Nanotechnology in Phytopharmaceuticals

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To

Prof. Syed Waseem Akhtar

Hon'ble Chancellor and Founder,

Integral University

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EPIGRAPH

Nanotechnology is on the verge of providing a wide range of innovations and ways to transform the approaches and treatment in medical science. Several diagnostic and medical sectors are already head stead in reaping the benefits from nanotechnology. The application of nanotechnology to the treatment, detection, monitoring, and management of biological systems has recently been referred to as nanomedicine. In pharmaceutical research, which includes nano-based formulations there are many benefits of phytomedicine, including improved melting and bioavailability, protection of youth, improved medical function, improved stability, increased proliferation of tissue macrophages, further delivery, protection, immunity, and protection, etc.

Herbal medicines have been widely used around the world since ancient times and have been recognized by doctors and patients for their superior therapeutic value as they have fewer side effects compared to modern medicines. Phyto therapeutics requires a scientific approach to delivering components in a continuous manner to increase patient compliance and avoid repeated administration. This can be achieved by designing novel drug delivery systems (NDDS) for drug components. NDDS not only reduces the frequency of administration to overcome complications, but also helps increase the amount of treatment by reducing toxicity and increasing bioavailability. One such new method is nanotechnology. Nano-sized drug delivery systems for herbal medicines have a strong future for improving performance and overcoming plant-related drug problems. Therefore, the integration of nanocarriers such as NDDS into the traditional medicine system is important in combating chronic diseases such as Chronic Obstructive Pulmonary Disorders (COPD), metabolic disorders, cancers and several others.

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FOREWORD

The amalgamation of Nanotechnology with Medical science is a subject that has continually intrigued many researchers in recent times. It is a unique discipline that opens the locks to various doors of formulation development like, poor bioavailability, sustainability and efficacy of any medicinal formulation, paving way for numerous untouched areas of research in the field of herbal pharmaceuticals.

A variety of nano-based technologies are being used in pharmaceutical sciences, for example, Quantum dots, Dendrimers, Carbon nanotubes, Liposomes, Polymeric nanoparticles, Metallic nanoparticles, Polymeric micelles and Nanocomposites, to name a few. Today, nanotechnology is applied to oral drug delivery, inhalational drug delivery, ocular drug deliver, gene therapy, cancer treatment, brain targeting, as a diagnostic tool and many more, making it a topic worthy of research. This book throws light on various aspects of a nanoform and hurdles one faces in developing a nanoformulation with measures to tackle them.

We, the editors of this book, **Dr. Tarique Mahmood Ansari** (PhD, Pharmacology), **Dr. Arshiya Shamim** (PhD, Pharmacology), **Prof. (Dr.) Mohammad Haris Siddiqui** (PhD, Bioengineering) and **Dr. Farogh Ahsan** (PhD, Pharmacology) have exhaustively worked in the said area and have traversed many novel paths in their journey. We have been working collaboratively, in developing standardised dosage forms for herbal extracts and formulations. Recently, *Dr. Tarique Mahmood Ansari,* Head, Department of Pharmacy, Integral University with *Dr. Arshiya Shamim*, Assistant Professor, Department of Pharmacy, Integral University have also worked as Investigators, in a research project, funded by the Ministry of AYUSH, Government of India, where we developed a novel nanoformulation of a traditional herbal formulation apart from having published numerous scientific papers on the same and related subject. *Prof. (Dr.) Mohammad Haris Siddiqui* is a man of great scientific and academic repute with recognition and accolades in the field of nanoscience and bioengineering to his credit, he has worked on various projects funded by different Government agencies and continues to strive for the best in the field of research as the Director, Integral Institute of Agricultural Sciences and Technology (IIAST), former Head, Department of Bioengineering. *Dr.*

Farogh Ahsan a budding, yet an exemplary academician and researcher has numerous publications in the journals of national and international repute and a German patent to his credit.

In the due course of compiling this book we came across many other contributors of this field who had their research journeys to share with rich experiences to cater the budding minds of pharmaceutical studies. Their contributions in form of different chapters to this book throws light on their vast knowledge and experience to cater budding researchers of this field. We are honored to have collaborated with various authors from different academic and research institutes across the country through the platform this book provided us.

This book is an honest attempt, to bring together the views and takes to share some pathbreaking results of various scientific studies that could help in merger of nanotechnology with herbal pharmaceuticals, a domain less touched but of great curiosity to researchers of medical and pharmaceutical field. This piece of work has been designed with sheer literature study, and thorough brainstorming to connect all the ends concerning nanotechnological interventions for herbal pharmaceuticals. The clinical scope of herbal nanoformulations, safety profile and future of novel nanoformulations, parameters governing the pharmacokinetics of herbal nanoformulation, Contemporary advancement in clinical applications of nanobiomedicines and nanotechnology based herbal cosmeceuticals are amongst the domains discussed in detail under different chapters of this book. Explicit experience of the editors in the field and rich knowledge has been the foundation to bring together a book for students, researchers, and academicians in the pharmaceutical technology, that holistically reviews all facets of ‘nanotechnology in phytomedicines’.

PREFACE

In recent years, applications of nanotechnology in herbal medicine have received increasing attention and emerged as a new branch of pharmaceutical science known as 'Phytonanotechnology'. Application of nanotechnology to herbal formulations deals with exploiting medicinal plants to provide solutions to various formulation and drug delivery related hurdles. Plant-mediated biological methods are being used by various researchers to synthesize nanoparticles of plant extracts and phytoconstituents in different size, shape, and quantity given to their easy availability and eco-friendliness. The appropriate application of nanoscience to medicinal herbs can provide improved outcomes and an exploration of their bioavailability and toxicity. These nanoparticles are explored for various applications as potent pharmacological products. They can also be used as electrochemical biomarkers and biosensors, in the field of diagnostic and therapeutic health care These nanoparticles can also be applied for pests, nutrients and plant hormones.

Herbal medicines face many challenges pertaining to formulation development that curb realising their full potential as pharmaceutical formulations. These problems include low solubility in water, low bioavailability, high toxicity, and instability. Nanotechnology has shown great promise in overcoming some difficulties encountered with using bulk variable drug molecules in their synthetic and natural forms. Nanomedicines have proven their potentials in cancer diagnosis, chemotherapeutic drug delivery, and diabetes treatments. Encapsulation of herbal extracts or active compounds into nanocarriers improves their activity through enhanced solubility, better stability for both *in vivo* and *in vitro* activities and better formulation. Currently, there are numerous nanoparticles are in clinical use, validating the ability of nanoparticles to improve the therapeutic index of herbal drugs as well.

Nanoparticles possess unusual characteristics due to their large surface area-to-volume ratio and extraordinary catalytic activity, electronic properties, optical properties, and antimicrobial activity while they are constructed at the atomic level. Because physical and chemical methods of nanoparticle synthesis are too expensive and environmentally unsound, there is a better

possibility of green synthesis of nanoparticles using plants, bacteria, and fungi, which are emerging as novel eco-friendly techniques. The growth rate of the bacterial culture, the extract of the plant secondary metabolites, and the mycelial surface area of fungus are the main comprehensible mechanisms in the green synthesis of nanoparticles. Phyto-nanotechnology has great potential to revolutionize pharma industry. Despite these promising perspectives, challenges are also pressing, including the impacts of diverse plant cellular structures on nanomaterial delivery and the induction of various levels of phytotoxicity.

The aim of this book is to bring together vies and take of experts in the field, focusing on the effects of nanotechnology on formulation development, drug delivery, biochemistry, pharmacokinetics and dynamics of the medicinal product and challenges faced in the journey towards achieving it. Different chapters in this book cover the impact on bioavailibility, techniques of developing nanoformulation, a review of positive and negative impacts and an overview of current policies regarding the use of nanotechnology on phytoconstituents. It will also cover medicinal applications including biomedical, biosensors, and drug delivery. We hope that this book highlights the promising applications and major obstacles of nanotechnology in phytomedicines.

Dr. Arshiya Shamim
(PhD. Pharmacology)

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We, the editors of the book, take this opportunity to express our gratitude first and foremost to the Almighty Allah, who guided and strengthened us to venture into this opportunity and make the best of it by sharing and spreading the knowledge on a global platform.

The editors wish to express their warm regards and heartfelt thanks to ***Prof. Syed Waseem Akhtar***, Founder and Hon'ble Chancellor, Integral University***. Prof. Javed Musarrat***, Vice-Chancellor, Integral University for endowing an indispensable infrastructure, facilities, resources, and a workplace to accomplish this work successfully. We are also grateful to ***Prof. Aqil Ahmad***, Pro Vice-Chancellor, Integral University and ***Prof. T. Usmani***, Dean, Faculty of Doctoral Studies & Research for their academic support, guidance and optimistic critique which helped in improvising our work at each step. We would like to express our heartfelt gratitude towards ***Prof. (Dr.) Syed Misbahul Hassan***, Dean, Faculty of Pharmacy, Integral University for his valuable inputs, support and motivation.

This note of thanks would be incomplete without expressing the gratitude to ***Prof. Hefazat Hussain Siddiqui***, Adjunct Professor Faculty of Pharmacy, Integral University, whose optimism and thorough encouragement has been of immense help to us. His knowledge & expertise in the field of pharmaceutical science was of great help, which encouraged dig and delve more and develop intensive logical piece of writing in form of this book.

With the support of an exemplary publishing house as '***Cambridge Scholars***' who believe in the spirit of divulging into the sea of currently relevant thrust areas of health and pharmaceutical science, it has been possible in shaping up this book to fetch maximum knowledge on the said topic. We find ourselves lucky to have found a fruitful association with 'Cambridge Scholars' and have tried to put our best foot forward in compiling this book for a global readership.

At last, but not the least we are highly thankful to all those people who have been a part of this journey as contributors/ authors in realisation of this piece of work through their contributions. In particular, the editors, express their

gratitude to ***Mr. Mohammad Shariq*** and ***Ms. Rufaida Wasim***, for assisting and providing their valuable inputs while proofreading the book.

LIST OF ABBREVIATIONS

AFM = Atomic Force Microscopy

$AgNO_3$ = Silver Nitrate

AgNPs= Silver Nanoparticles

API = Active Pharmaceutical Ingredient

AuNPs = Gold Nanoparticles

BET = Brunauer–Emmett–Teller

DCS = Differential Centrifugal Sedimentation

DLS = Dynamic Light Scattering

DNA = Deoxyribo Nucleic Acid

DOPE = Dioleylphosphatidyl Ethanolamine

DSPC = Distearoylphosphatidyl Choline

EBSD = Electron Backscatter Diffraction

EE = Entrapment Efficiency

EELS = Electron Energy Loss Spectroscopy

EPLS = Elliptically Polarized Light Scattering

EPM = Electrophoretic Mobility

EXAFS = Extended X-ray Absorption Fine Structure

FMR = Ferromagnetic Resonance

FTIR = Fourier Transform Infrared Spectroscopy

HPLC = High Performance Liquid Chromatography

HRTEM = High-Resolution Transmission Electron Microscopy

HRTEM = High-Resolution Transmission Electron Microscopy

ICP-MS = Inductively Coupled Plasma-Mass Spectroscopy

ICP-OES = Inductively Coupled Plasma Optical Emission Spectrometry

LDH = Lactate Dehydrogenase

LEIS = Low-Energy Ion Scattering

MALDI = Matrix-Assisted Laser Desorption/Ionisation

MFM = Magnetic Force Microscopy

MTT = 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide

MTX = Methotrexate

NaOH = Sodium Hydroxide

NMR = Nuclear Magnetic Resonance

NTA = Nanoparticle Tracking Analysis

PCL = Poly (ε- Caprolactone)

PEG = Poly Ethylene Glycol

PGA = Poly Glycolic Acid

PL = Photo Luminescence

PLA = Poly Lactic Acid

PLGA = Poly lactic-co-glycolic acid

PTA = Particle Tracking Analysis

RES = Reticulo Endothelial System

RMM- MEMS = Resonant Mass Measurement Microelectro-Mechanical System

SANS = Small Angle Neutron Scattering

SAXS = Small-Angle X-ray Scattering

SEM = Scanning Electron Microscopy

SEM-EDX = Scanning Electron Microscopy Energy-Dispersive X-ray Spectroscopy

SIMS = Secondary Ion Mass Spectrometry

SLNs = Solid Lipid Nanoparticles

SQUID = Superconducting Quantum Interference Device Magnetometry

STEM = Scanning Transmission Electron Microscopy

TEM = Transmission Electron Microscopy

TGA = Thermal Gravimetric Analysis

TRPS = Tunable Resistive Pulse Sensing

UV Vis-NIR = Ultraviolet Visible Near-Infrared

UV-Vis = Ultraviolet-Visible Spectrophotometry

VSM = Vibrating Sample Magnetometry

WHO = World Health Organization

XAS = X-ray Absorption Spectroscopy

XPS = X-ray Photoelectron Spectroscopy

XRD = X-ray Diffraction

XRMCD = X-ray Magnetic Circular Dichroism

XTT = 2, 3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilid

ZnO = Zinc Oxide

ZnONPs = Zinc Oxide Nanoparticles

CHAPTER I

NANOTECHNOLOGY IN PHYTOPHARMACEUTICALS

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Abstract

In recent times, nanotechnology has emerged as a powerful tool in the field of pharmaceutical sciences. Herbal nanoformulations have drawn a lot of attention for their extensive application, reduced dose and enhanced efficacy. Herbal nanoparticles are loaded with either plant extract or plant active isolate, set a higher benchmark than their modern counterparts, and hold an almost equivalent pharmacological potential with lower side effects, making them a lucrative option. This work is a detailed review of plant-based nanoparticles as an advanced therapeutic vehicle for the delivery of various herbal actives. It further elaborates different categories of nanoparticles such as polymeric nanoparticles, metallic nanoparticles, etc., along with their methods of preparation namely the hot homogenization method, co-precipitation method, cold homogenization method, salting out method, ionic gelation method, etc., including various characterization techniques such as ultraviolet spectroscopy, scanning electron microscopy, transmission electron microscopy, Fourier transform infrared spectroscopy, transmission electron microscopy, x-ray diffraction, zeta potential, etc. These are applied for exploring the size, size distribution, surface charge,

shape, density, nature, magnetic properties and surface area and this is mandatory for the successful development of herbal nanoparticles. This chapter also discusses the different *in-vitro* and *in-vivo* characterizations of nanoparticles that have been done so far to establish them as a novel formulation in pre-clinical and clinical studies. Hence, from this extensive compilation, it can be clearly understood that the implementation of nanotechnology in the herbal drug delivery system can enhance the efficacy of herbal drugs.

Keywords: Herbal Nanoparticles, Nanotechnology, Nanoformulations, characterization, Biomedical applications.

1.1 Introduction

The traditional system of medicine had been the only means of healthcare globally until the modern allopathic medicinal system saw daylight (Mamillapalli, 2016). Although in the current scenario, modern medicine overpowers traditional medicine in various aspects, when it comes to seeking holistic therapy or the management of several disorders facing multiple adverse effects, herbal or traditional medicine is preferable. There are different systems of traditional medicines practised worldwide which have gained recognition such as the Unani system of medicine, the Ayurvedic system of medicine, the Chinese system of medicine and homoeopathy. These systems mainly derive medicines from herbs, animals and mineral resources. The WHO defines herbal medicines as a product containing parts of plants, other plant materials, or combinations as active ingredients. The WHO listed around 21,000 plants that are useful for medicinal purposes around the world and among these 2500 species are found in the wild in India and approximately 150 species are cultivated on a fairly large scale. Approximately 80% of the world population directly approaches herbal or plant-derived medicines as their first-line treatment in primary healthcare (Pandey et al., 2013; Qadir et al., 2015). Nowadays, herbal pharmaceutical products have attracted the attention of researchers as well as pharmaceutical industries for their potential to cure diseases with effective pharmacological activity and minimal or no side effects (Mukeshwar et al., 2011).

Plants contain chemical compounds produced as a result of their normal metabolic pathways. These chemical compounds are primary metabolites such as carbohydrates, proteins, and fats and secondary metabolites such as alkaloids, phenols, glycosides, etc. These secondary metabolites produce

therapeutic actions when consumed by humans. For most bioactive compounds of herbal extracts like flavonoids, terpenoids, etc., although they are highly water-soluble, their limited absorption due to many reasons such as being unable to cross the lipid membrane, and their high molecular weight, makes them less bioavailable and less effective (Jimenez-Garcia et al., 2013; Bonifacio et al., 2014). So, these plants are good candidates to produce phytomedicines by refining through various techniques, the biochemicals or pharmacologically active compounds from their source plants (Mukeshwar et al., 2011; Jimenez-Garcia et al., 2013). Multiple studies have been done to identify the relation of a plant or a specific secondary metabolite or biochemical and their pharmacological activity. Some plants and their pharmacological effect in humans are illustrated in Table **1-1**.

S.No.	**Plant Name**	**Active Constituents**	**Use/ Pharmacological Activity**	**Ref.**
1	*Salsolaoppositefolia*	Salsoline (Isoquinoline alkaloid)	Alzeimer's disease	(Zhu et al., 2014)
2	*Areca catechu*	Arecoline (Pyridine alkaloid)	Schizophrenia	(Wang et al., 2015)
3	*Nandina domestica*	Nantenine (Aporphine alkaloid)	Epilepsy	(Dimatelis et al., 2012; Hussain et al 2018)
4	*Geissospermumvellosii*	Geissospermine (Indole alkaloid)	Alzeimers disease	(Hussain et al 2018)
5	*Digitalis purpurea* (Foxgloves)	Digoxin, Digitoxin, Digitonin (Cardiac glycosides)	Congestive heart failure	(Akinmoladunet al., 2014)
6	*Markhamia lutea*	Luteoside, verbacoside(Phenylpropanoid Glycosides)	Antiviral	(Kernan et al., 1998)
7	*Cassia angustifolia* (Senna)	Sennoside (Anthraquinone glycoside)	Purgative	(Anton and Haag-Berrurier, 1980)
8	Daphniphyllumcalycinum	Rutinoside, Kaempferol neohesperidoside	Antioxidant	(Gamez, Esperanza

		(Flavonoid Glycoside)		et al., 1998)
9	*Samanaea saman*	cyanidin, anthocyanin monoglycones, delphinidin and malidin (tannins)	Antimicrobial	(Ukoha et al., 2011)
10	*Allium cepa*	Quercetin (Flavanol)	Rheumatoid arthritis	(Hughes et al., 2017)

Table 1-1 List of plants with their pharmacological importance

Nanotechnology is an emerging field of research in the herbal biomedical field dealing with isolation, design, particle size and morphology alteration so they possess specific properties as compared to traditional technologies (Roy, 2017). Nanoparticles are the nanosized solid colloidal particles of macromolecular material which contain active compounds in various forms such as dissolved, entrapped, adsorbed or attached. Nanoparticles can be developed as targeted drug delivery systems for herbal medicines in accordance with their selective activity at specific sites making them more effective, with the fewest side effects, dose reduction and reduced dose frequency (Allen et al., 2004; Kostarelos and Kostas, 2003). Using a nanoparticulate system, herbal medicines can be targeted to deliver the active molecule to organs like the lungs, kidneys, gastrointestinal tract, brain, etc. (Lu et al., 2007). Herbal drugs with low water solubility or having a hydrophobic nature have enhanced systemic clearance and low bioavailability necessitating frequent and higher dosing to meet the therapeutic level. These lacunae of herbal molecules can be filled with the nanoparticulate system (Lu et al., 2007; Williamson and Elisabeth, 2001).

1.2. Types of nanoparticles

Nanoparticles are broadly categorized based on their chemical nature.

1.2.1 Polymeric Nanoparticles

Polymeric nanoparticles are a great choice for the development of herbal extract-based nanomedicines because of their high potential to retain the API and control its release, and their ability to safeguard labile drugs. Traditional herbal medications have a generalized action; the development of polymeric nanoparticles using specific polymers can make them suitable for targeted action (Bennet et al., 2014). Polymeric nanoparticles have many

advantages over conventional formulations, such as the solubility enhancement of constituents, the reduction in therapeutic doses and the improved absorption of active constituents. Polymeric nanoparticles can be directly administered into the bloodstream as these are non-toxic, biodegradable, and non-immunogenic and they avoid RES (Saraf, 2010; Alexis et al., 2008; Kumari et al., 2010). Herbal polymeric nanoparticles can be prepared in two aspects. One is the matrix type in which the API is distributed throughout the polymeric core or adsorbed onto the surface. These matrix-type polymeric nanoparticles are known as nanospheres. The other is the reservoir type where the API is dissolved in an oily core and surrounded by the network of the polymeric shell. These reservoir type polymeric nanoparticles are known as nanocapsules (Saraf, 2010; Zielińska et al., 2020; Mousavi et al., 2015). Polymers utilized for the development of polymeric nanoparticles are (Kumari et al., 2010; Martins et al., 2012):

Natural – chitosan, albumin, gelatine and heparin.

Synthetic – poly lactic acid (PLA), poly (lactic acid-co-glycolide), poly (ε-caprolactone) (PCL), and poly glycolic acid (PGA).

1.2.2 Solid Lipid Nanoparticles

SLNs are nanostructured lipid particles dispersed in an aqueous or water-based surfactant solution. SLNs have drawn the attention of researchers due to their high physical stability, biocompatibility and great potential for accommodating lipophilic as well as hydrophilic actives (Kolenyak et al., 2013; Martins et al., 2012). SLNs have more applications in the development of the nanoparticle formulation of various actives having aqueous solubility issues. Solid lipid allows the formulation to possess a high drug content and improved stability and no special solvent is required in development. Phospholipids are the irreplaceable component of SLNs due to their unique properties such as their amphiphilic nature, biocompatibility, and multifunctionality (Pardeike et al., 2009). The various phospholipids used for the development of SLNs are lecithin and its derivatives like lysolecithins and hydrogenated lecithins, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, glyceryl monostearate, DOPE, DSPC, etc. (Souto et al., 2011; Jenning et al., 2001).

1.2.3 Metallic Nanoparticles

Metals were used therapeutically in traditional therapy for a long time. Traditional systems of medicines like Unani and Ayurveda exploited the use of metals in the form of ashes for the treatment of various diseases (Sharma et al., 2016). Modern medicines further explored the use of metals for treatment as well as diagnostic purposes (Gielen et al., 2005; Frezza et al., 2010). The unique behaviour of metallic nanoparticles has encouraged researchers to further explore their development and application. Metal nanoparticles are nano-scale articles made of pure elements (e.g., gold, platinum, silver, titanium, zinc, iron, etc.) or their derivatives (e.g., oxides, hydroxides, sulphides, phosphates, fluorides, and chlorides). These metals can be synthesized into nanoforms and/or modified with different functional groups which can be conjugated with drugs, ligands, and antibodies making them potential candidates for biomedical application (Thakkar et al., 2010; Mody et al., 2010; Shah et al., 2015).

Plant extracts contain various biomolecules like alkaloids, and phenolic compounds, etc., along with coenzymes, which can reduce metal ions into nanoparticles without any external force. This single-step process is called the green synthesis of metallic nanoparticles. Silver and gold nanoparticles have been extensively explored for the green synthesis of nanoparticles of plant extract (Lee et al., 2020).

1.3 Techniques of nanoparticle preparation

Researchers have explored various preparation techniques for nanoparticles that are effective at controlling the particle size and the efficient entrapment of active agents. Techniques for the successful development of nanoparticles should be carefully selected as these are not universal. The various techniques employed by researchers are now discussed.

1.3.1 Hot homogenization method

The process of nanoparticle preparation needs a higher temperature above the melting point of the lipid. This technique is generally employed for the development of solid lipid nanoparticles. Hot lipid melt is loaded with the drug which is then mixed with the hot aqueous surfactant solution to form a pre-emulsion. This pre-emulsion is homogenized until the desired size of colloidal particles is achieved. Solid lipid nanoparticles of *Hibiscus rosa*

sinensis extract are developed using the hot homogenization method for antidepressant activity (Goyal et al., 2011; Campos et al., 2015).

1.3.2 Cold Homogenization method

This technique involves melting the drug or extract into the lipid melt which is then cooled spontaneously using cryogenic methods, for instance, using liquid nitrogen or ice nitrogen. The solid mass is then powdered and homogenized at low temperatures to get the nanoparticles. This technique is generally suitable to prepare solid lipid nanoparticles (Al Haj et al., 2008; Uner et al., 2007).

1.3.3 Complex coacervation (Ionic Gelation) method

This method is employed to prepare polymeric nanoparticles using biodegradable water-soluble polymers like gelatine and chitosan. In this method, spontaneous phase separation occurs in the colloidal system due to interaction between the charged polyelectrolyte aqueous solutions. The polymer is dissolved in an aqueous medium, and a crosslinking agent is separately dissolved in an aqueous medium; the two solutions are mixed at suitable rates and nanoparticles are formed which can be collected by centrifugation or lyophilization (Calvo et al., 1997).

1.3.4 Co-precipitation method

It can be said that the co-precipitation method is a modification of the complex coacervation method as it also involves spontaneous separation. The co-precipitation method is the simplest technique for the formation of iron oxide nanoparticles. In this process the precipitation of Fe^{2+} and Fe^{3+} salt (e.g., chlorides, sulphates, and nitrates) in aqueous solutions is achieved by adding a base (e.g., NaOH) solution (Sahni et al., 2011).

1.3.5 High-pressure homogenization method

High pressure is the external force used for the development of nanoparticles. This method is used for preparing lipid-based nanoformulations such as solid lipid nanoparticles, nanoemulsions and lipid carriers, etc. This technique involves passing the micro suspension through a micro-orifice using high pressure resulting in the disruption of particles to the desired size in the nanometer range. This method is very powerful and reliable, produces uniform size particles and is easily scaled up. This

technique can be commercially employed for the production of various nanoformulations (Sahni et al., 2011; Müller et al., 2000).

1.3.6 Salting-out method

This technique uses electrolytes to decrease the solubility of non-electrolytes in an aqueous medium. This technique is most suitable for thermolabile compounds. Nanospheres are prepared by this method. The polymer and drug are dissolved in a solvent, and then the salting-out agent is introduced which results in the formation of polymeric spheres containing drugs which can be separated by centrifugation (Allémann et al., 1993).

1.3.7 Solvent Displacement Method

This technique is simple and mostly employed for the preparation of nanoparticles with low solubility drugs. This technique involves the principle of interfacial adhesion of the polymeric coating by displacing low polarity water-miscible solvent from the lipophilic solution, and results in the formation of submicron droplets of organic solvent as the interfacial tension is reduced and the surface area is increased. The selection of the solvent and the concentration of the drug and polymers are the driving force (Fessi et al., 1989).

1.3.8 Supercritical fluid methods

The supercritical fluid technique has been employed for making various nanoformulations for the last two decades. A liquid or a gas can serve as a supercritical fluid above the thermodynamic critical point. Generally, supercritical water and carbon dioxide can be used to develop different nanomaterials. Several supercritical fluid-based techniques can be employed to develop various nanomaterials such as supercritical antisolvent preparation, supercritical emulsion extraction, supercritical gel drying, etc. (Jung et al., 2001).

1.3.9 Solvent emulsification diffusion method

In this technique an oil in water emulsion is first prepared; the oil phase containing the drug and polymer with an organic solvent and stabilizer is dissolved in the aqueous phase. A high shear mixer is used for emulsification, and then water is added to induce diffusion of the organic