3.1. Motivation

Copper is an essential trace element required for all living organisms. All biota utilize its redox properties to perform different biological functions. Copper homeostasis is regulated by homologous copper binding proteins in all biota. Copper metabolism in women is controlled by estrogen. Copper deficiency affects different biological functions and is associated with various physiological disorders leading to morbidity and mortality. Supplementation of copper improves biological function of copper binding proteins. However high amount of oral intake of copper can induce vomiting.

Postmenopausal osteoporosis is a severe bone disorder occurring in every second woman after menopause. Copper finds vital role in growth and development of bone and its deficiency can lead to bone disorders. Studies had found that postmenopausal women are lacking with required amount of copper. Supplementation of copper, in osteoporotic animal model, has shown remarkable improvement in bone mineral density. However it has not been developed as successful pharmaceutical product due to its pharmacokinetics and pharmacodynamics limitations.

3.2. Research gaps identified

- Osteoporosis is associated with copper deficiency and supplement therapy is needed.
- Most of the commonly used anti-osteoporotic drugs enhance bone mineral density by inhibiting bone resorption and require long duration of therapy which leads to side effects. So a drug/nutrient (copper) is needed which can enhance the bone mineral density by promoting bone formation.
- Bisphosphonates, such as risedronate sodium, are the commonly prescribed antiresorptive drugs. However their oral bioavailability is limited to 1-2% only. Therefore, its bioavailability needs to be improved.
- Both copper and risedronate find significant role in osteoporosis and could have synergistic effect if used as combination therapy. However they have physical incompatibility.
- Western diet is inadequate in copper and oral absorption of copper is inhibited in adults. So copper bioavailability needs to be improved. However high intake of copper induces vomiting.
- Copper nanoparticles (CuNps) have good penetration and surface properties and can improve bioavailability of copper. However they are reported for cytotoxic effect.
Chapter 3  

Research Objective

- Microbial synthesis of metal nanoparticle is reported as ecofriendly and green approach which synthesize biocompatible and safe nanoparticles. However yield of product is limited.
- Downstream processing of metal nanoparticles from extracellular biosynthesis is easier than intracellular biosynthesis. However extracellular synthesis is controlled by various biomolecules secreted by the microorganism.
- Extracellular biosynthesis of CuNps could be improved by optimizing media components to increase secretion of biomolecules and factors affecting biochemical process for biosynthesis of CuNps. However expression level of biomolecule is genetic attributes of organism.
- Copper binding proteins in all biota are homologous and evolved from prokaryotes to eukaryotes. Their expression is useful in reducing toxic effect of free form of copper in all biota. However their expression depends on genetic evolution of copper binding protein gene.

3.3. Research hypothesis

- Microorganisms growing in copper rich habitats would have evolved with copper binding proteins and would show higher expression and resistance to copper.
- Higher copper resistance would secrete higher amount of biomolecules, required for extracellular biosynthesis of CuNps, which would act as reducing and capping agent and provide higher yield of biosynthesized copper nanoparticles (BCuNps).
- Optimization studies would further improve the secretion of biomolecules, control over production conditions and yield of BCuNps.
- These BCuNps would be biocompatible with different physicochemical properties than native copper.
- These BCuNps would have better penetration and surface properties.
- Combination of BCuNps and risedronate sodium would remove physical incompatibility of native copper and improve bioavailability of risedronate.
- Combination therapy would have additive effect.
3.4. Objective

Objective of this research project was to explore various aspects for the development of copper as a pharmaceutical product for supplement therapy in post-menopausal women.

The study was designed to develop a copper formulation with following properties:

- The formulation should help to improve the absorption of copper in controlled manner
- The formulation should improve bioavailability of copper
- The formulation should be biocompatible and use biocompatible form of copper
- The formulation should be administered by topical route
- The formulation has to combine copper with risedronate to see any additive effects

Following tasks were envisaged to achieve the above desired properties:

- To isolate and characterize a copper resistant bacterium, capable of producing BCuNps
- To optimize cultural conditions and factors affecting biosynthesis of BCuNps
- To purify and characterize BCuNps
- To evaluate BCuNps for safety and efficacy through in vitro studies
- To develop, evaluate and compare the gel formulations of BCuNps, native copper and risedronate sodium and their combination
- To evaluate anti-osteoporotic effect of developed formulations in ovariectomized rat model
3.5. Plan of work

Collection of soil samples from copper mine

Screening for copper resistant bacteria

Characterization and identification of copper resistant bacteria

Bioprospecting for biosynthesis of copper nanoparticles

Optimization of cultural conditions and factors affecting biosynthesis using QbD

Biosynthesis, purification and characterization of copper nanoparticles

In vitro evaluation of biosynthesized copper nanoparticles (BCuNps)

Development and evaluation of gel formulation of BCuNps

Acute dermal toxicity studies for topical administration of developed formulation

Evaluation of anti-osteoporotic effect of developed formulation in ovariectomized rat model