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Research Article

Stability study in biological environment of Niosomes: Future targeted drug delivery system

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ABSTRACT

Niosomes (non-ionic surfactant vesicles) were biodegradable, biocompatible, and non- immunogenic in nature and having flexibility in structure & storage. These are chemicaly stable, ecently many researchers works on niosomes by oral drug delivery to provide better bioavailability to drug. Niosomes provides better encapsulation in biological membrain and maintain stability. Submicron sized vesicle consistin of single and double chain non-ionic surfactant mixtures which was prepared siply by dispertion of surfactants dessolved in aqueous medium or alternatively disolved surfactant were injected in organic solvant in to a aqu phase. Drug entraped values were measured by using flurosant markers like 5-6-Carboxyfluroscein and drug release rate is evaluated in biolgical media that is (serum & plasma) as a function of surfectant composition and in the presence or absence of cholesterol. Surfactant charge measurment is done by zeta potential as a function of pH, gel electrophoresis and immunoblotting were used to know the compatability study between biological fluid componant and prepared vesicles. It was found that all the vesicle carries negative charge & rapidly bound to the plasma protein which incluid albumin & imunoglobulin-G that affects the latency of entraped marker.

INTRODUCTION

The past 50 years have showed major advances in the control of disease brought about with the use of drugs. These advances were particularly apparent in the treatment of infectious disease by means of vaccines and antibiotics. Some successful anti-cancer drugs are available but the overall failure of cancer chemotherapy has been an important stimulus to drug delivery research.

Drug delivery systems modify drug release profile, absorption, distribution & elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Drug release is taking from, diffusion, degradation, swelling, and affinity-based mechanisms [1].

Targeted drug delivery is a method of delivering medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others. The goal of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue [2]. The conventional drug delivery system is the absorption of the drug across a biological membrane, whereas the targeted release system is when the drug is released in a dosage form. The advantages to the targeted release system is the reduction in the frequency of the dosages taken by the patient, maximum efficacy of drug, reduction of drug side effects, and loss of drug does not occurs. Different carriers have been used for targeting of drug such as immunoglobulin, serum protein, polymers, liposomes, microspheres, erythrocytes, & Niosomes [3,4].

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Aims & Objectives of Project [2,3,5]

- Preparation of niosomes from three non-ionic surfactants provided and investigation of physical properties in direct comparison with liposomes.
- Stability of vesicles in human plasma and serum, electrophoretic mobility measurements and surface charge calculations.
- Identification of proteins adsorbed to niosomes, a comparative study with liposomes.
- To make osmotically active and stable, as well as to increase the stability of entrapped drug.
- Handling and storage of surfactants requires no special conditions.
- To improve oral bioavailability of purely absorbed drugs and enhance skin penetration of drug.
- They can be made to reach the site of action by oral, parenteral as well as topical routes.
- To improve therapeutic performance of the drug molecule.
- Protecting the drug from biological environment and restricting effects to target cell.

MATERIALS AND METHODS

The Non-ionic surfactants, I, II and III, were a gift from L'Oreal, India, Chakan Pune. 5, 6-carboxyfluorscein (CF; sigmaaldrich, Powai, Mumbai.) was partially purified over activated charcoal before use. 4-chloro-l-napthol, dicetylphosphate (DCP), bovine serum albumin (BSA), Acrylamide, ammonium persulphate (APS), amidoblack (napthol blue black), bisacrylamide, bromophenol blue, calcein, carboxylicester hydrolase, cholesterol, , Coomassie blue R250, dipalmitoylphosphatidylcholine (DPPC), dimyristalphosphatidylcholine (DMPC), egg phosphatidy1choline (egg PC), glutaraldehyde, glycerol, glycine, phospholipase A2, sodium dodecyl sulphate (SDS), stearylamine, thrombin and trypsin were purchased from Hi Media Laboratories Pvt Ltd, Ghatkopar (West). EDTA was a gift sample from Bombay lubricants Oil Co. & all other reagents were of analytical lab grade.

Niosomes: Potential Drug Carriers

The discovery that non-ionic surfactant molecules, such as surfactant I, as shown in Fig. 1, are capable of forming vesicles, niosomes, entrapping an aqueous solution, was a lead to their potential use as drug carriers.

A system which could combine the advantages of liposomes with the ability to increase membrane permeability displayed by the non-ionic surfactants would be of great interest. An investigation to compare and contrast some of their relevant properties with the apparently similar and well characterized liposome system is vital to the development of a niosomal drug carrier system. A major prerequisite to the use of niosomes and other vesicles as drug carriers is their integrity in biological fluids. These are for example, interstitial fluid (sub-cutaneous administration), synovial fluid (intra –articular injection), contents of the stomach and intestine (oral route) and peritoneal cavity (intra-peritoneal administration). However, the great majority of potential in vivo applications involve intravenous administration so that stability in blood, especially plasma has been studied extensively. In a carrier role, niosomes must be able to both circulate in the body and retain drugs for significant periods of time to optimize access to, and interaction with, target tissue and in appropriate circumstances delivery of their contents to the interior of cells. An understanding of these processes which may affect niosome integrity in vivo is essential to a study of "niosome -encapsulated drugs".

Preliminary work within these laboratories has shown. That niosomes appear to be similar, in terms of their physical properties to liposomes. Studies, in mice, have shown modified tissue distribution and excretion of methotrexate entrapped in niosomes. Niosome formulation caused methotrexate accumulation in the liver and enhanced level of the drug in the brain after intravenous administration. Similar reports of brain accumulation have been reported for methotrexate entrapped in liposomes after intravenous injection into rats. Niosomally-entrapped doxorubicin has also been shown to have altered tissue distribution.

Intravenous injection of hand shaken niosomes containing doxorubicin into showed no apparent liver or spleen loading with drug but some evidence of accumulation of doxorubicin in the lungs. These results may be a direct consequence of the size distribution of the vesicles used, although the lung-loaded perhaps indicates intravenous aggregation of the niosomes. Increased anti- leishmanial activity after passive targeting of sodium stibogluconate to the liver using niosomally-entrapped drug is further evidence of the potential of drug-carrier role for niosomes. As part of an approach to the

optimization of this drug-carrying potential of niosomes, it is important to characterize their stability in terms of release of entrapped solute.

Production of Vesicles

Various methods are reported for the preparation of niosomes such as:

a) Ether injection method
b) Hand shaking method (Thin film hydration technique)
c) Sonication method
d) Reverse phase evaporation technique (REV)
e) Micro fluidization
f) Multiple membrane extrusion method
g) Trans membrane pH gradient (inside acidic) drug uptake process (remote loading)
h) Bubble method
i) Formation of niosomes from proniosomes.
j) "Negatively- Charged" Vesicles
k) "Positively- Charged" Vesicles
l) Purification of CF

a) Ether injection method

This method provides a means of making niosomes by slowly introducing a solution of surfactant (1.50 X 10-4M) dissolved in diethyl ether (volatile organic solvent) into warm water maintained at 60°C. The surfactant mixture in ether is injected through 14- gauge needle into an aqueous solution of material. Vaporization of ether (volatile organic solvent) leads to formation of single layered vesicles. Depending upon the conditions used the diameter of the vesicle range from 50 to 1000 nm.

b) Hand shaking method (Thin film hydration technique)

The mixture of vesicles forming ingredients like surfactant and cholesterol (1.50 X 10-4M) are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes. [2]

Purification of CF

Commercially available CF (25g) was treated with activated charcoal (10g) in boiling ethanol (300ml) contained in a round-bottomed flask (1 liter). After refluxing for 30 minutes the mixture was cooled and filtered through Whatman No. 50 filter paper. Cold distilled water (600ml) was added and solids allowed precipitating overnight at 00CT. he water was removed by filtration and the orange-colored precipitate was washed thoroughly (4 times, 50ml distilled water), dried at 500C in an airtight container and stored in the dark at room temperature.

CF Solution

Aliquots of this orange powder (0.2M) were weighed, made up to volume with the medium (distilled water or PBS) and adjusted to pH 7.4 with sodium hydroxide (NaOH, 4N).

Buffered CF

CF is usually made up in distilled water to the required molarity at pH 7.4 by adding sodium hydroxide solution (NaOH, 4N). In buffered CF, the distilled water was replaced with phosphate buffered saline (PBS) and the requisite amount of NaOH, to the final pH 7.4.

Separation of Free and Entrapped CF

Volumes of aqueous surfactant or phospholipid dispersions (5ml) from ether injection or hand shaken techniques were exhaustively dialyzed against PBS, (1.30 X 10-3 M, 0.9%w/v NaCl solution, pH 7.4). In experiments with T. elliotti, NaCl solution was omitted from the buffer.

Stability of Vesicles [6-8, 11, 12]

Leakage of CF

The CF entrapped within the vesicles is self-quenched at the working concentration of 0.2M at the wavelengths of measurement. Leakage and ensuing dilution into the extra-vesicular bulk volume, increases the fluorescence of CF markedly, which was measured (486 nm excitation, 514 nm analyzer wavelengths) using a Perkin ElmerR* 203 spectrofluorimeter.

Samples (2.5 X 10-2 ml) were added to the test media at "time zero" to give a final volume of 5ml. The fluorescence measured at these times was taken as zero percent, although in practice 15 seconds elapsed before these readings could be recorded. This amounted to 2-6% intensity of the total fluorescence. The samples were gently agitated throughout the experiments and further readings obtained at various time intervals. Maximum fluorescence (100%) for all niosomes and liposome suspensions, Ftot, was measured after vesicle disruption by addition of propan-l-ol (0.1ml), or Triton X-100 (0.1ml). The leakage of CF was corrected for background fluorescence at "time zero", Fo. The percentage of CF released in each sample was calculated as follows:

$$\% \, Realese = \frac{100(Ft - Fo)}{Ftot}$$

In which Ft - intensity at time "t".

Effect of pH

The leakage of CF was measured as described above, by challenging the vesicle suspensions (2.5 X 10-2ml) with a variety of different buffers (Mcllvaines citric phosphate buffer, pH 2.0 to 8.0) and incubating at 37°C. All the solutions used were of equal ionic strength (1.24) to that of the CF solution within the vesicle, thus preventing osmotically driven leakage of the CF solution from the vesicles.

Effect of temperature

The efflux of CF from the vesicles was measured after incubation of a suspension (2.5 X 10-2ml) in buffer (5ml, PBS at pH 7.4) at various temperatures (4°C, 22°C, 37°C and 50°C for various time intervals.

Preparation of Human Serum

Serum was prepared from plasma by treatment with thrombin (20 NIH units ml-1) at 370C for 10 minutes with gentle stirring, after which period the clot formed was removed. Serum was heat- inactivated by incubation at 510C for 30 minutes. Any dilutions of plasma or serum were made by addition of PBS (pH 7.4) to the correct volume.

Effect of Plasma

Leakage of CF from the vesicles was measured as above (page 52) after incubation of a suspension (2.5 X 10-2ml) of vesicles in human plasma (100%) at 370C. Total fluorescence (100%) was evaluated by disrupting all the vesicles using Triton X-100 (0.1ml). Leakage of CF was also monitored in the same way in the presence of serum and heat- inactivated serum.

Effect of 10% BSA

BSA (400mg) was dissolved in PBS (100ml, pH 7.4) at 370C. Leakage of vesicles was measured as above.

Measurement of Surface Potential

The electrophoretic mobility (μ) was measured as a function of pH in a laterally placed flat cell micro-electrophoresis apparatus with an optical assembly and constant temperature bath at 25^0C. The mobility was determined by measuring the time taken in seconds for the vesicles to travel a pre-determined distance (usually 2cm) under the influence of a constant known electric field (80 Volts). The vesicles were suspended in solution (NaCl, 2X 10-3M), the pH of which was varied by the addition of dilute hydrochloric acid (HCI) or sodium hydroxide (NaOH). The mobilities of at least 40 vesicles were measured at each pH and an average mobility obtained.

To measure the effect of plasma (human, 50%) on electrophoretic mobility, vesicles were prepared entrapping NaCl (2 X 10- 3 M) in glucose solution (0.2M). These vesicles were incubated in plasma for 2 hours, centrifuged at 210g for 5 minutes, washed twice and their electrophoretic mobility measured as described above. These measurements were used to calculate surface potentials.

Identification of Adsorbed Protiens

Gel Electrophoresis

Electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate (SDS; 0.1%) was carried out using a ShandonR* vertical slab unit 200.

Sample Preparation

Vesicle suspension (1ml) was incubated with plasma (2ml; 10% or 100%) for 1 hour, 2 hours or 24 hours. Samples were then diluted with PBS (10ml) and the vesicles centrifuged at 1000g for 5 minutes. The pellet was resuspended, washed 3 times and suspended in PBS (1ml). Measured quantities (2.5 X 10-2mg -7.0 X 10-2mg protein per 2.0 X 10-2 ml - 5.0 X 10-2 ml, sample buffer) were dissolved in sample buffer (6.25 X10-2 M Tris-HCI, plus 2% SDS, 10 % glycerol, 5% mercaptoethanol, 0.001%bromothymol blue, pH 6.8), heated (370C for 15 minutes) and cooled. Cleavage by Trypsin, EDTA, Urea and 1M NaOH Vesicles were incubated with plasma (1 hour, 37°C). These preparations were centrifuged and the resulting pellets washed in buffer (PBS, 1.3 X 10-3 M , pH7.4) and resuspended. Samples were reincubated with freshly prepared trypsin (1mM), EDTA (5mM), urea (4M) and NaCl (1M) for 30 minutes. These samples were then processed by centrifugation, and resuspended or analysis on polyacrylamide gel as previously described above.

Slab Gel Electrophoresis

Samples were carefully loaded onto a 7.5% acrylamide gel and run normally at 200V until the dye entered the separating gel. The voltage was then reduced to 100V and left to run for 5 to 6 hours. The run was terminated Mien the dye front reached 2cm from the bottom of the gel.

Staining and Destaining of Gels

Gel slabs were placed in a plastic tray containing Coomassie blue R250 (0.1 w/v in water: methanol: glacial acetic acid, 9:9:2) and agitated slowly. The gels were fully stained within 4 to 6 hours at room temperature (22°C). After staining was completed, excess stain was removed to allow protein bands to be seen clearly. Gels were

destained in several washes of water: methanol: glacial acetic acid (9:9:2).

Drying Gels

Gels were dried using a Bio-radR* drier (model 224) apparatus. Two sheets of filter paper (3mm) were placed on the stainless steel support screen of the dryer and wetted with water. The slab gel was aligned onto this, care being taken not to trap air bubbles under the gel since this would result in cracking during drying. The gel was overlaid with a sheet of pre-wetted Saren WrapR* (cling film), then a porous plastic sheet, and finally the silicon sheet (attached to the apparatus) forming a leak-proof seal. Vacuum was supplied by a water aspirator or vacuum pump fitted with a cold-finger water-trap and the heating block of the dryer was turned on. The exact time for drying depended on the size and concentration of the gel, generally 2 hours was sufficient. If air enters the assembly before drying is complete the gel will crack. The resulting dried gel was sandwiched between its filter paper and the protective Saren WrapR*.

Electro blotting

Proteins were first subjected to PAGE-SDS [9]. Then the proteins were transferred to nitrocellulose sheets. A sheet of nitrocellulose (0.45 prn pore size in roll form, MilliporeR) was wetted with water and laid on a scouring pad (Scotch- B riteR*) which was supported by a stiff plastic grid. The gel to be blotted was placed on the nitrocellulose sheet and any air bubbles were carefully removed. A second pad and plastic grid were added and secured in position by two strong rubber bands. The assembly was immersed vertically in an electrophoretic Trans-blot (Bio-RadR*) with the nitrocellulose sheet facing the cathode (-ve). The electrode buffer was 25 mM Tris 192 mM glycine in 20% methanol (vol/vol), pH 8.3. The electrophoretic run was at 100mA, conveniently overnight.

Staining and Destaining of Blots

The blot was sectioned and stained with freshly prepared amidoblack (0.1% w/v in 45% methanol/10% acetic acid) for 10 minutes. This blot was then immediately destained by washing in several changes of hot acetic acid (2% v/v, 900C). This manipulation was carried out in a ventilated fume hood.

Immunological Detection of Proteins on Nitrocellulose

Antigens (proteins) transferred from the gels to the nitrocellulose, paper were detected by Enzyme Linked Immuno Sorbent Assay (ELISA). The electrophoretic blots (not stained with amidoblack) were soaked in 3% BSA/5% GS (goat serum) in buffer (20mM Tris-HCl, l40mM NaCl solution, pH 7.4) at room temperature, for 1 hour to block non-specific protein binding sites. They were then washed in buffer containing Tween 20 (0.2% buffer/Tween) and incubated with the antibody, horseradish peroxidase-conjugated rabbit antihuman IgG for 2 hours at 40C. The antibody used was diluted I in 500 in BSA/GS/buffer before use. To detect the antibody reaction the nitrocellulose paper was incubated in the dark with 4-chloro-l-napthol (6mg/ml in methanol) and buffer containing 0.01% H202 (6ml

RESULT AND DISCUSSIONS

Vesicles of various compositions (Table 1) were prepared for use in this study using either the ether injection method [9] or, the original hand-shaken method [10]. The former method results, as reported for liposomes, in the formation of large unilamellar vesicles, with good entrapment efficiencies & greater stability in terms of leakage (graph 1).

The hand-shaken method was preferred for several reasons:

a) These vesicles were easier to produce and handle, with more reproducible entrapment efficiencies between batches;
b) The overall stability of liposomes and niosomes were easily compared due to their higher rates leakage, a major aim of this work; &
c) The components required to prepare the vesicles can be dissolved in suitable solvents,

The entrapped volume of CF (ml mol-1) was measured for 10 different types of vesicles and is shown in Table 2. Entrapment is greater for liposomes than in all types of niosomes but does not appear to depend on the surfactant used. These entrapment efficiencies are low but are comparable with previous reports in the literature for liposomes. However, these results suggest that manipulation of compositions may increase the entrapment of vesicles.

Removal of Unentrapped CF

Free drug or marker, can be removed after preparation of the vesicles in several ways (see introduction, page 16). Gel chromatography has been used in many laboratories and has proved an effective method of separation of free drug from entrapped drug.

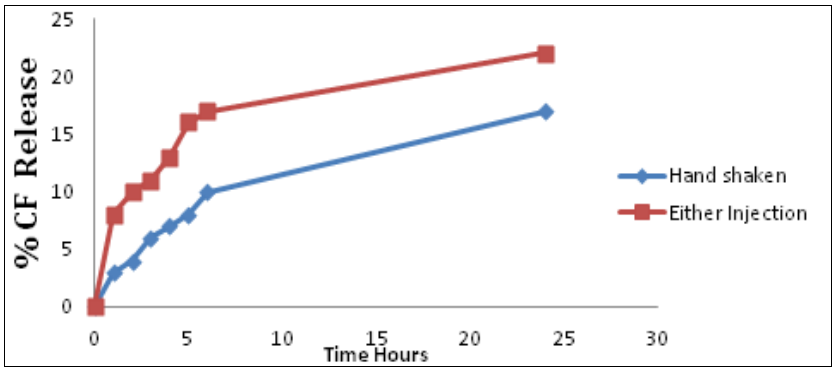
Table 1: Formulation of vesicles of various compositions

Sr. No.	Composition in mol %	Formulation Nomenclature
1	100% Surfactant I	I 100
2	50% Surfactant I + 50% Cholesterol	I 50 : CHOL 50
3	60% Surfactant I +30% Cholesterol +10% DCP	I 60 : CHOL 30: DCP 10
4	68%SurfactantI+30Cholesterol +2% Stearylamine	I 68 : CHOL 30: SA 2
5	50% Surfactant II + 50% Cholesterol	II 50 : CHOL 50
6	60% Surfactant II +30% Cholesterol +10% DCP	II 60 : CHOL 30: DCP 10
7	100% Surfactant III	III 100
8	50% Surfactant III + 50% Cholesterol	III 50 : CHOL 50
9	70% Surfactant III +20% Cholesterol +10% DCP	III 70:CHOL 30: DCP 10
10	100 DPPC	DPPC 100
11	50 DPPC+ 50 Cholesterol	DPPC 50: CHOL 50
12	70% DPPC +20% Cholesterol +10% DCP	DPPC70:CHOL 30: DCP 10
13	50 %Egg PC + 50 Cholesterol	Egg PC 50 + CHOL 50
14	50 %DMPC + 50 Cholesterol	DMPC 50 + CHOL 50

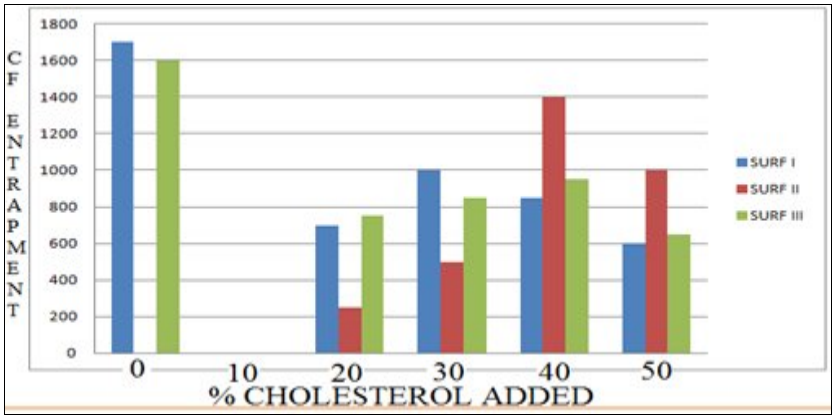
Table 2: Method of preparation

Vesicle type	Hand Shaken			Either injection		
	Entrapment	Efficiency		Entrapment	Efficiency	
	(ml. mol $^{-1}$)	(%)	(n*)	(ml. mol $^{-1}$)	(%)	(n*)
I100	1670± 255	6	6	1111± 200	4.2	3
I50:CHOL50	760±100	3	6	763±100	3	6
I60:CHOL30:DCP10	700±99	3	6	601±108	2.3	3
II50:CHOL50	979±124	4	4	1568±120	5.9	6
II60:CHOL30:DCP10	720±86	3	6	1212±99	4.5	3
III100	1580± 60	6	6	1480±200	5.6	4
III50:CHOL50	650±50	3	6	503±80	1.9	4
II70:CHOL20:DCP10	320±68	1	6	355±45	1.3	3
DPPC100	2300±320	9	4	NOT DETERMINED		
DPPC50:CHOL50	1140±90	5	2	NOT DETERMINED		

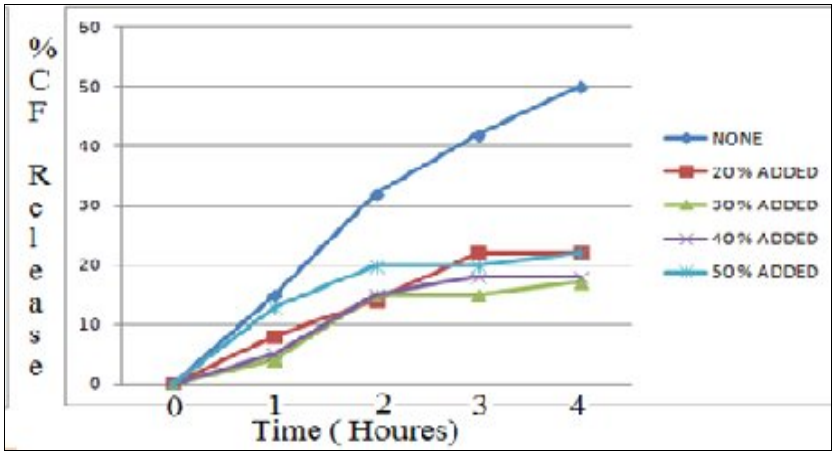
n* = number of determinations



Graph 1: CF release from niosomes, 150: CHLO50 Produced by Either injection and hand shaken method at 22°C.



Graph 2: Entrapment of CF as function of cholesterol content (mol%) for niosome from surfactant I, II & III (the entraped marker was CF at 200 mM & entrapment is expressed in liters/mol)



Graph 3: Leakage of CF from niosomes prepared fromsurfactant I containing varying amount of cholesterol (in mol%)

However, this method takes a considerable period of time and results in the collection of very dilute vesicle samples, therefore a concentration step is usually required, for example, Ultraflitration or centrifugation. After separation of vesicles from unentrapped CF, much of the free marker remains in the column and this is difficult to remove. Hence, cleaning the column after every use is time-consuming and the purchase of large quantities of column material (SephadexR*) is not economically sound. The process of centrifugation provides a quick, reliable method of separation requiring only a bench top centrifuge, for sedimenting medium to large vesicles, or an ultracentrifuge for smaller vesicles

Leakage of CF

Buffer

The rates of leakage from vesicles is dependent on the method of production, the nature of the entrapped substance, external factors such as, temperature and pH, osmotic pressure and their composition. Graph 1 illustrates the different rates of leakage from I50: CHOL50 vesicles (a representative vesicle preparation) produced by the ether injection (E. I.) technique and the hand-shaken (H. S.) method. The leakage rate was much higher with the latter method. In both cases the entrapped substance was CF (0.2M, pH 7.4) and the external medium was PBS (Sorensens, 1.3 X 10-2 M, pH 7.4, 220 C).

Niosomes were produced containing various percentages of cholesterol (0-50 mol %) and their entrapment values measured, as in graph 2. For all surfactants, II and III, optimal CF entrapment was found with the inclusion of 40 mot% cholesterol. The results indicate, for surfactant I niosomes, that leakage of CF is also greatly reduced with inclusion of 30-40 mot% cholesterol, as in graph 3.

Vesicles containing 100% surfactant have the highest entrapment efficiencies for niosomes but are the least stable and readily release their contents with time. Vesicles produced using surfactant II, had a minimum requirement of cholesterol (10 mol %) for their formation. This surfactant appeared to have several molecules of water present with it.

pH Effects

CF is a trivalent anion at neutral pH which becomes electrically neutral at acidic pH; pKa's at 6.7, 4.4 and 3.5 [13,14]. Calcein is more strongly charged than CF as a result of two methyliminodiacetic acid residues; carboxyl pKa <4.0; methylimino pKa 10-12. Calcein has been reported to be more resistant to changes in pH especially over the range pH 6 to 8. Niosomes, I50: CHOL50, were produced entrapping 200 mM CF or 200 mM calcein and their efflux as a function of external pH shown in graph 4, Graph 5, graph 6 & graph 7.

The effect of pH on CF leakage from 100% surfactant vesicles of all types is shown in figure graph 8, Graph 9, graph 10 & graph 11. For all vesicles examined, CF efflux was most rapid at pH 4.0 and below. Rapid efflux at low pH (4.0 and below) is probably a result of protonation of the carboxyl moiety of CF (at high H" concentration) which enhances CF diffusion across the bilayer to the external media.

Temperature

The effects of various temperatures, 4°C, 22°C, 37°C and 50°C were investigated using CF as the entrapped marker at pH 7.4 in eqi-osmolar PBS [15,16]. All vesicles examined showed a similar trend; that is, increased CF leakage with increased temperature. In all cases although leakage at 4°C and 22°C (room temperature) were similar, a significant increase was apparent at 370 C and 500C and this was most pronounced at lower incubation periods, for example, after I hour, as in graph 12, graph 13. Addition of cholesterol (50 mol%) significantly decreased temperature induced leakage for both surfactant I and III vesicles, as in graph 16.

Factors Affecting Plasma-Vesicle Interaction

Plasma protein-vesicle interaction is dependent on a number of factors which all relate to the "quality" of the lipid-water interface. A smooth, planar, homogeneous membrane cannot readily be penetrated by proteins; whereas any irregularity, such as the existence of phase boundaries or a high radius of curvature of the bilayer may effectively facilitate penetration [17]. Structural defects are also introduced into the bilayer on Sonication below the TC of the membrane components. This results in the formation of a vesicle with a rough surface [18].

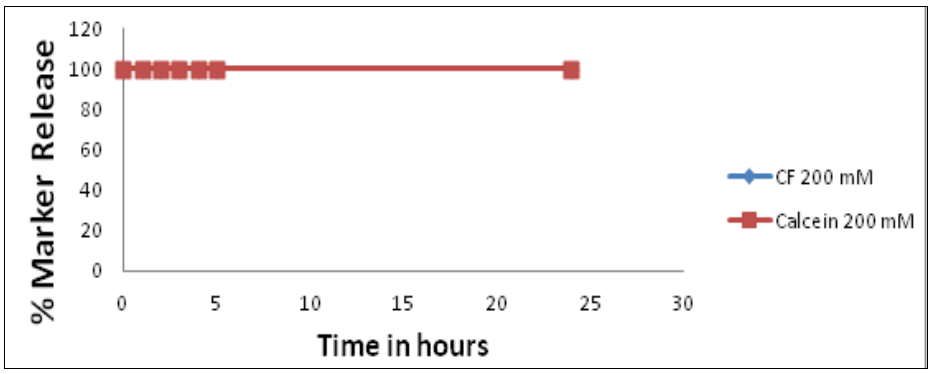
Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was used to provide more detailed information of the adsorbed proteins on vesicles after incubation with plasma. In this study gel electrophoresis as performed to investigate the nature of proteins bound or associated with different types of vesicle, under specific conditions. Three factors were studied:

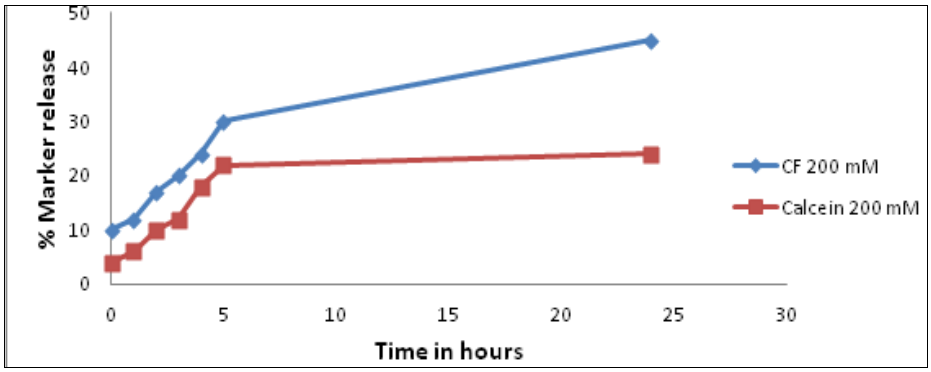
a) vesicle type and adsorbed protein;
b) effect of plasma concentration on the protein coat of vesicles; and
c) times of incubation.

A variety of vesicle types were incubated with plasma (10% and 100%) at various time intervals; 1, 2 and 24 hours, centrifuged at low speeds (1000g) and compared using gel electrophoresis. The gels were stained, dried and photographed as described.

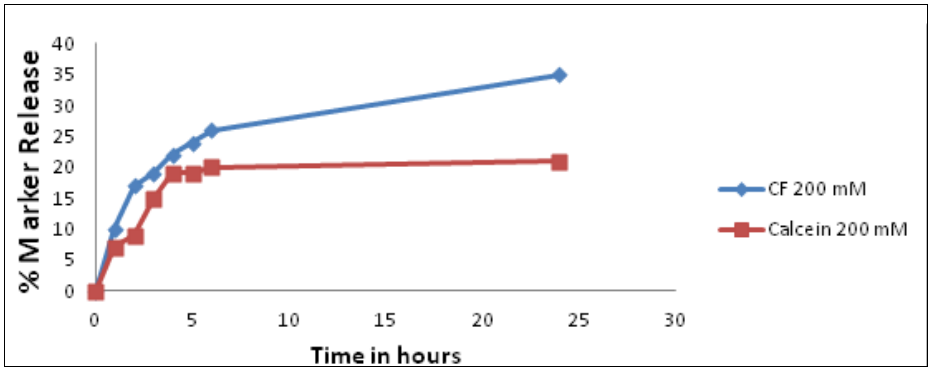
The adsorption of plasma protein to 3 different formulations of niosomes is illustrated in Fig. 1. This figure shows an increase in adsorption of proteins to all 3 types of vesicles with increasing time of incubation. Thus protein uptake by these vesicles appears to be time dependent.



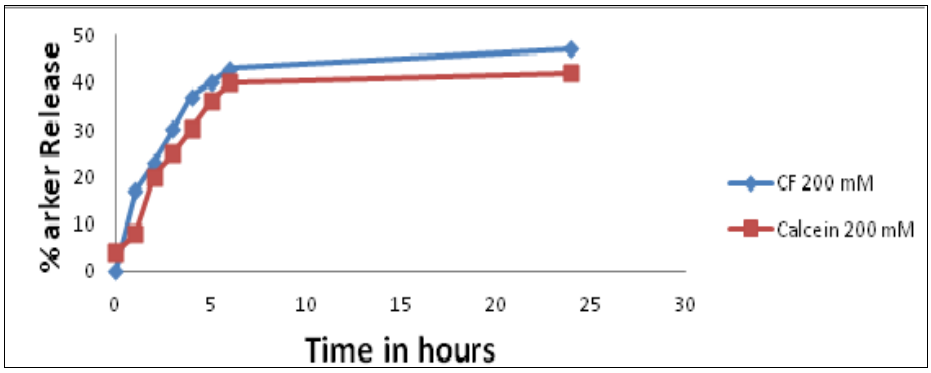
Graph 4: Release from niosomes at pH 2.0 containing CF & calcein as entraped marker



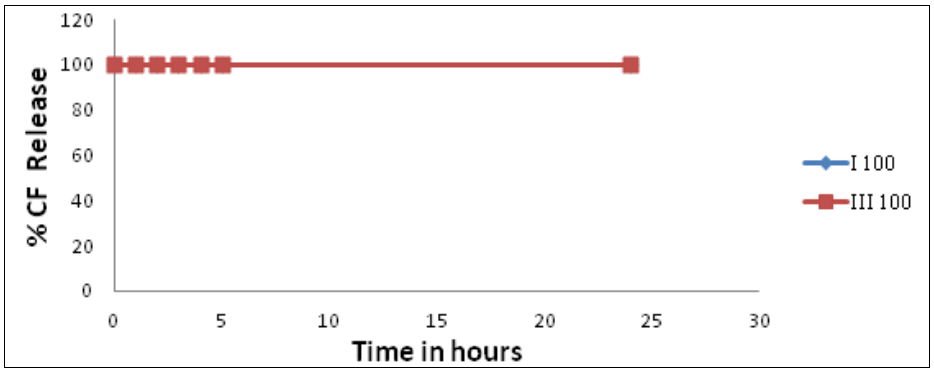
Graph 5: Release CF & calcein from surfactant I with added cholesterol at pH 4.0



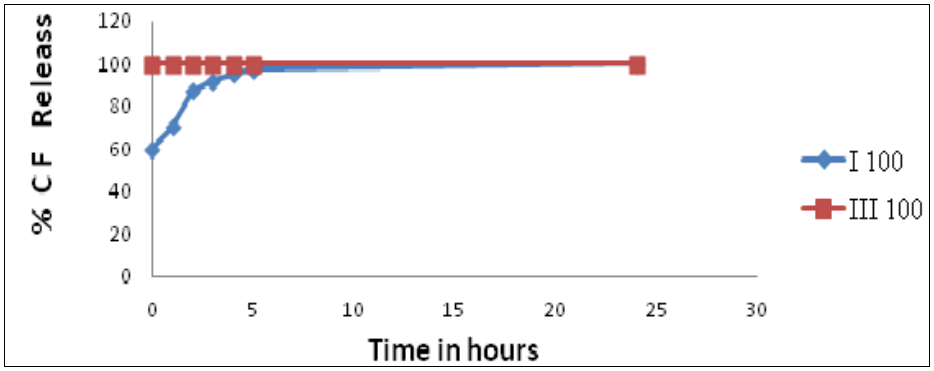
Graph 6: Release of marker from niosomes entraped with CF & calcein at 200 mM at pH 6.0



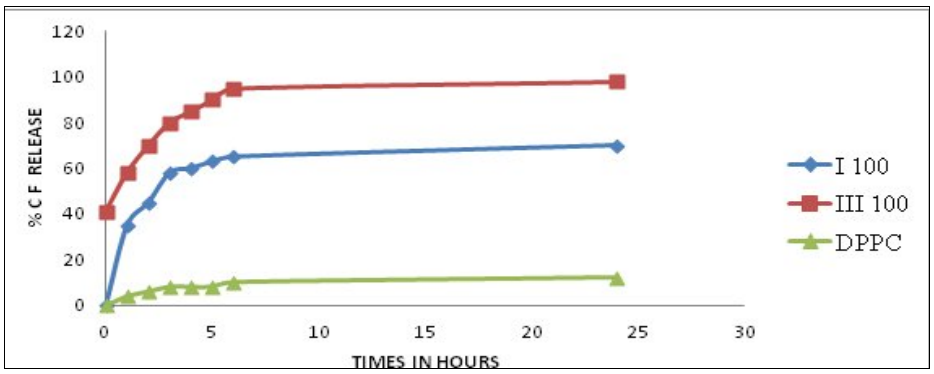
Graph 7: Release at pH 8.0 within niosomes of entraped CF & calcein used as marker



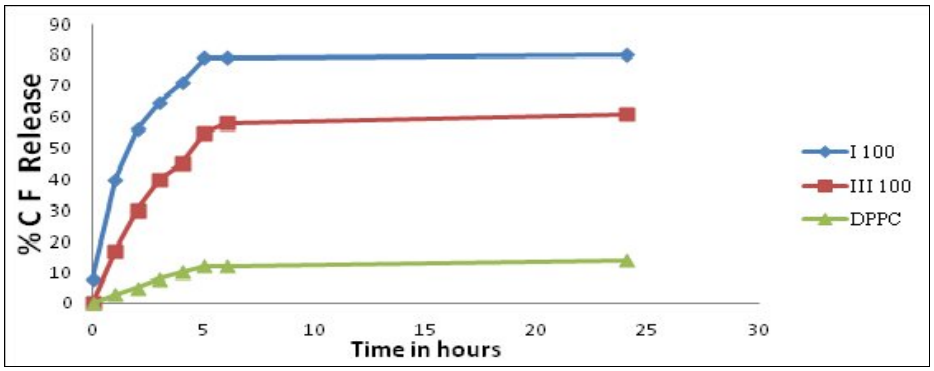
Graph 8: CF release from niosomes I 100 & I II 100 at pH 2.0



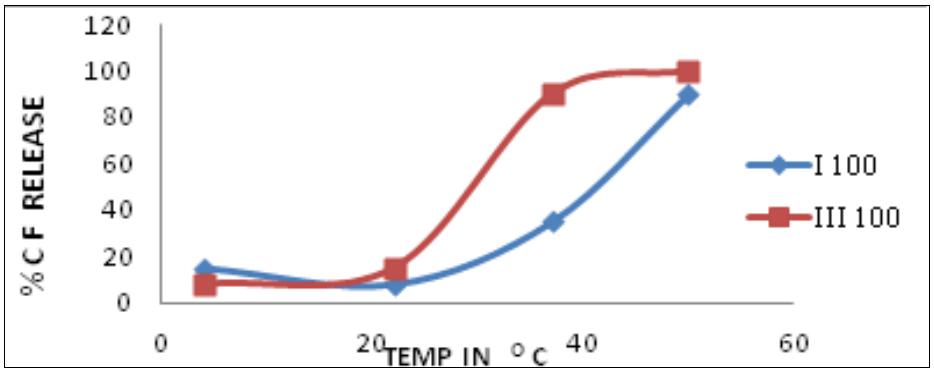
Graph 9: CF release from niosomes I 100 & I II 100 at pH 4.0



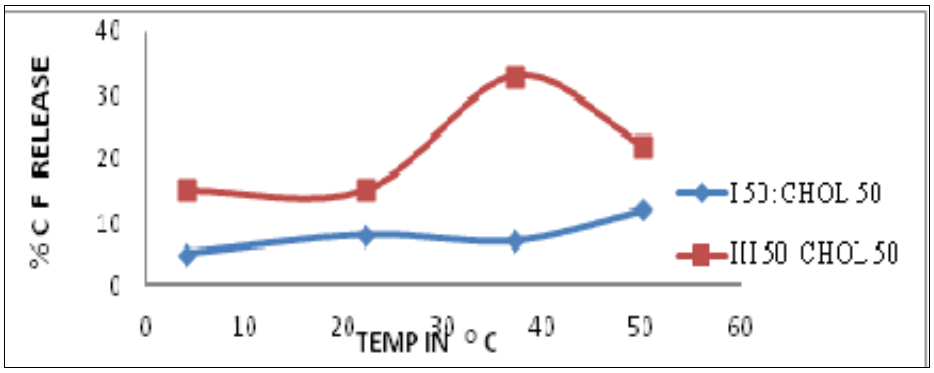
Graph 10: CF release from niosomes I 100 & I II 100 & liposomes DPCC100 at pH 6.0



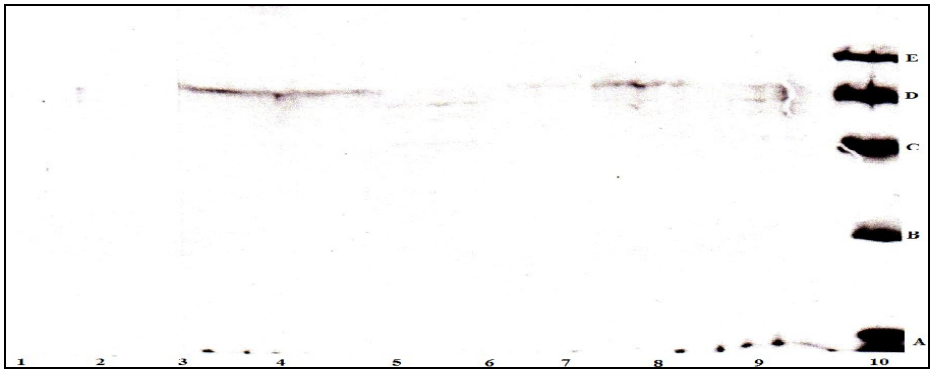
Graph 11: CF release from niosomes I 100 & I II 100 at pH 8.0



Graph 12: CF release from niosomes at various temperature after 1 hr for surfactant I, II & III (100%)

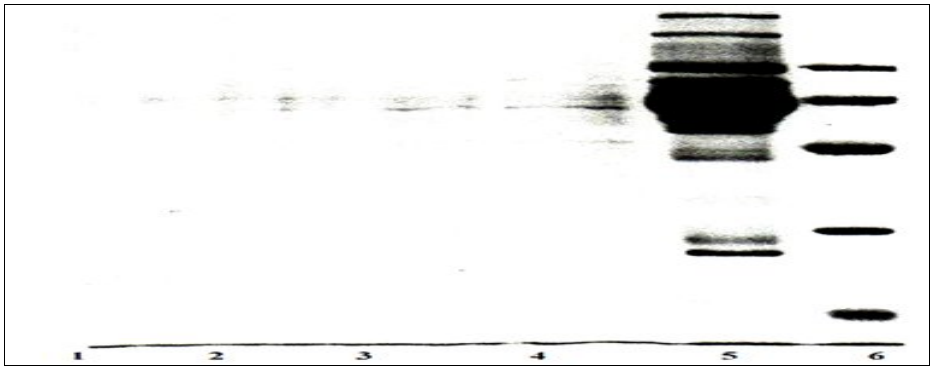


Graph 13: CF release from niosomes at various temperature after 1 hr containing cholesterol



A) Soyabean trypsin inhibitor (20000 Dalton); B) Carbonic anhydrase (30000 Dalton); C) Ovalbumin (43000 Dalton); D) Bovine serum albumin (67000); E) Phosporylase B (94000)

Figure 1: Niosomes II50:CHOL50 (1, 2, 3) III50:CHOL50 (4, 5, 6) & II50:CHOL50 (7, 8, 9) after incubation in plasma for 1hr (1, 4, 7) 2hrs (2, 5, 8) & 24 hrs (3, 6, 9) lane 10 are of marker proteins



Lane 1 & 2-liposomes types DPPC50:CHOL50; Lane 3 & 4-niosomes type III50:CHOL50; Lane 5-10% plasma; Lane 6-Marker

Figure 2: Incubation in 10% plasma for 24 hrs

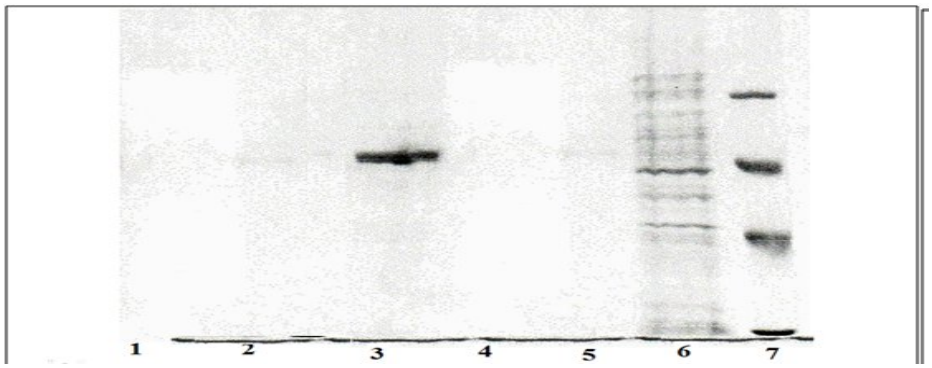


Figure 3: Effect of plasma concentration 10% (lane 1, 2, 3) & 100% (lane 4, 5, 6) & incubation time 1hr (lane 1 & 4) 2 hrs (lane 2 & 5) & 24 hrs (lane 3 & 6) on niosomes 150:CHOL50, lane 7 is marker

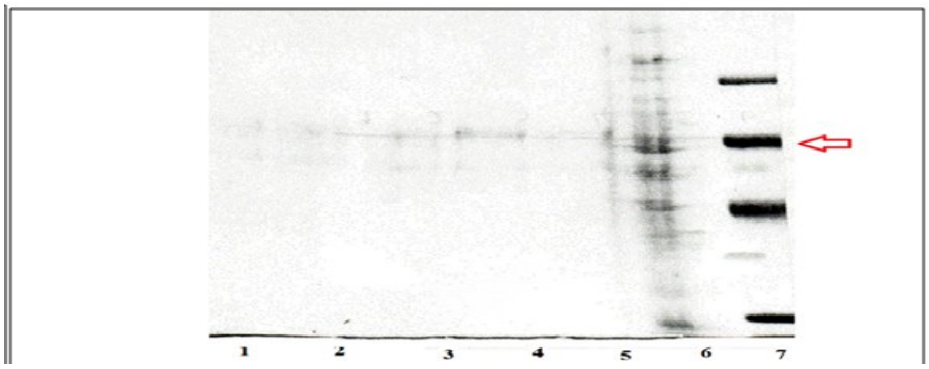


Figure 4: Niosomes III50:CHOL50 after incubation in 10% plasma (1, 2, 3) & 100% plasma (4, 5, 6) for 1hr (1, 4) 2 hrs (2, 5) & 24 hrs (3, 6) lane 7 shows the marker. The arrow denots position of albumin

Fig. 2 compares niosomes, III50:CHOL50, with liposomes, DPPC50:CHOL50, after incubation in plasma (10%) for 24 hours. The bands appear at similar locations to those observed previously indicating that the same types of proteins are adsorbed to both these vesicles. However, for the same quantity of vesicles and plasma proteins, niosomes, III50:CHOL50, show darker bands (lane 3 and 4, Fig. 2) than liposomes (lanes 1 and 2, Fig. 2), indicating the presence of more protein on the gel associated with the niosomes.

The effect of incubation times in plasma (10% and 100%) is shown in Fig. 3, and 4, for two separate vesicles. Both types of niosomes, I50:CHOL50 and III50:CHOL50, show an increase in adsorbed proteins with time and amount of plasma, that is, 10% and 100%. Again III50:CHOL50 vesicles, as in Fig. 4, showed a greater amount of protein adsorbed than I50:CHOL50 vesicles, Fig. 3, for reasons similar to above. There was an increase, in protein concentration and the number of different proteins adsorbed with time

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CONCLUSION

A study of niosomes, prepared from three different non-ionic surfactants, has been compared to liposomes. The niosomes have been fully characterized in terms of their composition, stability and behavior in a variety of systems, by analogy to the well-documented liposome delivery system. The ability of these bodies to interact with biological fluids has been studied thoroughly.

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