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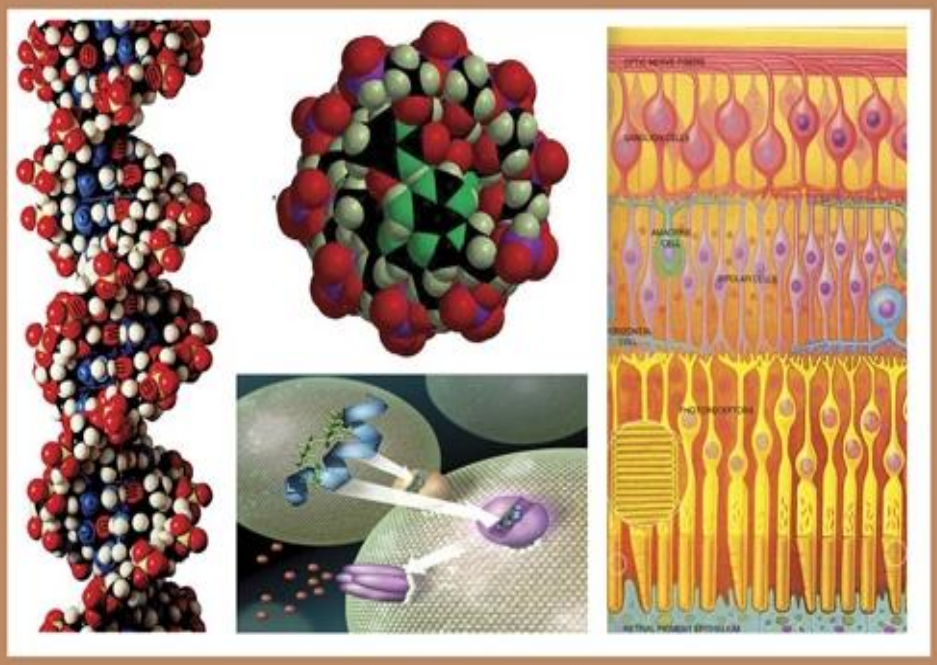

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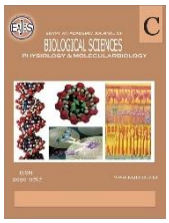

New Luminol Azo 1-(4 -sulfophenyl)-3-methyl-5- pyrazolone Reagent Preparation, Characterization, Biological Effectiveness and Enhancement Studies

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ABSTRACT

A new azo dye was produced by mixing 1-(4-sulfophenyl)-3-methyl-5-pyrazolone with diazonium salt from luminol. The produced dye was assessed using the FT-IR, ^{1}HNMR, and UV-Visible spectroscopy techniques. This dye was dissolved in five polar solvents, and its UV-visible absorption spectra were measured and spectroscopically analysed at room temperature. Additionally, an atomic force microscope (AFM) was used to measure the enhancement of CO_3O_4 and ZrO_2 nanoparticles in luminol azo 1-(4 -sulfophenyl)-3-methyl-5- pyrazolone solutions in aqueous solution.

INTRODUCTION

Ludwig Knorr coined the term "pyrazole" in 1883. Three carbon atoms and two nitrogen atoms close to each other make up the five-membered ring structure that characterises the family of simple aromatic rings of organic compounds known as pyrazoles. All of these constituents make up the heterocyclic compound. Despite being uncommon in nature, they are categorised as alkaloids due to their structure and pharmacological effects on humans. The first naturally occurring pyrazole, 1-pyrazolyl-alanine, was produced in 1959 using watermelon seeds.(Ardiansah, 2017)(Poudyal & Bharghav, 2021)(Eicher *et al.*, 2003)(Chauhan *et al.*, 2011).The most common form of pyrazol is a planar, colourless, or yellowish liquid or solid.(Saha *et al.*, 2021) The versatile lead component pyrazole was developed through chemical design for effective molecules with biological activity. Multiple synthetic techniques are used to create pyrazole-containing reactions, resulting in a distinctive molecule that opens up a lot of possibilities for medicinal chemistry (M. J. Alam *et al.*, 2015).

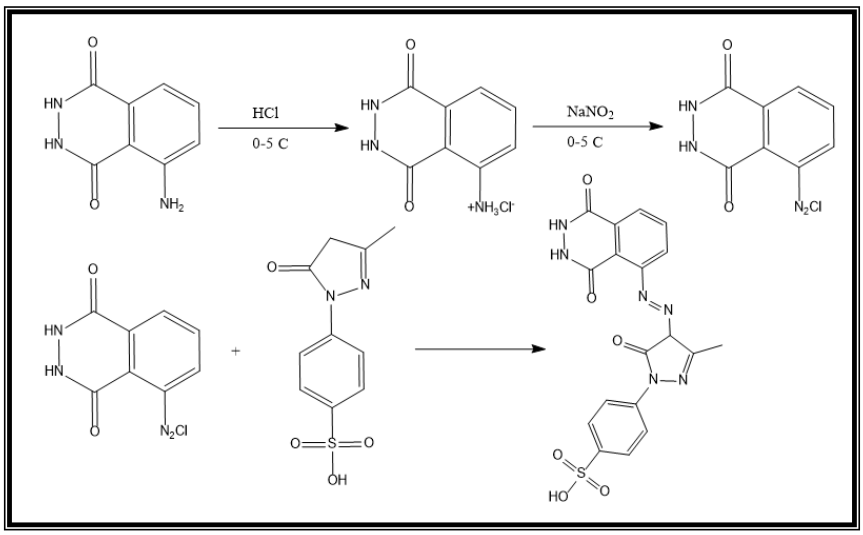
These medications successfully inhibit BRAF (V600E), GFR, telomerase, ROS Receptor Tyrosine Kinase, and Aurora-A Kinase. Anticancer medications heavily rely on pyrazole derivatives. Pyrazole compounds also have potent anti-inflammatory effects (Poudyal & Bharghav, 2021). The pyrazole family of heterocycles, one of the most well-known heterocycle groups, has a variety of biological effects, including anticancer (R. Alam *et al.*, 2016)(Shamsuzzaman et al., 2015), antitumor (Ismail *et al.*, 2016), anti-AIDS (K. & Ganguly, 2016), antibacterial (Surendra Kumar *et al.*, 2016)(Ningaiah *et al.*, 2014), antimalarial (Bekhit *et al.*, 2015), and antitubercular (Pathak *et al.*, 2014). Work on the synthesis and spectral analysis of heterocyclic azo compounds is currently ongoing (Mohammed, 2017)(Esraa & Hussain, 2018)(Fatma & Hussain, 2016)(Abdulhameed *et al.*, 2018).

Through the use of 3-methyl-1-(3-sulfoaminophenyl)-5-pyrazolone as the coupling component, a new hetaryl azo dye was created. FTIF, UV-Visible, and 1HNMR all verified the product. This dye's spectroscopic properties in various polar solvents were measured, and the enhancement of some nanoparticle metal oxides with this dye underwent a thorough analysis.

MATERIALS AND METHODS

Preparation of the Reagent (LASPMP):

1-(4-sulfophenyl)-3-methyl-5-pyrazolone, with diazotate in sodium hydroxide solution. The reagent was made by combining luminol. Diazonium solution was prepared by mixing luminol (0.5 g) with strong HCl (3.5 ml) and sodium nitrite solution at 0 to 5 °C 1-(4-sulfophenyl)-3-methyl-5-pyrazolone, with 0.5 g alkaline addition. The solution was made at temperature (0-5°C) and leave it overnight. Filtration was used to remove the precipitate, and it was crystallized using ethanol (YasirJ, 2021) as shown **(**Scheme 1**).**



Scheme 1: Preparation of reagent (LASPMP).

Apparatus:

Spectroscopic research was done in the UV-visible spectrum. Spectrophotometer with 1 cm glass cells and a T80 twin beam. Vibrational spectra were captured using the Shimadzu FT.IR 8000 series Test Scan. The pH was measured using an Inolab pH-meter WTW 720 with a glass-saturated calomel combination electrode. Reagent

Analytical-Grade Substances Were Used:

NaOH, HCl, Na2CO3, Ethanol, Methanol, Dimethyl sulfoxide (DMSO), N, N-Dimethyl formamide, Acetic acid, Polyvinylpyrrolidone luminol**,** Zirconium oxide nanoparticles, Cobalt oxide nanoparticles.

luminol azo 1-(4-sulfophenyl)-3-methyl-5-pyrazolone (8 mM):

200 ml of deionized water was used to dissolve 0.7078 g of reagent.

RESULTS AND DISCUSSION

In deionized water at room temperature, the Uv-VIS absorption spectra of the electronic structures of the synthesized compound were captured (Fig. 1). A high-intensity ($\pi \rightarrow \pi^*$) peak is produced by azo aromatic compounds in the ultraviolet region, and a low-intensity ($\pi \rightarrow \pi^*$) peak is produced in the visible spectrum.(Xia *et al.,* 2013)

The band of azo compounds changed to a larger resonance order when compared to azo phenyl pair. When the chemical Luminol azo3-methyl-1-(3-sulfoaminophenyl)-5-pyrazolone or substituted ortho azomethine is produced, it's possible that the azo group and C=N will form strong intermolecular hydrogen bonds. (Sıdır *et al.,* 2011).

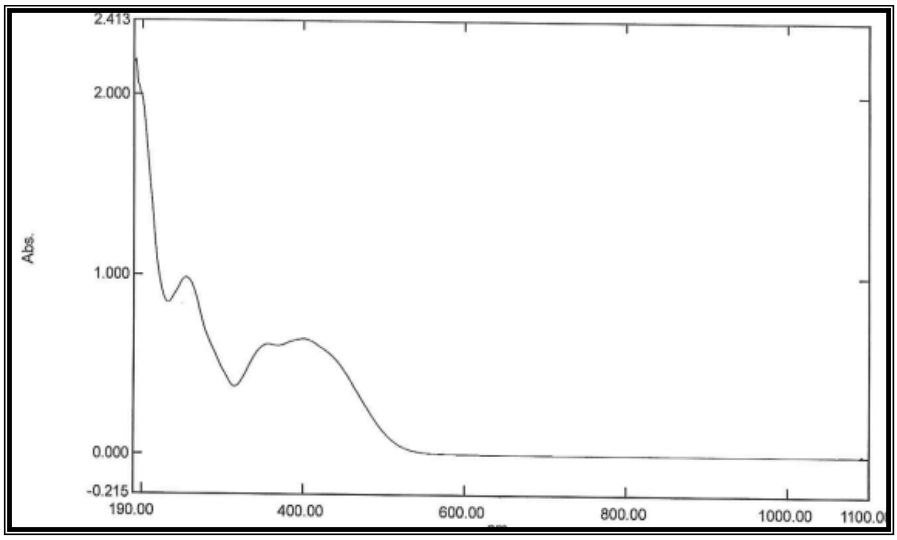


Fig. 1: The reagent solution's (LASPMP) absorption spectrum.

The band at 351 nm can be attributed to an intramolecular interaction with charge transfer and the presence of tautomeric equilibrium, while the band at 212-292 nm conforms to a low energy ($\pi \rightarrow \pi^*$) transition that includes the azomethine group.

FT.IR of Reagent*:*

The (LASPMP) reagent's FT-IR measurements are shown in Table 1 along with their likely assignments. The significant bands are seen in the reagent's spectra. A large band in The IR spectra of the free ligand at 3385.07 cm^{-1}, which may be caused by O-H stretching vibration, can be seen. The carbonyl group of ligands with the frequency of 1598.99 (s) cm^{-1}. The frequency at 1579.90 is equal to (N=N). (Mane *et al.*, 2011) (Fig.2**).**

Table 1: The most crucial spectral information for the reagent.

Ligand	**υ (NH_2)**	**υ (C=N)**	**υ (N=N)**	**υ (C=O)**
LASPMP	3385.07	1643.35	1579.90	1598.99

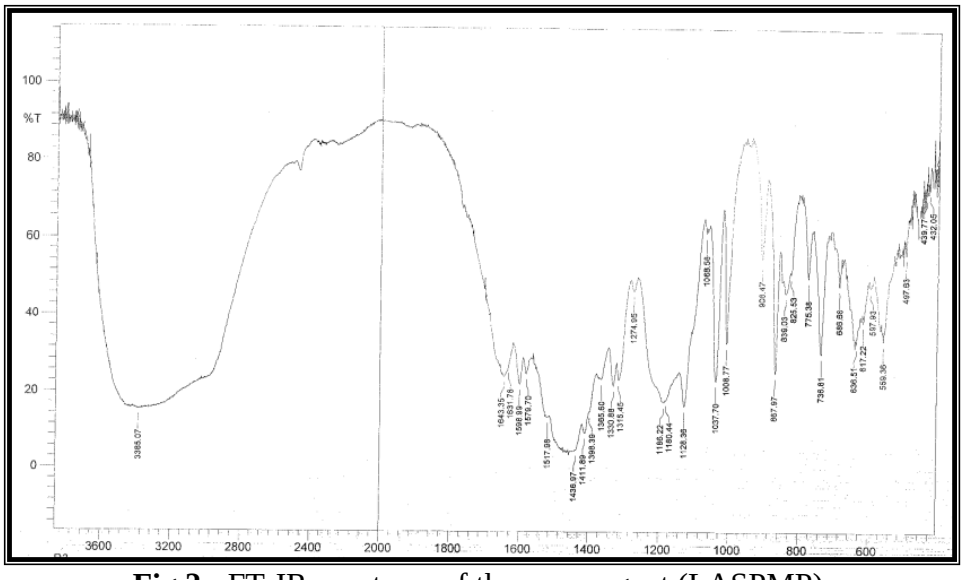


Fig 2: FT-IR spectrum of the azo reagent (LASPMP).

[1]HNMR Studies:

The produced azo compound's 1HNMR spectrum exhibits the following signals., which was dissolved in DMSO solutions with tetramethylsaline as an internal standard. A multiplet is seen in the ^{1}H NMR spectra for (L) at (8.06 - 7.99) ppm. (m, H Ar) due to the phenyl group, (S, H) for C-CH_3 at 1.65 ppm. (Fedorov, 1988) (Table 2 and Fig. 3).

Table 2: The ^{1}H NMR values of the reagent (LASPMP).

Compound	C-CH_3	CH-ring	H aromatic	-OH
LASPMP	1.65	1.16	7.65	8.06 - 7.99

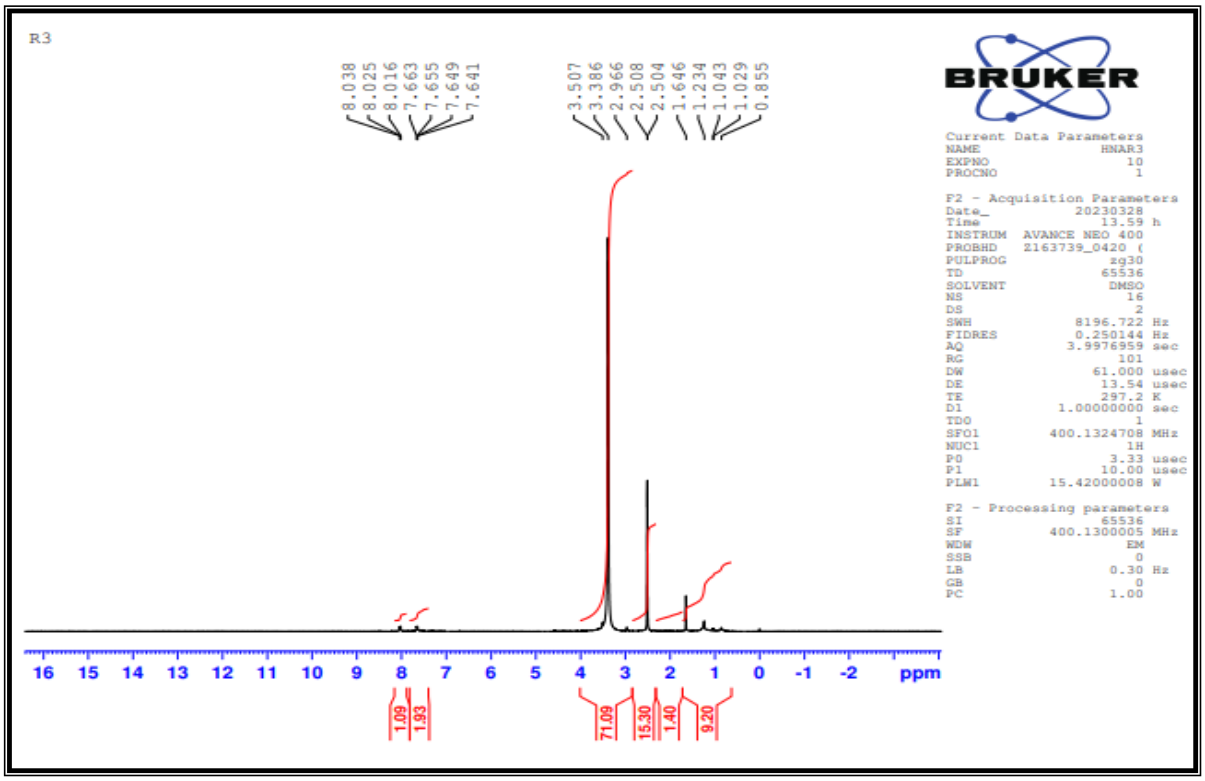


Fig 3: ^{1}HNMR spectrum of the azo reagent (LASPMP).

Solvent Effects on The Prepared Dye Luminol Azo 1-(4-sulfophenyl)-3-methyl-5-pyrazolone's Absorption Spectra:

To understand how the solvent affected tautomerism equilibrium and the interactions between the solvent and the solute, the ultraviolet-visible spectra of various solvents were analysed. The environment medium could affect the azo reagent's absorption spectra, and solvents could alter the type, location, and Because of interactions between solvent and solute molecules, absorption bands' strength. (Zhao *et al.*, 2020)

We examine the UV-visible spectra of the azo reagent in a variety of solvents, including (Ethanol, Methanol, Acetic Acid, Di methyl sulfoxide, and Di methyl formamide) at 25C, in order to ascertain the intermolecular forces between the solute molecule and solvent. Table 3 and Figure 4 show the results, respectively.

Table 3: Values of the reagent in various solvents.

Solvent	Wavelength	Molar absorption coefficient
Ethanol	343	730
Methanol	344	1024
DMF	339	684
DMSO	257	304
A. A	404	44

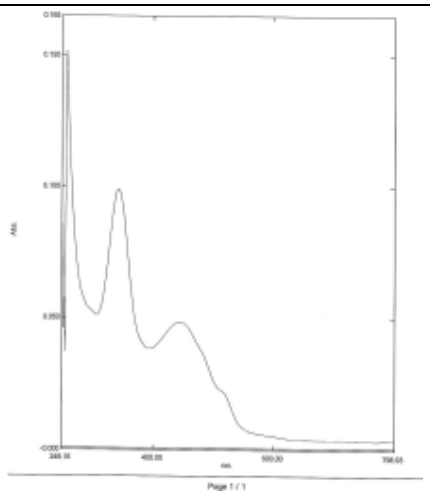


Fig. 4 A: R3 with DMSO

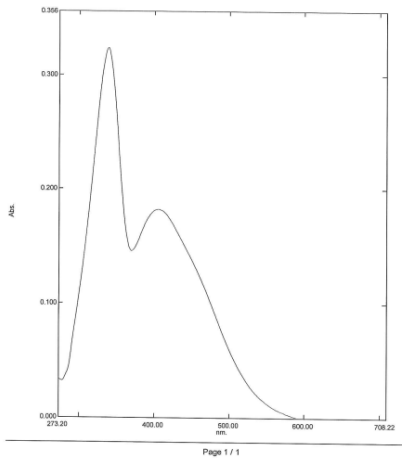


Fig.4B: R3 with DMF

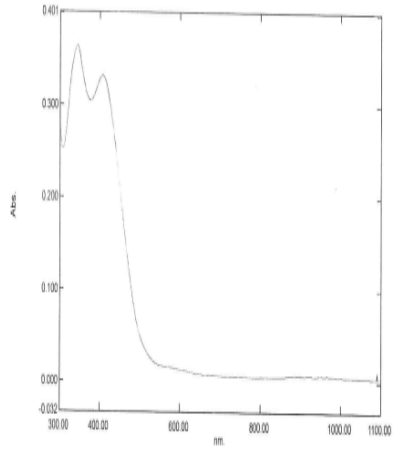


Fig.4 C: R3 with Ethanol

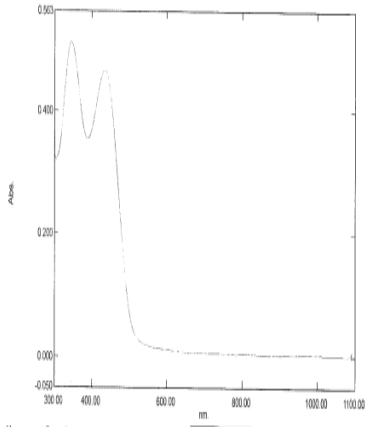


Fig. 4D: R3 with Methanol

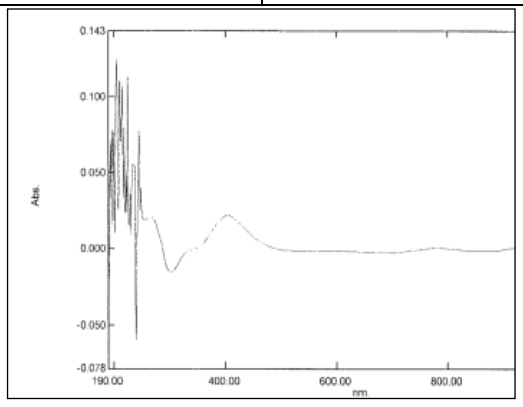


Fig. 4 E: R3 with Ac. Ac

Fig 4: Solvent effects on azo dye absorption spectra (LASPMP)..

It is clear that the luminol azo 1-(4-sulfophenyl)-3-methyl-5-pyrazolone shows three bands at 255, 357, and 399 nm in deionized water. It was discovered that as the solvent's polarity is raised, the band at 255 nm exhibits a red shift. the impact of dipole moment charge and hydrogen bonding strength on the positive solvatochromism of this dye in polar solvents. (Reichardt, 1994)

Enhancement of Nanoparticles of ZrO_2 and Co_3O_4 by Using the Reagent Luminol Azo 1-(4-sulfophenyl)-3-methyl-5-pyrazolone:

I- **Enhancing the Properties of Zirconium Oxide Nanoparticles:**

By reducing their diameter through a ligand exchange procedure, the ZrO_2 nanoparticles used in this study ensure enhanced characteristics (Ravindran *et al.*, 2013). Alternative ligands, the ligand molecules on the surface of particular nanoparticles can be changed to increase stability, which may be responsible for new functionality or properties. The accumulation of nanoparticle compounds over time is caused by the physical nature of the interaction between organic compounds and nanoparticles (Luo *et al.*, 2013). New ligands and nanoparticles consequently bind with greater vigour. Ligand exchange on nanoparticles, which combines free ligands with a compound of nanoparticles, is the technique used for this. The purified ZrO2 nanocrystals were agitated while ligands were dissolved in the mixture at room temperature. An AFM investigation of the ZrO2 nanocrystals' anatomy and morphology was performed. (Rahdar, 2013) The results for nano-Zirconium oxide using the AFM technique are shown in Table 4 and Figures 5& 6, before and after the ligand exchange, respectively.

Table 4: Shows the diameters of ZrO2 before and after ligand of LASPMP exchange**.**

Status	Grain size (G.S.)	Roughness average (Ron.)	Root mean square (R.M.S)
Before	63.59 nm	41.28 nm	74.34nm
After	89.25 nm	1.273 nm	2.587 nm

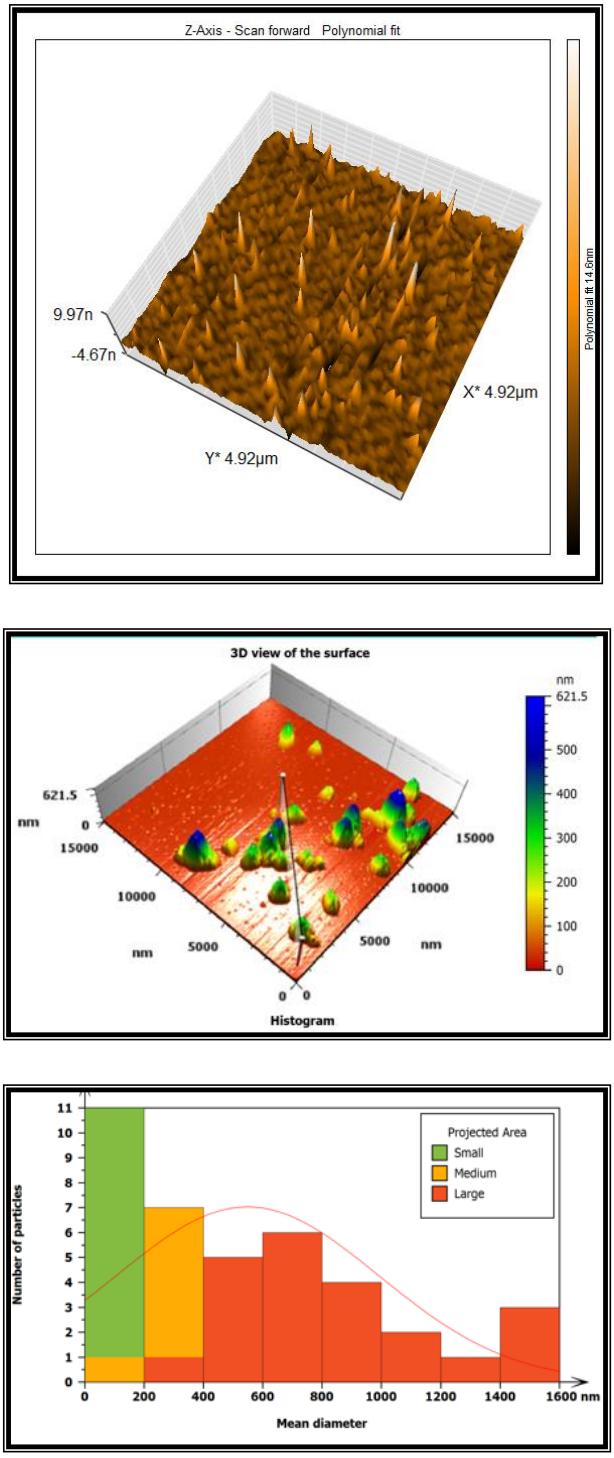


Fig. 5: The average diameters determined by the AFM for ZrO_2(63.59) nm before LASPMP ligand exchange.

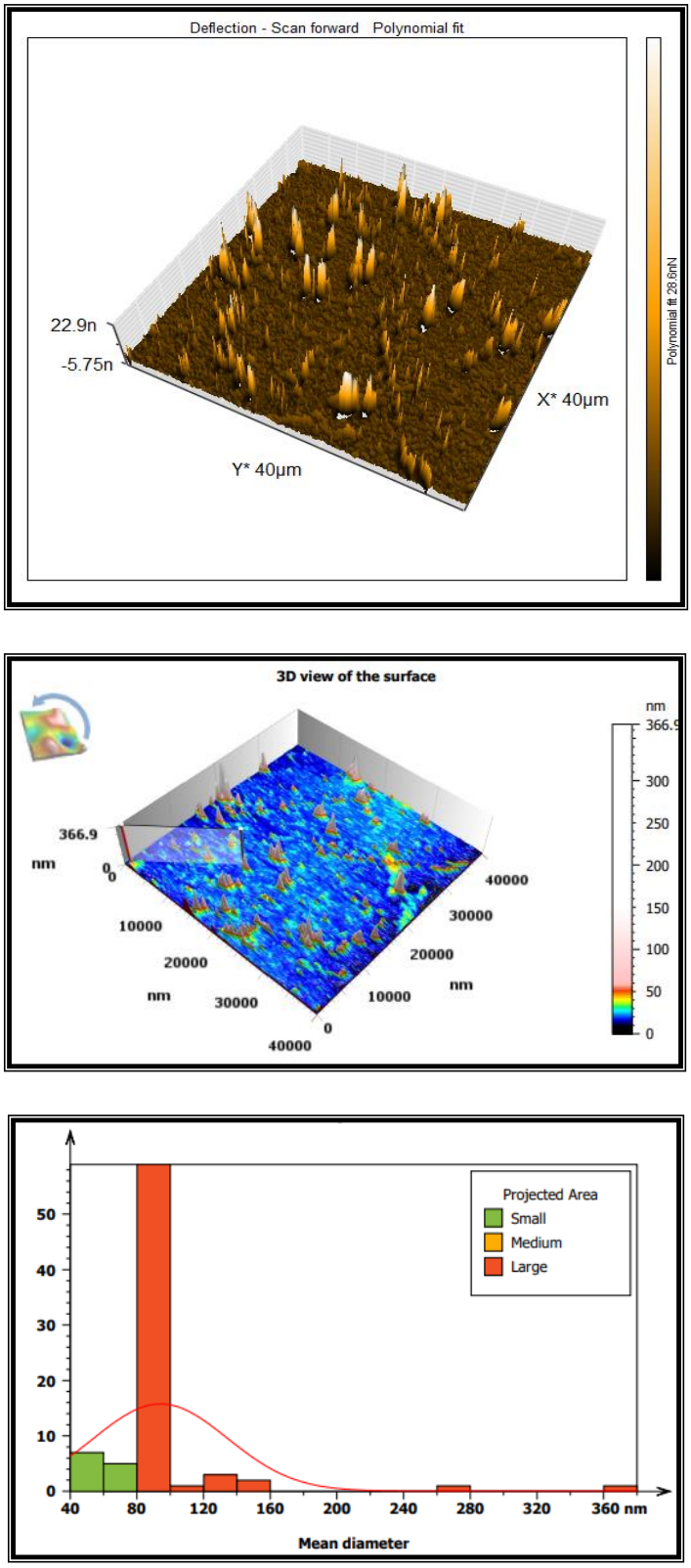


Fig 6: The average diameterS determined by the AFM for ZrO_2(89.25) nm after LASPMP ligand exchange.

II- Enhancing the Properties of Cobalt Oxide Nanoparticles:

To stop the centre of the particles from aggregating, the ligands cover the nanoparticle surfaces. However, there are dynamic binding and unbinding processes that take place between the stabilising ligands and the inorganic nanoparticle surface, for example, when an end group ligand molecule with an electron-donating end group, such as a thiol, amine, or phosphine, is present. This might cause the particles to group together (Bruchez *et al.*, 1998). To improve a nanoparticle's stability, It can exchange the ligand molecules on its surface for others that give the particles new characteristics or abilities (Daniel & Astruc, 2004). As a result, the ligand molecule that enters the inorganic nanoparticle always forms stronger bonds with its surface. (33The "ligand exchange reaction" is a popular technique for adding new ligands to nanoparticles that have bound ligands. Simply combining free ligands and nanoparticles causes this reaction. Consequently, ligand exchange is a quick and adaptable way to change the ligand shell on nanoparticles. The protective ligands are additionally exposed to outside chemicals and solvents, which alters the solubility, chemical composition, and physical properties of the nanoparticles. They can be utilized as biosensors, catalysts, or applications of optoelectronic after the exchange of ligands. By changing the ligands that are attached to the particles, the particle size can also be changed (Canumalla *et al.*, 2001). After being dissolved with the prepared ligand, the purified cobalt oxide nanocrystals were stirred for 24 hours at room temperature.(Luo *et al.*, 2013) The surface Cobalt oxide, which was bonded to the initial ligands at the extremely reactive sites, may have contributed to the morphological change in part. Table (5) and Figures 7&8 show the results obtained with the technique AFM for nano Cobalt oxide before and after the ligand exchange, respectively.

Table 5: Shows the diameters of Co_3O_4 before and after ligand of LASPMP exchange**.**

Status	Grain size (G.S.)	Roughness average (Ron.)	Root mean square (R.M.S)
Before	61.28 nm	28.38 nm	36.88 nm
After	60.72 nm	9.816 nm	15.99 nm

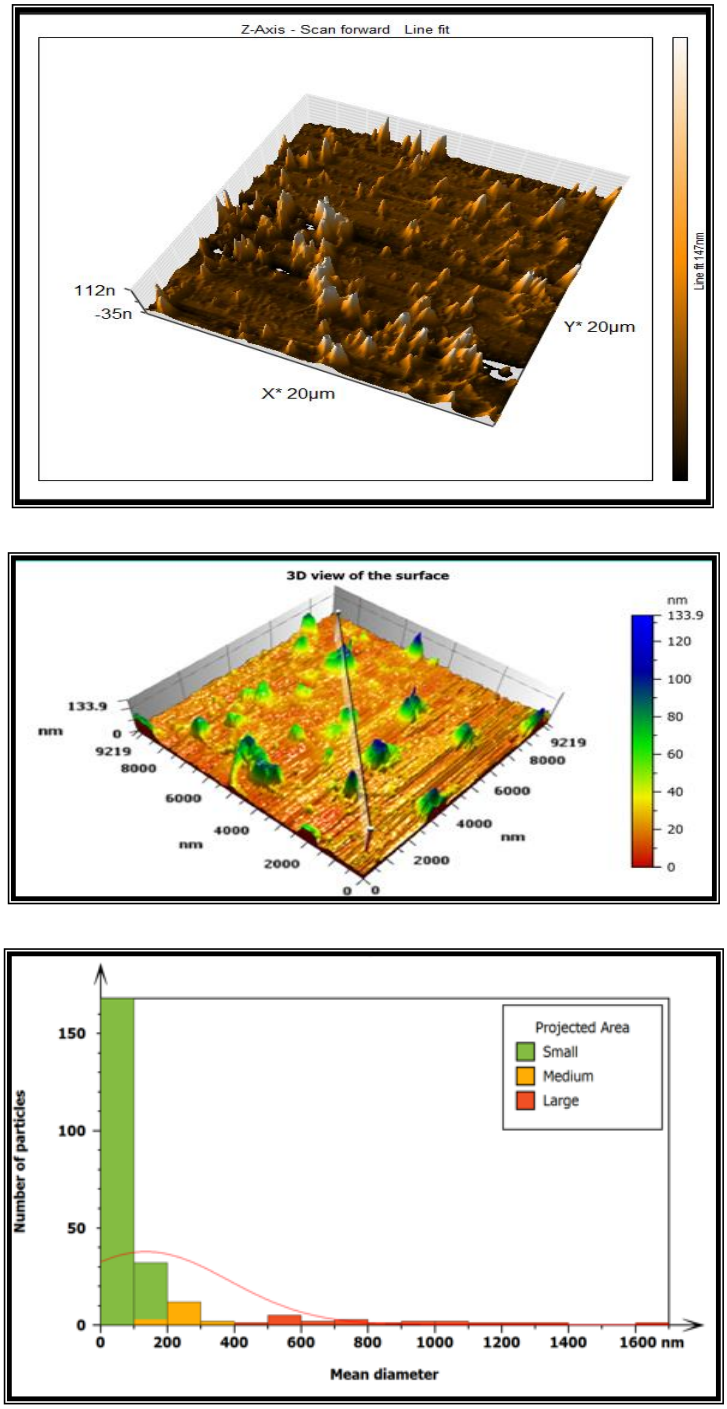


Fig. 7: The average diameterS determined by the AFM for Co2O3 (61.28) nm before LASPMP ligand exchange.

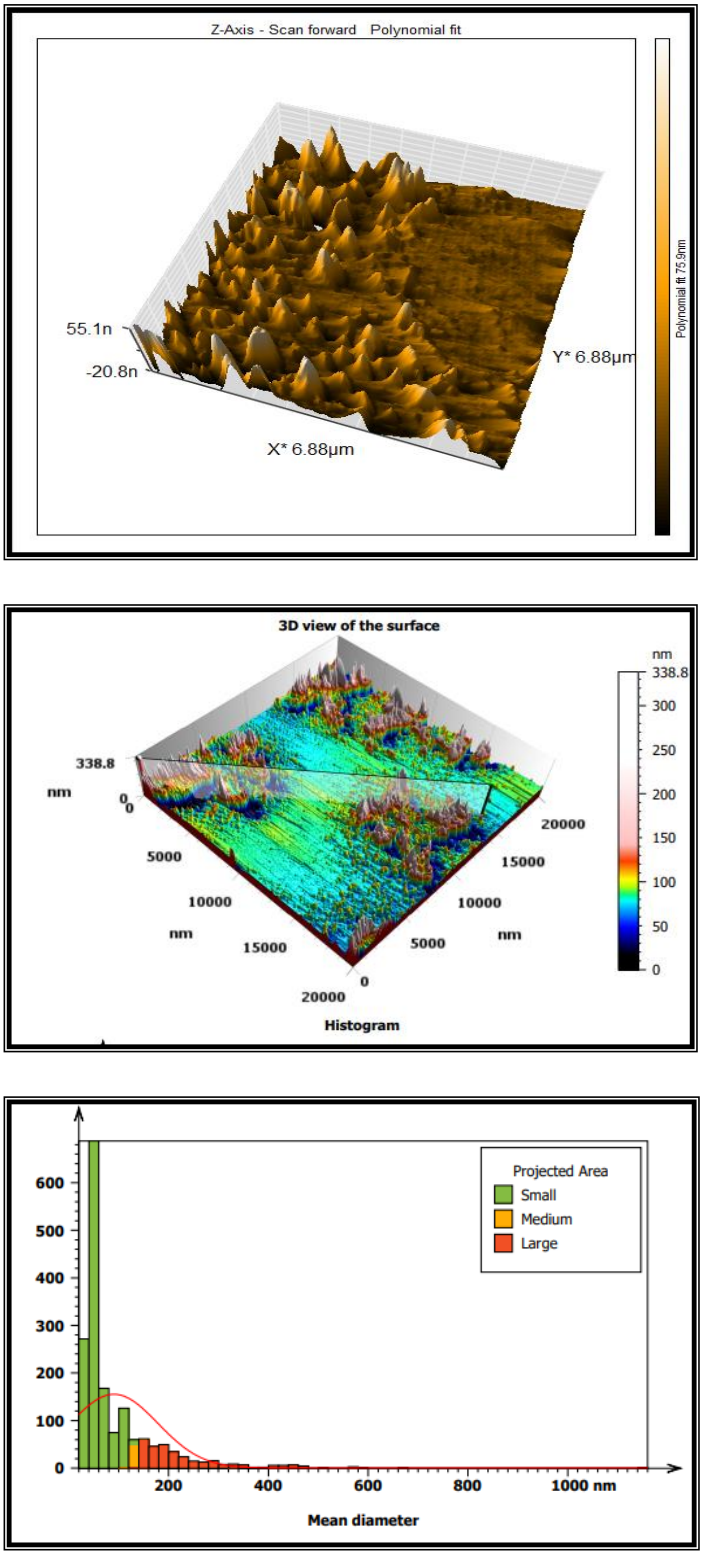


Fig.8: The average diameters of Co2O3 (60.72) nm as measured by the AFM following LASPMP ligand exchange.

Biological Effectiveness:

The biological activity of the prepared reagent luminol azo 1-(4-sulfophenyl)-3-methyl-5-pyrazolone was studied against two types of bacteria, *E. Coli* Gram-negative bacteria and *Staph aureus* Gram-positive bacteria. The reagent did not give clear effectiveness against these two types of bacteria (Fig. 9).

Fig.9: Biological activity of the reagent with bacteria E Coli Gram-negative bacteria and Staph aureus Gram-positive bacteria.

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