National Journal of Biological Sciences

Received: 20th January, 2025
Revised: 4th March, 2025
Accepted: 22nd May, 2025
Published: 24th June, 2025

DOI: https://doi.org/10.37605/v6i1/3

REVIEW ARTICLE

TITLE:

SYNTHESIS OF ZINC SULFIDE NANOPARTICLES AND CONJUGATION WITH FOLATE AND SORAFENIB DRUG FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

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SYNTHESIS OF ZINC SULFIDE NANOPARTICLES AND CONJUGATION WITH FOLATE AND SORAFENIB DRUG FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

ABSTRACT

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It is a cancer that originates in the hepatocytes, which are the main functional cells of the liver. HCC typically develops in the context of chronic liver diseases, such as cirrhosis (scarring of the liver tissue) or chronic hepatitis B or C infection. In its early stages, HCC may not cause noticeable symptoms. As the cancer progresses, symptoms may include abdominal pain or discomfort, unexplained weight loss, jaundice, fatigue, and a feeling of fullness in the abdomen. Treatment options for HCC depend on the stage of the cancer and the overall health of the patient. They may include, radiation therapy, targeted therapy, immunotherapy, and in some cases, chemotherapy. Liver transplantation can be curative for early-stage HCC in select patients.

Keywords: Hepatocellular carcinoma (HCC), Cirrhosis, Chronic hepatitis B and C, Liver transplantation, Targeted therapy and immunotherapy

1. INTRODUCTION

Nanobiotechnology is an interdisciplinary subject that mixes each the nanotechnology and biotechnology in its regard. It includes all kind of packages of the nanoscale substances and equipment for the organic processing. It has diverse of packages in addition processing the synthesis of its main forms – nano debris that have massive effect withinside the variety of body structure of beings. The manipulation of count at the atomic, molecular scale and supramolecular scale commonly beneath the one hundred nanometers is called as Nanotechnology. The use of organic structures or organisms for the synthesis of merchandise that offer diverse packages withinside the subject of technological know-how is called as Biotechnology. Nanobiotechnology own the epic and simply extra special residences of nanoscale substances to narrate with the organic structures in methods that aren't viable at large scales. This can consist of more desirable reactivity, electric conductivity and the functionality to have interaction with organic molecules in novel methods [1]. Its key additives consist of the nano substances, biomolecules and strategies.

The nano substances consist of the nanowires, nano debris, nano rods and nano fibers for the packages of medication and industry, they may be frequently synthetic with the aid of using the metals, semiconductors and polymers. The biomolecules consist of the DNA, RNA – nucleic acids, proteins and lipids alongside one-of-a-kind enzymes for the diverse packages. The strategies consist of the extensive techniques which include lithography for the synthesis of nano systems for one of a kind uses. The nano biotechnology has large packages and it covers all the associated fields as Targeted drug delivery, Diagnostics and therapeutics, Nano fertilizer and Nano insecticides in agriculture, Pollution control and Industrial packages which include meals protection and packaging.

Nanobiotechnology holds mammoth ability for revolutionizing diverse fields, specifically medication and environmental technological know-how, with the aid of using offering novel answers to massive problems. Besides nanobiotechnology, we're searching ahead in the direction of the synthesis of nanoparticles which comes below the class of this rising subject. The nanotechnology has splendid

packages in one of a kind associated fields of medication, industry, agriculture and lots more [2].

The metal core nanoparticles are comprised of natural metallic or alloy-based core structure. They own precise traits of bodily chemical and biological. Their length as much as the nanometers, with their smaller length and huge floor area, they showcase the precise traits for distinctive applications. Their reactivity is more suitable and interplay with different factors and substances. They own precise Optical traits including Surface plasmon resonance wherein the conduction electrons at the metallic floor oscillate in resonance with incident light. They act as catalyst in chemical reactions because of excessive floor area. They showcase magnetic homes for the drug delivery [3].

Hepatocellular carcinoma is the maximum not unusual place form of liver most cancers and it progresses a number of the sufferers of persistent liver disorder as Hepatitis B & C infection [4]. The sufferers are at an excessive chance of happening the Hepatocellular carcinoma. They are a number of the sufferers which are excessive alcoholic, having a record of colossal alcoholic intake. Their signs and symptoms include; the unexpected weight reduction and stomach ache frequently accompanied via way of means of fatigue and jaundice. They are frequently recognized via way of means of the blood exams of alpha and beta feta proteins, the picture research as MRI and CT scan [5]. Liver biopsy is accomplished for showed diagnosis. This cited most cancers HCC is advanced via the cirrhosis, the liver disorder of Hepatitis B&C infection, whilst it turns into excessive then it results in the tissue damage, infection, then via genetic mutation the pathway contributes to the conversion of hepatocytes into the tumor cells [6][7]. They impede the float of blood to liver via way of means of developing portal hypertension, fluid accumulation withinside the stomach and bleeding withinside the tissues. The ongoing infection and fibrosis result in the out-of-control department of hundreds of cells.

The Hepatocellular carcinoma whilst interacted with the nanoparticles offer the centered drug transport except different remedy strategies as liver transplant and chemotherapy [8]. The receptor mediated endocytosis wherein the nanoparticles engage with the over expressed receptors at the HCC cells, main to closing internalization. Tumor frequently have leaky vasculature which complements permeability and retention for the Nanoparticles to build up greater in tumor cells. The floor amendment via way of means of binding to peptide offer the improved specificity and uptake of the medicine whilst they may be bind to changed antibodies or different molecules [9]. In this manner the nanoparticles carry out the desired and centered drug transport. Gold nanoparticles including AuNPs, for imaging, drug transport and may be functionalized with ligands. Liposomal nanoparticles, encapsulate the chemotherapeutic tablets for his or her transport performance and decreasing aspect effects. Iron oxide nanoparticles, in MRI and imaging for visualization of tumors [10].

2. MATERIAL AND METHODS

2.1 Synthesis of Zinc Sulfide Nanoparticles

The First step is to prepare the solution of Zin Acetate and Sodium Sulfide, to prepare these solutions add 6.61 g of zinc acetate in the 50 ml deionized water and add 8.7 g of the sodium sulfide in the 50 ml de-ionized water. After making these solutions, mix them well by stirring continuously for 5 hours at Room temperature by using the hot plate or magnetic stirrer. Now add 10 ml cysteine solution in the mixture and mix it well. After this add the prepared solution in Falcon tubes and centrifuge it at 3000 rpm for 30 minutes. After centrifugation discard the supernatant

and dry the nanoparticles in the incubator for 15 hours at 37° Celsius. Store them in PBS buffer [11].

2.2 Conjugation with Sorafenib

The attachment of sorafenib with Zinc Sulfide nanoparticle is done via glutaraldehyde method. A functionalized nanoparticle having amino group is cross-linked with the amino group of sorafenib drug. These both are incubated together in the presence of glutaraldehyde, which acts as a cross-linking agent. The glutaraldehyde causes stable linkage between the amino group of ZnS nanoparticles and amino group of sorafenib drug. In this method 40 mg of drug and 0.2 ml pyridine is dissolved in 25 ml of water. After mixing shake this solution at 100 rpm for 10 minutes, add 1.3 ml of glutaraldehyde and shake it for 3 hours. Now add 25 µl of synthesized nanoparticles in it at and shake at 100 rpm for 15 hours. Store some solution for pre-coupling. Now add this prepared solution in 10 Eppendorf and centrifuge at 7000 rpm for 10 minutes. After centrifugation store some of the solution for post coupling, discard the supernatant and incubate the pallet in Glycine for 30 minutes at room temperature. Again, centrifuge the solution at 7000 rpm for 10 minutes. Discard the supernatant and wash it with buffer (0.01M Tris & 0.15M NaCl). Now store the solution in PBS Buffer. FTIR and NMR Studies can check the efficiency of conjugation [12].

2.3 Conjugation with Folic Acid

The conjugation of folic acid with nanocomposite is done via Carbodiimide method. Through this process, a covalent bond is formed between carboxyl and ammino groups. This method involves the use of a bifunctional cross-linker, N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide (EDC), in combination with N-hydroxy succinimide (NHS). This method facilitates the covalent bonding between the folate ligand and the amino groups present on the surface of zinc sulfide nanoparticles. For this method 5 mg of folic acid has weighted and then dissolved in 25 ml of distilled water. By using 1M NaOH adjust the pH at 8. Out of this take 2 ml in separate falcon tube as pre coupling. Now mix the remaining solution well after adding 20mg of synthesized particles. Put this mixture in Ice bath for 10 minutes. After this incubate the solution at 37°C for 15 minutes. After that add 400 mg of carbodiimide and pH was set to 6 by 1N HCL. Now shake at room temperature for 6 hours at shaking incubator. After 6 hours again add 400 mg of carbodiimide in it and place for stirring at shaking incubator set at room temperature for 15 hours. After this centrifuge the reaction mixture and collect the pallet and store it in PBS buffer at 4°C. The efficiency of this conjugation can be checked by FTIR and other techniques.

3. RESULTS

3.1 Characterization

Characterization is done to ensure the synthesis and conjugation of Nanoparticles. Characterization Techniques involves Microscopy, FTIR, UV Spectrometer and X-Ray Crystallography. First indication in the synthesis of Nanoparticles is the color change of the solution. As Shown in Figure, the formation of Black color characterizes the formation of Zinc Sulfide nanoparticles.

3.1.1 Zinc Sulfide Nanoparticles

Figure 3.1: ZnS nanoparticles conjugated with cysteine

3.2.2 Ninhydrin Test

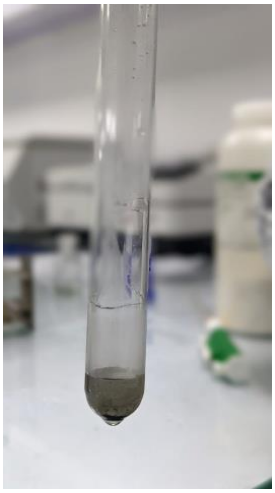

Figure 3.2:
Test sample without Cysteine

Figure 3.3:
Final color after Ninhydrin Test

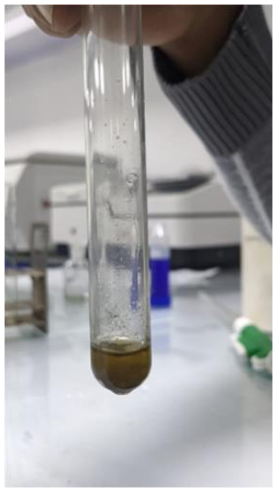

Figure 3.4:
Initial color of ZnS nanoparticles

3.2.3 Figure at 4X and 10X Zoom

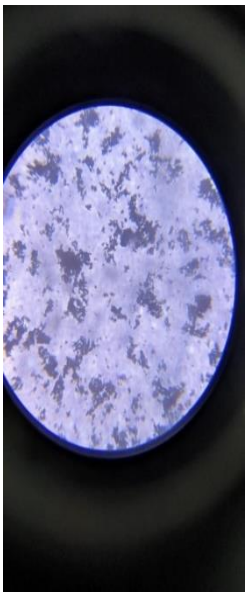

Figure 3.5(a): 4x Zoom

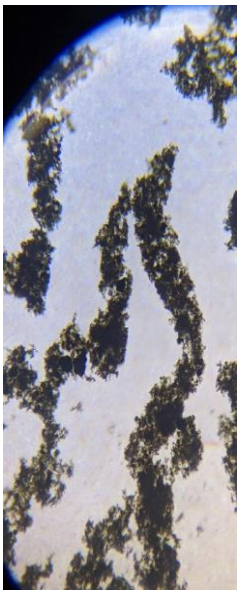

Figure 3.5(b): 10x Zoom

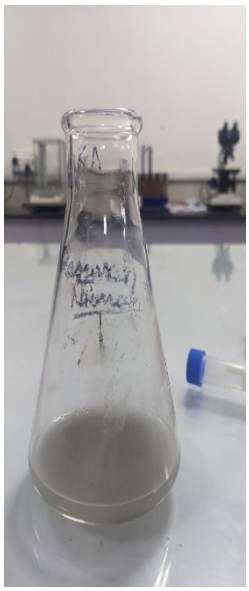

Figure 3.6: ZnS nanoparticles Conjugated with Sorafenib

Figure 3.7: ZnS nanoparticles Conjugated with Folic Acid

3.2 UV spectrophotometer

UV spectrophotometer is one of the confirmatory tests for the synthesis ZnS nanoparticles and its conjugation with Sorafenib and Folic Acid. The average peak of Zinc Sulfide nanoparticles lies between 250-350 nm with a common peak around 340 nm. ZnS nanoparticles conjugated with Sorafenib drug shows a peak around 260 nm after performing tests with every 20 minutes interval. While the ZnS nanoparticles conjugated with Folic Acid shows peak at about 290 nm [11].

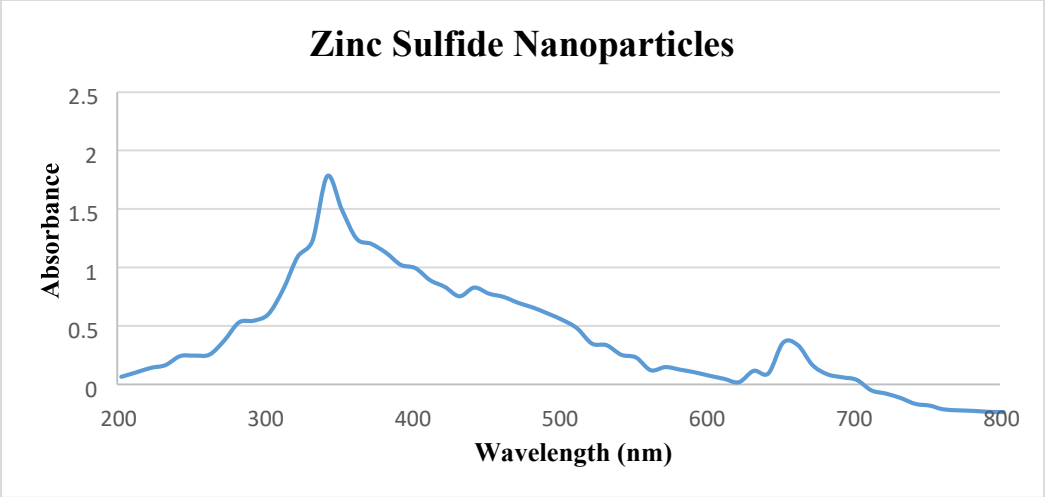

Figure 3.8:UV analysis of ZnS NPs

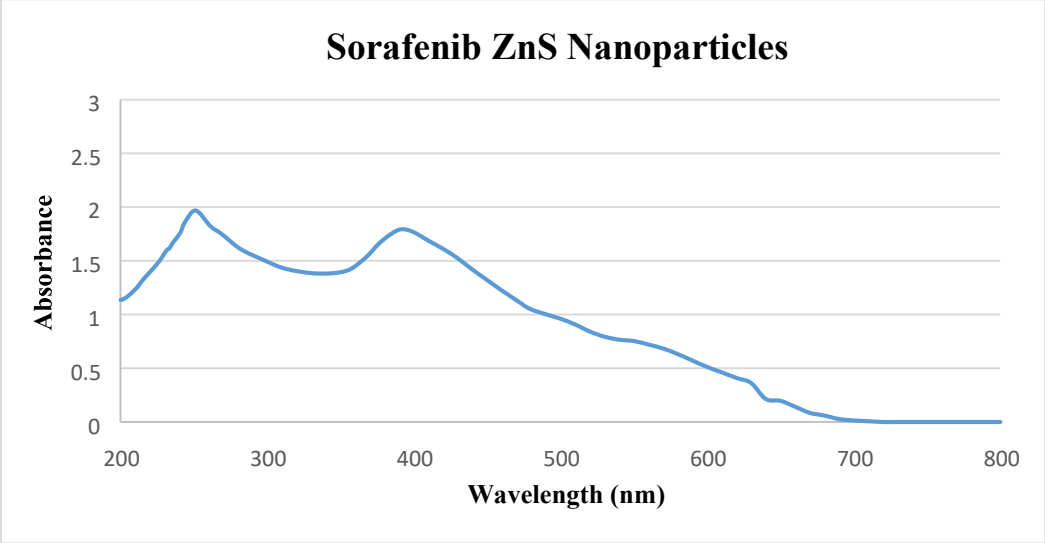

Figure 3.9: UV analysis of Sorafenib-ZnS NPs

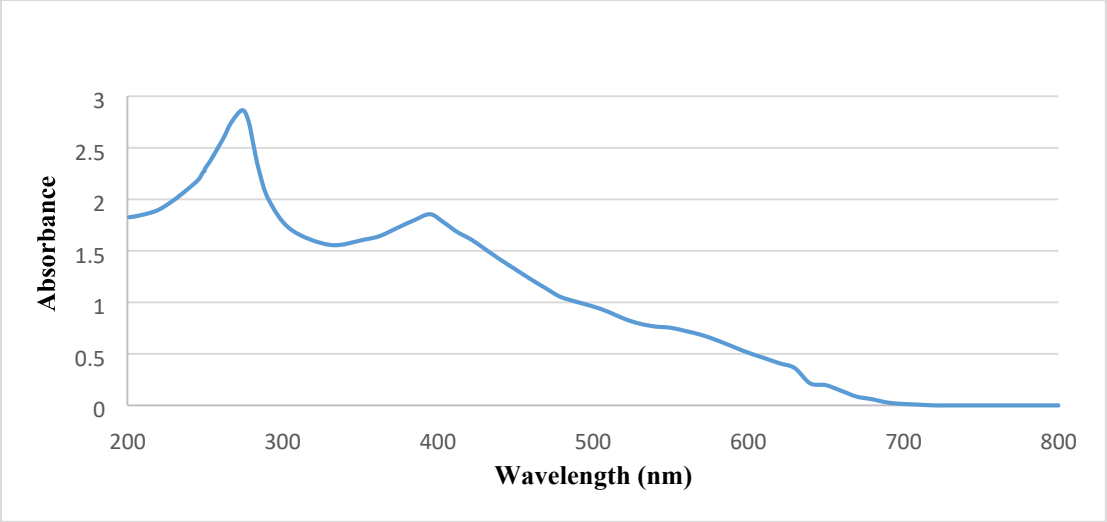

Figure 3.10: UV analysis of sorafenib-Folic Acid-ZnS NPs

3.3 FTIR Analysis

FTIR Analysis is done to observe the binding of the functional Groups. It is the confirmatory test to observe the binding of the Sorafenib drug and Folic Acid with Zinc Sulfide nanoparticles. The vibrations that occur when ZnS bonds stretch is usually found within the range of 400-600 cm^3. This specific region plays a role, in detecting the presence of ZnS in your sample. Once folic acid and sorafenib are combined the aromatic C=C bonds can be observed around 1600-1580 cm^3 [11].

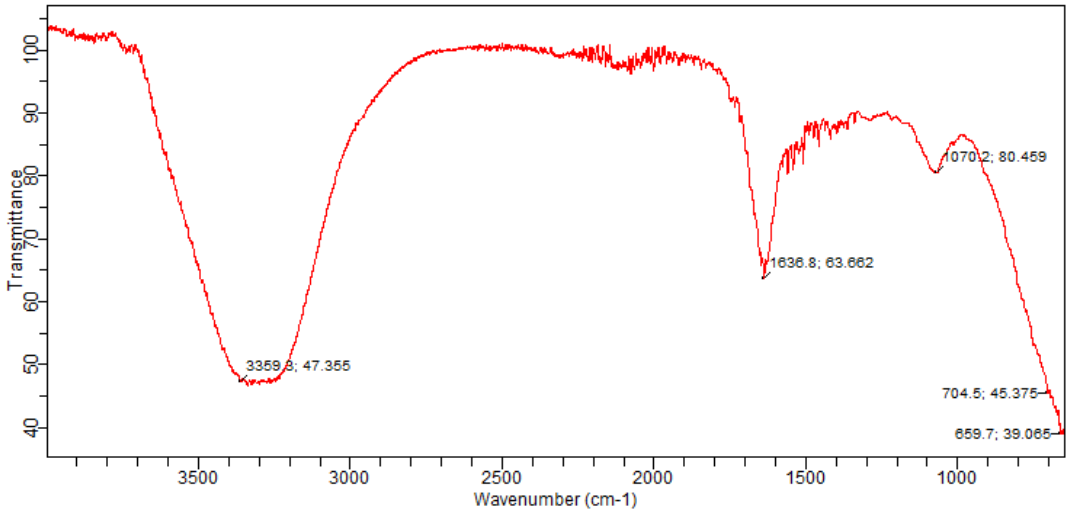

Figure 3.11: ZnS capped with Cysteine

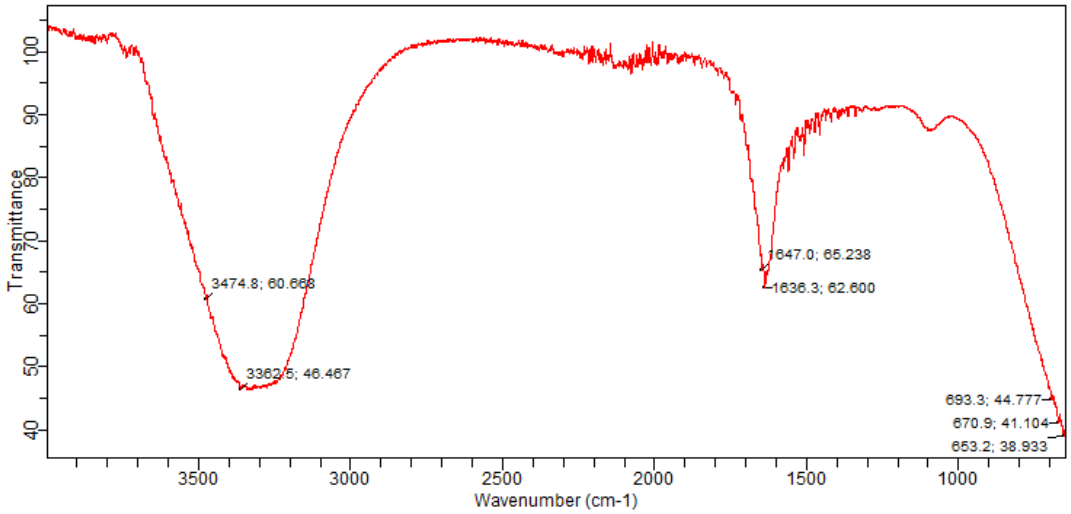

Figure 3.12: ZnS NPs conjugated with sorafenib drug

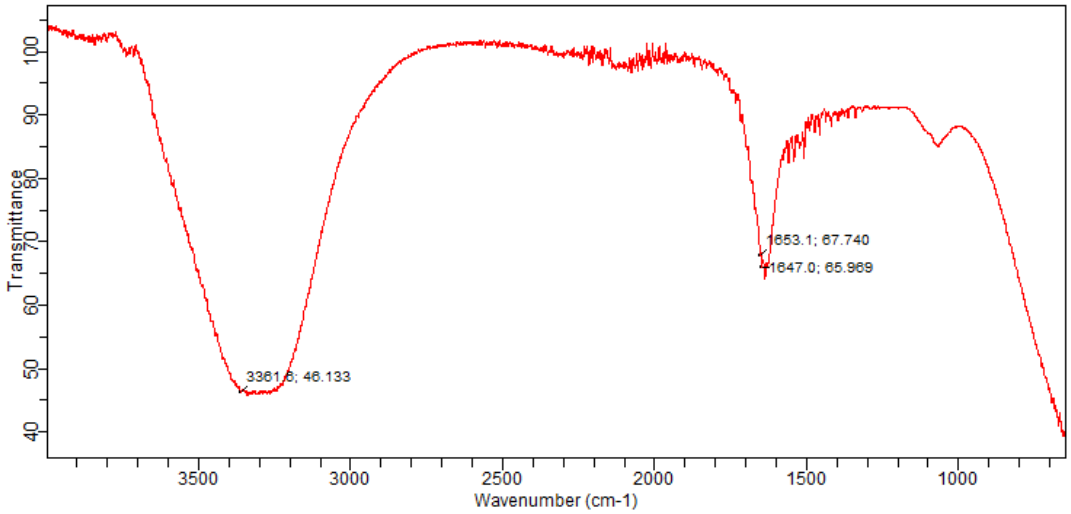

Figure 3.13: ZnS NPs-Sorafenib-Folic Acid

3.4 Immunochemistry

Immunochemistry is the method in biological techniques to detect the presence, localization and abundance of specific proteins in cell or tissue samples, antibodies are used in this type of study to detect the protein in tissue sample allowing visualizing the distribution and localization of the target molecules. In this method tissue samples of cancer cell lines are attached to the surface via formalin. Then this prepared nanocomposite is applied on this tissue sample, parallel to this a control tissue sample is also prepared. As shown in fig 3.14a & 3.14b there was no particles bound to this tissue sample while figure 3.15a & 3.15b shows that nanoparticles are bound to the receptor on the cancer cells present in the tissue sample.

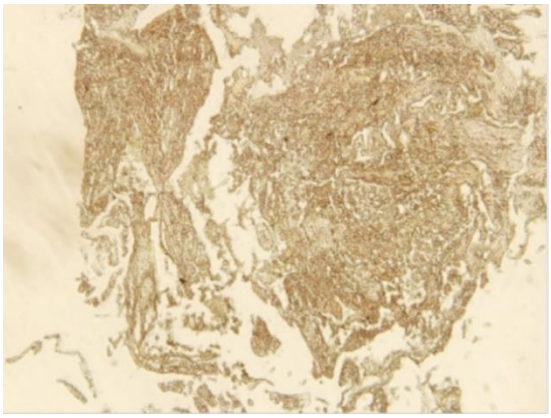

Figure 3.14a: Control sample of tissues showing no binding

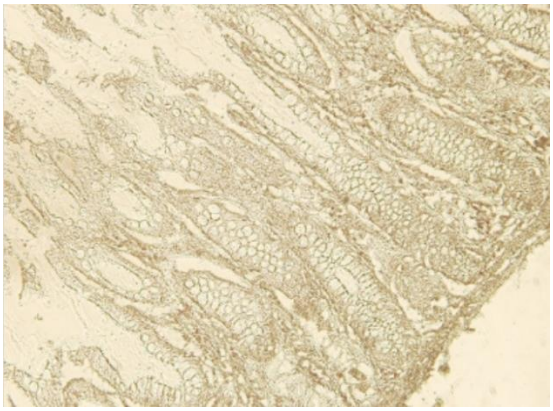

Figure 3.14b: Control sample of tissues showing no binding

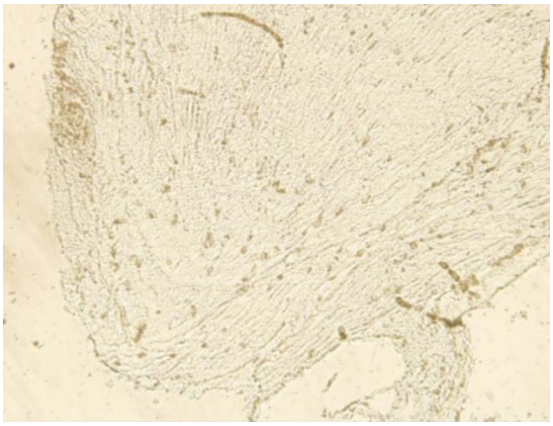

Figure 3.15a: Active binding of Nanocomposite to cancer cells

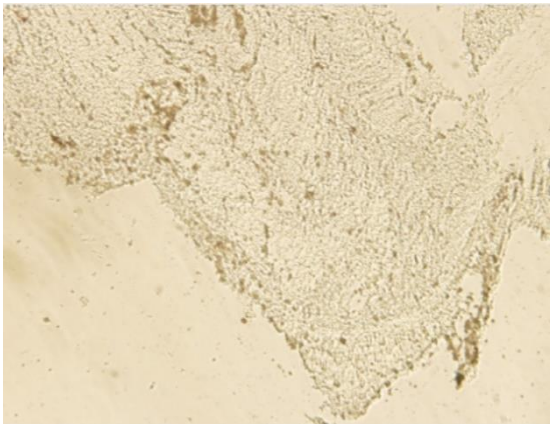

Figure 3.15b: Active binding of Nanocomposite to cancer cells

4. DISCUSSION

This study investigated the efficacy of hepatocellular carcinoma drug named sorafenib conjugated with zinc sulfide nanoparticles. It demonstrates that the nanoparticles will target the drug and enhance its efficacy as a chemotherapeutic agent to HCC while it reduces the off target and normal cells effect. Use of folic acid as a ligand on the nanoparticles surface increases the specificity of the drug targeted delivery to cancer cells, increased the drug accumulation and binding on the specific receptors of the cancer cells and reduce the toxicity [9][12].

Our results find out that the zinc sulfide nanoparticle based sorafenib drug delivery enhance the therapeutic index of it to HCC. The sorafenib drug is conjugated through glutamate and folic acid method [13]. The FTIR results show the conjugation of the drug with nanoparticles [11]. The nanoparticle-based drug is then tested on the mice model. HCC is introduced in the mice and then implement nanoparticle-based drug on it. The microscopic results demonstrate that the drug concentration on the HCC is more than normal or local cells. It shows that the drug is targeted and bind to the HCC due to the receptors of folic acid on HCC. There are limitations of this study that mouse model is used as the trail but it may give different results in the human due the difference in

physiological environment of humans [14].
With the aid of nanoparticles, the ability to target cancer cell is more precisely lead to the better clinical outcomes such as improved patient survival and higher remission rates. Reducing toxicity while using nano particle-based drug also improve the quality of life for patients that undergo chemotherapy. There are many researches that show significant positive result in treating the cancer cell using nanoparticles for making the drugs targeted [15].
Future research should focus on the clinical trials to evaluate the efficacy and safety of the nanoparticles-based system in human patient with HCC. Investigate the long-term biocompatibility and potential side effects of nanoparticles will be important for their successful translation into clinical practice.

5. CONCLUSION

Zinc sulfide nanoparticles conjugated with the sorafenib drug used for centered remedy of hepatocellular carcinoma. Zinc Sulfide nanoparticles conjugated with folate and Sorafenib drug have fine effect at the treatment of the Hepatocellular carcinoma due to the fact the presence of folate guarantees its shipping to most cancers cells and Zinc guarantees its shipping to HCC cells because of the presence of Alfa-feto protein. Characterization turned into finished with the aid of using FTIR, UV spectrophotometer and microscopy. In the prevailing studies the conjugation of zinc sulfide with sorafenib drug turned into showed with the aid of using FTIR and the use of UV spectrophotometer that confirmed the height variety turned into 340 nm and 370 nm. Higher awareness of drug in HCC cells of mouse version suggests that the drug grow to be centered with the aid of using unique folic acid ligand on nanoparticles.

ACKNOWLEDGEMENTS

We highly appreciate the School of Science, University of Management & Technology, Lahore for allowing and providing us the essential workspace for this study.

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