Synthesis of Nickel Nanoparticles by Using Collagen Extract of Emperor Fish Waste and Biological Applications

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Abstract:

The biosynthesis method of nanoparticles is a alternative, inexpensive and eco-friendly as compared to physical and chemical methods. The aim of this work is the biosynthesis of Nickel nanoparticles from collagen extract of emperor fish wastes such as bone, skin, scales. Fishes are the alternative sources of collagen and it is extracted by acid soluble soluble method (ASC). The green synthesized nickel nanoparticles from collagen extract were characterized by UV-Vis spectroscopy (UV-Vis), X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM).

Keywords: *Collagen, Fish waste, Acid soluble collagen extraction method (ACS)*

INTRODUCTION

COLLAGEN

Collagen is a naturally occurring protein present in the animal body, it is fibrous in nature and found in the flesh and connective tissue such as cartilage, bones, tendons, ligaments and skin of invertebrates and vertebrates of mammals.[1,2] Collagen consist of amino acid linkage results triple helix of elongated fibril known as collagen helix.[3] Collagen presents 25 to 35% of the protein content.[4] It is an important material which is used for pharmaceutical and cosmetic applications.[5] The collagen produced by organisms is defined as endogenous, in which it consists of three long helicoidally shaped chains of amino acids.[6] Chains of polypeptide constituted by the repeated sequence $(Gly\text{-}X\text{-}Y)_n$ form collagens, where X and Y can be occupied by any amino acid, although these positions are commonly occupied by proline and 4-hydroxyproline.[7] Collagen breaks down due to aging, the degradation of collagen results in wrinkles, sagging skin, stiff joints and dry skin.[8]

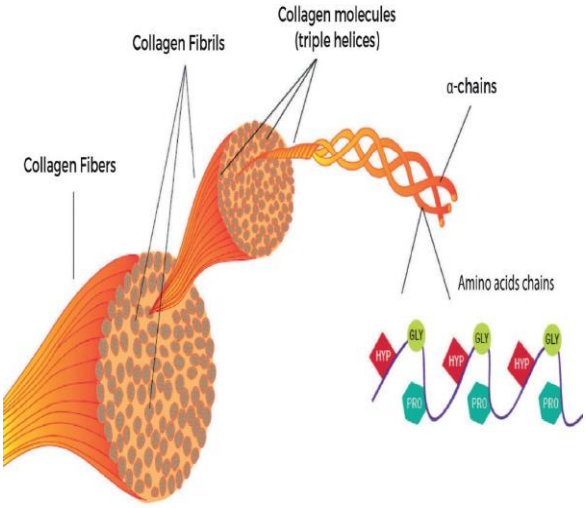

Fig 1.1 Structure of collagen fibers, collagen fibrils and triple helix structure

Sources of Collagen

The main sources of collagen are pig, deer, chicken, egg whites fruits and vegetables like citrus fruits, berries, red and yellow vegetables, garlic, cashew, etc., Fishes are found to be the alternative excellent source for collagen which is the most abundant protein in the human body.[9]

Marine Collagen

Marine collagens such as fish skins, scales, cartilage and bones, other marine invertebrates and vertebrates are bioavailable sources as compared to bovine collagens and it have higher absorption capacity.[10] The extraction of collagen involves in two methods namely Acid soluble method and Pepsin soluble method.[11,12]

Nanotechnology

Nanotechnology is the creation of nano dimension particles which are potentially used for human benefits.[13] The nanoparticles synthesized from biological methods can be the alternative, inexpensive and eco-friendly as compared to the chemical and physical preparation method.[14] The biosynthesis of nanoparticles is a bottom up approach and it involves in both oxidation and reduction reaction.[15] The nanotechnology has a wide range of applications mainly in the field of biomedical applications.[16]

Marine based nanoparticles

The marine based nanoparticles synthesis is very rich in nature due to the presence of some renewable sources in the marine animals.[17] The presence of nanostructure on shark skin gave a new advancements in the marine nanotechonology in synthesis and which is used for biomedical applications.[18] Fish is the one of the predominant source of human diet and having protein contents in it. Fishes serves a vital healthy food due to the higher protein, beneficial fats and various micro nutrients.[19]

Metal nanoparticles

Generally metal nanoparticles are used as catalyst for many chemical reactions. And this metal nanoparticle catalyst has several advantages like easily dispersed nature in solutions, controlled size and shape, etc.

Nickel nanoparticles

Nickel nanoparticles have gained much attention due to their unique magnetic, chemical, and physical properties as well as their potential applications in various technological fields such as catalysis, battery manufacture, novel ink for nanotube-printing, incorporation in textile, enhanced pseudo-capacitance, field-modulated gratings and optical switches, direct immobilization of biomolecules through magnetic force of nickel nanoparticles, and adsorption of yellow dyes[20,21]. In comparison to other magnetic nanoparticles, Ni nanoparticles possess great potential as a catalyst in reactions and propellant and sintering additive in coatings, plastics, and fibres. Due to its relative abundance in the earth's crust, Ni is more cost-effective than most of the metals in use as a catalyst[22]. The electrical conductivity of nickel enables its use in several applications. Nickel nanoparticles can be used as nano fluids in high purity, ultra-high purity, passivated, coated, and distributed forms [23]

MATERIALS AND METHODS

Collection of Fish Wastes

Fish wastes (skin, bone, scales, fins) of Emperor fish were collected from local fish market. The material was washed with tap water and distilled water. The collected fish waste were dried for about two weeks.

Preparation of Collagen Extract

The 10g of the dried sample was treated with 1000ml of 0.1M NaOH solution for 24h stirring, to remove non-collagenous protein. The residue from the above treatment was stirred with 1000ml of 0.5M Acetic acid for 24h and then centrifuged to 3000rpm for 20 mins. The supernatant from the above solution is the Acid Soluble Collagen Extract.

Synthesis of Nickel Nanoparticles

The 2g of $NiCO_3$ was dissolved in 100ml of distilled water, then 10ml from the nickel solution was added to the 10ml of the Acid Soluble Collagen Extract. Then the mixture was stirred with 100^{o}C refluxing for 1 hour until the colour of the solution becomes dark, that indicates the formation of nanoparticles. Then the solution was centrifuged and the yielded the The precipitate was washed with distilled waster and dried.

Characterizations

UV-Vis spectra analysis

The extracted collagen and synthesized Nickel nanoparticles was characterized by the UV-Visible spectrum. This analysis was carried out by diluting a small amount of the samples with water. The UV-Visible analysis of the collagen and nickel naoparticles was done by using UV-Visiblespectrometer.

Scanning Electron Microscope (SEM)

The surface analysis of the prepared Nickel nanoparticles was characterized by using a scanning electron microscope. The small amount of the synthesized nickel nanoparticle was sent for Scanning Electron Microscope(SEM) analysis.

Energy Dispersive X-ray Analysis (EDAX)

Energy-dispersive X-ray analysis (EDAX) is a technique used for the measurement of nanoparticles by SEM. In this technique, the nanoparticles are analyzed by using an EDS X-ray spectrophotometer present in modern SEM.

X-Ray Diffraction study

The XRD patterns of the sample were analyzed to determine peak intensity, position and width. The particle size of the sample was calculated by using Debye Scherrer's formula.

$$D=K\lambda / \beta COS\theta$$

Where, D is the particle size to be determined, **λ** is the wavelength of X-ray radiation and β is the full width half maximum (FWHM) for diffracted peak at the diffraction angle θ. The X-ray Diffraction (XRD) pattern for the synthesized nanoparticles was done by using X-ray Diffractometer.

Protein test for Collagen extract

a) Xanthoprotic test

To the few drops of the extract, add 2 – 4 drops of conc. HNO_3 and note the changes.

b) Biuret test

To the few drops of the extract, add few drops of $CuSO_4$ solution followed by NaOH and the changes were observed.

REULTS AND DISCUSSION

The nanoparticles were characterized by various analytical techniques.

Characterization of Collagen

Protein test

a) Xanthoproteic Test

The yellow colour change in the xanthoproteic test showed the confirmation of presence of protein in the collagen extract.

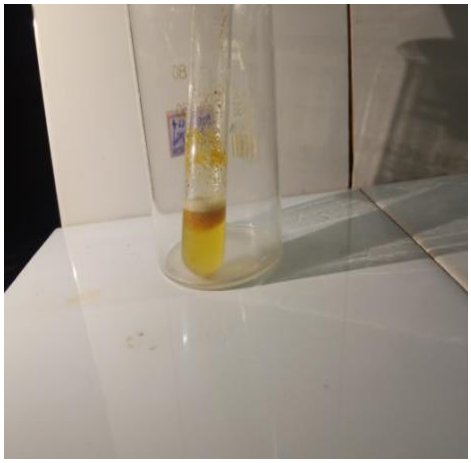

Fig 2. Xanthoproteic test

b) Biuret Test

The formation of violet colour solution in biurette rest indicates the presence of protein in the collagen extract.

Fig 3. Biuret test

UV-Visible Analysis

The collagen extract from fish waste was characterized by UV-Visible spectrophotometer. The maximum absorption peak was obtained at 192 nm. The peaks attributed to the n→ π^* transition of the carbonyl groups and amino groups.

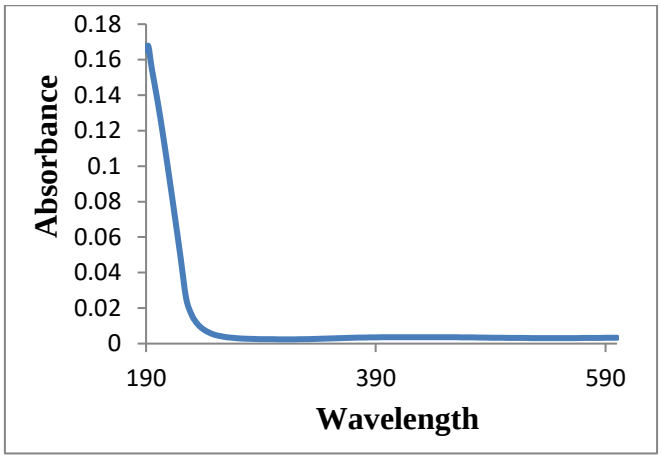

Fig 4. UV-Vis absorption of collagen

Characterization of Nickel nanoparticles

The synthesized Nickel nanoparticles were characterization by UV-Vis, XRD, SEM and EDAX.

UV-Visible spectroscopy (UV-Vis)

The synthesized nickel nanoparticles from collagen extract were characterized by using UV-Vis spectrophotometer. The maximum absorption peak was obtained at the range Of 270nm, the peak corresponds to the $\pi \rightarrow \pi^*$ transition.

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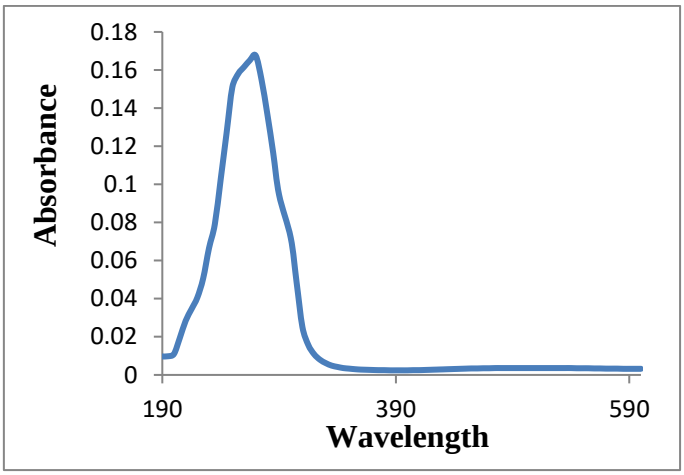

Fig 5. UV-Vis absorption of NiNPs

Scanning Electron Microscope (SEM) Analysis

SEM Analysis was performed to observe the surface morphology and particle size distribution of the synthesized Nickel nanoparticles from the collagen extract. The shape of the synthesized nickel nanoparticles is spherical in shape. The SEM image of the nickel nanoparticles are shown in fig.

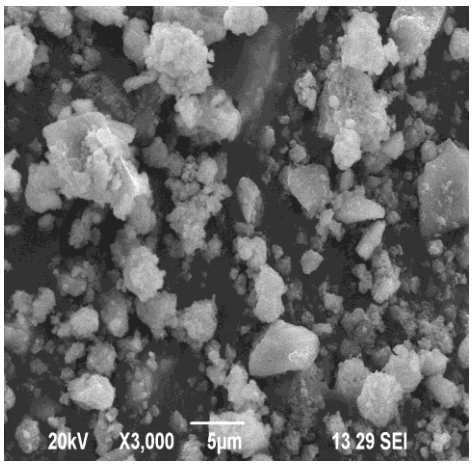

Fig 6. SEM image of NiNPs at 5µm

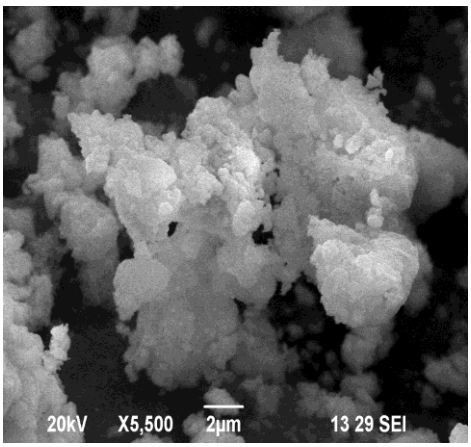

Fig 7. SEM image of NiNPs at 2 µm

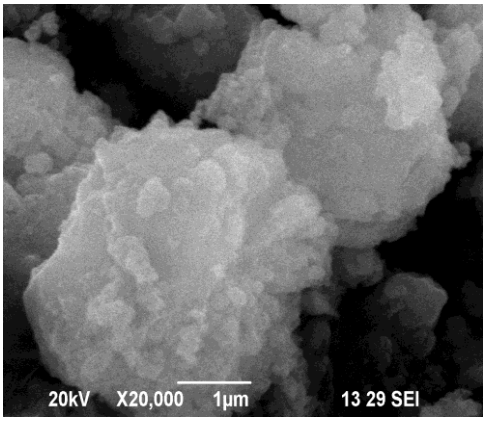

Fig 8. SEM image of NiNPs at 1 µm

Energy Dispersive X-ray Analysis (EDAX)

EDAX was performed for the measurement of nanoparticles by SEM. The EDAX analysis confirms the nanoparticles synthesized from collagen extract contains Ni peak along with C and O peak. The image is shown in the fig.

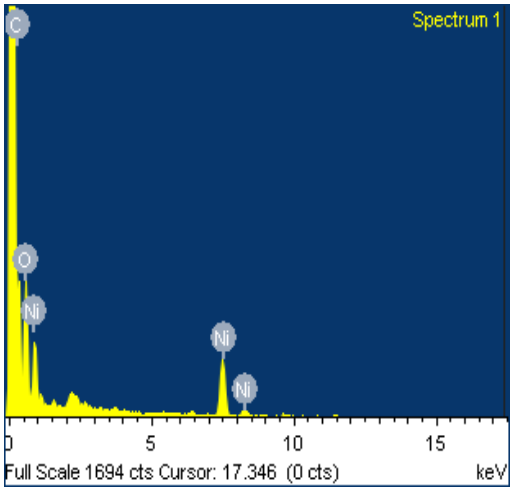

Fig 9. Image of the EDAX

X-ray Diffraction Study

The x-ray diffraction pattern of synthesized Nickel nanoparticles were analysed by using an x-ray diffractometer. The XRD pattern of the synthesized Nickel nanoparticles results the size of the nanoparticles. The experimentally obtained x-ray diffraction angle was compared with the standard copper metal. The XRD pattern for the nickel nanoparticles gives 2θ value at 11.8079^{o}.

The size of the nanoparticles can be calculated by using **Debye-Scherrer's** equation.

$$D=K\lambda / \beta COS\theta$$

Where,

D is the size that can be calculated.

K is the scherrer's constant (0.94).

λ is the wavelength having value 1.5406.

β is the full width for half maximum for the diffracted peak.

θ is the Bragg's angle for the peak.

$$D = 0.94 \times 1.5406 / 1.1424 \times 0.9947$$

$$D = 1.2743 \text{ nm}$$

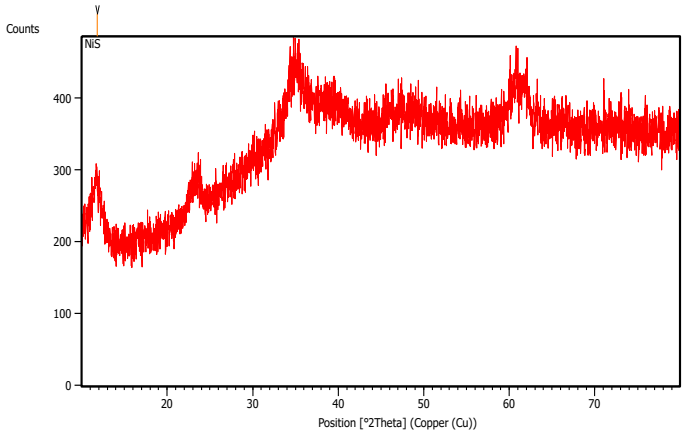

Fig 10. XRD pattern of NiNPs

Table 1. Size of the synthesized Nickel nanoparticles

S.No	2θ(deg)	FWHM (deg)	Particle size(nm)
1.	11.8079	1.1214	1.2743

BIOLOGICAL APPLICATIONS

Anti-microbial Activity

The collagen extract and the synthesized Nickel nanoparticles was tested with two bacterial pathogens, one-gram positive pathogen *Bacillus subtilis* and one gram negative pathogen *Escherichia coli* and one yeast pathogen *Candida albicans* by diffusion assay.

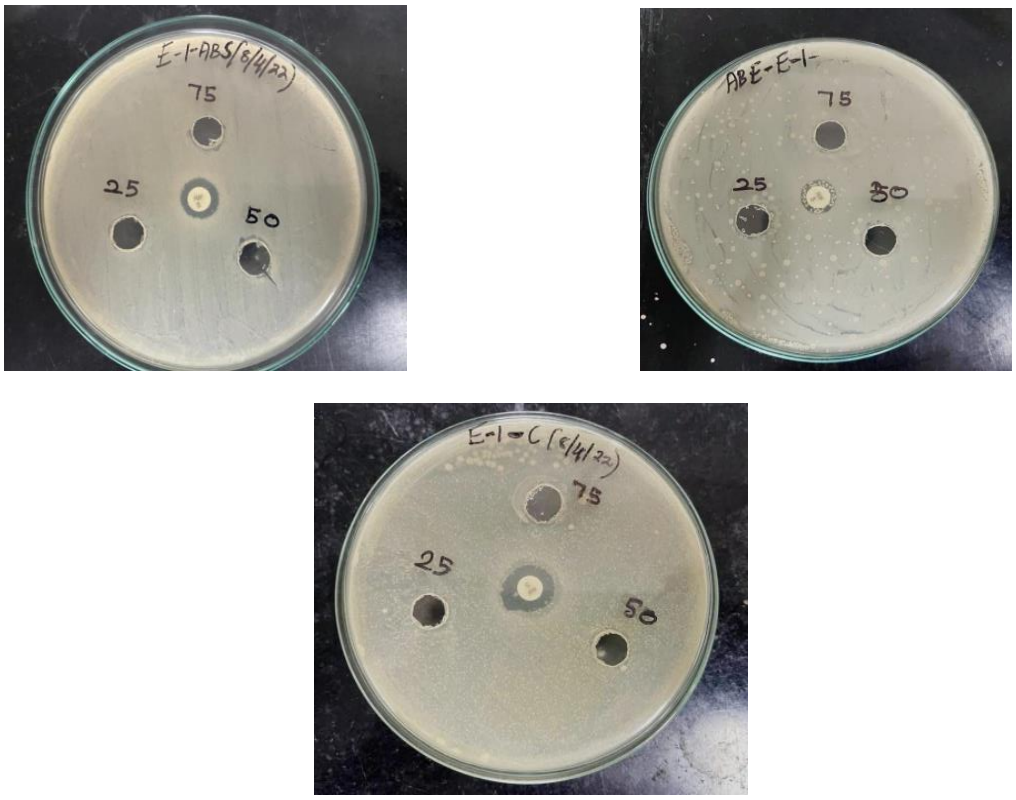

Fig 11. Microbial activity of Collagen at different concentration

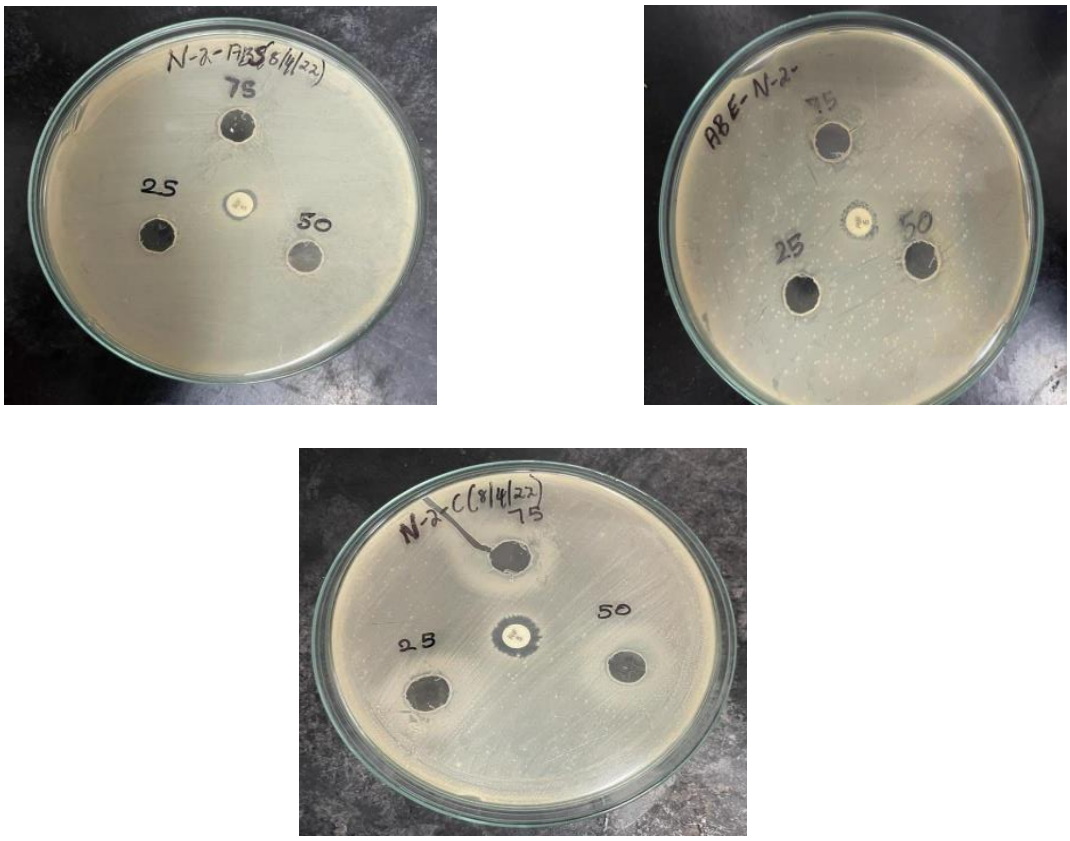

Fig 12. Microbial activity of Synthesized Nickel nanoparticles at different concentration

Table 2 Antimicrobial activity of Collagen extract

Name of the Microorganism	Concentration / Zone if Inhibition			
	25µl	50 µl	75 µl	Standard
Bacillus subtilis	-	-	-	9
Escherichian coli	-	-	-	10
Candida albicans	-	-	-	12

Table 3 Antimicrobial activity of Nickel Nanoparticles

Name of the Microorganism	Concentration / Zone if Inhibition			
	25µl	50 µl	75 µl	Standard
Bacillus subtilis	-	-	-	8
Escherichian coli	-	-	-	9
Candida albicans	-	-	-	11

Antioxidant property

The inhibition of the oxidation activity of nickel nanoparticles were decreasing as concentration of the sample increases. The inhibition of the sample was compared with the standard Ascorbic acid values. The image of the inhibition of nickel nanoparticles sample was shown in the **Fig.4.10** and **Fig.4.11.**

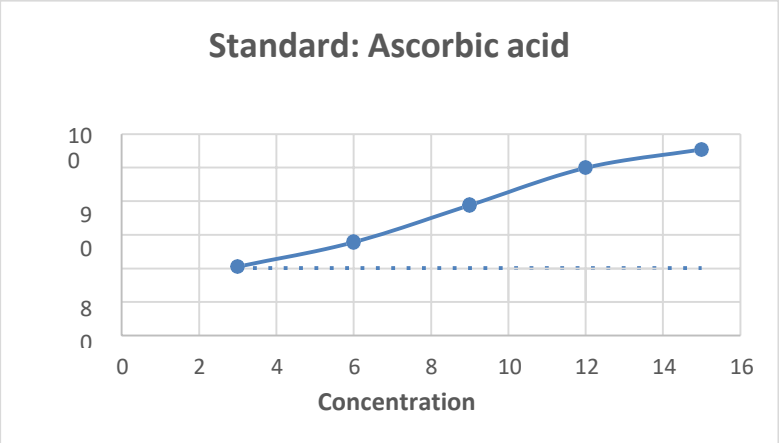

Fig.4.10 Inhibition of Standard Ascorbic acid

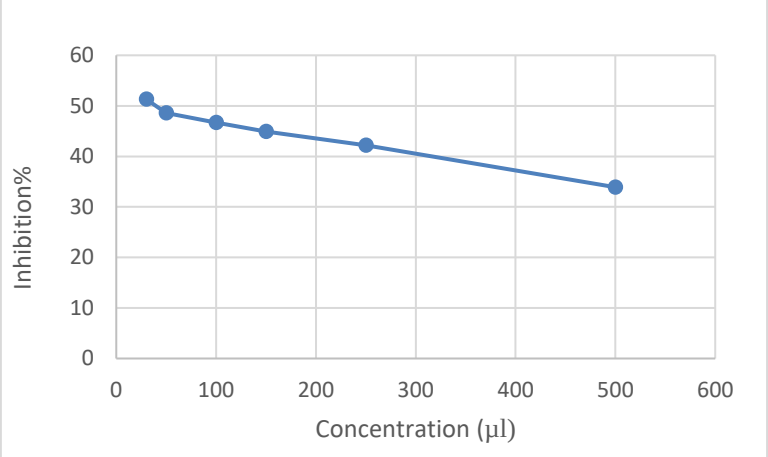

Fig.4.10 Inhibition of synthesized nickel nanoparticles

CONCLUSION

In this paper, we have successfully synthesized nickel nanoparticles using a collagen extract of emperor fish waste, in which many applications can be observed. The UV-Vis spectra obtained from the synthesized nickel Nanoparticles shows the absorption band around 200nm. The synthesized nickel Nanoparticles displays a spherical morphology according to the micrographs obtained from the SEM technique. In addition, the XRD patterns helps to identify the size of the synthesized nickel nanoparticles as reported for Nickel. We concluded that the synthesis of Nickel Nanoparticles from collagen extract is eco-friendly, green, cheap and simple method.

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