



Formulation Development and Evaluation of Allicin loaded Niosomes

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Abstract

Allicin is a potent bioactive compound derived from Garlic, with diverse biological properties and potential health benefits. The biological properties of allicin, such as its antimicrobial effects against bacteria and fungi, as well as its antioxidant capabilities in scavenging reactive oxygen species, underscore its potential in promoting human health. As scientific understanding deepens and research progresses, allicin may become an essential component in the development of novel therapeutics and preventive measures, offering a natural and versatile solution to various health challenges. From ancient topical cream formulations to novel topical drug delivery methods, the pharmaceutical industry has undergone a revolutionary transformation. In the present paper Allicin loaded niosomes were formulated by using thin film hydration technique and the different nonionic surfactants (span 20, span 60, tween 40 & tween 60) grades in different drug:surfactant: Cholesterol ratios as 1:1:1, 1:2:1, 1:1:2 and was further evaluated.

Key-words: Allicin, Niosomes, Formulation

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Introduction

Drug delivery system ensures the reach of adequate amount of drug to the targeted part of the body. It also ensures therapeutic response, while minimizing the adverse effects of the drug. Topical drug delivery system is a localized drug delivery system that involves introduction of drug on any surface of the body, from where, the drug can be absorbed and produce action. Skin, vaginal, rectal and ophthalmic are some of the routes for the topical delivery of the drugs. [1] Traditional and conventional medicinal approaches have certain limitations, such as, drug degradation and loss, harmful adverse reactions, lesser bioavailability and, accumulation of adequate amount of drug at the required target area. These limitations can be addressed by novel drug delivery

systems (NDDS). NDDS is presently used widely in allopathic and other medicinal systems. Integrating the knowledge of traditional herbal medicines and NDDS concepts can provide safer and more effective formulations for the disease. [2]

Due to presence of excellent barrier, active principles cannot easily pass through the skin. Nanoparticle and nanocarriers are developed to address this problem. These nanoformulations include solid nanoparticles, nanoemulsions, polymeric nanoparticles, ethosomes and niosomes. [3] Due to their small particle size feature, it provide better drug targeting, specificity and retention. It allows passage of macromolecular drugs through skin barrier in painless way and without disturbing skin barrier function. [4]

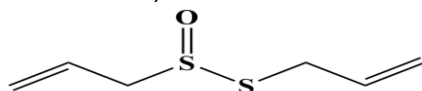


Liposomes and niosomes are novel drug delivery systems that enhances permeation of drugs across skin. In addition, proliposomes and proniosomes are also developed that provides advantage of better stability and can be converted into liposomes and niosomes respectively on hydration. [5]

Phytobioactive compounds are secondary metabolites present in abundant quantity in medicinal plants, and contributes in adaptation of plants to the environment. These phytobioactive compounds also possess high therapeutic potential, and are used by mankind as remedies for numerous diseases from time immemoria. Phytobioactive compounds with excellent antifungal properties are have already been reported. Development of drugs using these phytobioactive compounds can be possible. Polyenes and echinocandins are two of the major classes of antifungal drugs, that are derived from natural products. Griseofulvin,

undecylenic acid, flucytosine and allylamines are few more natural or naturally-derived antifungal compounds that are already approved by the FDA. [6-8]

Allicin (**Fig. 1**) is a sulphur containing compound, commonly present in Garlic as a defence molecule. Along with other useful pharmacological activities, allicin was found to be a potent antifungal agent against *Candida* infections. Group of researchers used pure allicin derived from garlic and confirmed its activity against dermatophytes. Another research demonstrated antifungal effects of allicin against phytopathogenic fungi, such as, *Fusarium oxysporum*, *Phytophthora casici*, *Verticilium dahlia* and *Botrytis cinerea*. Allicin was found to be responsible for the accumulation of reactive oxygen species and disturbing biosynthesis of cell walls of fungal species, thus showing a cidal effect. [9-12]



3-prop-2-enylsulfanylprop-1-ene

Figure 1: Molecular structure and IUPAC name of Allicin

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Material and Methods

Selection and Procurement of Phytobioactive Compounds

The anti-fungal phytobioactive compounds i.e., Allicin (diallyl thiosulfinate) was selected for the present investigation. The sample was obtained as gift sample from the Care Medicine, Indore

Methodology

Development of Niosomes

Selection of surfactants for niosomes formation

The different nonionic surfactants viz., span 20, span 60, tween 40 & tween 60 grades was selected for present study.

Preparation of Drug Loaded Niosome

Allicin loaded niosomes were formulated (table 1) by using thin film hydration technique and the different nonionic surfactants (span 20, span 60, tween 40 & tween 60) grades in different drug: surfactant :Cholesterol ratios as 1:1:1, 1:2:1, 1:1:2. Accurately weighted quantities of surfactant and Cholesterol were dissolved in 5 ml chloroform using a 100 ml round bottom flask. The lipid solution was evaporated by rotary shaker. The flask was rotated at 135 rpm until a smooth and dry lipid film was obtained. The film was hydrated with 5 ml phosphate buffer saline (PBS) of pH 7.4 containing drug for 3 hours with gentle shaking. The niosomal suspension was further stabilized by keeping at 2-8°C for 24 hours.

Table 1: Formulation and Composition of Niosomal of Allicin

Formulation Code	Surfactant used	Allicin: Surfactant:Cholesterol Ratio	Solvent	Weight taken (mg)
AN1	Span 20	1:1:1	Chloroform	100:100:100
AN2		1:2:1	Chloroform	100:200:100



AN3		1:1:2	Chloroform	100:100:200
AN4	Span 60	1:1:1	Chloroform	100:100:100
AN5		1:2:1	Chloroform	100:200:100
AN6		1:1:2	Chloroform	100:100:200
AN7	Tween 40	1:1:1	Chloroform	100:100:100
AN8		1:2:1	Chloroform	100:200:100
AN9		1:1:2	Chloroform	100:100:200
AN10	Tween 60	1:1:1	Chloroform	100:100:100
AN11		1:2:1	Chloroform	100:200:100
AN12		1:1:2	Chloroform	100:100:200

Evaluation of Niosomes

Niosomes formulations were characterized with respect to shape, particle size distribution, entrapment efficiency and *in vitro* release studies.

Vesicle shape and morphology

Shape and morphology of niosomal formulations were determined by optical microscopy and Scanning Electron Microscopy (SEM)⁴⁴ and result were shown.

Particle size

The particle size of the niosomal suspension was determined by optical microscopy. A drop of niosomal suspension was placed on a glass slide. A cover slip was placed over the niosomes suspension and evaluated the average vesicle size by an ordinary optical microscope using a pre calibrated ocular eye piece micrometer.⁴⁵

Entrapment Efficiency

Entrapment efficiency of niosomal formulations was determined by centrifugation method. 10mL niosomal suspension was poured into a stopper test tube and centrifuged by using cooling centrifuge at 10,000 rpm maintained at 4°C for 90 minutes and then filtered by using Whatman filter paper to obtain clear fraction. The clear fraction was used for the determination of free drug by using UV spectrophotometer at 240 nm.⁴⁶ The encapsulation efficiency was calculated using the formula

$$EE (\%) = [(Ct - Cf) / Ct] \times 100$$

Where, *Ct* is concentration of total drug ; *Cf* is concentration of untrapped drug.

Drug Content

Drug content was determined by disrupting the niosomal formulation by propane-1-ol, diluted suitably using phosphate buffer pH 6.8 and analysed for the drug content spectrophotometrically at 240 nm.⁴⁷

In vitro diffusion study

The *in vitro* drug diffusion study was conducted by using Franz diffusion cell assembly. Niosomal formulation was placed on dialysis membrane between donor and receptor compartment of diffusion assembly. The receptor compartment was filled with (Phosphate buffer pH 6.8) which was maintained at 35° ± 1°, magnetically stirred at 50 rpm. The drug content was determined by collecting the receptor fluid (1ml) every h for 24 h, the volume withdrawn was replaced with 1ml of fresh buffer. After suitable dilution, the samples were analyzed spectrophotometrically at 240 nm.⁴⁸

Results and Discussion

Numerous phytoactive compounds offer a potential solution to the challenges of resistance development and toxicity associated with conventional medications. Several of these phytochemicals have already gained clinical approval, while others remain under investigation. The healthcare industry faces a growing challenge of resistance development in fungal species, which could be effectively tackled through the development of novel formulations based on phytoactive principles. Although this process may be challenging and complex, it is crucial for advancing drug development towards safer and improved outcomes. The Allicin loaded niosomes were formulated in different



drug:surfactant:Cholesterol ratios as 1:1:1, 1:2:1, 1:1:2 concentration and was evaluated. The particle size, % EE and drug content of the niosomal formulation of allicin was given in table 2. The results indicate that the particle size was found in between 1.76 to 6.4 μm , % EE in the range of 68.32 to 85.28 and drug content in between 96.39 to 98.85. The results of *in vitro* dissolution profile of niosomes formulation containing allicin was given in table 3.

Conclusion

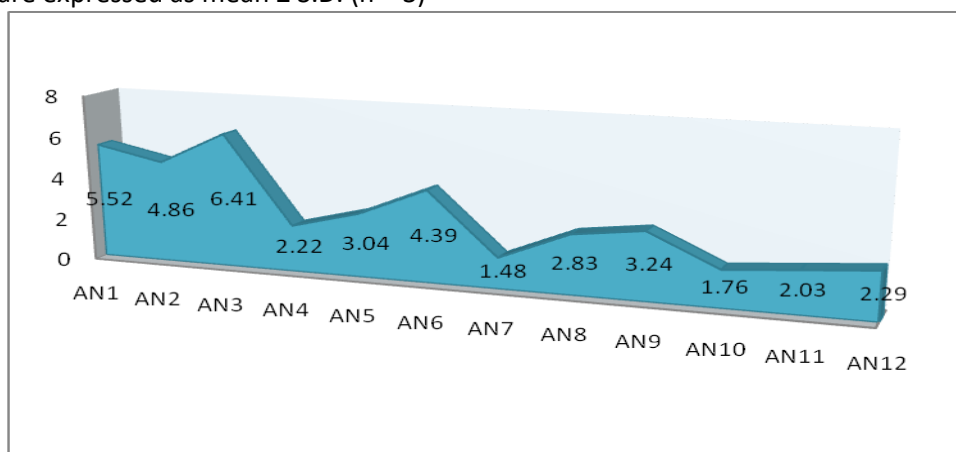
12 batches of allicin loaded Niosomes were prepared (AN1 to AN12) and was evaluated for PS, %EE, DC and % DR and from the results obtained it was concluded that all the formulation code AN8 having drug content and % drug release was found to be 98.85 ± 0.18 and 59.12 respectively. The AN8 having Allicin: Surfactant: Cholesterol Ratio (1:2:1) and surfactant Tween 40 showed better results than other compared formulation.

Table 2: Evaluation Parameters of Niosomal formulation containing Allicin

Formulation Code	Particle size (μm)	EE(%)	Drug content (%)
AN1	5.52 \pm 0.53	68.32 \pm 0.32	96.39 \pm 0.28
AN2	4.86 \pm 0.83	72.39 \pm 0.12	97.29 \pm 0.12
AN3	6.41 \pm 0.70	74.33 \pm 0.29	98.03 \pm 0.10
AN4	2.22 \pm 0.21	69.59 \pm 0.04	96.28 \pm 0.25
AN5	3.04 \pm 0.27	74.42 \pm 0.46	97.10 \pm 0.37
AN6	4.39 \pm 0.05	77.39 \pm 0.02	98.36 \pm 0.24
AN7	1.48 \pm 0.32	82.03 \pm 0.37	98.46 \pm 0.33
AN8	2.83 \pm 0.77	85.28 \pm 0.41	98.85 \pm 0.18
AN9	3.24 \pm 0.54	78.20 \pm 0.29	98.73 \pm 0.97
AN10	1.76 \pm 0.68	72.13 \pm 0.07	97.84 \pm 0.18
AN11	2.03 \pm 0.49	70.29 \pm 0.38	98.32 \pm 0.12
AN12	2.29 \pm 0.39	79.82 \pm 0.22	98.31 \pm 0.32

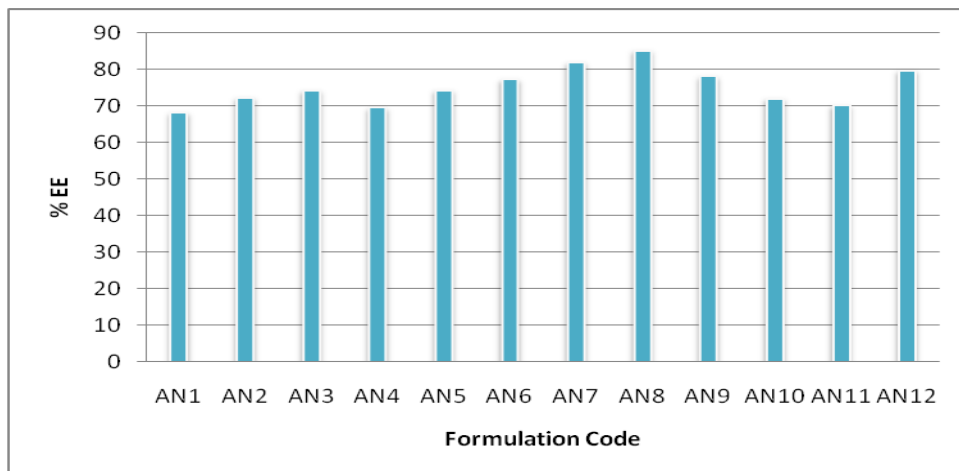
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All reading are expressed as mean \pm S.D. (n = 3)

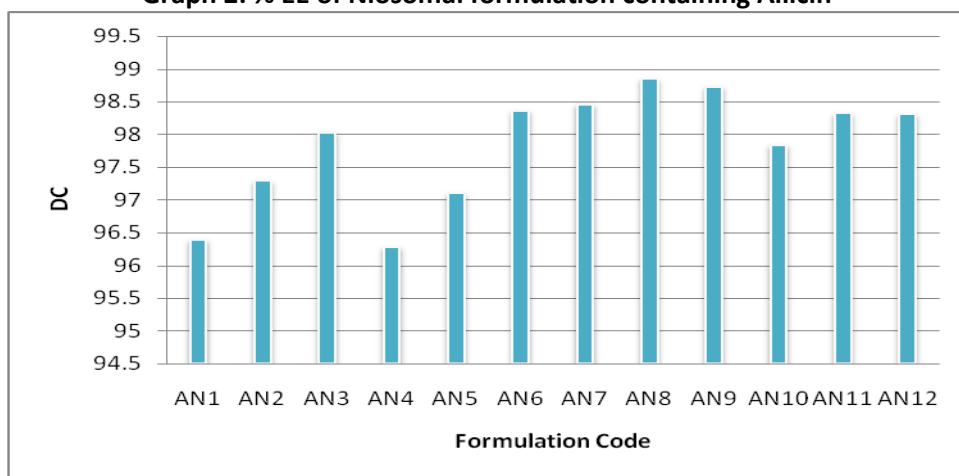


Graph 1: Particle size of Niosomal formulation containing Allicin





Graph 2: % EE of Niosomal formulation containing Allucin



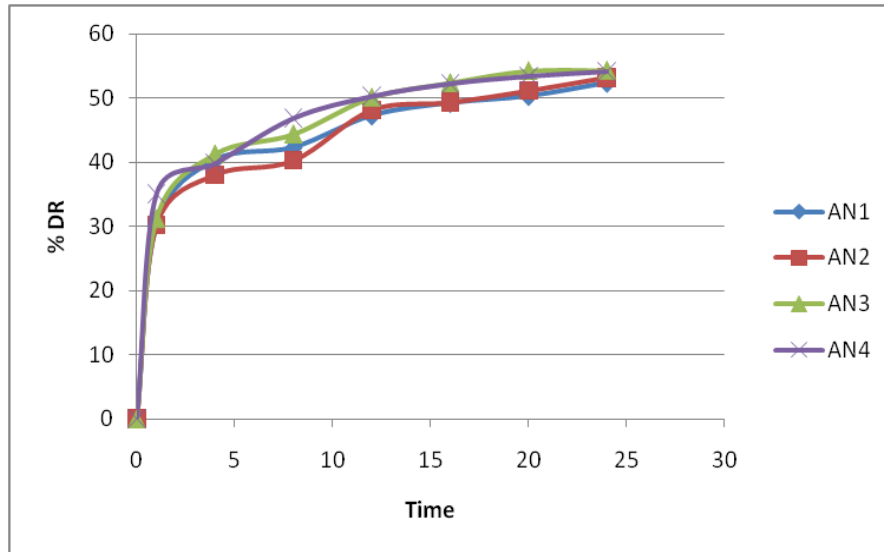
Graph 3: Drug Content of Niosomal formulation containing Allucin

Table 3: *In Vitro* Dissolution Profile of Niosomes formulation containing Allucin

Time (h)	Cumulative % of Drug Release											
	AN1	AN2	AN3	AN4	AN5	AN6	AN7	AN8	AN9	AN10	AN11	AN12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	30.21	30.22	31.20	35.11	39.10	33.72	36.20	37.42	36.41	35.12	36.12	35.48
4	40.33	38.10	41.28	39.84	41.27	44.29	39.18	40.12	40.12	38.48	37.48	36.47
8	42.32	40.32	44.38	46.92	46.98	48.01	47.29	48.12	47.84	46.28	47.24	48.20
12	47.28	48.20	50.10	50.33	50.10	50.92	50.81	52.12	51.89	50.41	50.22	50.48
16	49.24	49.37	52.37	52.31	52.37	51.67	52.18	53.24	52.37	52.12	51.28	52.18
20	50.32	51.20	54.24	53.47	52.98	53.27	53.47	55.12	54.27	52.22	52.34	52.87
24	52.37	53.27	54.31	54.21	53.74	54.36	55.46	59.12	55.86	54.27	54.21	53.47

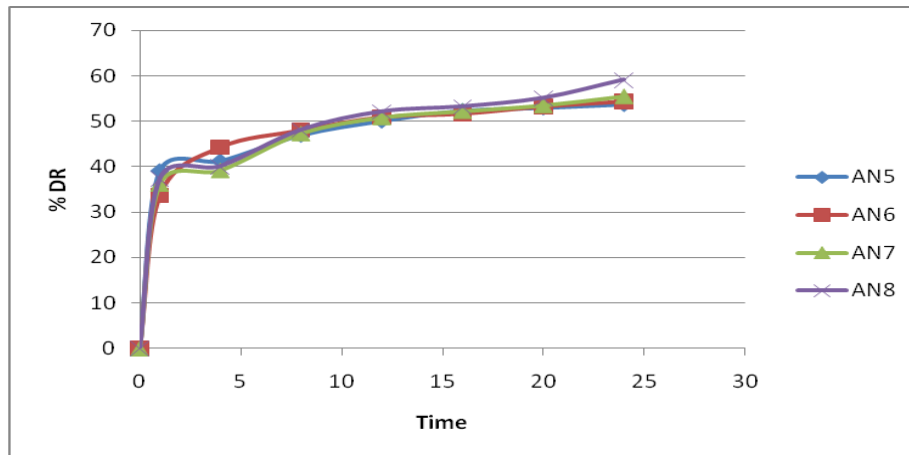
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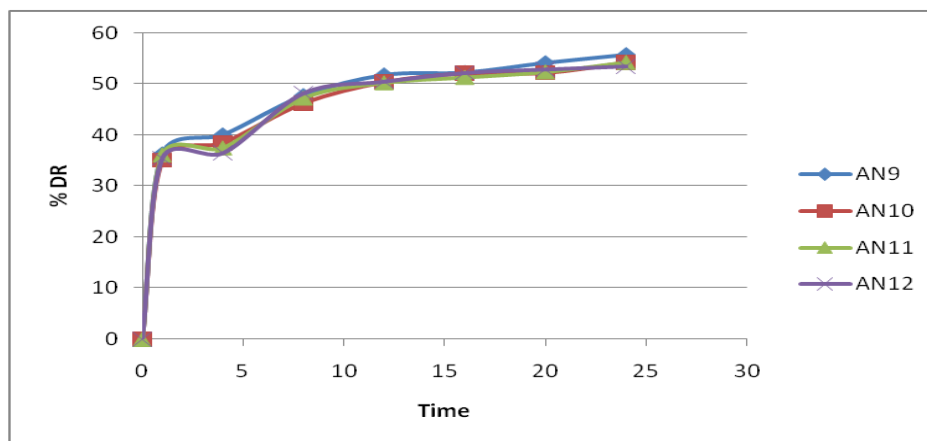


Graph 4: % Drug release of Niosomal formulation containing Allucin (AN1 to AN4)

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Graph 5: % Drug release of Niosomal formulation containing Allucin (AN5 to AN8)



Graph 6: % Drug release of Niosomal formulation containing Allucin (AN9 to AN12)



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