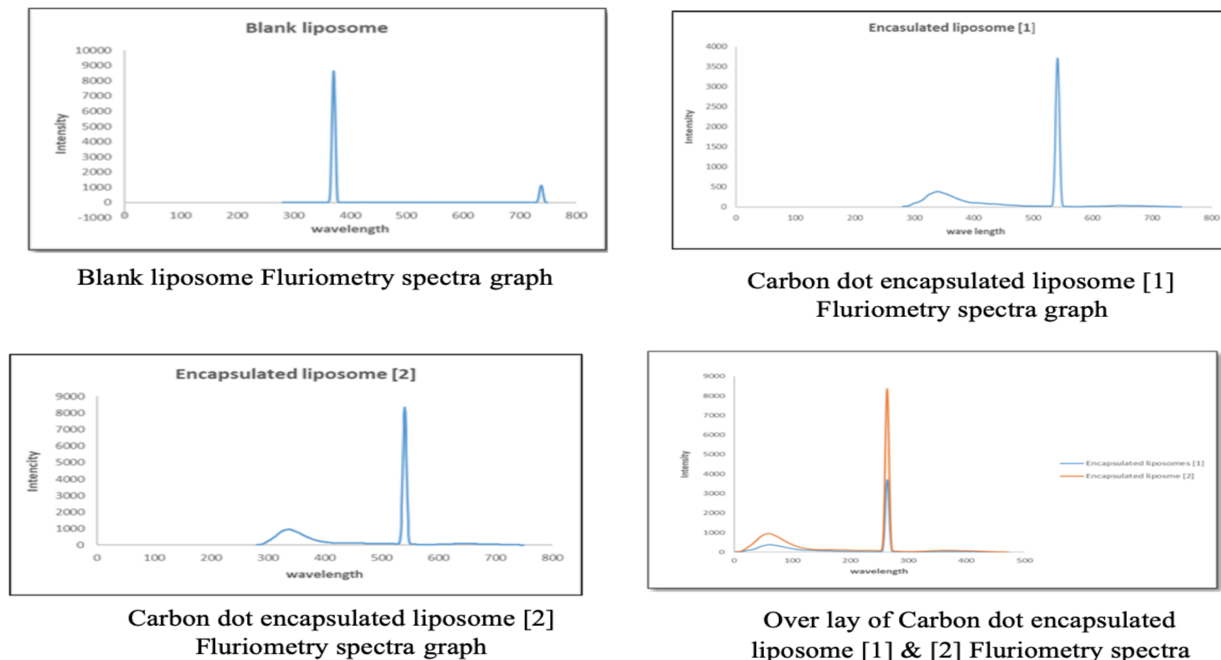
Encapsulation efficiency of carbon dot encapsulated liposomes [1] & [2] were 50.4 % and 53.46%. That means the carbon dot was encapsulated well. The opti1mization formulation products of encapsulated liposomes encapsulation efficiency varied due to the amount of material used. Hence, the carbon dot is entrapped properly.

Fluriometry Spectroscopy Analysis –

Sample preparation for Fluriometry spectroscopic:-

After the dialysis process, Carbon dot encapsulated liposomes were diluted 5000 times. Then calculate these parts made quinine sulphate solution of the same optical density as the carbon dot encapsulated liposomes they were btmeasured by FP-8200 spectro fluorometer. Spectroscopy was done at an excitation wavelength of 280 nm and an emission wavelength of 543 nm [14,15].

FluriomertySpectroscopy Analysis



Blank liposome Fluriometry spectra graph

Carbon dot encapsulated liposome [1] Fluriometry spectra graph

Carbon dot encapsulated liposome [2] Fluriometry spectra graph

Over lay of Carbon dot encapsulated liposome [1] & [2] Fluriometry spectra

Figure – 9: Graph of Fluriometry spectra of bank liposomes and C-dots encapsulated liposomes

Here we measure all products in an excitation wavelength of 280 nm and emission wavelength of 543 nm. Then blank liposome was not show any range. So, here as a result we can see that all encapsulated liposomes have a proper fluriometry range.

Quantification of carbon Nano dots encapsulated liposome

Encapsulated liposome [1]

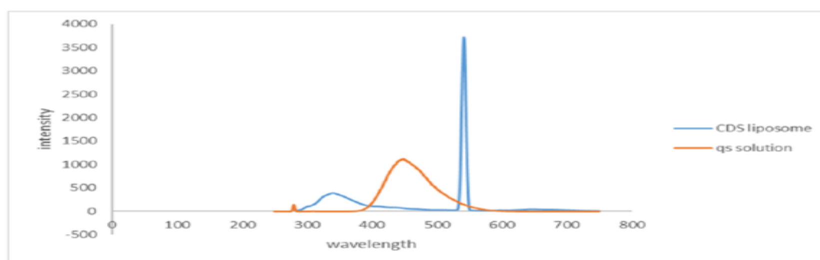


Figure 10: Over lay of carbon dot encapsulated liposomes and quinine sulphate solution

Area under the curve

CNDs Liposome	Quinine Sulphate (QS) Solution
36869.26	92839.5

Quantum Yield = [54*(36869.26/92839.5)*1] = 21.44 %

Encapsulated liposome [2]

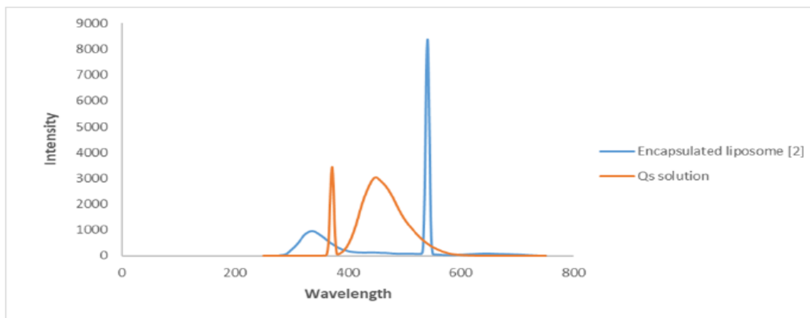


Figure 11: Over lay of carbon dot encapsulated liposomes and quinine sulphate solution

Area Under the curve

CNDs Liposome	Quinine Sulphate (QS) Solution
83640.90	258726.50

Quantum Yield = [54*(83640.90/258726.50)*1] = 17.5 %

Here, carbon dot encapsulated liposomes [1] got 21.44% and encapsulated liposomes [2] got 17.5% so here 21.44% is the best quantum yield because of excitation and emission of spectra range.

All optimize formulations Encapsulated liposome fluriometryspectra,

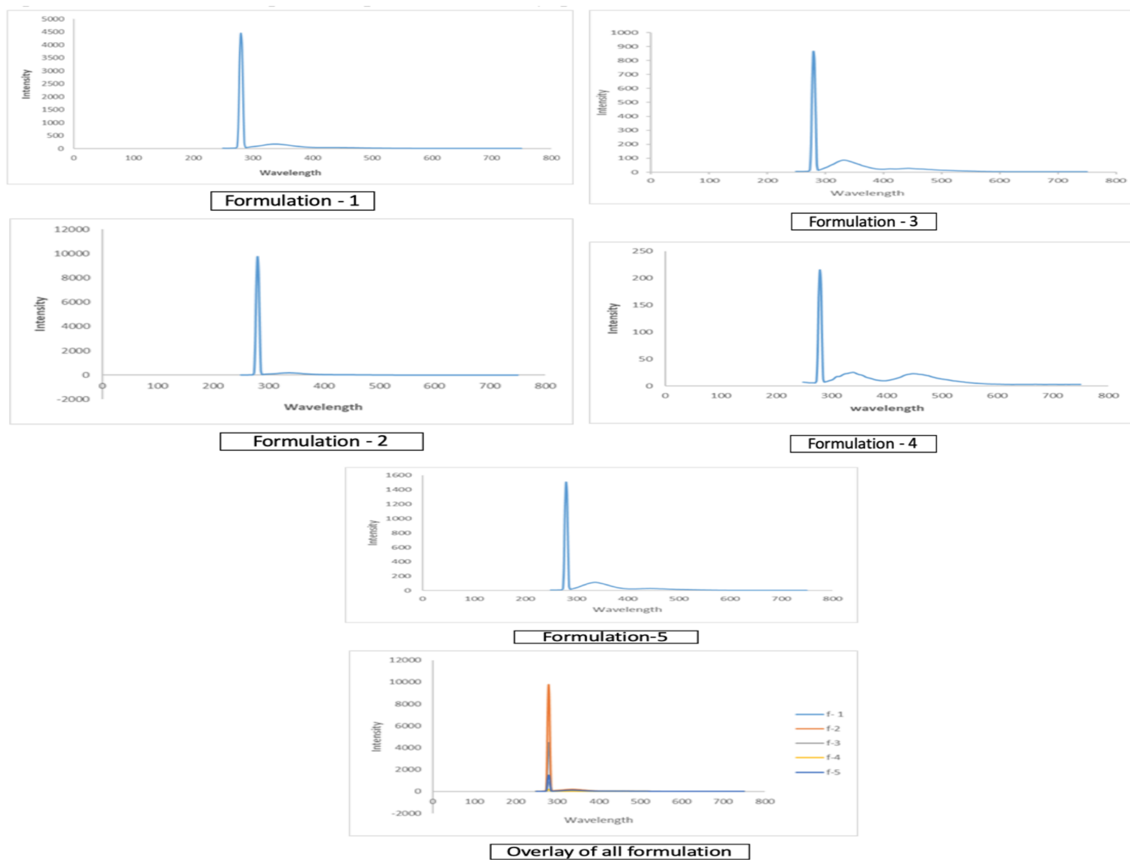
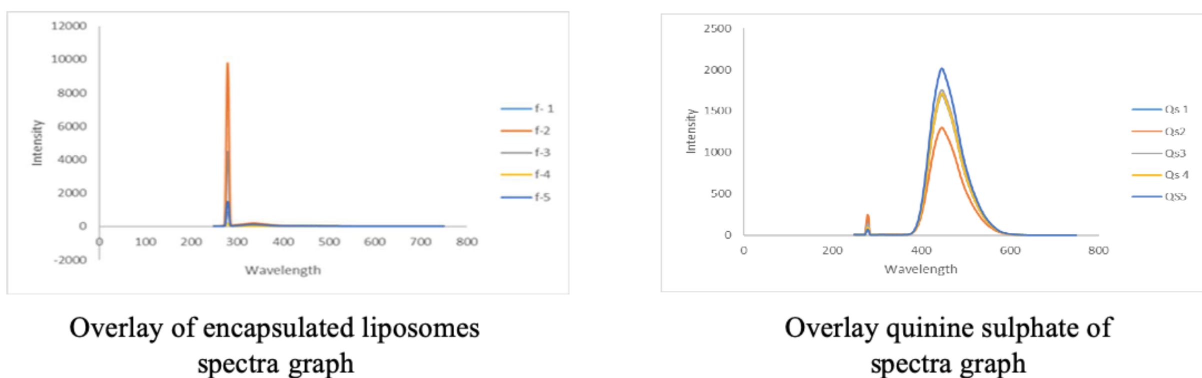


Figure -12: Fluriometry graph of all formulation of encapsulated liposomes



Overlay of encapsulated liposomes spectra graph

Overlay quinine sulphate of spectra graph

Figure 13: Overlaygraphs of encapsulated liposomes and quinine sulphate

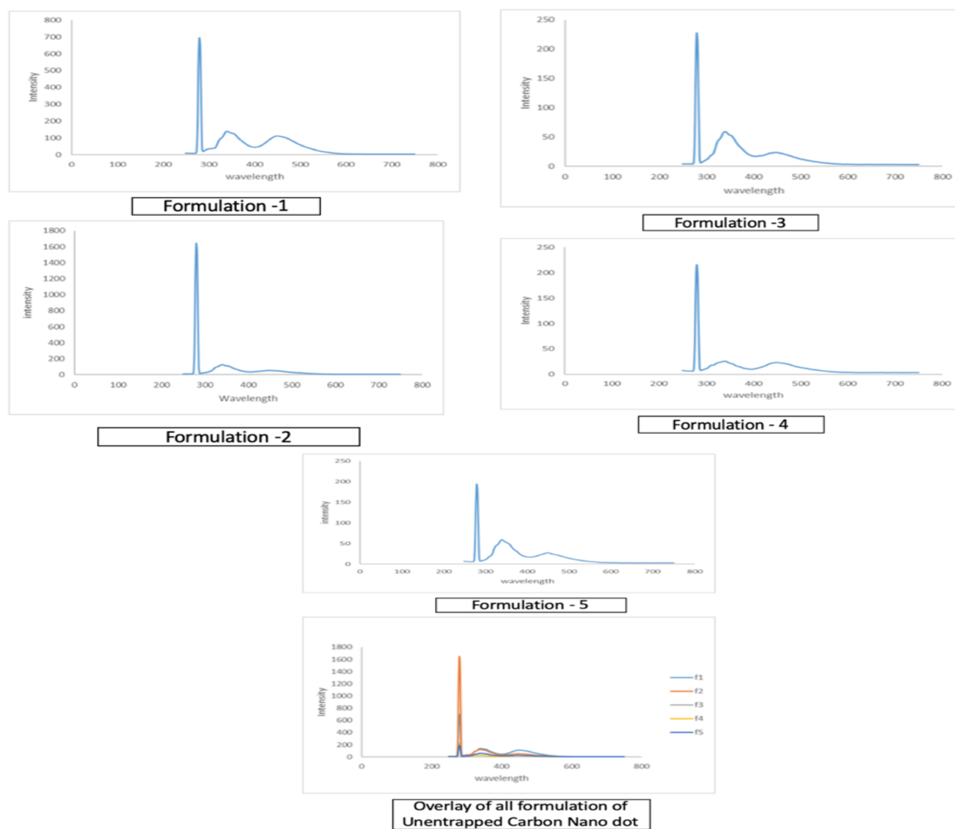


Figure 14: Fluriometry graph of all formulation of unentrapped Carbon Dots liposomes

Here we measure all products withan excitation wavelength of 280 nm and emission wavelength of 543 nm. Then all optimization shows range. So, here as a result we can see that all encapsulated liposomes have a proper fluriometry range.

Quantification result of all carbon dot encapsulated liposomes optimization products are,

Table 09: All formulation quantum yield result

Formulation No.	CNDs liposome	QS solution	Quantum Yield
1	15569.9	144338.9	5.9 %
2	17232	11392.8	8.1 %
3	8285.03	149664.5	2.9 %
4	4322.95	145535.6	1.6 %
5	1248.10	144674.2	0.50 %

Here, carbon dot encapsulated liposomes all formulations have quantum yield got less yield due to changes to dilution so here best is 8.1%

Lyophilization: Sample preparation for lyophilization

As the CDNs encapsulated liposomes and blank liposomes added the lyophilization tube. After free drying the powder form of liposomes were observed.

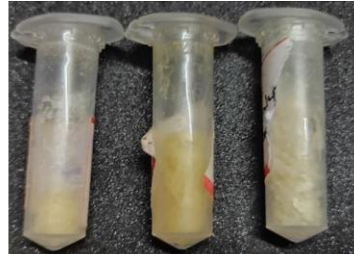


Figure 15: Lyophilization product

Presence of Functional Group

The presence of the functional group was determined by Fourier-transform infrared spectroscopy. (FTIR)

Sample preparation for FTIR:-

Ft-IR study of blank Liposomes, CNDs loaded liposomes [1] & [2] and Carbon Dot was analyzed by spectrophotometer (Perkin Elmer 100 Spectrum). Each sample was combined with KBr before being compressed to disk. All measurements were taken at room temperature. The spectra of water, carbon dioxide and KBr were subtracted from the sample spectrum and the procedure was done under nitrogen gas to prevent humidity interface. For Ft-IR 3 to 5 mg sample was needed. The Ft-IR peaks were analyzed by Spectrograph Version 1.2 Software

All FTIR graphs are below,

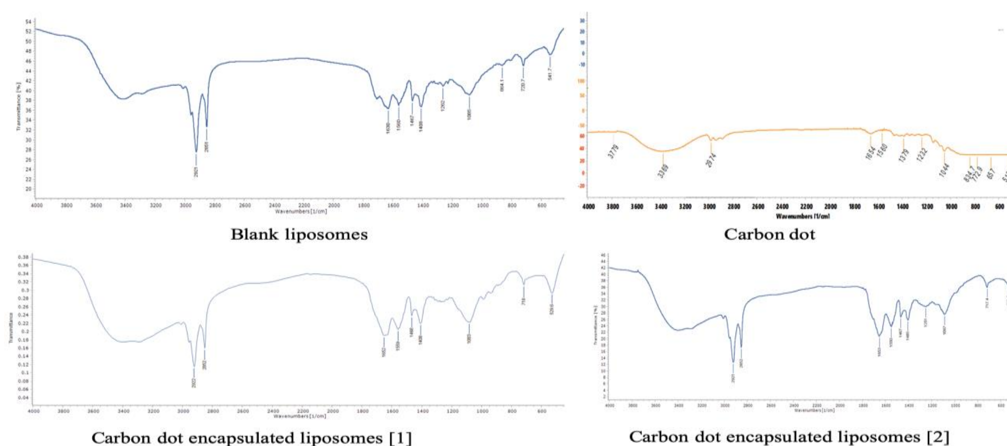


Figure 16: Graph of FT-IR study of Blank liposome, carbon dot and encapsulated

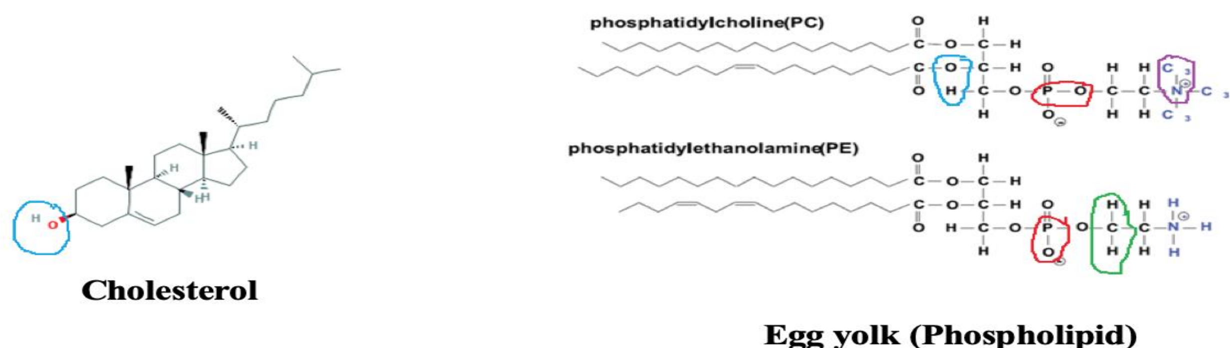


Figure 17: FTIR study related to cholesterol and phospholipid

In Outcome of spectra, the wave number 2952 has strong C-H bond and dimer O-H bond, 2922 has strong C-H bond and dimer O-H bond and as well as strong CH_2 bond, 1630 has C=C stretch,strong c=o stretch(H-bond),C=Nbond, 1251 has Strong C-O stretch, P= O phosphonate, P=O phosphormide.

Result: - Here, wave – number present in blank liposomes 1630 in the graph has a C=C stretch strong bond in cholesterol and phospholipid. Encapsulated liposomes both 1653 wave numbers which are present in both carbon dot FTIR graphs also. Then it can conclude that carbon dotsare present in liposomes.

Liposome size is 20nm - 5µm, here we made large unilamellar vesicles (LUV).Normally LUV size is 100nm – 100nm. So here blank liposome size we get 134nm and after carbon dot [1] (size -14nm) encapsulation size was 323.5nm and carbon dot [2] (Size – 0.9nm -1.4nm) encapsulated liposome size was 423.8nm. Here, two encapsulated liposomes were diluted 500 times and5000 times. All five optimization products' sizes were 245.0nm, 216.3nm, 263. nm, 227.8nm, 296.4nm. These all products were encapsulated by Carbon dot [1] (Size-14) and all were done by 5000 times dilution. For timing of dilution liposomes size show less. But large unilamellar vesicles (LUV) were formed. The encapsulation efficiency of carbon dot encapsulated liposomes (1) & (2) were 50.4 % and 53.46%. That means the carbon dot was encapsulated well. The optimization formulation products of encapsulated liposome encapsulation efficiency varied due to the amount of material used. Hence, the carbon dot is entrapped properly. Here, carbon dot encapsulated liposomes (1) got 21.44% and encapsulated liposomes (2) got 17.5% so here 21.44% is the best quantum yield because of excitation and emission of spectra range [5]. Here, carbon dot encapsulated liposomes all formulations have quantum yield got less yield due to changes to dilution so here best is 8.1%. In FTIR wave – number present in blank liposomes 1630 which present in the graph have C=C stretch strong bond in both cholesterol and phospholipid [16,17].

Encapsulated liposomes both 1651 wave numbers which are present in both carbon dot FTIR graphs also. Then it can conclude that carbon dotsare present in liposomes. Thin-film hydration and reverse-phase evaporation procedures were examined in terms of liposome stability and QD loading efficiency.

Conclusion

The liposomal formulation has significant advantages over other encapsulation techniques that already exist. Its stable and lipoidal structure gives better efficacy of the encapsulated carbon dot for the intended application and is environment-friendly and biodegradable. The study presented here is noble as very few formulations are available like this as per the literature survey. In terms of the prospects of this formulation, it can be used in in vivo bioimaging purposes because of its tunable fluorescence property, it can be used in biosensors and medical devices as it has optical and sensing properties, and it can also be used in wastewater treatment.

Reference

1. Mehran A, Naser K and Mohsen S: Application of Various Types of Liposomes in Drug Delivery Systems. Adv Pharm Bull 2017; 7(1): 3–9.
2. Ravichandiran V, Masilamani K and Senthilnathan B: Liposome- A Versatile Drug Delivery System. Der Pharmacia Sinica 2011; 2(1): 19-30.
3. A. Schnyder and J. Huwyler, “Drug transport to brain with targeted liposomes,” *NeuroRx*, vol. 2, no. 1, pp. 99–107, 2005.
4. Allen TM: Liposomes. Opportunities in drug delivery. Drugs 1997, 54(Suppl 4):8–14.
5. Andreas W, Karola VU: Liposome technology for industrial purposes. J Drug Deliv 2011, 2011:9.
6. Hofheinz RD, Gnad-Vogt SU, Beyer U, Hochhaus A: Liposomal encapsulated anti-cancer drugs. Anticancer Drugs 2005, 16:691–707.
7. Maria Laura I, Franco D, Cattel L: Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int J Nanomedicine 2006, 1(3):297–315.
8. Kunisawa J, Mayumi T: Fusogenic liposome delivers encapsulated nanoparticles for cytosolic controlled gene release. J Control Release 2005, 105:344–353.
9. Surface functionalization of porous chitosan microsphere with silver nanoparticle and carbon dot DG Dastidar, S Saha, G Dutta, S Abat, N Guha, D Ghosh Materials Research Express 7 (1), 015031

10. "Quantum Dot Loaded Liposomes As Fluorescent Labels forImmunoassay."N. V. Beloglazova, †,# P. S. Shmelin,‡,# E. S. Speranskaya,§ B. Lucas,∥ C. Helmbrecht,⊥ D. Knopp,⊥ and S. D. S. R. Niessner, †,# and I. Yu. Goryacheva§,#
11. Arcadio C, Cullis PR: Recent advances in liposomal drug-delivery systems. CurrOpinBiotechnol 1995, 6:698–708.
12. Pick U: Liposomes with a large trapping capacity prepared by freezing and thawing of sonicated phospholipid mixtures. Arch BiochemBiophys 1981, 212:186–194.
13. Liu L, Yonetaini T: Preparation and characterization of liposome- encapsulated haemoglobin by a freeze-thaw method. J Microencapsulation 1994, 11(4):409–421.
14. Batzri S, Korn ED: Single bilayer liposomes prepared without sonication. BiochimBiophy Acta 1973, 298(4):1015–1019.
15. Kirby CJ, Gregoriadis G: A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions. In Liposome Technology. 1st edition. Edited by Gregoriadis G. Boca Raton: CRC; 1984:19–27.
16. Mehta K, Sadeghi T, McQueen T, Lopez-Berestein G: Liposome encapsulation circumvents the hepatic clearance mechanisms of all- trans-retinoic acid. Leuk Res 1994, 18:587–596.
17. Hamilton RL, Guo LSS: Liposomes preparation methods. J ClinBiochem Nut 1984, 7:175.