Formulation And characterization of Niosomes Using Herbal Extract For Immunity Booster.

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Abstract:

Niosomes composed of non-ionic surfactant vesicles they are prepared by hydrating mixture of cholesterol and non-ionic surfactant. The basic component of drug delivery systems is an appropriate carrier that protects the drug from rapid degradation or clearance and thereby enhances drug concentration in target tissues. The release kinetics was found to be first order initially followed by mixed order. More drugs were released from charged niosomes than the neutral niosome's. A prolonged duration of response was observed during in vivo studies of niosome formulation. Primary chemical constituents amino acid, protein, carbohydrate. Secondary chemical constituent alkaloids, glycosides, tannins, flavanoids, terpinoids etc. Positively charged niosome showed greater duration of action (DR 468min) and AUC (23.5) compared to negatively charged niosome's (DR 396 min) and AUC (20.8) The results obtained are in accordance with the results obtained by Schaeffer. In the drug entrapment 48.08%, particle size of the niosomes 6.7±0.062, and invitro release study shows 92.88% which follows the Zero order and pepass order of reaction.

Keywords: Herbal Niosomes, Encapsulation herbal extract, Innate immunity, Drug entrapment,. Lipid film method.

Introduction

In the year 1909 the researcher name Paul Ehrlich started the work of establishment of targeted delivery when he thought that a Drug Delivery mechanism that would target directly to infective cells. We will now study what is drug targeting.[1] Immune System Is a Network of Cells,Tissues and Organs that work together to Protectbody from Infection and viral effect. In the environment for many microbes, such as viruses,bacteria,fungi, and parasites and the immune system prevent sand limits their entry and growthto maintain optimal health.[2] Biological products and animals and plant sources havebeen used by human thousands of years either in the pure formor crude extracts to treat muchdisease. Herbs are used as bases of medicine in many ways inhuman being in their life. Research interests have focused on various drugs that possess immune stimulating properties asuseful features in helping diminish the risk of cancer.[3] In a different herb, a wide ranging of phytochemical have been identified such as the flavonoids, lignans, terpenoids, polyphenolics, Sulfides, saponins, carotenoids, Cummins, plant sterol and phthalides. “Immune boosting” is a trending topic correlated with the corona virus pandemic, appearing long side numerous perculative cures, treatments, and preventative strategies.[4] There is no current evidence that any product or practice will contribute toenhanced “immune boosting” protection against COVID-195. The photo-andvideo-sharing social networking service owned by has over 500 million users active daily[6-7]. Immune booster allowusers to categorize their posts and to search for topic-related content. Research has shown increasingly commercialized, generating profits for both companies and users. “Attention economy”, A good Niosome formulation of clinical utility must fulfill twoimportant criteria. The Niosomal drug carrier must incorporate CPT in the Niosomal bilayer in arelevant therapeutic concentration and be able to retain the drug within the noisome.[8] The 1,2-dioleoyl-3-trimethyl ammonium-propane containing formulations exhibited as well a trend toward higher retention ability inserum compared to theother formulations.[9] Although they showed better retention ability, only 25 % of the drug was associated with the Niosomes, which is far from being optimal. One of theimportant criteria mentioned above for Niosomes as drug delivery systems is their ability toremain stable in blood circulation for prolonged time in order to reach the specific target and to avoid rapid clearance.[10]

ROLE OF MEDICINAL PLANT IN IMMUNITY

The human history that medicinal plants have been the treatment regimento cure variety ofdiseases, including diseases caused by insects, fungi, bacteria ,and viruses. The effects shown bythe plants are due to the chemicals present in them and they work in the same manner as theconventional

drugs. However, there are equally chances for theseplant to have same potential harmful and toxic effect also. The term immunity defines body's natural defense system against disease and disorders. The complex immune system is capable to generate a limitless variety of cells and molecules to arrest enormous spectrum of infections and undesirable substances.[12]

TYPES OF IMMUNE SYSTEM

Innate immunity

Innate immunity refers to nonspecific defense mechanisms that come into play immediately or within hours of an antigens 'appearance in the body.[12] These mechanisms include physical barrierssuch as skin chemicals in the blood, and immune system cells that attack foreign cells in the body.

Adaptive immunity

The adaptive immune system, also referred as the acquired immune system, is a sub system of the immune system that is composed of specialized, systemic cells and processes that eliminatespathogens by preventing their growth.

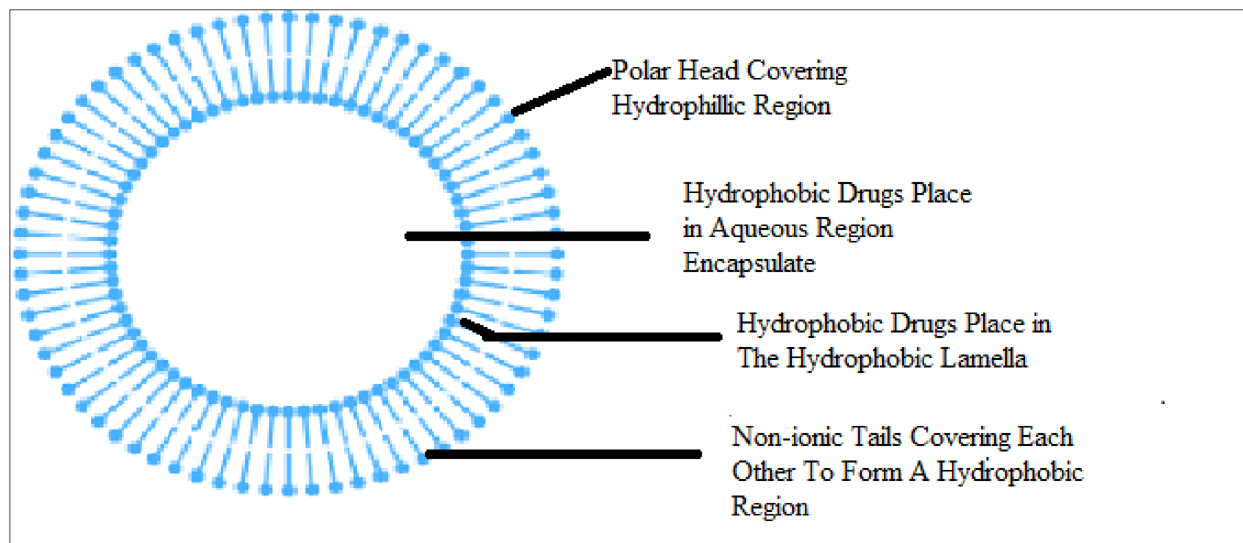


Fig.No.1: Structure of Niosomes

MATERIALS AND METHODS

Ashwagandha, Giloy and Tusi Fresh leaves were dried at 35° C for 48 hours and powdered using electric mixer grinder and stored in a desicator. Care was taken to avoid fungal contamination while drying.

Fig.2:Giloy leaves

Fig.3: Tulsi Leaves

Fig.4: Ashwagandha

PHYTOCHEMICAL SCREENING

Phytochemical screening was carried out to investigate the various classes of natural compounds present in the extract. Phytochemical tests were performed for primary and secondary chemical constituents.[13]

Primary chemical constituents:-Amino acid, protein , carbohydrate.

Secondary chemical constituents:-Alkaloids, glycosides, tannins, flavanoids, terpinoids etc.

Qualitative profiling*:* extract of *Giloy, Tulsi, Ashwagandha* was used for qualitative assessment for the major classes of phytochemicals namely steroids ,tannin, acids, esters, terpenoids, alkaloid, carbohydrate and protein etc. The tests were performed according to various standard methods. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents as shown in the following table.

Tablen- 1. Phytochemical constituents of *Giloy, Tulsi and Ashwagandha*

Sr.No.	Chemical Test	F1	F2	F3
1.	Carbohydrates	-	+	+
2.	Proteins	+	+	+
3.	Amino acids	+	-	-
4.	Alkaloids	+	+	+
5.	Glycosides	-	+	+
6.	Tannins	-	+	+
7.	Flavonoids	+	-	+
8.	Saponins	+	+	-
9.	Steroids	+	+	-
10.	Terpenoids	-	+	+

(F1)Water extraction(F2)Ethanol extraction (F3)

Hydroalcohalic(+)present.(-)Absent

Preparation of Niosomes

Preparation of MLVs using thin lipid film method:

Stock solution containing phosphatidyl choline and cholesterol in 10:4 molar ratios were prepared chloroform. Appropriate volume of these solution and 10 g glass beads were transferred to a 250ml round bottom flask and attached to the rotary vacuum evaporator. The flask was kept immersed in at her most at water bath, with the temperature set at 300and rotated at about 100 rpm. Process was allowed to continue till all the liquid had evaporated from the solution and a dry lipid film had deposited on the wall of the flask. Flask was rotated under vacuum for another 15 min and then flushed with nitrogen to remove the last traces of solvent. Aqueous phase (5mlcontaining drug) was added to the flask and the flask was rotated with the same speed as before for 30 min or until all the lipid had been removed from the wall of the flask. The suspension was allowed to stand for an optimized period of 2 h at room temperature in order to complete the hydration.

Incorporation of charged species:

The inclusion of negatively charged lipid such as dicetyl phosphate or positively charged surfactant such as stearyl amine tend to increase the inter lamellar repeat distances between successive bilayers in the MLV, swelling the structure with the greatest proportion of the aqueous phase. These effects lead to a greater overall entrapped volume. Hence two batches of ni somes were prepared containing phosphatidyl choline, cholesterol, stearylamine and phosphatidy choline, cholesterol and dicetyl phosphate in the ratio 10:4:1 and percent drug incorporation was calculated. Antioxidant such as 1mol %α- tocopherol was used to prevent peroxidation of lipid during sonication of MLVs.

Size reduction of MLVs system:

Vibronics-250W probe type ultra sonicator was used for size reduction of MLV dispersion. Sample (5 ml) was placed in a 50 ml beaker and the probe was dipped into it. The process was carried out at low temperature using ice bath. Total ultra-sonication period was 3 min including intermittent stoppage of 30s.

Separation of non-entrapped drug:

Sample (4 ml) was placed in ultracentrifuge tubes at 20,000 rpm. Ice cold water was added to enhance centrifugation and mixture was centrifuged for 20min. Solid particles left in the tube was collected and suitable volume of aqueous medium was added. Nitrogen gas was flushed to avoid per oxidation of lipids.

Niosomes size and stability

Niosomes are formed on the admixture of non-ionic surfactant of the alkyl or dialkylpolyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Niosomes may be unilamellar or multilamellar depending on the method used to prepare them. The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle while the hydrophobic chains face each other within the bilayer. Hence, the vesicle holds hydrophilic drugs within the space enclosed in the vesicle while the hydrophobic drugs are embedded within the bilayer itself. The application of niosomal technology is widely varied and can be used to treat a number of diseases. One of the most useful aspects of niosomes is their ability to target vaccines and drugs to the reticulo-endothelial system. The reticulo-endothelial system (RES) preferentially takes up niosomal vesicles. The uptake of niosomes is controlled by circulating serum factors called opsonins, which mark the niosomes for clearance while delivering the cargo to the antigen presenting cells Multilamellar vesicles (MLV) were prepared using a technique based on lipid hydration method. Briefly, Span 20, cholesterol and dicetyl phosphate in a molar ratio of (10:10:1) were weighed and dissolved in chloroform/methanol system (2:1) in a 100 ml round bottom flask. The solvent mixture was evaporated to obtain a thin dry film. The film was hydrated with 2 ml of PBS pH 7.4 containing 0.2 ml/dose of the Newcastle disease vaccine at 4o with gentle shaking during which MLVs were formed. The vesicles were allowed to anneal for 30 min Different molar ratios of Span 20-based niosomes were evaluated for vaccine entrapment efficiency. Non-entrapped antigen was separated from vesicle entrapped antigen by centrifugation for 10 min at 3000 rpm. The free (un entrapped) antigen was determined in the supernatant by haem agglutination test.

Table no 2. : Composition of Formulation by Varying Drug To Surfactant Ratio

Formulation	Herbal Extract (Giloy, Tulsi, Ashwagandha)	surfactant	Cholesterol	Solvent (chloroform)	Buffer
F1	20 ML	20 mg	12 mg	7:2	5 ml
F2	20 ML	30 mg	10 mg	7:1	5 ml
F3	20 ML	40 mg	10 mg	7:0	5 ml
F4	20 ML	50 mg	9 mg	7:3	5 ml

EVALUATION OF NIOSOMES

Drug entrapment efficiency of Niosomes

Entrapment efficiency of Niosomes was determined by centrifugation method. Aliquots (1 ml) ofliposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 3500 rpm for a period of 90 min. The clear supernatants were removed carefully to separate non entrapped and absorbance recorded at 245nm. The sediment in the centrifugation tube was diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance of this solution was recorded at 245 nm. % entrapment of drug was calculated by the following formula

$$\%\text{Drug Entrapment} = \frac{\text{Drug Loading}}{\text{Theoretical Drug loading}} * 100$$

Table No 3 : Results of entrapment efficiency of Niosomes of formulations

Dissolution Batch Code			
Sr.No	**F1**	**F2**	**F3**
1	48.23	47.62	47.47
2	47.38	48.70	49.15
3	49.73	46.80	47.63
Mean	48.33	47.71	48.08
Mean ± S.D.	48.33±1.000	47.70±0.566	48.09±1.545

Now, let H_0 be the hypothesis that there is no significant difference between the batches.

Particle size analysis:

All the prepared batches of Niosomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined.

Vesicle shape

Vesicle shape of the prepared formulation was found to be spherical from the SEM (scanning electron microscope) analysis at 15.00kV

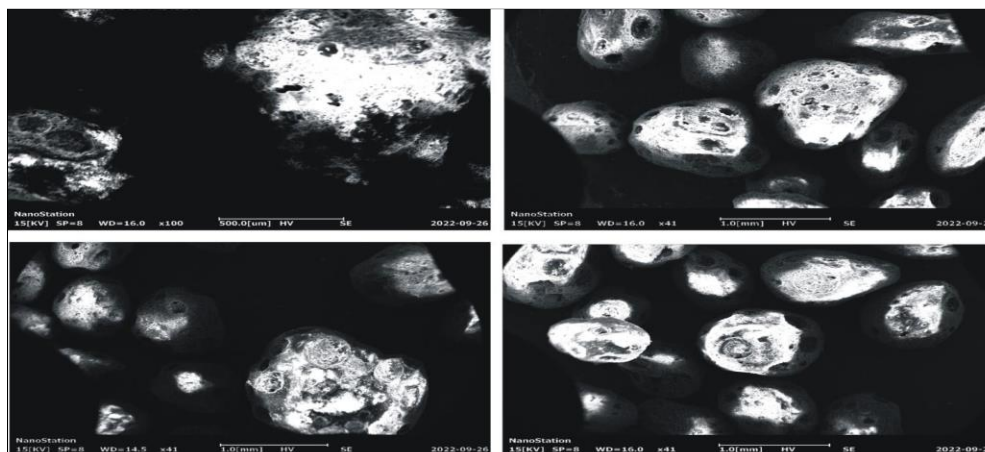


Fig. No.5: Particle size of Prepared Niosomes

VESICAL SIZE

Table no 4: Vesicle size

Sr.no	Formulation	Size (µm)
1	F1	144
2	F2	152
3	F3	269

Table No 5: Results of particle

Sr.No	F1	F2	F3
1	6.43	7.22	6.08
2	7.15	6.74	7.20

size of Niosomes

3	6.62	7.43	6.70
Mean	6.72	7.22	6.5
Mean ± S.D.	6.76±0.097	7.24±0.050	6.7±0.062

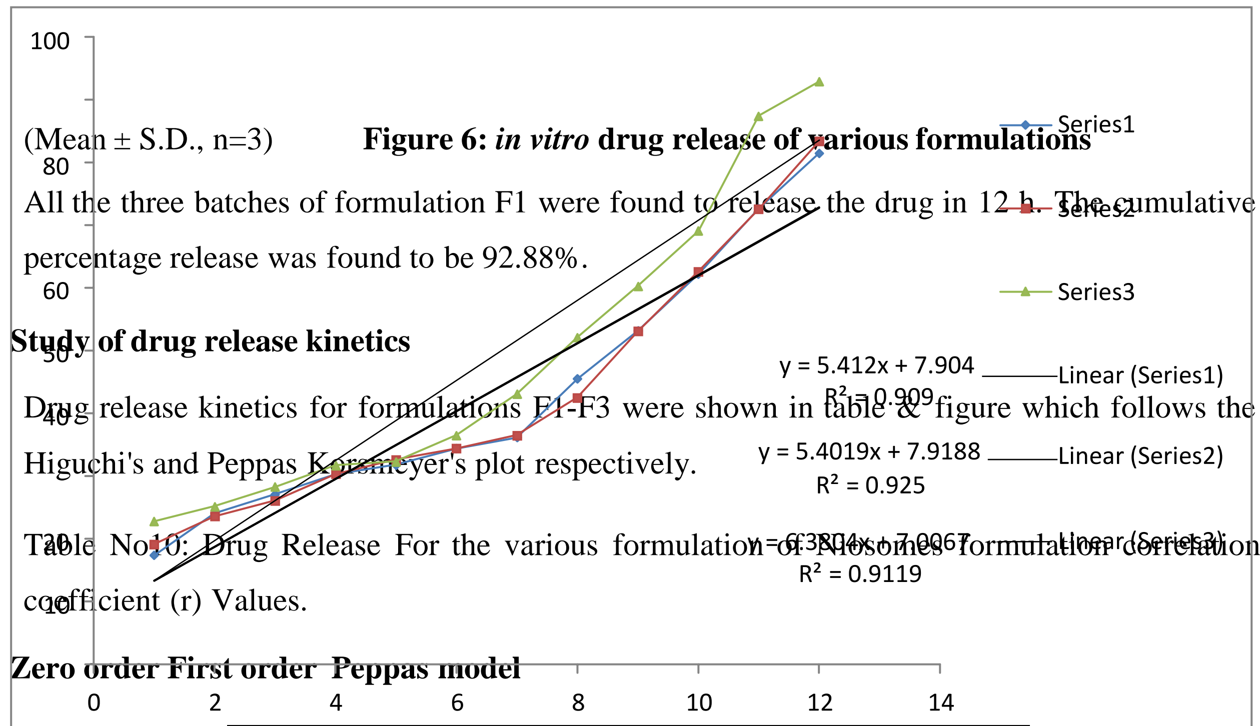
Now, let H0 be the hypothesis that there is no significant difference between the batches.

***In Vitro* Drug release study**

The release studies were carried out in 250 ml beaker containing 100 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 250 ml beaker. The beaker was assembled on amagnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 5ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Table 6: Cumulative percentage drug release from various formulation of Niosomes

Time (HRS)	**Batch**	**code**	
0	**F1**	**F2**	**F3**
1	17.38	19. 17	22.80
2	24.07	23.63	25.18
3	27.14	26.10	28.32
4	30.32	30.35	31.72
5	31.85	32.62	32.35
6	34.41	34.44	36.54
7	36.14	36.53	43.09
8	45.52	42.51	52.12
	53.21	53.10	60.26
10	62.20	62.61	69.10
11	72.65	72.58	87.39
12	81.48	83.38	92.88



(Mean ± S.D., n=3) **Figure 6: *in vitro* drug release of various formulations**

All the three batches of formulation F1 were found to release the drug in 12 h. The cumulative percentage release was found to be 92.88%.

Study of drug release kinetics

Drug release kinetics for formulations F1-F3 were shown in table & figure which follows the Higuchi's and Peppas Korsmeyer's plot respectively.

Table No 10: Drug Release For the various formulation of Niosomes formulation correlation coefficient (r) Values.

Zero order First order Peppas model

Sr.No	Batch Code			
1	F1	0.8350	0.9156	0.9420
2	F2	0.8623	0.8632	0.9217
3	F3	0.7965	0.8722	0.9422

Table.7: order of reaction

The correlation coefficient (r) values showed that formulations follow Pepas

Reference

1. TheKeyRoleOfTumor-SelectiveMacromolecularDrugTargeting.Adv.EnzymeRegul.,41,189
2. Aulton, M. E. Aulton's Pharmaceutics: "The Design And Manufacture Of Medicines, Edinburgh", *Churchill Living stone*. 2007.
3. Bishayi B, Roychowdhury S, Ghosh S, Sengupta M. "Hepatoprotective and Immuno modulatory effects of Tinospora cordifolia in CCl4 Intoxicated Mature Albino Rats". *J Toxicol Sci* 2002;27(3):139-46
4. Brgles,M.,Jurasin,D.,Sikiric,M.D.,Frkanec,R.& Tomasic, J. "Entrapment of Ovalbumin In to Liposomes- Factors Affecting Entrapment Efficiency, Liposome Size, And Zeta Potential". *J. Liposome Res*., 2008, 18, 235-248.
5. Burke, T. G. & Bom, D. "Camptothecin Design And Delivery Approaches For Elevating Anti-Topoisomerase Activities In Vivo". *Ann. N.Y. Acad. Sci*., 2000, 922, 36-45.
6. Fahr, A., Van, H. P., Kuntsche, J. & Leigh, M. L. S. "Lipophilic Drug Transfer Between Liposomal And Biological Membranes: What Does It Mean For Parenteral And Oral Drug Delivery" *J.LiposomeRes*.,2009, 16,281-301.
7. Flaten, G. E. Incorporation Of Camptothecin In Liposomes, Method Development And in corporation Efficacy Screening Using Different Liposome Formulation. Master Thesis, University of Tromson. 2003.
8. Ingebrigtsen, L. "Size Analysis Of Submicron Particles And Liposomes By Size ExclusionChromatography And Photon Correlation Spectroscopy Thesis For The Degree CandidatusPharmaciae", University Of Tromson. 2008
9. Labhasetwar, V., Mohan, M. S. & Dorle, A. K. 1994. A Study On Zeta Potential And DielectricConstantOfLiposomes.J.Microencapsulation,11,663-8.
10. Maurer,N.,Fenske,D.B.&Cullis,P.R.2001.DevelopmentsInLiposomalDrugDeliverySystems Med.Chem.,13,2021-2039.
11. Zou, J.; et al. Naturally occurring anti-cancer compounds: Shining from Chinese herbal medicine. Chin. Med. 2019, 14, 1–58.
12. Azmin MN, Florence AT, Handjani-villa RM, Stuart JEB, Valerberghe G, Wittaker JS. The effect of non ionic surfactant vesicle (noisome) entrapment on the absorption and distribution of methotrexate in mice. J Pharm Pharmacol. 1985;37:237–42

13. Handjani-Vila RM, Ribier A, Rondot B, Vanlerberghe G. Dispersions of lamellar phases of non-ionic lipids in cosmetic products. Int J Cosmetic Sci. 2003;1:303–14