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FORMULATION AND EVALUATION OF NOVEL N-ACETYLCYSTEINE NANOPARTICLES

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Abstract

With the development of technology in the last two decades, the particle size of materials ranges from the micro- to nano-scale. The reduction in the particle size of materials at the nanometer scale increases their overall surface area by several orders of magnitude. Particles with a size in the range of 1 nm to 1000 nm are known as nanoparticles. The word nano can be easily defined, but it covers numerous areas of application. However, nanomaterials with excellent biodegradability and biocompatibility are considered to be the best vehicles for drug delivery systems in biomedical applications. Currently, scientists and researchers are focused on discovering new methods/routes to control the pharmacokinetics (ADME), pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and drug efficacy of drugs. These new strategies are often called novel drug delivery systems (NDDS) and are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. Some of the different approaches for novel drug delivery include transdermal patches, sustained and controlled release by polymeric and magnetic control, liposomes, hydrogels, implants, microspheres, erythrocytes, and nanoparticles. Nanoparticular drug delivery systems are a successful approach in the treatment of chronic human diseases, which have excellent function in satisfying the biopharmaceutical and pharmacological considerations.

Keywords: Nanoparticles, N-acetylcysteine, Bioavailability and Melt emulsification.

Introduction

The emergence of nanotechnology and the growing capabilities of functional proteomics, genomics, and bioinformatics combined with combinatorial chemistry have driven scientists to become more enthusiastic to express their technical expertise to discover, invent and explore novel approaches for drug delivery systems through new techniques. Novel drug delivery systems remain the foundation to deliver drugs having complications that cannot be minimized by conventional drug delivery systems, where the therapeutic effectiveness of drugs depends on their pharmacokinetics and site of administration. Pharmacokinetics are also based on physico-chemical properties such as solubility, crystallinity, toxicity, and HLB value. After understanding the biopharmaceutics and pharmacokinetics, the administration route, absorptive surface area, and transportation of drugs in the body are the key points for their absorption and distribution. Furthermore, metabolism and elimination depend on the aforementioned properties [1].

The formulation design has a major impact on the effective delivery of the active pharmaceutical ingredient (API), and thus all the above parameters are crucial challenges. Drugs based on the HLB scale are categorized into two classes, hydrophilic and lipophilic molecules. Lipophilic molecules exhibit very poor solubility, and depending on this, they produce a great challenge to design safe, efficacious, and cost-effective drug delivery systems and have been a source of frustration for pharmaceutical scientists [2]. Lipophilic molecules allow the design of formulations for hydrophobic drug molecules, and despite all the problems confronted by pharmaceutical scientists, the current solid lipid nanoparticles are the result of their great effort. Traditionally, lipid-based novel drug delivery systems have focused on the delivery of lipophilic molecules, but recently, lipoid drug delivery systems have received attention due to their inherent properties such as biocompatibility, self-assembly capabilities and ability to cross the blood brain barrier, particle size variability and finally cost effectiveness, making lipid-based delivery systems much more attractive [3].

Over the past few years, nanomaterials have emerged as drug carriers. Liposomes are important biological molecules, which have been used for many years, but currently, there are various alternative molecules. Niosomes are one of the promising economical alternatives to liposomes. Niosomes are highly stable and slightly leakier than liposomes. The size of niosomes decreases substantially upon freezing in liquid nitrogen and subsequent thawing, as evident by cryo-EM and dynamic light scattering.

The successful delivery of drugs through nanoparticles depends on their ability to penetrate barriers, continuously release drugs and their stability. However, the scarcity of regulatory approved polymers, i.e. the Food and Drug Administration (FDA), and their expensive costs have limited their clinical application [4]. Thus, to overcome these

limitations, scientists and researchers have proposed lipids as alternative carriers. These lipid-based nanoparticles are known as solid lipid nanoparticles (SLNs), which have attracted worldwide interest due to their advantages [5-7]

Solid lipid nanoparticle overview:

For lipid and lipid-based drug delivery systems, phospholipids are an important constituent because of their various properties, such as amphiphilic nature, biocompatibility and multifunctionality. However, liposomes, lipospheres, and microsimulation carrier systems have many drawbacks such as their complicated production method, low percentage entrapment efficiency (% EE), difficult large-scale manufacture, and thus the SLN delivery system has emerged [8]. SLNs are commonly spherical in shape with a diameter in the range of 50 to 1000 nm.

The key ingredients of SLN formulations include lipids, which are in the solid state at room temperature, emulsifiers and sometimes a mixture of both, active pharmaceutical ingredients (APIs) and an adequate solvent system. Nanocarrier-based drug delivery systems can be subcategorized in many aspects depending on the route of administration, degree of degradability, etc. The route of administration includes nanoparticles for parenteral administration, oral administration, ocular administration, and topical administration, and nanoparticles for protein peptide delivery. Nanocarrier systems can also be subcategorized based on the degree of their degradability as follows.

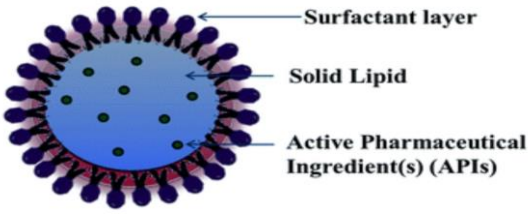


Fig 1.3 Schematic presentation of the complete structure of solid lipid nanoparticles.

An ideal nanoparticulate drug delivery system must contain the following characteristics:

(1) Maximum drug bioavailability [9].

(2) Tissue targeting.

(3) Controlled release kinetics.

(4) Minimal immune response.

(5) Ability to deliver traditionally difficult drugs such as lipophiles, Amphiphiles and biomolecules.

(6) Sufficient drug loading capacity [10].

(7) Good patient compliance.

Solid lipid nanoparticles have changed the dimension of drug delivery by combining all the advantageous characteristics of polymeric nanoparticles, liposomes and microemulsion. All the properties of lipid nanoparticles are upgraded with surface modification, better pharmacokinetic acceptability, formation of inclusion complexes, improved stability pattern and incorporation of chemotherapeutic agents. SLNs are appropriate for intravenous applications because of their effortless dispersion in solution, which are aqueous or aqueous-surfactant [11].

Nanoparticles undergo phagocytic uptake, and thus by surface modification, their phagocytic uptake can be minimized. A pharmacokinetic study also showed a good increase in the of concentration doxorubicin in with solid lipid nanoparticles compared with conventional commercial drug solutions, and it was found that the drug concentrations were higher in the lungs, spleen and brain of rats. In drug delivery technology, cyclodextrin is used as a complex agent, which can be used to increase aqueous solubility, bioavailability and improve the physicochemical properties of drugs by forming inclusion complexes.

The incorporation of these inclusion complexes into solid lipid nanoparticles increases their release profile compared to solid lipid nanoparticles without cyclodextrin. Furthermore, the stability pattern of solid lipid nanoparticles (SLNs) is more attractive than that of other nanoparticulate formulations. Aqueous SLNs can be stored for up to 3 years or longer, and their gelling tendency due to long term storage and light exposure can be stabilized by inhibiting the transitions by lipid modification. The major aim of solid lipid nanoparticles (SLN) in terms of drug delivery is to enhance the bioavailability and efficacy of drugs, and control the non-specific toxicity, immunogenicity, pharmacokinetics and pharmacodynamics of drugs. This review focuses on the potential of SLNs in various types of chemotherapy such as cancer, where conventional chemotherapy is hindered by different obstacles such as drug resistance, low specificity and poor stability of chemotherapeutic compounds.

These issues may be partly overcome by encapsulating drugs as SLNs. The new generations of SLN such as nanostructured lipid carriers (NLC), lipid drug conjugates (LDC), polymeric lipid hybrid nanoparticles (PLN), and long-circulating SLNs, improve the role of SLNs as versatile drug carriers for various types of chemotherapy, and treatment of parasitic infections and tuberculosis [12].

Cell line studies have shown that SLNs can be easily internalized and may be designed as surrogate colloidal drug carriers for the administration of chemotherapeutic agents, especially for the treatment of malignant melanoma and colorectal cancer. Besides their antitumor activities, SLNs are also capable of hindering the adhesive interactions between cancerous cells (resulting from human breast, prostate cancers, melanoma, etc.) with the cells present on human umbilical vein endothelium [13]. Furthermore, since SLNs are based on nontoxic and non-irritating materials, they are ideal for use in topical formulations. Accordingly, there has been extensive research on the topical applications of SLNs (containing lipids such as glyceryl palmitostearate and glyceryl behenate) to treat several skin diseases since SLNs adhere strongly because of their greater surface area as a result of their smaller sizes. The coenzyme Q10 penetrated the stratum corneum more effectively as SLNs in comparison with liquid paraffin and isopropanol the extent of drug release was higher and more rapid for SLNs of Compritol ® (Retinol-loaded) compared to conventional carriers.Also, SLNs were found to be significant vehicles for numerous sunscreen agents.

The delivery of genetic material via nanotechnology is now gaining significant attention. Cationically modified SLNs can effectively deliver DNA to binding sites, where the transfection efficiency and cytotoxicity are also very low. Furthermore, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have been considered as effective and safe alternatives to potentially treat both genetic and non-genetic diseases [14]. Lipid nanoparticles (LNs) easily overcome the main biological barriers for cell transfection, including degradation by nucleases, cell internalization, intracellular trafficking, and selective targeting to a specific cell type. SLNs and NLCs can effectively be used for gene therapy, and the treatment of ocular diseases, infectious diseases, and lysosomal storage disorders. SLNs and NLCs have been established to be very effective in the topical delivery of antifungals such as clotrimazole and ketoconazole.

Various studies have shown that because of several factors such as stability, complete release, and low toxicity, SLNs can also be considered as new potential vehicles for the pulmonary delivery of antitubercular drugs. Claus-Michael Lehr and co-workers showed that a two-tail cationic lipid had a greater transfection efficiency than a one-

tail cationic lipid, and concluded that higher tolerability and transfection efficiency can be achieved with SLNs. Ocular drug delivery is one of the most critical drug delivery technologies, which is still lacking regarding sensitivity. Accordingly, since SLNs contain no inflammatory lipid material, they may be suitable for ocular drug delivery [15].

Tobramycin was incorporated in SLNs and compared to a reference eye drop, showing a 1.5-fold and 8-fold increase in Cmax and tmax value with respect to the reference solution. SLNs show occlusive properties and UV blocking potential, which are ideal for cosmetic preparation, resulting in excellent skin hydration.Thus, SLNs are interesting for drug delivery, where they mostly cover all the sites for drug delivery and have numerous applications with respect to the route of administration. Furthermore, stability-related issues are not a major problem, and drugs, proteins and peptides can also be deliverable to the target site. Thus, SLNs are potential carriers for bioactive materials [16].

MATERIALS AND METHODS

Materials used:

S.No	Materials	Supplier
1	N-Acetyl Cysteine	Sigma Aldrich pvt.ltd
2	Stearic acid	Himedia,Mumbai
3	Tween 80	Himedia,Mumbai
4	Acetone	Rankem
5	Methanol	Rankem
6	Potassium di hydrogen phosphate	Rankem
7	Ortho phosphoric acid	Rankem

Equipment list:

S.No	EQUIPMENTS	MODEL
1.	Electronic balance	Metler Toledo AG 135.
2.	Ultra centrifuge	Remi instruments, Mumbai.
3.	Mechanical stirrer	Remi instrument.
4.	DSC	Schimadzu DSC-60.
5.	Particle size analyzer	Malveran master sizer.
6.	UV spectrophotometer	Schimadzu 1710, Mumbai.
7.	USP dissolution apparatus	Lab india, DS8000.

METHODS:

PREFORMULATION STUDIES:

Preparation of calibration graph for N-Acetyl Cysteine:

Preparation of calibration curve in pH 1.2, pH 7.4 and pH 6.8 buffer solutions:

An accurately weighed amount of N-Acetyl Cysteine 100mg was dissolved in small volume of buffer solutions in each of three 100 ml volumetric flask and the volume was adjusted to 100 ml with 1.2 pH buffer in first volumetric flask, 7.4 pH buffer in second volumetric flask and the third one was adjusted to 100 ml with 6.8 pH buffer. A series of standard solution containing in the concentration range from 10-50 µg/ml of N-Acetyl Cysteine were prepared for 1.2 pH buffer solution, 7.4 pH buffer solution and 6.8 pH buffer solution separately, absorbance was measured at 200.3 nm and calibration graph was plotted using concentration versus absorbance.

Drug-excipient compatibility study by DSC:

Samples of individual components as well as each drug-excipient were weighed (Mettler Electronic balance) directly in pierced aluminum crucible pans (5-10 mg) and scanned in the 50-300°C temperature range under static air, with heating rate of 10 ºC /min, using shimadzu DSC-60 equipment.

METHOD OF PREPARATION:

PREPARATION OF SLNs LOADED WITH N-ACETYLCYSTEINE BY MELT EMULSIFICATION AND LOW-TEMPERATURE SOLIDIFICATION METHOD [17]:

SLNs loaded with N-Acetylcysteine were prepared by melt emulsification and low-temperature Solidification method.

- N-Acetylcysteine was dissolved in methanol, Stearic acid in various concentrations were dissolved with actone and mixed well.
- The above mixtures were sonicated for 15 minutes, and then added drop wise in to Tween 80 solution, stirred at 3000 rpm for 30 minutes at 70°C.
- The mixed solution was transferred to icy water bath and stirring for four hour at 3000 rpm.
- Different formulations of SLNs loaded with N-Acetylcysteine were prepared by varying concentrations of stearic acid as shown in the Table **(NACSLN1- NACSLN5)**
- The SLNs loaded with N-Acetylcysteine were used for further characterization studies.

Particle size and Surface charge

Surface charge is important in adhesion and interaction of particle with cells. The zeta- potential is used to measure the cell surface charge density. It can be measured using Malvern-Zeta sizer. The prepared SLNs loaded with N-Acetylcysteine were evaluated for their particle size and surface charge by photon correlation spectroscopy (PCS) using zeta sizer [18]. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was carried out at 25°C with an angle of detection of 90°. In this experiment six replicates were taken for the measurement. The results were given in results and discussion section.

Drug content

1gm of SLNs loaded with N-Acetylcysteine were accurately weighed and transferred into a 25ml volumetric standard flask. The sample was dissolved with methanol .1ml of this solution was diluted to 25ml with the purified water. The standard N-Acetyl Cysteine was dissolved and diluted with same methanol and water respectively [20]. Then the standard and sample absorbance was measured at 200.3 nm using UV-Visible spectrophotometer. The percentage of drug content was calculated. The results were given in results and discussion section.

Entrapment efficiency

The SLNs loaded with N-Acetylcysteine in buffer solutions were subjected to centrifugation at 15000 rpm for 30 min. The supernatant liquid was separated and 1ml of this solution was diluted with buffer solution and the absorbance was measured at 200.3 nm. The amount of N-Acetyl Cysteine unentrapped in the supernatant was calculated. The amount of N-Acetyl Cysteine entrapped was determined by subtracting amount of free unentrapped N-Acetyl Cysteine from the total amount of N-Acetyl Cysteine taken for the preparation [21]. The results were given in results and discussion section.

Scanning Electron Microscopy (SEM)

For the external morphology studies, air dried particles were visualized using scanning electron microscopy (FEI-Quanta 200F) operating at 15 Kv. The optimized SLNs loaded with N-Acetylcysteine formulation was mounted on a metal slab with double adhesive tape and coated with platinum under vacuum and the SEM images were recorded.

In-vitro release

In vitro release studies were performed for 24 h using dialysis membrane by using the Franz diffusion cell. The prepared SLNs loaded with N-Acetylcysteine formulations (**NACSLN1- NACSLN5)** were placed inside a dialysis membrane and immersed in buffer pH 6.8. At predetermined time intervals the sample was withdrawn and the amount of N-Acetyl Cysteine released was determined by measuring the absorbance at 200.3 nm using a UV-Visible spectrophotometer. From the absorbance values the cumulative percentage drug release was calculated. The results were given in results and discussion section.

RESULTS AND DSCUSSION:

Pre-formulation studies

Preparation of Calibration graph for N-Acetyl Cysteine

Standard calibration data of N-Acetyl Cysteine in pH 1.2, 7.4 and 6.8 buffers at 200.3 nm Standard calibration curve of N-Acetyl Cysteine was carried out in 1.2 pH, 7.4 pH and 6.8 pH buffer at 200.3 nm. The r^2 value in the entire medium shows nearly 1, which signifies linearity.

DSC analysis

DSC of N-Acetyl Cysteine showed a sharp endothermic peak at about 105.96°C (melting point). The physical mixture of N-Acetyl Cysteine with other excipients also showed the same thermal behavior (106.93°C) as the individual component. DSC results also revealed that the physical mixture of N-Acetyl Cysteine with excipients showed superimposition of the thermogram. There was no significant change observed in melting endotherm of physical mixture of N-Acetyl Cysteine and other excipients used in the formulation. Hence from the DSC study, it was found that there was no interaction between N-Acetyl Cysteine and other excipients used in the formulation.

Drug –Excipients accelerated compatibility study - Physical observation and assay

Upon analysis of the drug excipient mixture for their physical characteristics no colour change was observed. Based on the chemical evaluation it was found that there was no significant change observed indicating that the drug is compatible with the added ingredients.Particle size and entrapment efficiency of the SLNs loaded with N-Acetylcysteine (NACSLN1- NACSLN5) were increased with increasing Stearic acid concentration.

This may be due to high amount of availability of Stearic acid to encapsulate the drug, upon increasing the Stearic acid concentration, number of layers coated the drug was increased, this resulted in increase in particle size and increase in entrapment efficiency. Further increase in the Stearic acid concentration (NACSLN1- NACSLN5), there was no increase in the entrapment efficiency due to the availability of the drug to be incorporated is low therefore it is not enough for further encapsulation of drug by Stearic acid. Based on the results it was found that the particle sizes of formulations were increases as the concentration of Stearic acid increases.

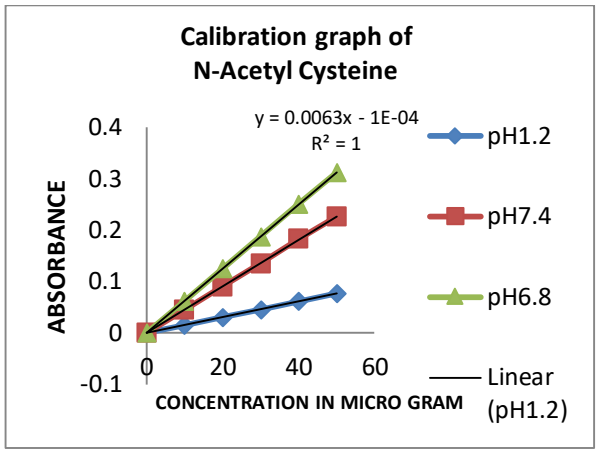
TABLES AND GRAPHS:

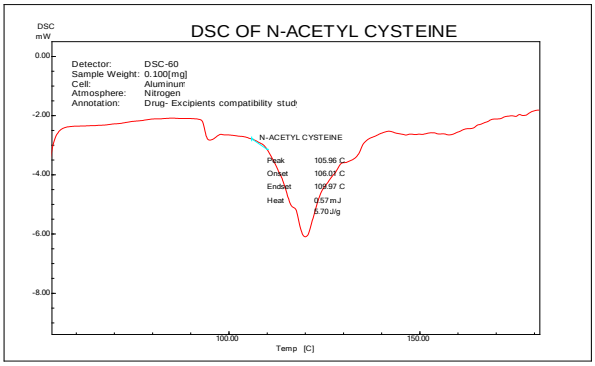
Formula used for the preparation of SLNs loaded with N-Acetylcysteine

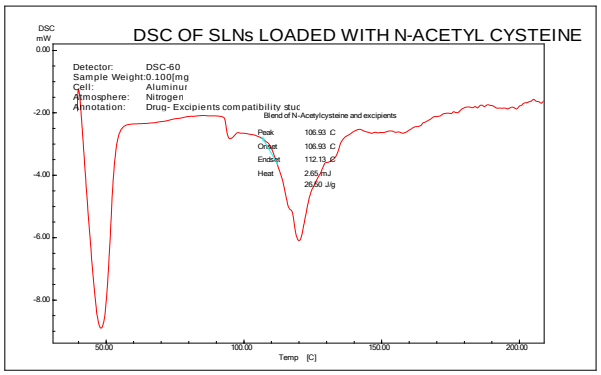
S.NO	FORMULATION	DRUG (mg)	Stearic acid (mg)	Tween 80 (%)
1.	**NACSLN1**	100	500	2.5
2.	**NACSLN 2**	100	600	2.5
3.	**NACSLN 3**	100	700	2.5
4.	**NACSLN 4**	100	800	2.5
5.	**NACSLN 5**	100	900	2.5

Absorbance of N-Acetyl Cysteine in buffer solutions

S.NO	Concentration (in micro gram)	Absorbance		
		pH 1.2	pH 7.4	pH 6.8
1	**0**	**0**	**0**	**0**
2	**10**	0.015	0.045	0.062
3	**20**	0.031	0.091	0.125
4	**30**	0.046	0.136	0.188
5	**40**	0.062	0.183	0.251
6	**50**	0.078	0.227	0.312







CONCLUSION:

The active pharmaceutical ingredient **N-Acetyl Cysteine** was selected for the study due its hydrphilicity and poor bioavaialabilty. It was evaluated for its organoleptic properties, solubility and assay as per the standard procedures. The results obtained were found to be within the limits as given in the standard specification for **N-Acetyl Cysteine.**

N-Acetyl Cysteine solid lipid nanoparticles were prepared by melt emulsification and low-temperature Solidification method and the **Stearic acid** concentrations was optimized by conducting different trials using various concentrations of stearic acid.

In the present study **N-Acetyl Cysteine** solid lipid nanoparticles was prepared. The effect of increase in **Stearic acid** concentration in various parameters like **particle size, zeta potential and *in vitro*** release profile were studied.

The **N-Acetyl Cysteine** solid lipid nanoparticles were formulated and evaluated for its Drug content, Entrapment efficiency, Particle size analysis, Zeta potential and *In vitro* drug release profile.

Based on the results of **N-Acetyl Cysteine** solid lipid nanoparticle formulations **(NACSLN1-NACSLN5)** formulation **NACSLN5** was selected as the best formulation in which the particle size was 332.2 nm and the entrapment was **88.75%**.

The results of SEM analysis confirms the prepared **N-Acetyl Cysteine** solid lipid nanoparticles were spherical in shape and nano in size.

The *in vitro* % drug release of **NACSLN5** formulation was **99.58 %** and it was found to be suitable formulation for the treatment of Paracetamol Poisoning. Hence it can be concluded that the newly formulated controlled release N-Acetyl Cysteine solid lipid nanoparticles may be ideal and effective in the treatment of Paracetamol poisoning which releases the drug continuously for the period of 24 hrs in order to maintain the steady state blood level concentration of drug.

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