Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Kerbala
College of Education for Pure Sciences
Department of Chemistry

Adsorption Study Of The Interaction Between Zinc Oxide Nanoparticles With Albumin And Creatinine

A Thesis Submitted to
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In Partial Fulfillment of the Requirements for the Master Degree in Chemistry

By

Mayes Ahmed kadhim

Supervised by

Prof. Dr. Hamida Edan Salman **Prof. Dr. Hana'a Addai Ali**

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صدق الله العلي العظيم

سورة الفاتحة

Dedication

To those who have supported me throughout my life and cared for me more than themselves

To those who spent Sleepless nights For my comfort

To the most precious thing in my life

To light of my eyes My dear parents

My mother and my father

♥

MAYES

2019

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I will certainly not forget to extend my thanks and gratitude to all my friend. And in particular my dear friend who urged me to complete this scientific journey from the first step so far and continued with my support, my friend **Tayseer Jalil**.

MAYES

Abstract

The subject of this thesis is concerned with the adsorption of albumin and creatinine onto the surface of zinc oxide nanoparticles.

Initially the surface was prepared and characterized by using; Fourier infrared spectroscopy (FT-IR), atomic force microscope (AFM), scanning electron microscopy (SEM), and X-ray diffraction Spectroscopy (XRD).

A series of batch adsorption experiments were carried out under various operating conditions; equilibrium time, amount of adsorbent, pH, temperature, initial concentration of albumin and creatinine and shacking speed.

The results showed that the creatinine required a shortest equilibrium time (2.5 min.), while albumin has (30min.) to reach equilibrium time, and found that the adsorption percentage of albumin was higher than creatinine by 28% .

Following optimization of variables, the relation between concentration of albumin and creatinine remained in aqueous and onto ZnO nanoparticles has been evaluated using various adsorption isotherm models, such as Langmuir, Freundich and Temkin.

The values of correlation coefficient (R^2) showed that the Langmuir and Temkin equations appear to fit the equilibrium data better of adsorption of albumin and creatinine than Freundlich equation.

Thermodynamic parameters such as enthalpy (ΔH), entropy (ΔS) and Gibb's free energy (ΔG) were also calculated. It was found that the adsorption process of albumin and creatinine onto ZnO nanoparticles were feasible, spontaneous, endothermic, increasing randomness in a system and physisorption.

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Abbreviation and Symbols	The Meaning
NPs	Nanoparticles
ΔG	Change of free energy
ΔS	Change of entropy
ΔH	Change of enthalpy
λ	Wavelength
C_o	Initial Concentration
Ce	Concentration at equilibrium
Qe	Amount of adsorbate
T	Temperature
dl	Dici liter
J	Joule
R	Universal gas constant

Chapter One

Introduction

1. General introduction

In recent times, the interest in nanotechnology has increased because it has special characteristics that enable it to enter many fields and applications. They have been used in the chemical, mechanical and technological industries, also entered the medical field and the pharmaceutical industry.

Organisms may be exposed to nanomaterials by different ways like in consumer products like cosmetics, foods and their possible applications in drug therapy, bioelectronics and other uses(1).

Because of the wide nanoscale applications, especially in the biomedical field, the extent of biological compatibility and the risk of exposure to nanomaterials should be known. It's also important to recognize the molecular mechanisms of the interaction of the nanoparticle and biological system.

In the biological medium, there are many biomolecules that can interact with nanomaterials including proteins that may be adsorbed on a nanoparticle. that leads to the formation of nanoparticles-protein complexes. See figure [1-1] or so-called nanoparticles-protein corona. The adsorption of proteins by nanoparticles is done by different forces, including the hydrogen bonds, salvation forces and Vander Waals interactions(2).

The composition of protein corona is determined by the concentrations of an over (3700) proteins in plasma. It is also determined by the kinetic and equilibrium binding constants of the protein for particular nanoparticle. Balance it is not reached immediately when Corona is exposed to the biological liquid, high-concentration proteins and high correlation constants will first occupy nanoparticles surface but may also separated quickly and exchanged by proteins that have lower concentration, higher affinity and lower exchange rate(3).

There are many factors that affect on the nature of NPs-biomolecule corona like NPs size, surface charge, NPs shape, and solubility all playing important role in the interaction of NPs with proteins, and also nanoscale surface curvature have strongly effects on protein corona adsorption(4).

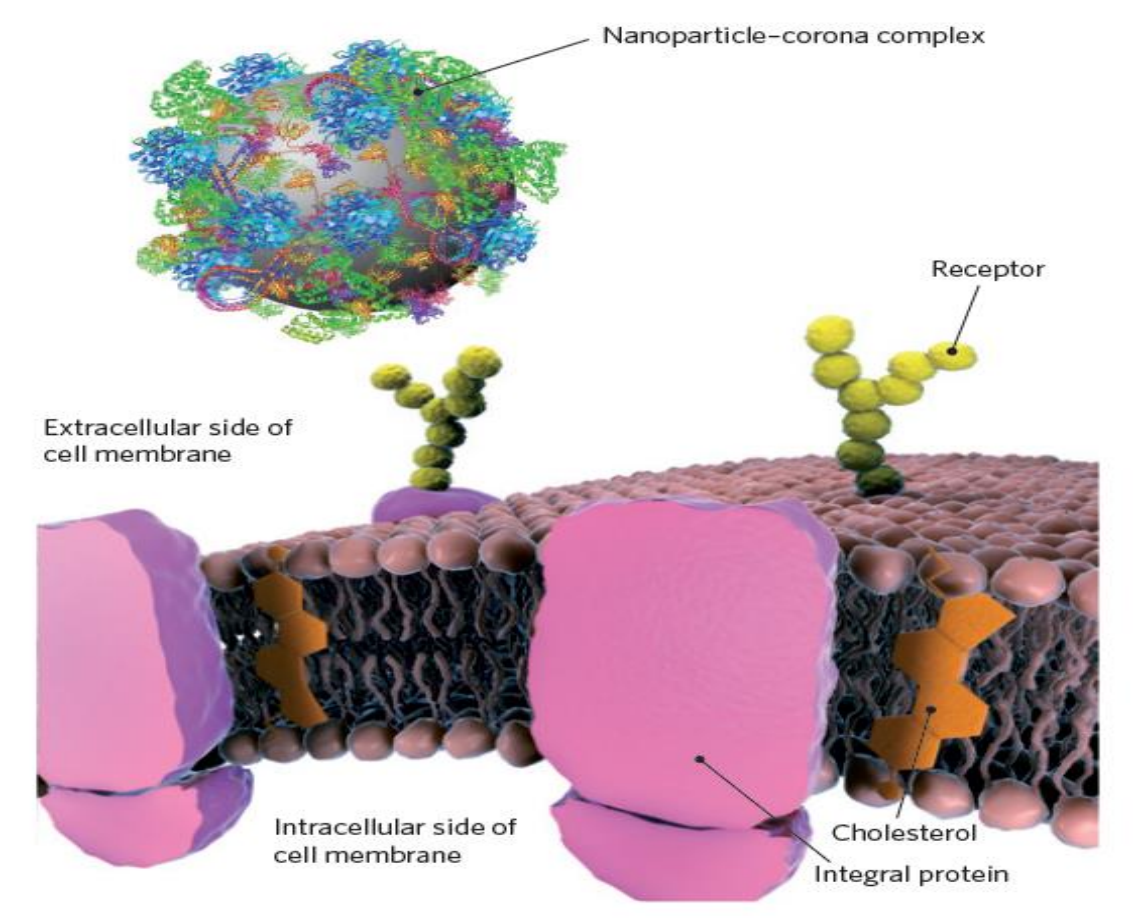


Figure 1-1: The nanoparticle-corona complex In a biological environment[5].

Since the advent of nanotechnology, the interactions of nanomaterials such as semiconductor NPs ZnO, have attracted much interest because it has unique optical properties, and thus, they have wide applications in various areas such as drug delivery, bio-imaging, and gas sensing. ZnO nanostructure show high catalytic efficiency, strong adsorption ability and are more used in the manufacture of sunscreens, ceramics and rubber processing. It can also be used as a fungicide and wastewater treatment[1,6].

Due to the importance of zinc oxide and its wide applications, it was selected in this study as a nanoparticle and it was reactivate with protein and metabolic product of protein.

1.1 Nanochemistry

The term "nanochemistry" which consists of two sections: chemistry and nano; the word ʿnanoʾ in the metric system is equal to 10^{-9} [7]. Nanochemistry, it is a new branch of solid state chemistry that focuses on synthesis rather than the engineering aspects of preparing little small pieces of material of nano-size in one, two or three dimensions. Nanoparticles have got a great attention that due to their small size .These small objects can be made from inorganic, organic or organometallic components to produce new materials with optical, electronic and magnetic properties [8]

1.1.1 Nanotechnology

Nanotechnology is a technique that deals with the study of the treatment of matter on the atomic and molecular scale and a creation of new means measured in nanometers.

The nanometer is about one billionth of a meter, or it's about 100,000 of the width of a human hair and be equal to 10 hydrogen atoms with some. Also the red blood cell volume is up to 2000 nm. Nanotechnology deals with particles in measurements between 1 and 100 nanometers[9].

It can be used efficiently in a different fields such as chemistry, medicine, biology etc..

Researchers have recently made a great effort to design and build nanomaterials to make use of advantage of their increased unique features like light, weight, high strength, enhanced control of the light spectrum and perfect chemical reaction compared to their counter parts at micro scale[10].

1.1.2 History of Nanotechnology

Thousands of years ago, nanotechnology was used before the term was introduced. Humans introduced nanomaterials in the rubber, glass and steel industry. We cannot define a specific date for the beginning of

nanotechnology, but it was found that glass was painted in the middle Ages using colloidal gold nanoparticles.

As in the Roman cup that was made for the king (Lycurgus), which is located in the British Museum since the fourth century AD, the color of the cup changes between green and red when exposed to light, the cup was made using silver particles and gold nanoparticles[11]. It was also found in Japan that (samurai) used nanoparticles in the process of painting swords to obtain high quality properties[12].

In 1974 a word "nanotechnology" was first used by researcher "Norio Taniguchi" in Tokyo; although it was not widespread[13]. in 1986 "K. Eric Drexler" also used the word "nanotechnology" in his book (Engines of Creation: The Coming Era of Nanotechnology), which proposed the idea of a nanoscale "assembler" that would be able to produce a copy of itself and other items of arbitrary complexity with atomic control. Also in 1986, Drexler participated in establishing. The Foresight Institute to help the increase general information and understanding of nanotechnology concepts and implications, since 1980 Most of nanotechnologies have included studying several ways of manufacturing mechanical devices from a small number of atoms[14].

1.1.3 Nanomaterials Synthesis

There are two main approaches to synthesize nanomaterial. One is top-down approach, and another is bottom-up approach[15].

[Top-down] approach : From larger to smaller where nanomaterials can be yielded from large-scale materials. These structures are gradually being destroyed and through special transformation resulting in reduced particle size to the nano-scale, several techniques are used to achieve nanomaterials, such as grinding techniques, mechanical milling [16].

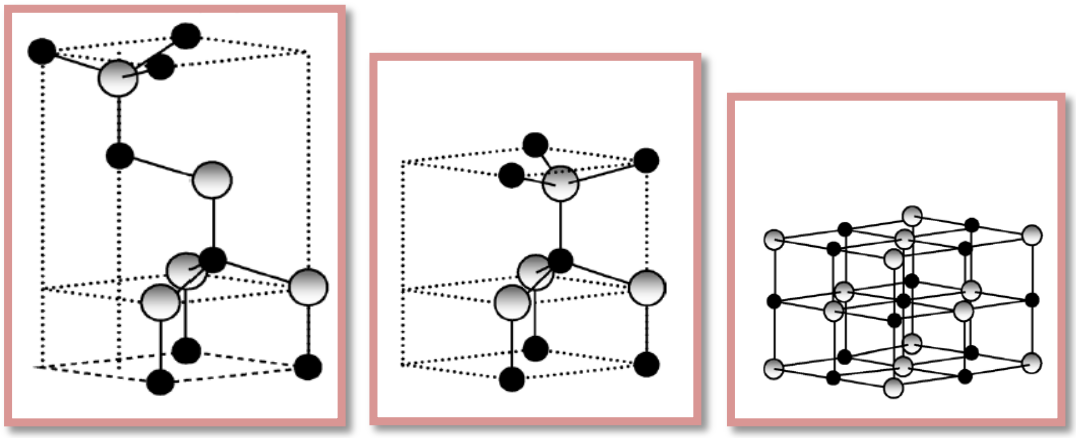
[Bottom-up] approach from the smallest to the largest, this method is within chemical methods and is characterized by the small size of the resulting materials.

Where nanomaterials consist of atoms and molecules that are arranged through control and accurately on the chemical reactions are involved in the collecting of these components[17]to reach the nanoscale.

There are many different methods used in this technique, like chemical vapor deposition, sol-gel, electro deposition, solvothermal method[18].

1.2 Zinc oxide

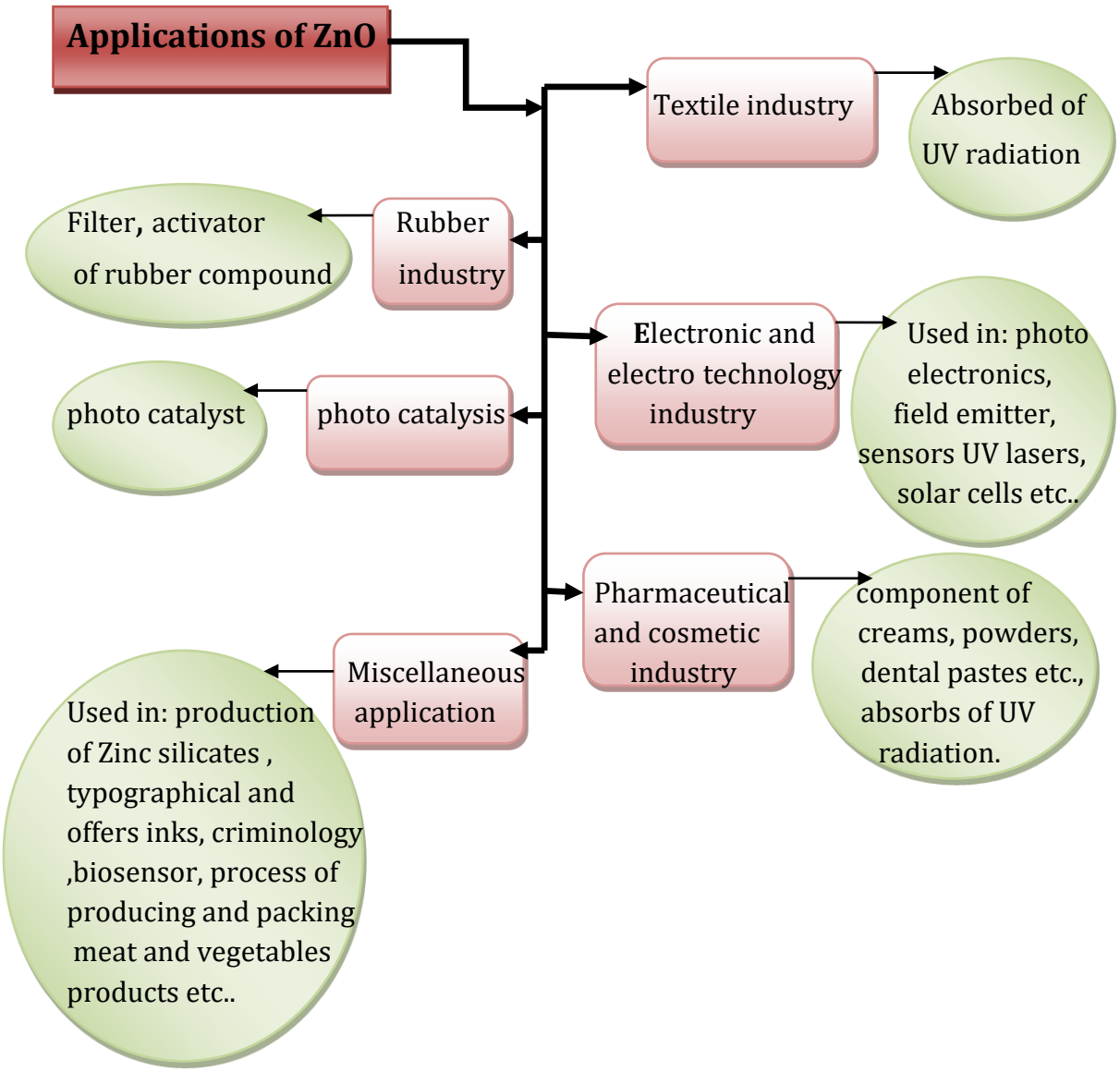
Zinc oxide is an inorganic compound have the formula ZnO in the form of white powder, and it's insoluble substance in water. ZnO is having two main forms: hexagonal wurtzite[19]and cubic zinc blende. The wurtzite structure is a most stable form at natural conditions, and this makes it most common. ZnO can be changed to the rocksalt form at relatively high pressures about 10 GPa[20]. See the figure[1-2].



(A)Cubic Zinc blende. **(B) Wurtztie.** **(C) Rocksalt.**

Figure 1-2: The Different forms as stick and ball representation of ZnO crystal structures[20].

Zinc oxide is one of the most widely used semiconductors in different fields such as flat panel displays, solar cells , electro and surface acoustic devices , luminescent , UV lasers and photo catalysis [21].This is explained in detail in schem[1-1] that shows most important applications of zinc oxide.



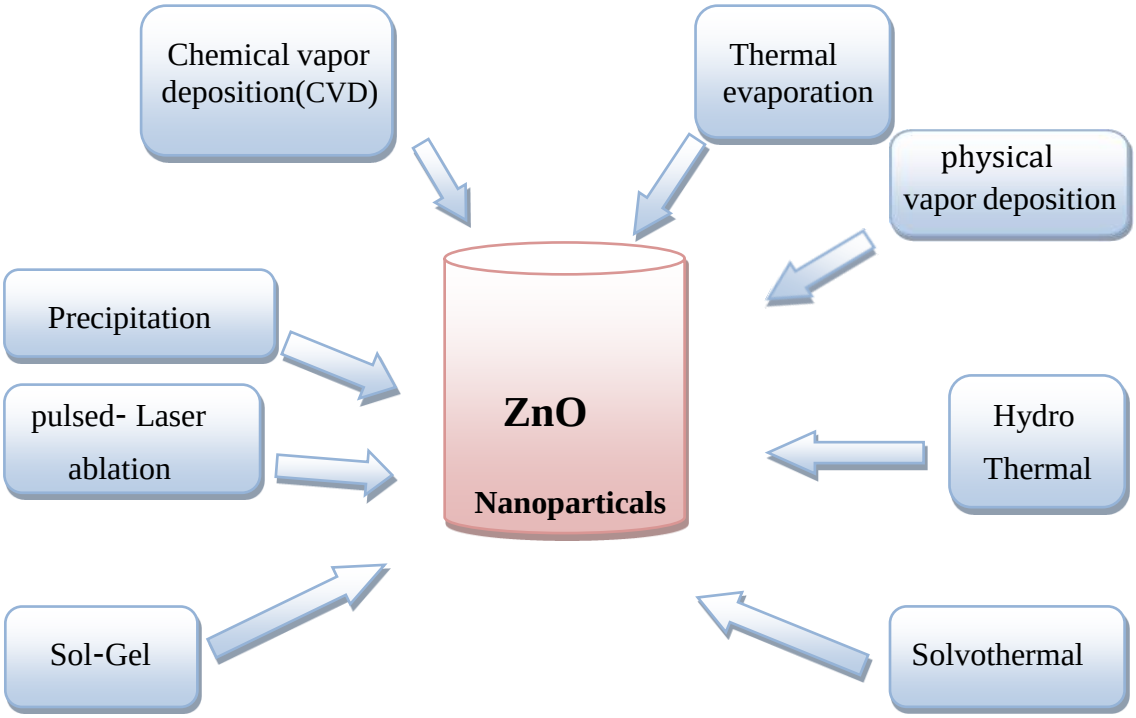
schem1-1: Schematic representation the application of ZnO[22] .

1.2.1 Zinc oxide Nanoparticals

Zinc oxide (ZnO) is known as the most important material for the manufacture of nanosystems and nanoscale devices because of its various characteristics such as piezoelectric, electromagnetic and semiconductor properties[23]. ZnO Can be found in one- (1D), two- (2D), and three-dimensional (3D) structures. In one dimensional structures are more than others , including nanorods[23-26] nano ribbons[27] ,nano helixes, -springs and –rings[28], nano needles[29] , nanowires[30] nanotubes[31-33] nano

belts[34], and nano combs[35-37].Zinc oxide can be found in 2D- structures, such as nano sheet /nano plate and nano pellets[38], and in 3D structures of zinc oxide include flower, snowflakes, dandelion, coniferous urchin-like[39-41], etc ..

Zinc oxide nanoparticles can be made from many different methods, the schem[1-2] explain some of them briefly.[32][42-45]



Schem1-2: different methods of preparation of zinc oxide nanoparticals .

Precipitation : is a widely used method to obtain ZnO NPs, since it makes it possible to obtain a product with repeatable properties. This method involves fast and spontaneous reduction of a solution of Zn salt by using a reducing agent that for limitation the growth of particles with specified dimensions. followed by precipitation of ZnO from the solution and the next stage this precursor succumb to thermal treatment followed by milling to remove impurities.

Sol-Gel Method : this method is characterized by the simplicity, low cost, repeatability, reliability and mild conditions of synthesis, and involve a transformation from liquid precursors to a (sol), which is a stable suspension of colloidal particles (NPs) in a liquid and finally to a (gel).

The hydrothermal method : this synthesis takes place in an autoclave, where the mixture is heated at a temperature of (100–300) °C and left for period of time, followed by cooling, Where the crystal nuclei are formed. Characterized by the diverse shapes of the crystals and the high degree of crystalline and purity[22].

Solvothermal processes : are characterized by being simple, mild synthesis conditions, and capability to mass production.

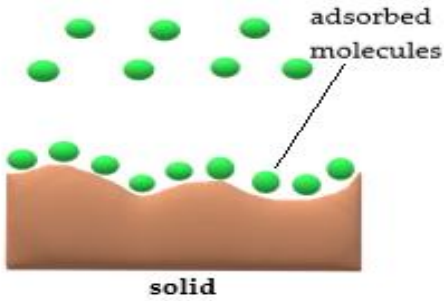
Pulsed-laser : in this method, high power laser pulses are used in order to evaporate material from a specific surface and the stoichiometry of the material is preserved.

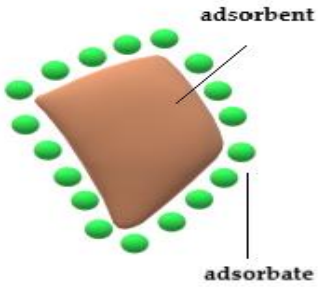
Chemical-vapor deposition : it produces high-quality films and it is applicable to large-scale production In this method, Zinc oxid deposition occurs as a result of some chemical reactions of vapor-phase precursors on a substrate, that delivered into the growth region by the carrier gas[20].

Thermal Evaporation : Is a process in which vaporized or condensed at a high temperature and the resultant vapor phase condense under certain conditions to form the product, this process is usually conducted in a tube furnace[46].

1.3 Adsorption

Adsorption is a process of accumulating atoms or molecules of gas or a liquid (called adsorbate material), on another surface (Adsorbent)[47]. This process occurs in the following systems: liquid gas, liquid liquid, solid liquid and solid gas[48].





Adsorption process is due to presence of unsaturated fields of forces because of the lack of consistency or contact of sufficient number of particles with the surface particles, and be the case as in the inside of the solid phase or liquid phase where adsorption leads to saturation field of these forces on the surface and thus causes a decrease in the free energy of the surface (ΔG).

Adsorption process is accompanied by decreasing in degrees of freedom of the adsorbate that expressed thermodynamically by the decrease of the entropy (ΔS). If this process was performed under isothermic conditions and according to the following thermodynamic relationship[49,50]:

$$\Delta G = \Delta H - T\Delta S$$

1.3.1 Types of Adsorption

Adsorption studies have shown two types of adsorption: physical adsorption (Vander Vaal's adsorption), and the chemical adsorption in which the bonding occurs in a manner that is similar to the chemical reaction and is characterized by specifically, which requires that this adsorption has a certain surface, and certain conditions , it needs activation energy and obtained at temperatures above the boiling point of the adsorbate substance[51-53], It occurs in a single layer (Unimolecular adsorption) on the surface.

As for physical adsorption, the adsorbate molecule is associated with the surface by weak forces (Vander Vaal's forces), it is sometimes called natural adsorption, which is not specific and tends to occur at low temperatures , and it does not need activation energy. The adsorption shall be the thickness of several layers (Multi molecular adsorption)[54].

Table(1-1) Some differences between chemical and physical adsorption.[47,54]

Physical adsorption	Chemical adsorption
1. Caused by weak van der Waal's forces.	1. Caused by chemical bond.
2. Small heat adsorption (about 20-40 kJ mol^{-1}).	2. Large heat adsorption (40-400 kJ mol^{-1}).
3. Reversible.	3. Irreversible.
4. This type is not specific.	4. Much more specific.
5. This type occurs rapidly at low temperature .	5. Increase of temperature lead to increases of adsorption.
6. Increase of pressure lead to increases adsorption.	6. Not affected by pressure change.
7. Multimolecular layers are made up in this type.	7. Forms unimolecular layer.

1.3.2 Adsorption Isotherms

It is defined as the relationship between the amount of the adsorbate material (Qe) on one gram of the adsorbent material and the concentration of the adsorbate material at the equilibrium state (Ce), at a constant temperature[55].Giles[56]presented a classification of the adsorption curves and the isothermal section of adsorption from solutions on the surface of adsorbent to four main categories shown in figure (1-3).

Type S: the isotherm appears as S letter shape, which is the orientation of the molecules that are adsorbed on the surface of the substrate, either tilted or vertically.

Type L: custom for isotherm langmuir, The orientation of the adsorbed particles is horizontally on the surface and the adsorption is monolayer.

Type H: specific for very diluted solutions.

Type C: indicates a high probability of chemical adsorption. These curves are used to determine the position of the molecules in the surface and the type of reaction. Among the main varieties there are secondary types referred to as 1,2,3,4 and max, which are related to the form of isotherm to high concentrations.

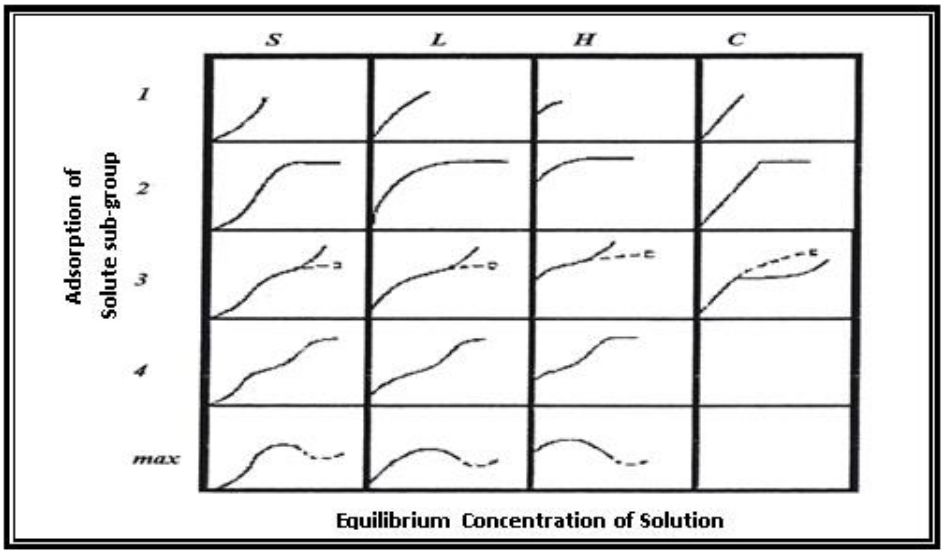


Figure 1-3: Different varieties of isotherm by Giles classification.

1.3.3 Langmuir equations for adsorption:

This equation spread widely since it developed by (Langmuir) in 1918 to describe adsorption of gas molecules on a solid surface. Langmuir assumed that adsorption of a single molecular layer on the surface of the adsorbent material excludes interactions between the adsorbed molecules in the low surface coverage. Thus, the amount of adsorbed material increases rapidly at the beginning of adsorption and then gradually stabilizes due to the desorption process[57].

The Langmuir equation can be expressed mathematically as follows[58] :

A	**+**	**B**	⇆	**A**	**+**	**B**
Dissolved in solution N_2		Solvent suffers adsorption N^S_1		Melted suffers from adsorption N^S_2		Solvent in solution N_1

N_1,N_2: Concentrations in the terms of mole fraction of solvent and solute

Respectively.

N^S_1,N^S_2: Effectiveness of the adsorption layer in the terms of mole fraction of solvent and dissolved, and the letter (s) refers to adsorption on the surface.

The equilibrium constant K can be expressed as follows:

$$K= \frac{N_1\, N_2^S}{N_2\, N_1^S} = \frac{a_1\, N_2^S}{a_2\, N_1^S} \Longrightarrow \frac{K}{a_1} = \frac{N_2^S}{a_2\, N_1^S} \quad \ldots\ldots\ldots(1\text{- }1)$$

a_1,a_2 : effectiveness of solvent and solute, respectively.

Since adsorption usually occurs in dilute solutions, it is appropriate to express concentration at equilibrium (Ce) rather than (a_2) and to consider the effectiveness of the solvent (a_1) constant, and if imposed b= K / a_1.

$N^S_1 + N^S_2 = 1$ and by introducing these relationships to equation (1-1):

$$N_2^S = \frac{b\ Ce}{1 + b\ Ce} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1\text{- }2)$$

And when

$$N_2^S = \frac{n_2^S}{n_1^S} = \theta \quad \ldots\ldots\ldots\ldots\ldots\ldots(1\text{- }3)$$

Since n^S represents the number of adsorption site moles per gram and (θ) is the part of the surface occupied by the adsorbed molecules, substitute equation (1-3) in equation (1-2) :

$$\theta = \frac{b\ Ce}{1+b\ Ce} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1\text{- }4)$$

The amount of adsorbate material (Qe) is expressed in mg / g units that is proportional to the surface coverage θ :

$$Qe \ \alpha \ \theta \implies Qe = a.\theta$$

$$Qe = \frac{a\ b\ Ce}{1+b\ Ce} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(1\text{-}5)$$

Equation (1-5) represents the Langmuir equation for adsorption of solution[59].

where as:

Qe: Adsorption capacity at equilibrium (mg / g).

Ce: Concentration of solute at equilibrium in units (mg / L).

a: Proportionality constant (is the theoretical maximum adsorption capacity(mg/g)).

b: Langmuir adsorption constant (L /mg). As in equation (1-5).

Equation (1-5) can be written in linear form to become as follows:

$$\frac{Ce}{Qe} = \frac{1}{ab} + \frac{Ce}{a} \qquad \ldots\ldots\ldots(1\text{-}6)$$

When drawing Ce / Q vs. Ce the slope is equal to 1/a and its intercept equals to 1/ab , as in figure (1-4)[58].

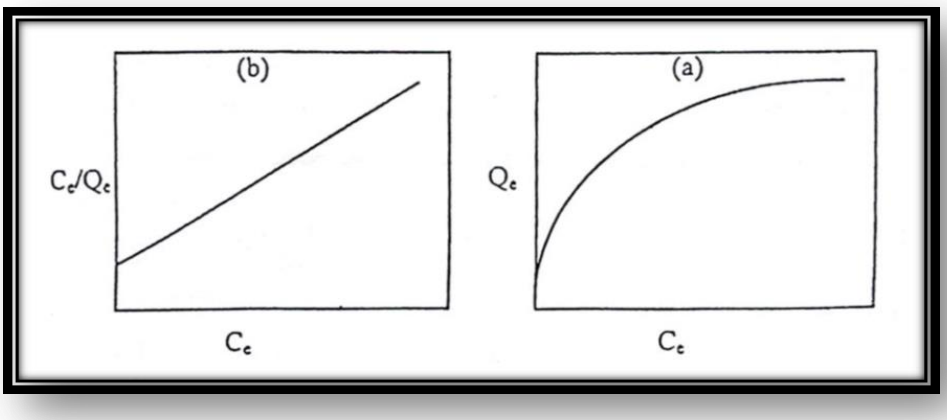


Figure 1-4: a- Isotherm Langmuir, b- The linear equation of isotherm Langmuir.

1.3.4 Freundlich equation for adsorption:

The German (Freundlich) put in 1926 an equation used to explain the adsorption of solution on heterogeneous surfaces, where potential energy changes are irregular due to the occurrence of adsorption sites at varying levels of energy[60].

The mathematical formula of the Frindlish equation is as follows:

$$Qe = K_f Ce^{\frac{1}{n}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1-7)$$

By taking logarithm equation (1-7) we get:

$$\log Qe = \log K_f + \frac{1}{n}\log Ce \quad \ldots\ldots\ldots\ldots\ldots (1\text{-}8)$$

Ce : Concentration of the adsorbate at equilibrium (mg/L).

Qe : Quantity of the adsorbate material at equilibrium (mg/g).

K_f (L/mg), n: are isotherm constants indicate the capacity and intensity of adsorption respectively. The 1/n factor also indicate heterogeneity factor. Figure (1-5) shows the Freundlich (log Qe vs. log Ce) plots for adsorption process.

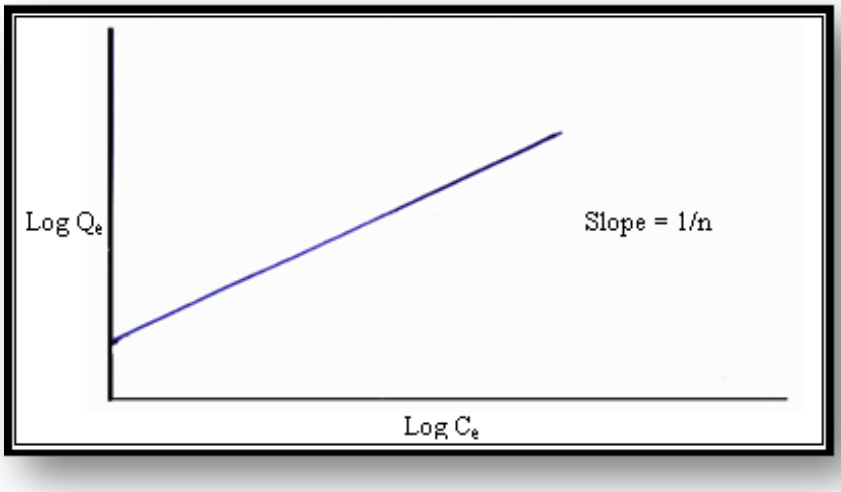


Figure 1-5: Isotherm freundlich for adsorption.

1.3.5 Temkin equation for adsorption:

Temkin isotherm : is based on the assumption that the decline of the heat associated with adsorption process is linear rather than logarithmic, as implied in the Freundlich equation.

and its equation is given as[61]:

$$q_e = B \ln (A_T.Ce) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1\text{-}9)$$

where

A_T : is the equilibrium binding constant corresponding to the maximum binding energy(L/g).

B : is Temkin constant that equal : $\Longrightarrow$ $B = \frac{RT}{b}$ and related to the heat of adsorption.

R : the gas constant (8.314 J/mol. K).

T : the absolute temperature (K).

b : the heat adsorption constant (J/mol).

The linear form of the Temkin equation :

$$q_e = B \ln A_T + B \ln Ce \quad \ldots\ldots\ldots\ldots\ldots\ldots (1\text{-}10)$$

the constants (A_T,B) can be obtained from the slope and intercept of the straight line plot of (qe) versus (lnCe)[62].

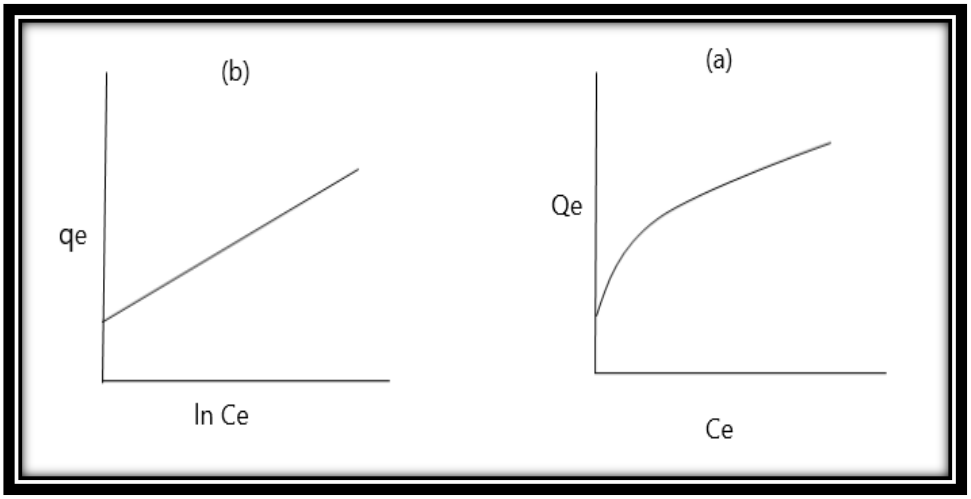


Figure 1-6: a- Isotherm Temkin, b- The linear equation of isotherm Temkin.

1.4 proteins

Proteins are big biomolecules, that consisting of one or more long chains of amino acid residues up to 20 different L-α- amino acids. carboxyl group, an amino group, and a diverse of the side chain are bonded, the side chains possess a large various chemical structures and properties. The (20) amino acids are joined together by peptide bonds.

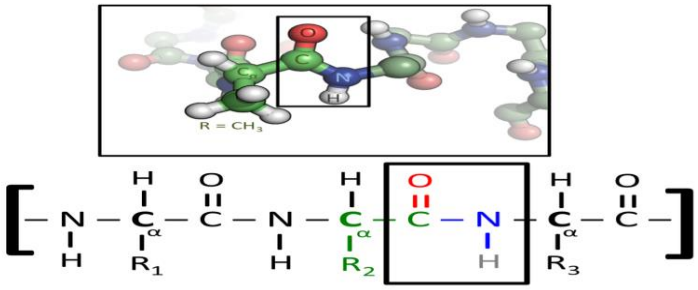


Figure 1-8: Chemical structure of the peptide bond and the three-dimensional structure of a peptide bond between an alanine and an adjacent amino acid.

Biochemists refer to four distinct aspects of a protein's structure: primary, secondary, tertiary, and quaternary[63,64]. Proteins perform different functions, like catalyzing metabolic reactions, transporting molecules and ions , nutrients, contractile system of muscles, cartilage, antibodies, tendons, and regulating physiological and cellular activities. The primary structure describe amino acid sequence. Secondary structure show regular, recurring arrangements in space of neighboring amino acid residues in a polypeptide chain. It is maintained by hydrogen bonds between carbonyl oxygen’s of the peptide backbone and amide hydrogen. Tertiary structure show the overall three dimensional arrangement of a polypeptide chain, including the folding of secondary structural elements with respect to one another. Quaternary structure are formed by several protein molecules [polypeptide chains].[64,65]

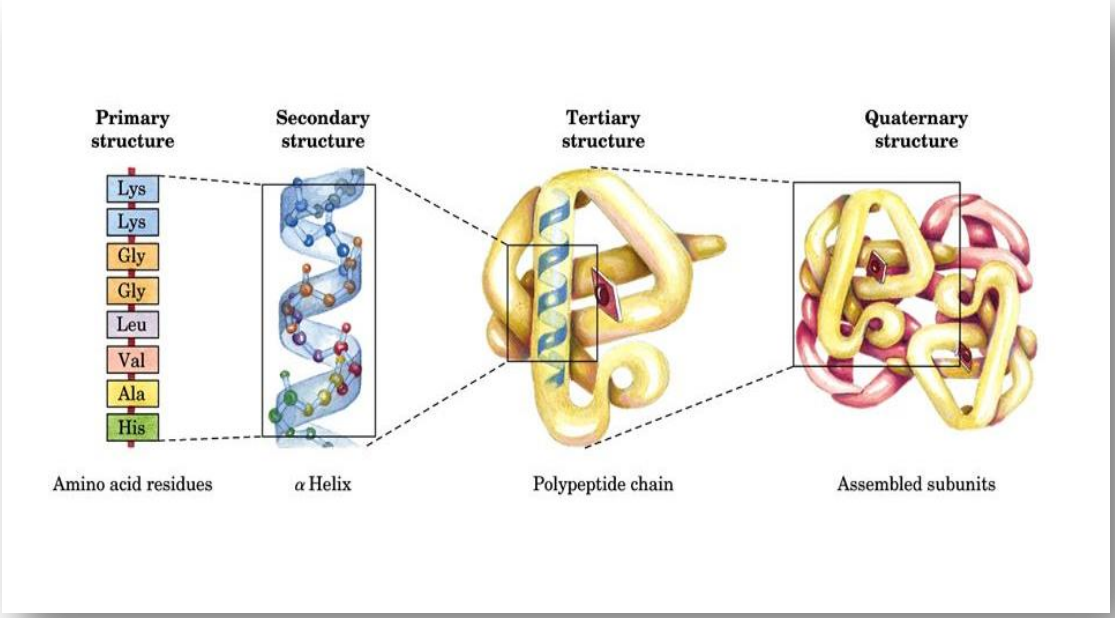


Figure 1-9: the four kinds of protein's structure[66].

1.4.1 Albumin

Human serum albumin is a small globular protein Includes 585 amino acids, with a few tryptophan or methionine residues, but plenty of charged residues such as lysine, and aspartic acids [67] .

Albumin is the most copious in blood plasma protein, and it normally constitutes about 50% of human plasma protein[68].

In physiological conditions, about 10–15 grams of human albumin are synthesis in the liver every day, with none or very low intracellular storage, and its product is stimulated by hormones, like cortisol, insulin and growth hormone[69]. Also in egg whites we find albumin protein called ovalbumin.

Ovalbumin or (OVA)[70] is the main protein in egg whites, accounting for about (55%) of total protein. Ovalbumin in chickens consists of (385) amino acids, with a molecular mass of (45 Dalton)[71, 72].

1.5 Nitrogen Compounds

Nitrogen compounds contain one or more nitrogen atoms. Nitrogen existing in all organisms, especially in amino acids (and thus proteins) and in the nucleic acids DNA and RNA. The human body contains about 3% nitrogen by mass. Although over (78%) of the atmosphere is composed of nitrogen, for a long-time source of nitrogen compounds were limited but nitrogen fixation by industrial processes like the Haber–Bosch process (1908–1913) and Frank–Caro process (1895–1899) eased this shortage of nitrogen compounds[73].

1.5.1 Creatinine

It is a nitrogen compounds produced by the breakdown of creatine phosphate in muscle, It is also known as the dehydrated final product of the creatine compound.[74] Creatinine its molecular formula ($C_4H_7N_3O$), and it's removed from blood by the kidneys, by glomerular filtration. If kidney filtration is not adjusted, the level of creatinine in the blood will rise. The creatinine ratio in the blood and urine can be used to calculate the creatinine clearance (CrCl) that associated with the glomerular filtration rate (GFR), The GFR is clinically important, this is due to being a measurement of kidney function[75]. Men have a higher proportion of creatinine than women because they are more powerful in muscle and skeletal structure, whereas vegetarians have shown that their creatinine ratio is low. The normal level of creatinine in the blood is about 0.5 - 1.5 mg per 100ml of blood[74,76].

1.6 Protein Adsorption

Biophysical mechanism of protein adsorption have substantial importance because adsorbed proteins mediate, catalyze, or moderate the biological response to artificial materials. Protein adsorption is a complex phenomena for a different reasons , like that proteins are comparatively large polyelectrolytes with adsorption properties that depend on interrelated factors like protein type , size, (structure, molecular weight, conformation, etc..), adsorbent surface chemistry, solution concentration, and interaction time between protein and surface . Thus, protein adsorption is versatile problem in surface physical chemistry. As a

consequence of these factors, exacerbated for having a range of analytical methods employed in order to measure protein adsorption, and it has proven difficult to recognized the generalities underlying protein adsorption [77]. Protein adsorption goes through some of steps. The first step : transmission of proteins from bulk solution to the interface and the second step : initial adsorption of protein , and conformational changes of protein on the surface When there is a mixture of proteins, the proteins that have the highest concentration, will control on the adsorption at the first time. Later, these proteins will be replaced by other proteins that have higher affinity to the surface. The surface property have important role on the adsorption of proteins [78-80]. Protein adsorption it becomes a spontaneous process when (ΔG) Gibbs free energy of the system is negative[81] $\Delta G_{ads} = \Delta H_{ads} - T \Delta S_{ads} < 0$. where (H) is the enthalpy and (S) entropy of the system and (Δ_{ads}.) is the operator.

1.6.1 Protein adsorption on nano structured surface

Nanoscale surface topography possesses an effect on the protein adsorption, It is important to understand and control the physicochemical interaction between nanostructured surfaces and proteins for biomedical applications. The adsorption depends on the surface chemistry and surface roughness and the concentration of the adsorbed proteins. In the nanoscale reported that the surface roughness has an affects the wettability [82]. nanoparticles causes changes in the structure of adsorbed proteins, and can modify the structure and therefore the function of the adsorbed protein thus affecting the overall bioreactivity of the nano particles . Curved nano particles surfaces compared to the planar surfaces provide extra flexibility and enhanced surface area to the adsorbed protein molecules[83]. Curved nano particles surfaces also can affect the secondary structures of proteins, and in the some cases cause irreversible changes [84]. When the protein is about the same size as the surface curvature the latter is not altered, as shown in figure [1-7]. P Roach et al., showed that albumin and fibrinogen they go through different interactions due to their differing shapes. where Albumin is stabilized by high surface curvature, while, fibrinogen, is distorted by wrapping around surface curvature, inducing secondary structure loss [85].

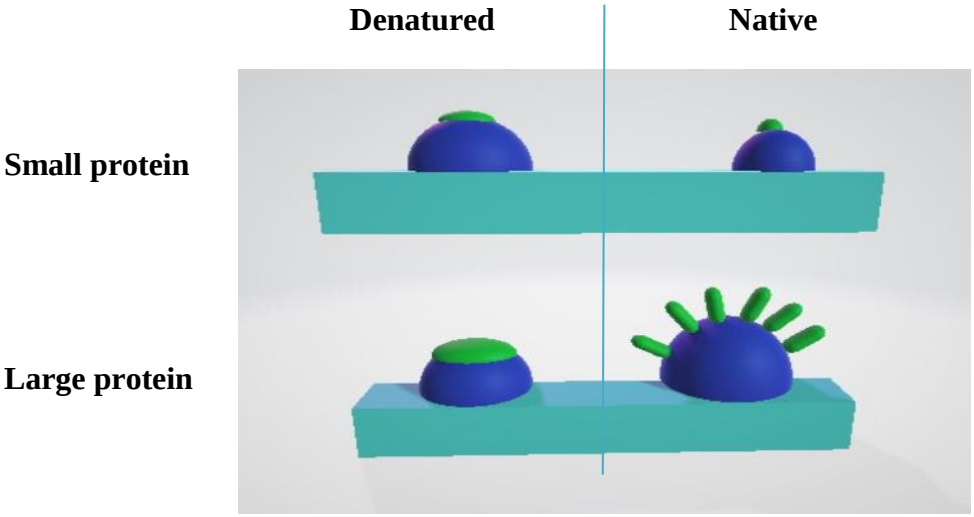


Figure 1-7: Schematic to show the impact of size difference, between nanostructured surface and protein[85]

1.6.2 Size effect of nanoparticles

The amount of adsorbed protein varies according to the size of the nanoparticle, and the installation of protein corona varies depending on size. Cerdervall et al, was studied the role of the surface area and particle size on the protein adsorption. The nanoparticle size is diverse, between (70 - 700)nm. the amount of bound proteins diverse with size of nanoparticles, and with the amount of article available surface area, but the surface curvature does not affect the relative affinities of proteins for the particles[86]. Rafaela et al, studied the protein adsorption on gold nanostars (AuNSs) and gold nanorods (AuNRs) with average sizes of (40 and 70)nm, the results explain that, both the total amount of protein adsorbed on AuNPs and the protein corona composition, were affected by AuNP shape and size [87].

1.7 The Literature Review :

Many scientists and researchers are interested in nanotechnology and study the effects of the interaction with different types of proteins due to the importance of the consequences of this interaction in the bodies of organisms, especially after the development in the medical field and increase the use of nanotechnology in this area so must know the variables that result from this Interaction.

In[2009] Chakraborti et al.[88]studied the interaction of ZnO NPs with lysozyme by using calorimetric and spectro photometric techniques ,this study has been clarified that ZnONPs are capable of disrupting protein-protein association. ZnONPs bind to the biggest cleft on the protein surface, so it's helping it to keep the secondary structures to a better degree and show enzymatic activity under denaturing conditions.

In[2009] Deng et al.[89] studied the effects of physicochemical properties on the plasma proteins. and have been clarified that the adsorption of proteins onto ZnO ,TiO_2 and SiO_2 NPs reached to the equilibrium at the first few minutes of incubation. This study has also shown that the agglomeration state has largely effected on the protein binding pattern .Because protein binding to the nanoparticles may be important in determining the extent of interaction with the cells and the tissues in vivo.

In[2009] Bardhan et al.[90] the interaction of ZnO NPs with the protein bovine serum albumin (BSA) was studied by the different spectroscopic methods. in this study that stimulated the BSA-ZnO conjugate spectroscopically, found that the protein keep most of its helical structure after the interaction. Therefore (BSA-ZnO) bio conjugate can play an important role in different biomedical applications without any loss of the original structure of BSA.

In[2010] Chatterjee et al.[91] In this study, ToxR protein of Vibrio cholerae adsorption was carried out on a ZnO NPs surface with a diameter of (2.5 nm).the result showed that binding to ZnO NPs can leads to changes in major structural of the ToxRp protein of V. cholerae.

1n[2012] Chakraborti et al.[92] have carried out a detailed investigation on the interaction of bovine serum albumin (BSA) with poly ethylene imine-

functionalized ZnO nanoparticles (ZnO-PEI). ZnO-PEI was synthesized using a wet chemical method with a core size of ∼3−7 nm , and the result indicating the possible involvement of electrostatic interaction, and shows that the protein surface can bind the nanoparticle. On binding ZnOPEI, the protein gets destabilized to some extent, when carried out in the presence of ZnO-PEI, also indicated a small perturbation in the protein structure.

In [2013] Bhogale et al.[93] synthesized (ZnO) nanoparticles with average size of (75 nm) to study their interaction with bovine serum albumin (BSA) at different temperatures .The results confirmed that the ZnO(NPs) quench the fluorophore of BSA by forming ground state complex in the solution , and inferred on the formation of aggregates of BSA-Zno NPs to induce slight conformational modification in BSA.

In[2013] Saptarshi et al.[2]studied the interaction of proteins with various nanoparticles, The search has shown that size, shape, and surface characteristics of NPs effect on the protein adsorption and also have the susceptibility to change the structure of the adsorbed protein molecules, this has a great impact on the reaction of the NPs with cells .

In[2014] Rosana et al.[94] work on the interaction of metal oxides NPs (ZnO , TiO_2, CeO_2 and Al_2O_3) with the main protein (albumin, fibrinogen and globulins), and found that the pattern of Np-protein interaction was clear up to vary depending on the type of nanoparticles.

In[2014] Soo Jin et al.[95] this study focuses on physicochemical factors affecting the bio kinetics of ZnO NPs ,and also understanding bio available fates and their interaction with proteins.

In [2016] Satzer et al.[96] studied the interaction of nanoparticles with nine proteins and demonstrated conformational changes for myoglobin and BSA upon adsorption to nanoparticles, that dependent on the size of the nanoparticle they are adsorbing to. also they uncovered significant differences between the unfolding kinetics of myoglobin and BSA.

1.18 The aim of this work

- Preparation and characterization of ZnO NPs and use batch adsorption experiments to evaluate the adsorption process.

- Study the influence of several parameters such as incubation time, adsorbent dosage, temperature, effect of pH, initial protein concentrations and shaking speed on the adsorption capacity of protein and biomolecules metabolic product of proteins.

- Determination of adsorption isotherms such as Langmuir, Freundlich and Temkin to know which of them are the most appropriate for fit of equilibrium experimental data.

- Calculate the thermodynamic parameters values: free energy (ΔG), enthalpy (ΔH), and entropy (ΔS).

Chapter Two

Experimental part

2.1Chemicals

The chemicals used in this work are listed in Table (2-2).

No.	Chemicals	Purity	Company supplied
1	zinc sulphate heptahydrate (ZnSO4.7H2O)	99%	Laboratory reagent thomas baker, India.
2	sodium hydroxide (NaOH)	98%	BDH chemicals Ltd, Poole England
3	Hydrochloric acid (HCl)	37%	SD Fine_Chemical limited- India
4	Ovalbumin	99%	BDH chemicals Ltd, Poole England
5	Creatinine	99%	BDH chemicals Ltd, Poole England

2.2 Instruments

Table (2-1) shows the Instruments used in this work.

No.	Instrument	Type, Company	Place
1	Electric Sensitive, Balance.	Lab.BL210, Sartorius median- Germany.	University of Karbala
2	Centrifuge.	Megafuge1.0,Herouse Spartech-Germany.	University of Karbala
3	Oven Memort.	LOD-080+N-Labtech ,Korea.	University of Karbala
4	pH-Meter.	pH-Meter WTW-720 ionlab- Germany.	University of Karbala
5	Shaking water bath	SWB-25 Hysc-Korea.	University of Karbala
6	Centrifuge	Universal 320R, Hettich-Germany	University of Karbala
7	Spectrophotometer Uv –Visible.	Uv-Visible 1800 Shimadzu-Japan.	University of Karbala
8	X-Ray diffraction.	XRD-6000 Shimadzu-Japan.	University of Baghdad
9	Fourier transform infrared (FT-IR).	Bruker - Optice Germany.	University of Babylon
10	Scanning electron microscope (SEM).	FEI Quanta 400	University of Babylon
11	Atomic Force Microscope (AFM).	A A 3000 Scanning probe Microscope (SPM) –USA.	University of Baghdad

2.3 Preparation of Standard Solutions

Zinc sulphate heptahydrate (ZnSO4.7H2O) solution was prepared with a concentration of 0.3 M by dissolving 40 g of (ZnSO4.7H2O) in 500ml of distilled water.

Sodium hydroxide solution (2M) was prepared by dissolving 8g of NaOH in 100ml of distilled water. From this solution, diluted solution was prepared with concentration of (0.5) by dilution process using volumetric flask.

The albumin solution was prepared with a concentration of 0.001M(4.5g/dl) by dissolving 0.45g of albumin in 10ml of distilled water. From this solution, diluted solutions were prepared with concentrations ranging from 0.45 - 4.5 g/dl by dilution process using volumetric flasks.

Creatinine solution 0.00088M ($10x10^{-3}$g/dl) was prepared by dissolving 0.001g of creatinine in 10ml of distilled water. From this solution, diluted solutions were prepared with concentrations ranging from 0.1- 2 g/dl by dilution process using volumetric flasks.

HCl solutions was prepared with a concentration of (0.5M): 1ml from (11.4M) HCl solution was diluted by 25ml of distilled water.

2.4 Preparation of ZnO Nanoparticles[97]

The nanoparticle was prepared by adding a solution of sodium hydroxide (NaOH) (2M) to the zinc sulphate heptahydrate (ZnSO4.7H2O) solution (0.5M) in droplets with continuous stirring until the pH=10.

The precipitate was then separated using a centrifuge and washed by deionized water three times with the return of the separation after each wash.

The resulting precipitate was dried at 70 °C.

2.5The interaction of zinc oxide NPs with albumin and creatinine.

In this work we studied the effect of many factors on the adsorption of (albumin, creatinine) on the surface of ZnO nanoparticles, including:

2.5.1 Effect of equilibrium time on adsorption of albumin and creatinine on ZnO NPs.

Equilibrium time effect on adsorption was studied by preparing a number of solutions by mixing 40 mg of ZnO NPs with 100µl of the albumin solution (2.25g/dl), creatinine ($0.5x\ 10^{-3}$g/dl). And complete the volume to 1000µl with distilled water, these solutions were placed in the thermostated shaker for several times (10-60 min) for albumin and (2.5-60min) for creatinine. The solutions were then separated using a centrifuge for 20 min (4000 rpm) and the solution was then withdrawn by syringe needle and it was again separated using centrifuges for 20min , the absorption of each solution for this effect was measured at λ_{max} 630nm for albumin and at λ_{max} 510nm for creatinine by colorimetric method using the working method of the commercial kit (Cromatest, Linear Chemicals-Spain).

2.5.1.1 Albumin.

Principle: The method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acidic pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.

$$\text{BCG + Albumin} \xrightarrow{\text{pH 4.3}} \text{BCG- albumin complex.}$$

Reagent composition R1 ➡ Bromocresol reagent. CAL. ➡ Albumin standard 5g/dl.

Procedure:

1. reagents and samples were brought to room temperature.

2. The sizes listed below were taken into labelled tubes:

Tubes	Blank	Sample	CAL. Standard
• R1.Reagent • Sample • CAL. Standard	2.0 ml - -	2.0 ml 0.01 ml -	2.0 ml - 0.01 ml

3. The solutions were mixed and left the tubes stand (1 min) at room temperature.

4. Absorption was read (A) of the samples and the standard at (630)nm against the reagent blank.

Calculations:

$$\frac{\text{A sample}}{\text{A standard}} \times \mathbf{C}\ \text{standard} = \mathbf{Ce}\ \text{of albumin.}$$

Where:

A sample : absorption of sample.

A standard : absorption of standard.

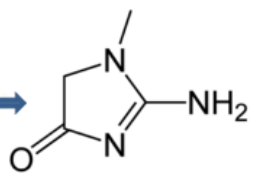
Ce : concentration (g/dl) at equilibrium.

2.5.1.2 Creatinine.

Principle: This procedure is based upon a modification of the original picrate reaction (jaffe). Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid $\xrightarrow{\text{PH} >12}$ Reddish complex.

Reagent composition: R1 ➡Picric acid. R2 ➡Alkaline buffer.

CAL ➡Creatinine standard (2×10^{-3})g/dl ➡ 

Procedure:

1. Working reagents (The resulting mixing of equal volumes of R1+R2), samples and standard were brought to reaction temperature 37℃.

2. The photometer is set to 0 absorbance with distilled water.

3. The sizes listed below were taken into labelled tubes:

Working reagents	1.0 ml
Samples or Standard	0.1 ml

4. The solutions were mixed gently and the absorption was read at 510 nm after 30sec (A1) and after 90sec (A2) of the samples and the standard addition.

Calculations:

$$\frac{(A2-A1)\ \text{sample}}{(A2-A1)\ \text{standard}}\ \text{x}\ \mathbf{C}\ \text{standard} = \mathbf{Ce}\ \text{of creatinine.}$$

2.5.2 Effect of zinc oxide NPs weight on adsorption of albumin and creatinine.

The effect of different weight of ZnO NPs (10-50)mg was studied by the preparation of several solutions of (100µl) albumin and creatinine mixing with weights of ZnO NPs and completed the volume to 1000µl, This work was completed in the same manner as previously mentioned in (2.5.1) with install optimal conditions for adsorption(time of adsorption about 30min for albumin and 2.5min for creatinine).

2.5.3 Effect of pH on adsorption of albumin and creatinine on ZnO NPs.

Several experiments have been done to study this effect on adsorption by changing the pH of solutions to the following values (pH= 2,5,7,9,11) by 0.5M NaOH or 0.5M HCl, and completed the work in the same manner as previously

mentioned in (2.5.1) with install optimal conditions for adsorption(time of adsorption about 30, 2.5min and 30,10 mg weight of ZnO NPs for albumin and creatinine respectively).

2.5.4 Effect of temperature on adsorption of albumin and creatinine on ZnO NPs.

For this purpose we studied the effect of temperature on adsorption of albumin and creatinine on ZnO NPs , the reaction was conducted at different temperatures (15,25,35,45)℃ and completed the work in the same manner as previously mentioned in (2.5.1) with install optimal conditions for adsorption(time of adsorption about 30, 2.5min and 30,10 mg weight of ZnO NPs for albumin and creatinine respectively and at pH=7).

2.5.5 Effect of albumin and creatinine concentration on adsorption.

Amount of solutions of albumin and creatinine were prepared with different concentrations (0.45, 1.12, 2.25, 3.37, 4.5) g/dl for albumin and (0.1, 0.5, 1, 1.5, 2) $x10^{-3}$g/dl for creatinine for study the effect of changing the concentration of the adsorbent material at optimal conditions for adsorption (time of adsorption about 30, 2.5min and 30,10 mg weight of ZnO NPs for albumin and creatinine respectively and at pH=7, temperature of 25℃).

2.5.6 Effect of shaking speed on adsorption of albumin and creatinine on ZnO NPs.

To know the effect of the speed of shaking we placed albumin and creatinine solutions in the thermostatic shaker, the speed has been changed from (40-280) rpm, and completed the work in the same manner as previously mentioned in (2.5.1) with install optimal conditions for adsorption(time of adsorption about 30, 2.5min , (30,10) mg weight of ZnO NPs and concentration 2.25, $0.5x10^{-3}$ g/dl for albumin and creatinine respectively at pH=7 and temperature of 25℃).

2.6 Adsorption Isotherm

Different weights of zinc oxide were used (10-50 mg) with the concentrations of 2.25 g/dl for albumin and $0.5x10^{-3}$g/dl for creatinine, at pH=7. the solutions were placed in the thermostated shaker at 140 rpm for 30, 2.5 min for both albumin and creatinine respectively, at 298 K. Then separated using a centrifuge for 20 min at 4000 rpm, the solution was then withdrawn by syringe needle and it was again separated using centrifuges for 20min. The concentration (Ce) was extracted by commercial kit (Cromatest, Linear Chemicals-Spain). But the Qe value was measured from this equation[59] :

$Qe = (C_o - Ce)*V/ m$ ……(2-1)

Where:

Qe : amount of adsorbate (mg/g).

Ce : concentration of protein at equilibrium (mg/L).

C_o : initial concentration of protein (mg/L).

V: volume of adsorption (L).

m : weight of adsorbent (g).

The Langmuir , Freundlich and Temkin adsorption equation was then applied to study the interaction between zinc oxide and protein. The Langmuir equation can be written in the following form[58]:

$Ce/Qe = 1/ (ab) + Ce/ a$ …..……..…(2- 2)

The Freundlich equation can be written in this form[60] :

$Log\ Qe = Log\ K_f + 1/n\ Log\ Ce$……..(2-3)

The Temkin equation can be written in the following form[62]:

$Qe = B\ lnAT + B\ lnCe$ ……..……(2-4).

2.7 Thermodynamic parameters for adsorption

The thermodynamic parameters (free energy change ΔG, entropy change ΔS and enthalpy change ΔH) can be determined by studying the effect of temperature. (ΔG) values can be calculated from the following equation:

ΔG = - RT ln Keq ……..…..…(2-5)

Where:

ΔG : free energy change ($J.mol^{-1}$)

R : universal gas constant (8.314 $J. mol^{-1}. K^{-1}$)

Keq : Equilibrium constant.

(K_{eq}) can be calculated at each temperature by the following equation[98]:

$$\mathbf{K_{eq}} = \frac{Qe\ m}{Ce\ V} \qquad \text{…..……(2 - 6)}$$

(ΔH) calculated by drawing the relationship between ln Keq and 1/T according to Vant - Hoff - Arrhenius equation:

ln Keq = (-ΔH/RT) + constant. .....(2 - 7)

(ΔS) values are calculated from this equation :

ΔG = ΔH - TΔS …….(2 - 8).

2.8 The activation energy

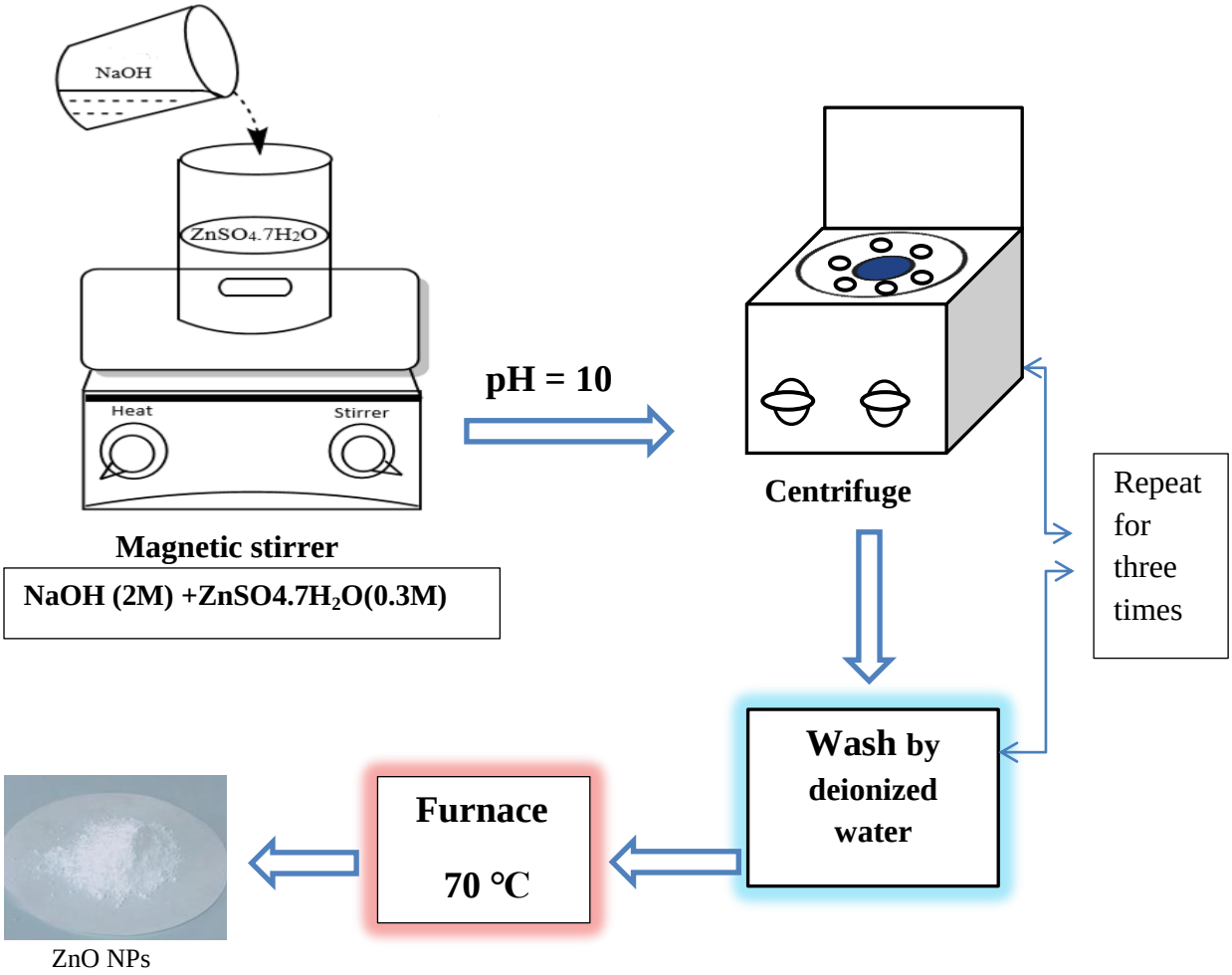
The activation energy was calculated after calculate the surface coverage(Ɵ) according to equation (1-4) and then by drawing the relationship between 1/ T and ln (1-Ɵ) according to the following equation:

Ln(1- Ɵ) = ln S^{*} + Ea / RT……(2 - 9).

Chapter Three

Results And Discussion

3.1 Preparation and Characterization Of Zinc Oxide NPs :



Scheme3-1: showing the method of preparing zinc oxide NPs.

Zinc oxide NPs was characterized by Fourier Transform Infrared Spectroscopy FTIR, Scanning Electron Microscopy SEM, Atomic Force Microscopy AFM and X-Ray Diffraction Spectroscopy XRD.

3.1.1 Fourier Transform Infrared Spectroscopy (FTIR)

The samples were measured within (4000-400cm^{-1}) at room temperature. ZnO spectra in Figure (3-1) show that the essential peaks of ZnO Appeared as a strong band around (500cm^{-1}) was assigned to the stretching band of Zn-O, those previous results referred to create of ZnO[99] , strong band around (1100cm^{-1}) also referred to create of Zn-O while (3400cm^{-1}) referred to the bond of O-H.

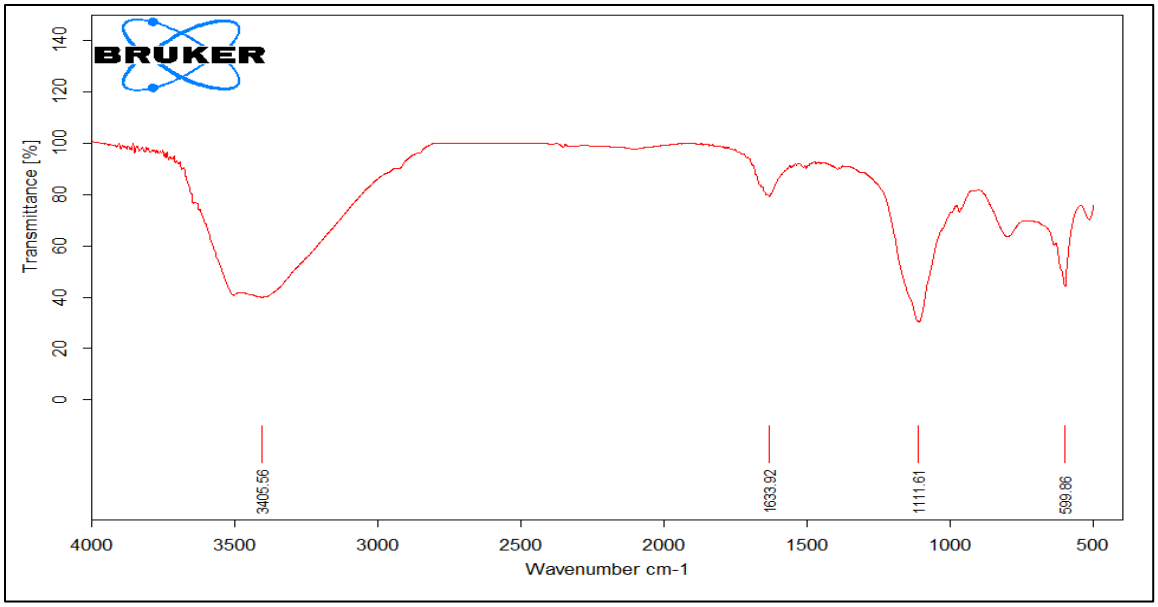


Figure 3-1: The FTIR characterization of ZnO NPs.

3.1.2 Scanning Electron Microscopy (SEM)

Figure (3-2) shows scanning electron micrographic of zinc oxide nanoparticles with different scales. The microstructural features of all the images of ZnO nanoparticles revealed a smooth particles morphology with high dense aggregation. The image shows the array of nanoparticles as arranged in several layers at different size, varied shape, and many pores in the surface were observed.

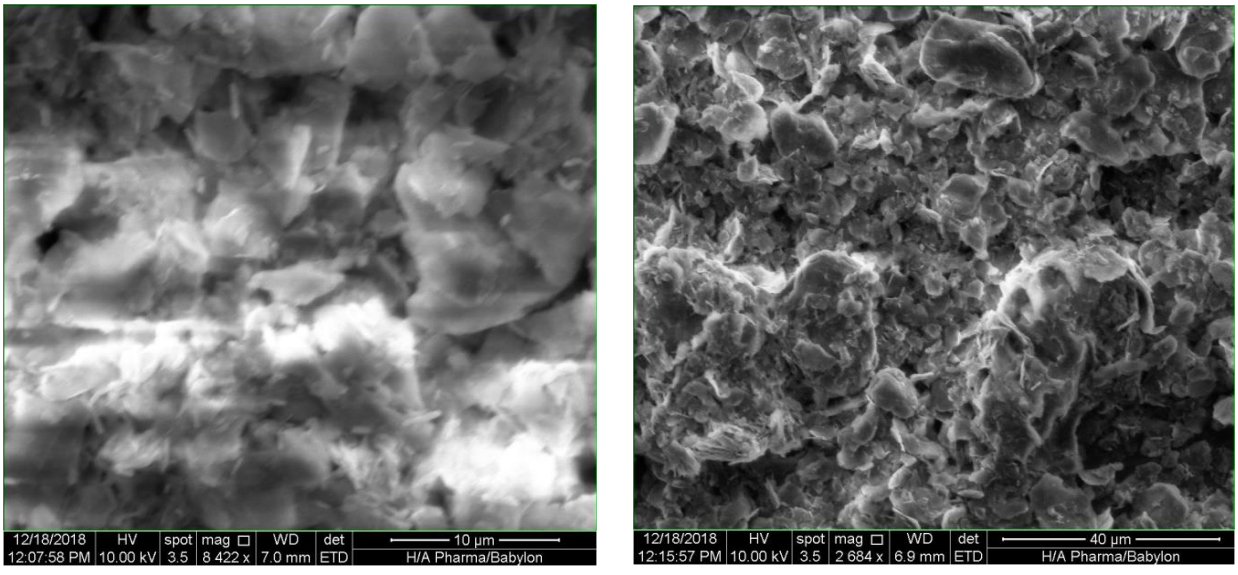


Figure 3-2: The SEM characterization of ZnO NPs.

3.1.3 Atomic Force Microscopy (AFM)

Zinc oxide NPs surface was studied using an atomic force microscope. Figure (3-3)a shows a two dimensional picture of nanomaterials in which molecular clusters appear. Figure (3-3)b shows a three dimensional image of a section of the surface of nano materials showing the rise of molecular clusters that are about (48.55nm) and that the average particle size is about (73.97nm).

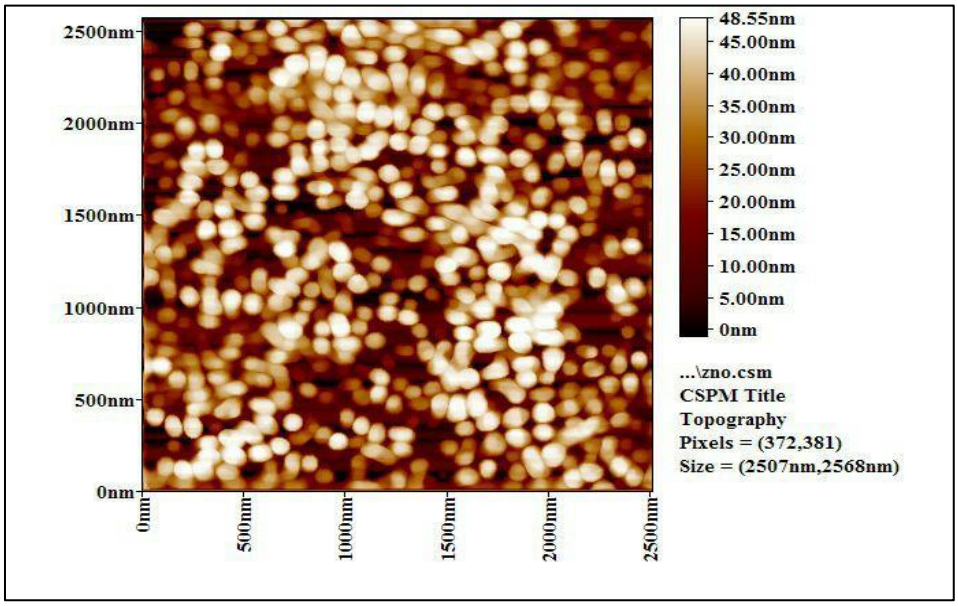


Figure 3-3: a- The AFM characterization of ZnO NPs show the two-dimensional picture.

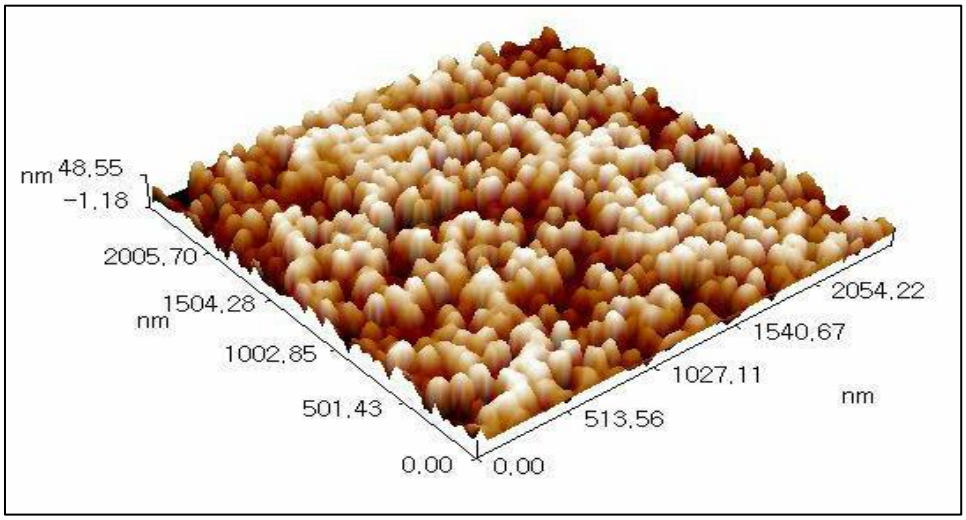


Figure 3-3: b- The AFM characterization of ZnO NPs show the three-dimensional picture.

Table(3-1): Some information from AFM characterization of ZnO NPs

Amplitude parameters		**Hybrid parameters**	
Sa (Roughness Average)	11.9 nm	Ssc (Mean Summit Curvature)	-0.0277[1/nm]
Sq (Root Mean Square)	13.9 nm	Sdq(Root Mean Square Slope)	0.771 [1/nm]
Ssk(Surface Skewness)	-0.257	Sdr (Surface Area Ratio)	23.4
Sku (Surface Kurtosis)	1.9		
Sy (peak-peak)	49.7 nm		
Sz (Ten point Height)	49.7 nm		
Functional parameters		**Spatial parameters**	
Sbi(Surface Bearing Index)	6.07	Sds (Density of Summits)	144 [1/nm2]
Sci(Core Fluid Retention Index)	1.33	Fractal Dimension	2.32
Svi(Valley Fluid Retention Index)	0.0897		
Spk(Reduced Summit Height)	0 nm		
Sk(Core Roughness Depth)	38.5nm		
Svk (Reduced Valley Depth)	10.7 nm		
Sdc 0-5(0-5% height intervals of Bearing Curve)	2.28 nm		
Sdc 5-10(5-10% height intervals of Bearing Curve)	1.65 nm		
Sdc 10-50(10-50% height intervals of Bearing Curve)	16.7 nm		
Sdc 50-95(50-95% height intervals of Bearing Curve)	25.3 nm		

Table (3-2): The total rate of particle size of ZnO nanoparticle.

Sample: ZnO **Line No.:** lineno **Instrument:** CSPM	**Code:** Sample Code **Grain No.:** 417 **Date:** 2018-12-30
Avg. Diameter: 73.97 nm **<=50% Diameter:75.00 nm**	**<=10% Diameter:45.00 nm** **<=90% Diameter:100.00 nm**

Diameter(nm)<	Volume(%)	Cumulation(%)	Diameter(nm)<	Volume(%)	Cumulation(%)	Diameter(nm)<	Volume(%)	Cumulation(%)
25.00	0.72	0.72	55.00	6.95	20.38	85.00	8.39	65.47
30.00	0.96	1.68	60.00	7.19	27.58	90.00	9.59	75.06
35.00	1.20	2.88	65.00	7.43	35.01	95.00	5.52	80.58
40.00	1.92	4.80	70.00	5.76	40.77	100.00	9.11	89.69
45.00	4.32	9.11	75.00	8.63	49.40	105.00	9.59	99.28
50.00	4.32	13.43	80.00	7.67	57.07	110.00	0.72	100.00

3.1.4 X-Ray Diffraction Spectroscopy (XRD)

Prepared zinc oxide NPs surface was studied using X-Ray diffraction spectroscopy, the results in figure (3-4)b show the strong diffraction peaks at (31.78°), (34.33°), and (36.17°), which correspond to (100, 002 and 101) planes respectively show hexagonal wurtzite structure of Zinc oxide NPs[100,101], the crystallite size of ZnO NPs was calculated to be 33.1 nm by Debye-Scherrer's formula[101] that shows in the following equation.

$$\mathbf{L=}\frac{\mathbf{K\,\lambda}}{\boldsymbol{\beta\, Cos\theta}}$$

Where : [L] indicates to the crystallite size. [K] is the Scherrer's constant that depends on the shape of the crystal used (0.94),[λ] is the wavelength 1.54056Å (Cu/K-alpha1), β (FWHM) indicates to the full width of half-maximum intensity (it's measured in degrees then multiply by ((π/180)) to convert to radians) and [θ] is a diffraction angle[102]. The results were identical to the peaks shown in the figure (3-4)a of standard XRD pattern of zinc oxide NPs[103].

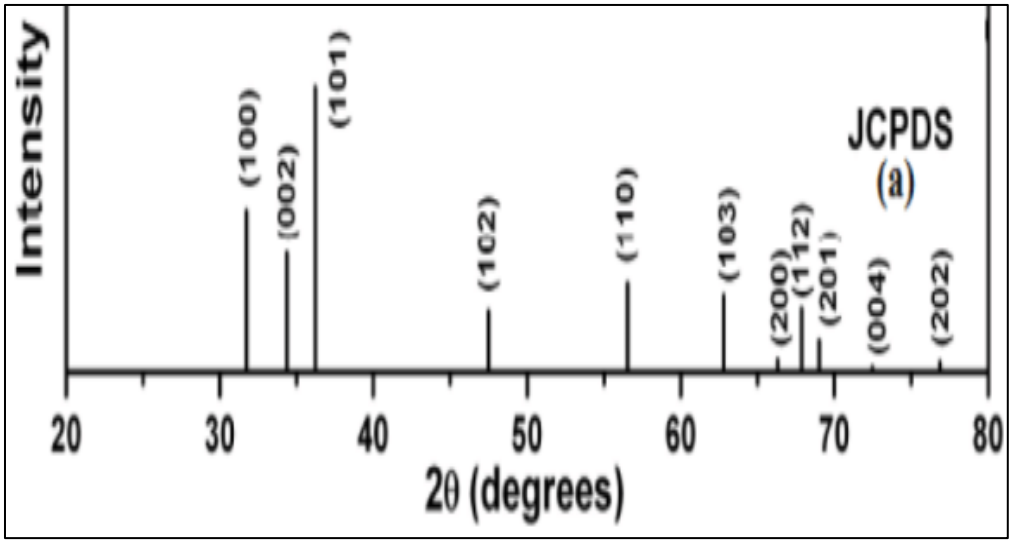


Figure 3-4: a- The XRD characterization of standard ZnO NPs.

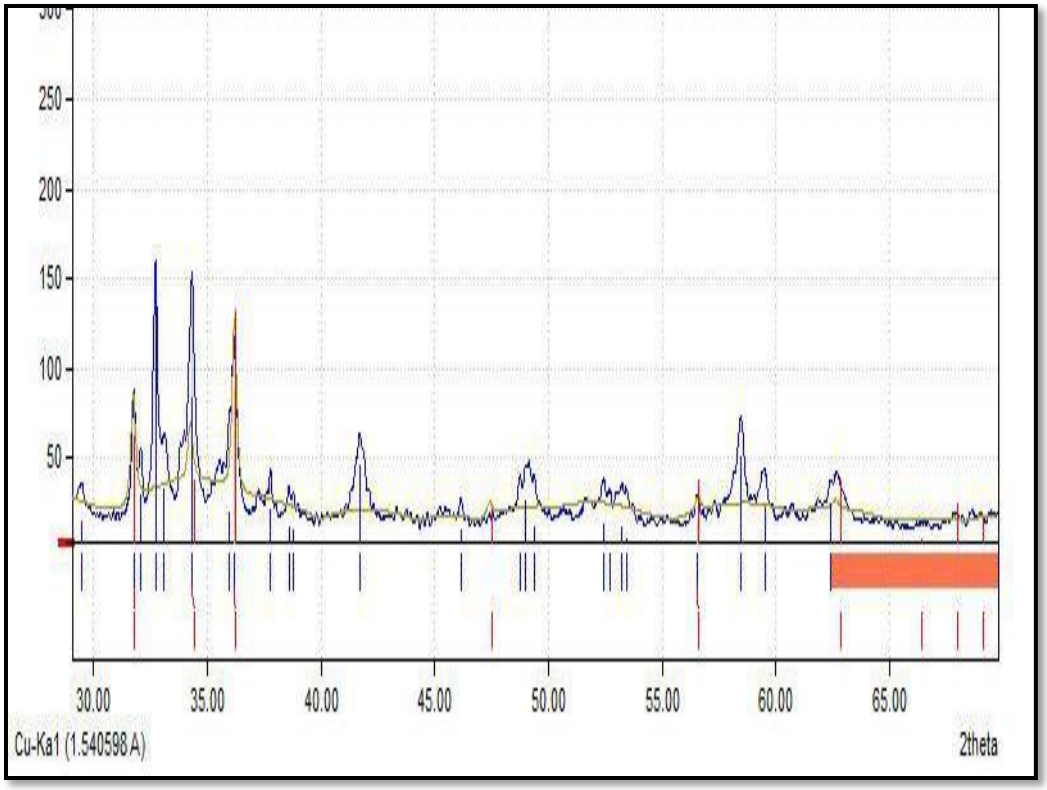


Figure 3-4: b- The XRD characterization of Prepared ZnO NPs.

3.2 Effect of equilibrium time on adsorption of albumin and creatinine on ZnO NPs :

The effect of the equilibrium time on adsorption was studied by using a weight of 40mg zinc oxide NPs to concentrations of 2.25g/dl of albumin and $0.5x10^{-3}$g/dl of creatinine for different time (1.5-60min). The results shown in Figure (3-5) show that time 30min and 2.5min is the best equilibrium time for both albumin and creatinine respectively because zinc oxide NPs reached saturation at this time, and it reached the highest adsorption value. The results corresponded to the percentage of adsorption as shown in Figure (3-6) for the same reasons and for all studied parameter.

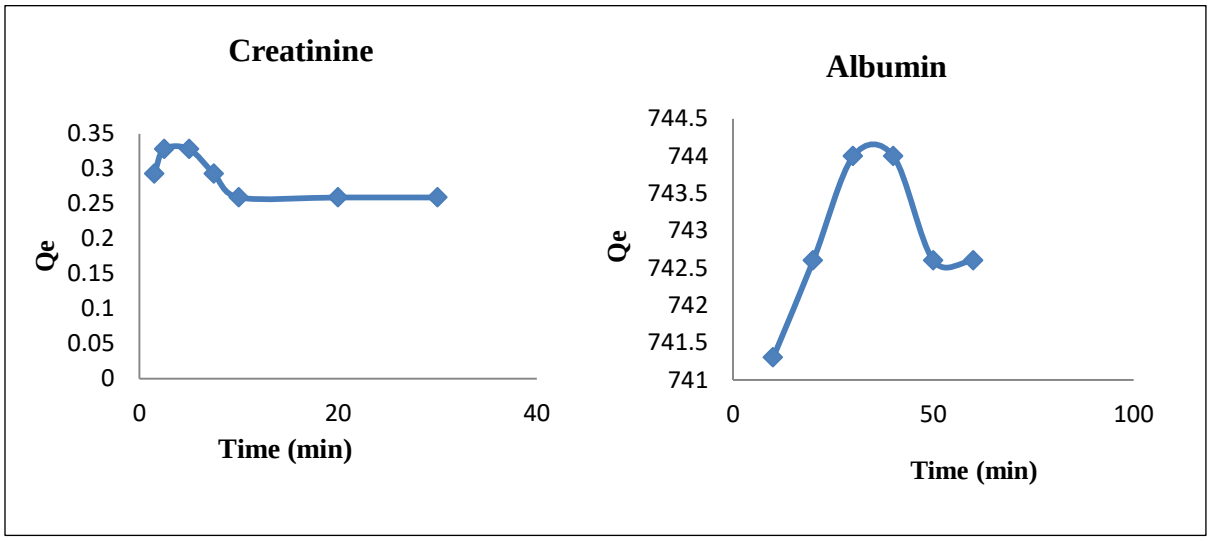


Figure 3-5: Effect of equilibrium time on amount of adsorption of albumin and creatinine by ZnO NPs.

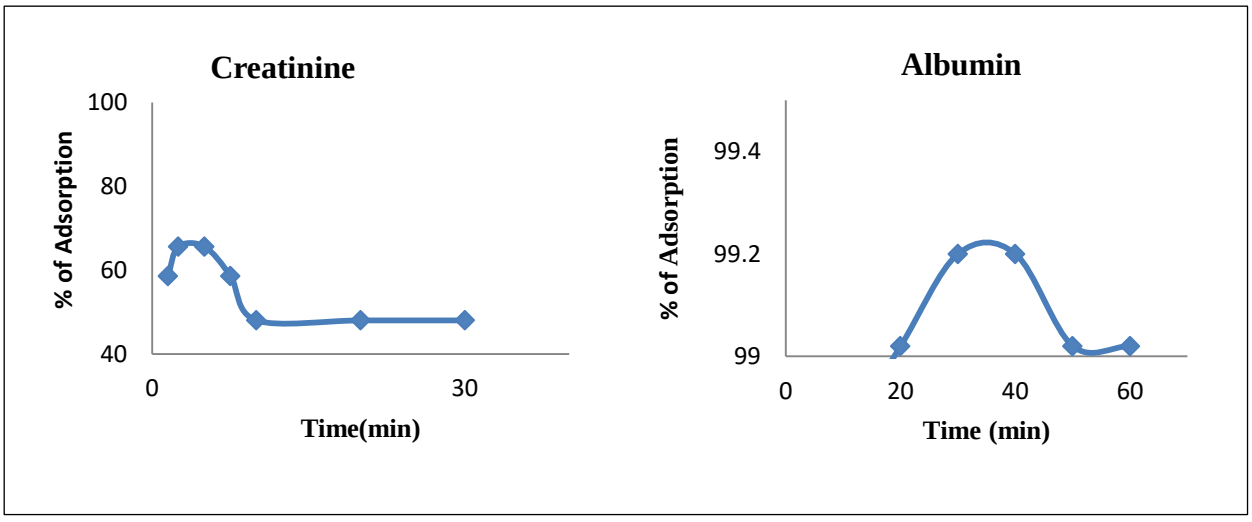


Figure 3-6: Effect of equilibrium time on percentage of adsorption of albumin and creatinine by ZnO NPs.

3.3 Effect of adsorbent weight(ZnO NPs) on adsorption of albumin and creatinine:

The effect of weight of zinc oxide NPs on adsorption was studied by adding different weights (10-50mg) to the concentrations of (2.25g/dl and 0.5x10-3g /dl) and at (30, 2.5) min for albumin and creatinine respectively and, the adsorption percentage of albumin increases with increasing weight. This is due to the availability of a larger surface area with an increase in the number of active sites of adsorption, thus increasing the amount of adsorption from the solution[(104)], until it reaches a state of equilibrium and there is no noticeable increase in the amount of adsorption, while the adsorption of creatinine reached a state of equilibrium since the start of the interaction as a result of the saturation of active sites in creatinine until it reaches a state of equilibrium. The reason for this difference between the result of albumin and creatinine, it can be attributed to the difference in molecular weight and of the two substances. 10 mg and 30mg gave the best adsorption percentage for creatinine and albumin respectively as shown in Figure (3-7), (3-8) :

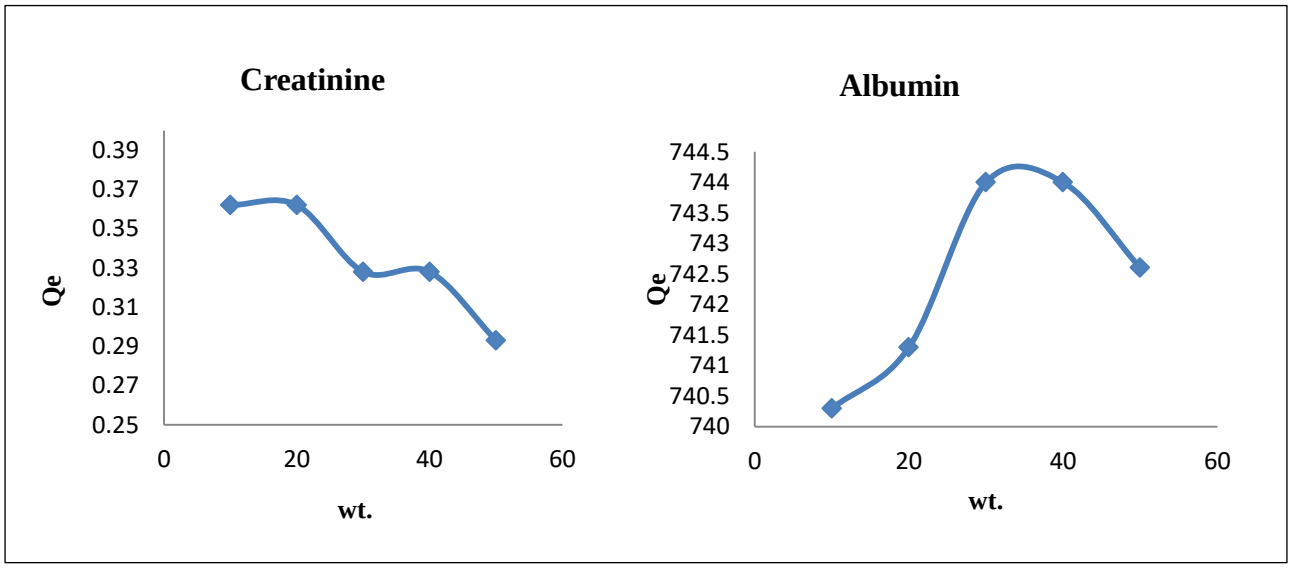


Figure 3-7: Effect of weight of ZnO NPs on amount of adsorption of albumin and creatinine.

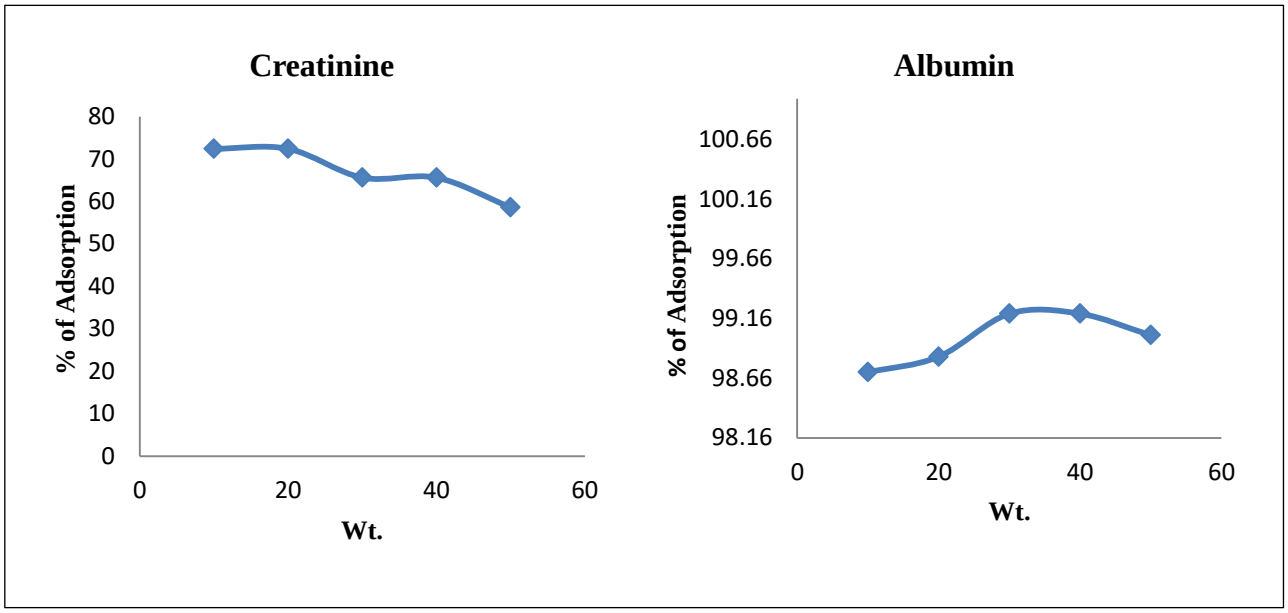


Figure 3-8: Effect of weight of ZnO NPs on percentage of adsorption of albumin and creatinine.

3.4 Effect of pH on adsorption of albumin and creatinine:

The effect of various pH on adsorption process was investigated by using (30,10) mg of zinc oxide NPs with (2.25 , $0.5x10^{-3}$)g /dl and(30,2.5)min for albumin and creatinine respectively. By using solutions with pH ranging from (2-11), the results shown in Figure (3-9),(3-10). In the acidic medium adsorption sites of zinc oxide NPs are charged with a positive charge (protons provide). while in the basic medium adsorption sites are charged with a negative charge (due to an increase in hydroxide ions).So we find that with albumin, the amount of adsorption is high in the acidic medium because albumin carries a negative charge, while in the basic medium, repulsion occurs between negative surface sites and albumin molecules. In the case of creatinine, the amount of adsorption increases in the basic medium, because the binding of this molecule to the sites of the surface that prepared for adsorption occurs at the side that has a shortage of negative charge. The opposite occurs in the acidic medium due to repulsion between the adsorption sites of the surface that is charged with a positive charge with the positive molecule tip.

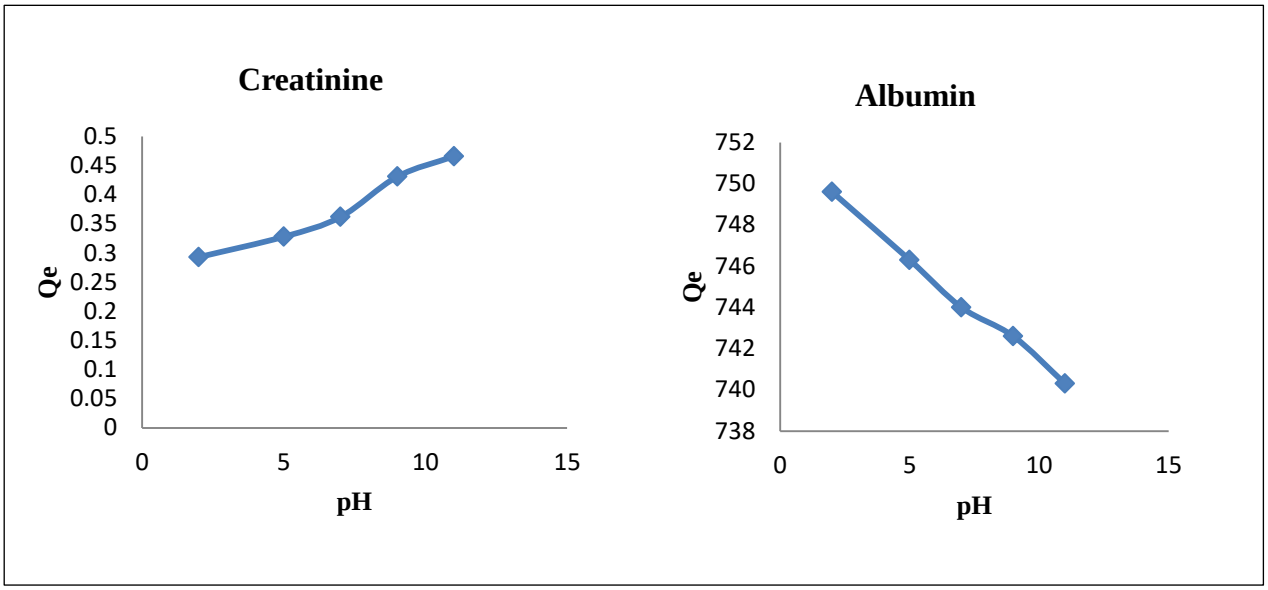


Figure 3-9: Effect of pH on amount of adsorption of albumin and creatinine on ZnO NPs.

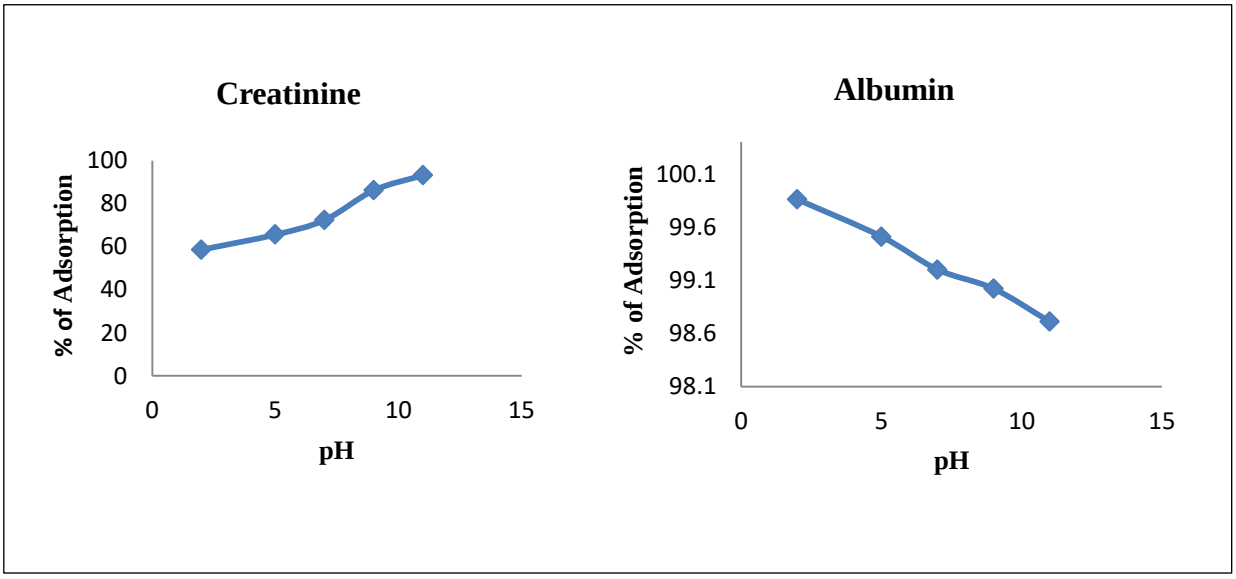


Figure 3-10: Effect of pH on percentage of adsorption of albumin and creatinine on ZnO NPs.

3.5 Effect of temperature on adsorption of albumin and creatinine:

The effect of temperature on the adsorption process was studied by applying different temperatures (15-45℃) and using 10,30 mg of zinc oxide NPs and concentrations of (2.25 , $0.5x10^{-3}$) g /dl,(30,2.5min) and pH=7 for albumin and creatinine respectively. The results shown in Figure (3-11),(3-12) showed that the amount of adsorption increases with temperature increase due to increasing kinetic energy of molecules that helps to enter the pores on the surface of the zinc oxide and thus increase the amount of adsorption. Temperatures greater than 50℃ have not been studied because the bio compounds including proteins, denatured at temperatures above 50℃.

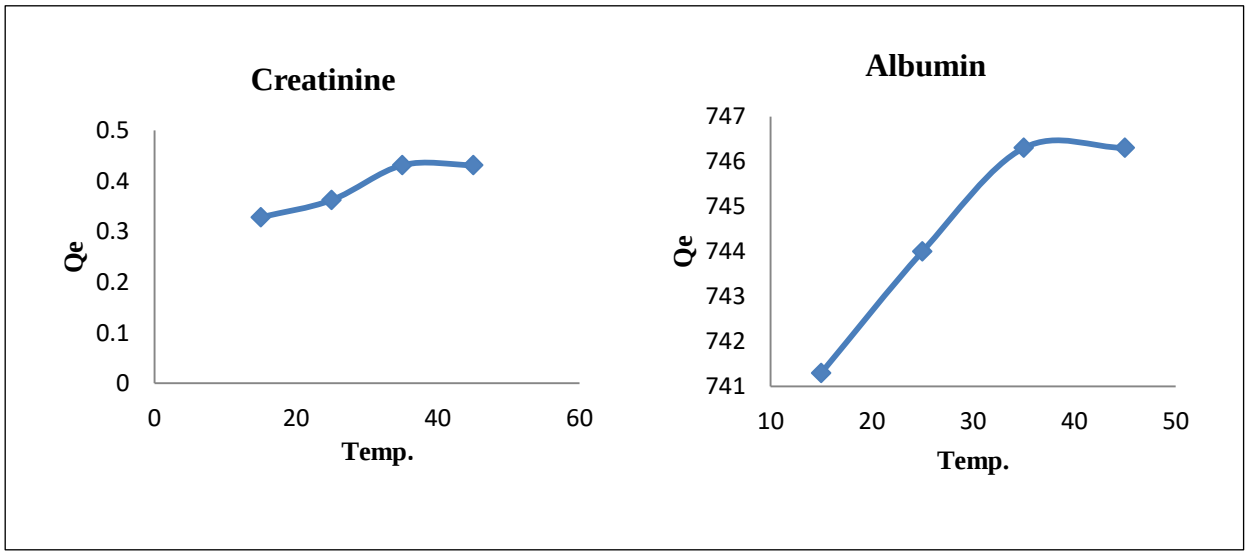


Figure 3-11: Effect of temperature on amount of adsorption of albumin and creatinine on ZnO NPs.

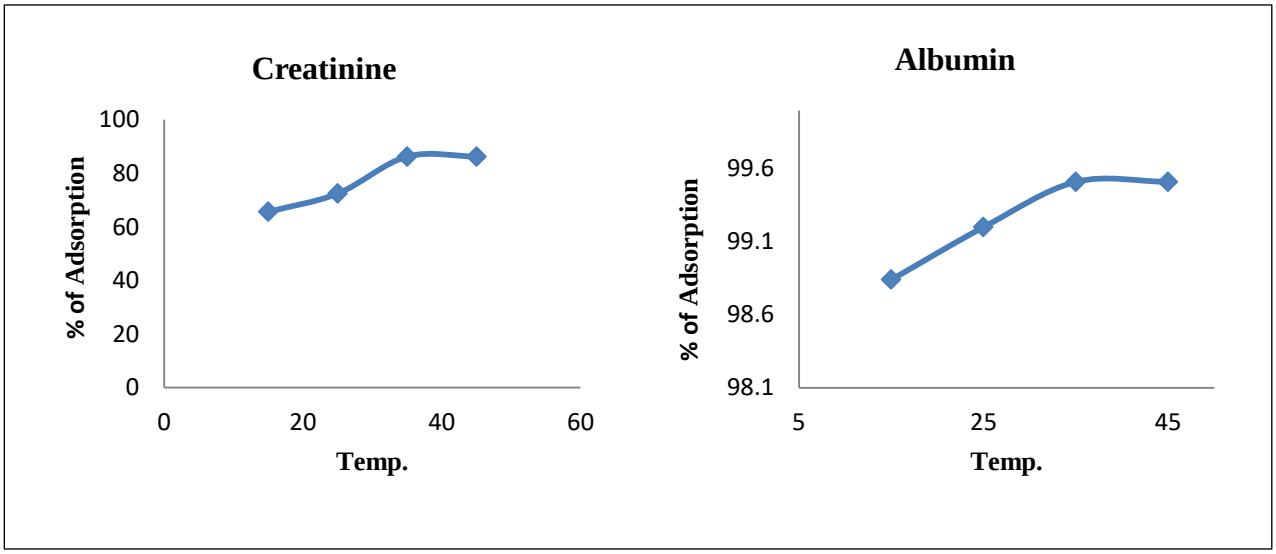


Figure 3-12: Effect of temperature on percentage of adsorption of albumin and creatinine on ZnO NPs.

3.6 Effect of concentration of albumin and creatinine on adorption:

This effect on adsorption process was investigated by using different concentrations of albumin and creatinine at (30,10) mg of zinc oxide NPs with albumin and creatinine respectively. The results shown in Figure (3-13),(3-14) we showed that the amount of adsorption increases with increasing concentration due to the increase in the active groups which are associated with the active sites on the surface of the ZnO NPs by increasing the concentration leading to increased adsorption.

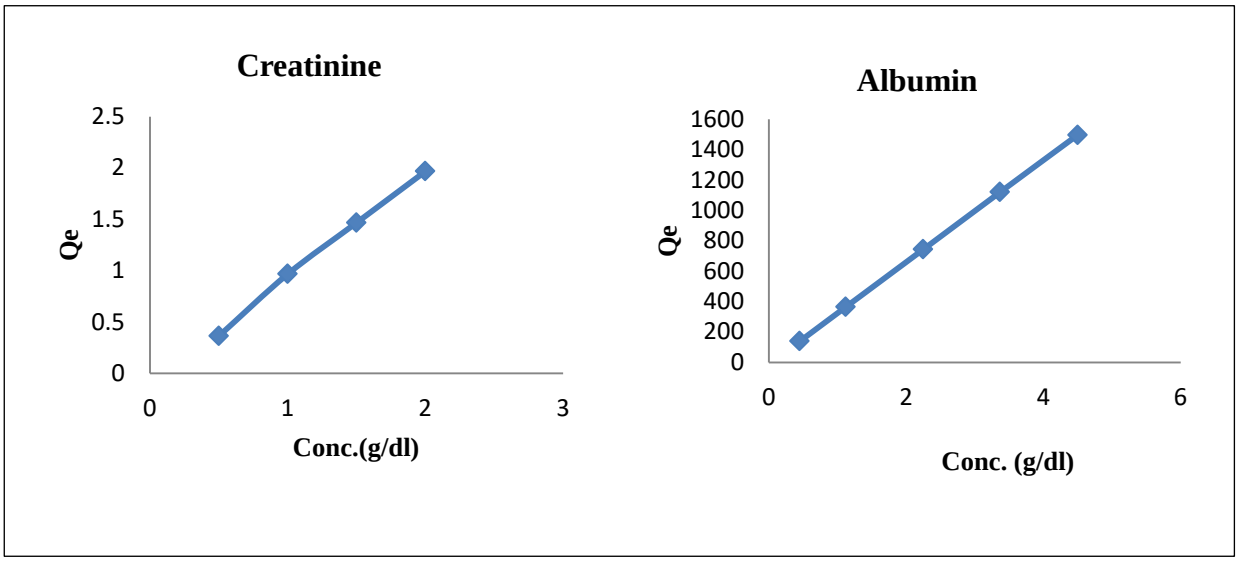


Figure 3-13: Effect of concentration of albumin and creatinine on amount of adsorption on ZnO NPs.

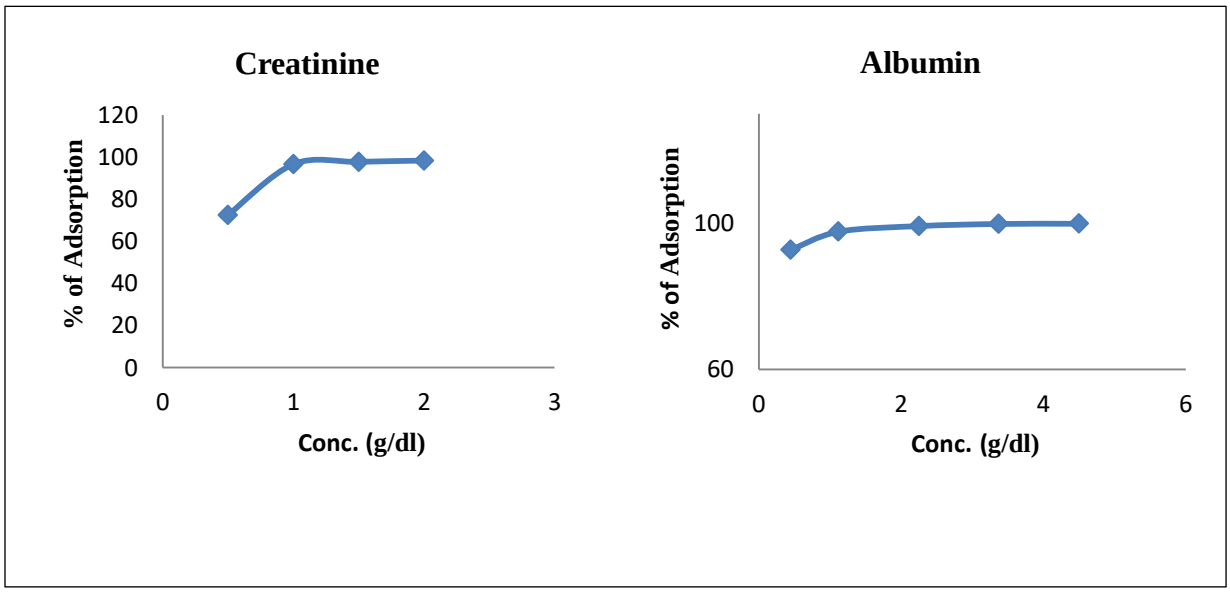


Figure 3-14: Effect of concentration of albumin and creatinine on percentage of adsorption on ZnO NPs.

3.7 Effect of shaking rate on adsorption of albumin and creatinine:

The results in Figure (3-15),(3-16) show the effect of shaking speed on the amount of adsorption when (30, 10) mg of zinc oxide NPs at 30,2.5 min and concentrations of 2.25 g/dl and $0.5x10^{-3}$g /dl for both albumin and creatinine respectively at pH =7 and 298k. we found that adsorption increases with increasing the shaking rate until it reaches a state of equilibrium and that is due to increasing kinetic energy, this speeds up the link with effective sites on ZnO NPS.

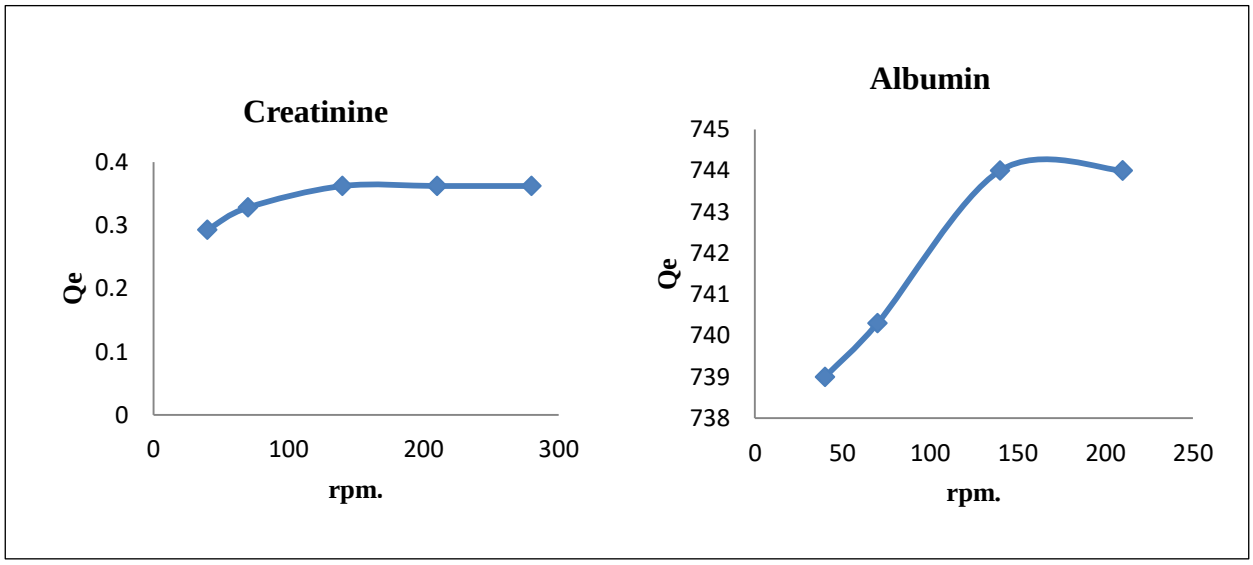


Figure 3-15: Effect of shaking rate on amount of adsorption of albumin and creatinine on ZnO NPs.

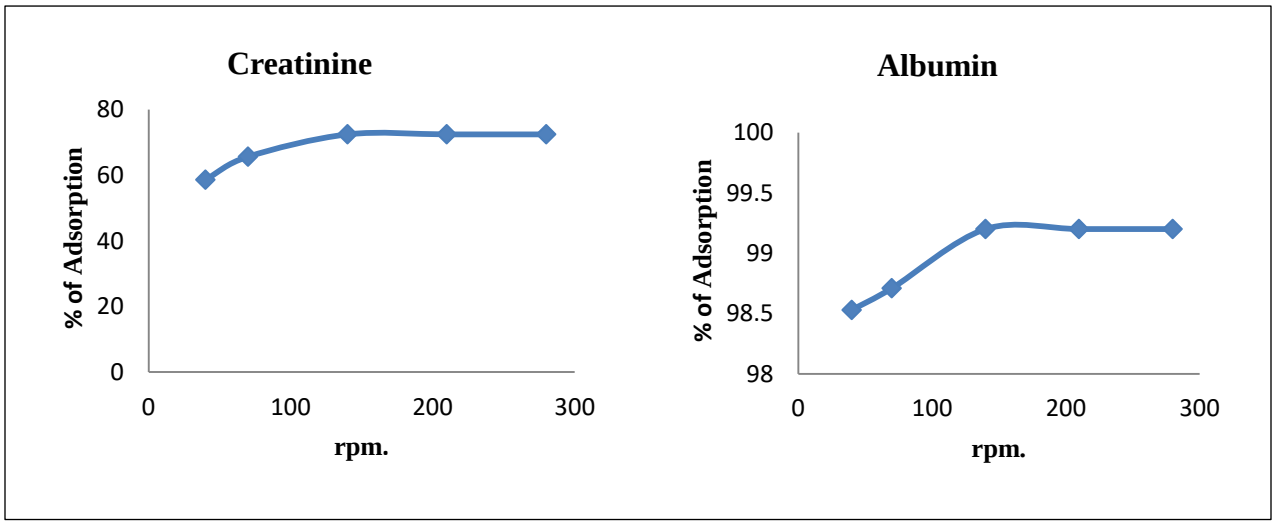


Figure 3-16: Effect of shaking rate on percentage of adsorption of albumin and creatinine on ZnO NPs.

3.8 Comparison study for adsorption ratio of albumin and creatinine on ZnO NPs:

By comparing the results obtained from this study for the different parameter which studied at the same condition for the adsorption on zinc oxide NPs, the results listed in Table (3-2) show that the percentage of adsorption of albumin was higher than the percentage of adsorption of creatinine by 28%. This is due to the high molecular weight of albumin compared to creatinine, as well as the high surface area and large molecular structure being a protein compound all lead to higher adsorption ratio.

Table (3-3): Comparison of adsorption ratio of albumin and creatinine.

	% Adsorption of Albumin	**% Adsorption of Creatinine**
Effect of Contact Time	99.2	65.6
Effect of Adsorbent Dose	99.2	72.4
Effect of pH	99.2	72.4
Effect of temperature	99.2	72.4
Effect of concentration	99.2	72.4
Effect of shaking rate	99.2	72.4
Rate of adsorption ratio	**99.2**	**71.2**

3.9 Adsorption Isotherms

Langmuir, Freundlich and Temkin isotherm constants are shown in Table (3-3). From this table we can see that the results of Albumin are more compatible with the Langmuir equation and that is clearly when drawing the linear equation because (R^2=1) is considered to be the best value compared to the Freundlich and Temkin equation. While Creatinine showed more compatible with the Temkin equation since the value of R^2 is the highest in the Temkin equation.

Table(3-4): Coefficient isotherm parameters for albumin and creatinine adsorption onto ZnO NPs.

Langmuir			
Adsorbate	**a**	**b**	**R^2**
Albumin	714.2	-0.333	1
Creatinine	0.215	-1.756	0.9953
Freundlich			
Adsorbate	**1/n**	**K_f**	**R^2**
Albumin	-0.0074	772.1	0.9443
Creatinine	-0.5083	0.428	0.9902
Temkin			
Adsorbate	**B**	**A_T**	**R^2**
Albumin	-7.6309	2.511×10^{-45}	0.9975
Creatinine	-0.1674	0.0829	0.9959

By applying the linear equation to each isotherm, the constants of Langmuir (a,b) were obtained: (a) represents a constant that is associated with the adsorption capacity the higher its value, the better adsorption capacity. The values of constant (b) are related to the adsorption energy. Freundlich constants(K_f, 1/n): K_f are approximate indicators of adsorption capacity and (1/n) indicates the intensity of adsorption. (A_T,B) are Temkin constants: A_T indicate equilibrium binding constant that corresponding to the maximum binding energy, while (B) is related to the heat of sorption.

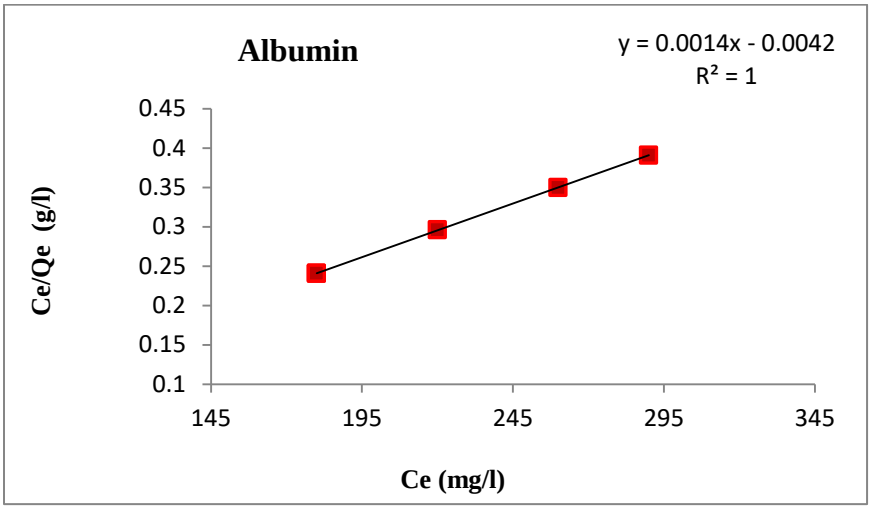


Figure 3-17: Langmuir isotherm for adsorption of Albumin on ZnO NPs.

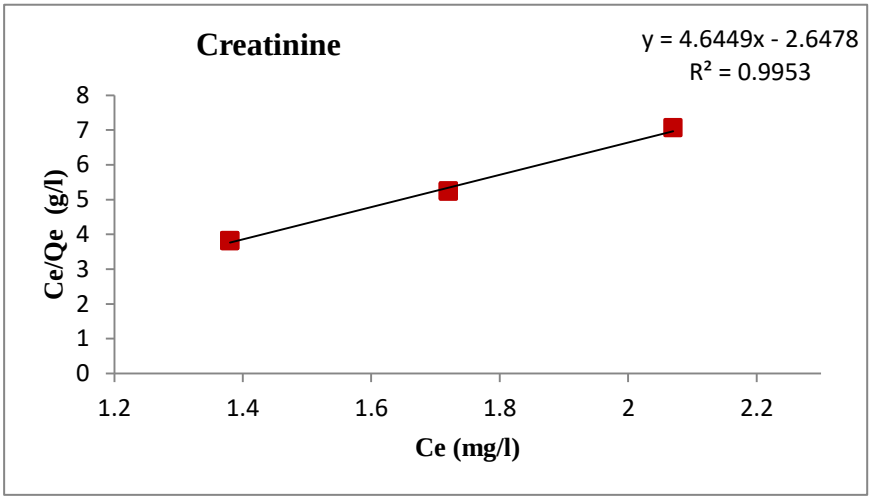


Figure 3-18: Langmuir isotherm for adsorption of Creatinine on ZnO NPs.

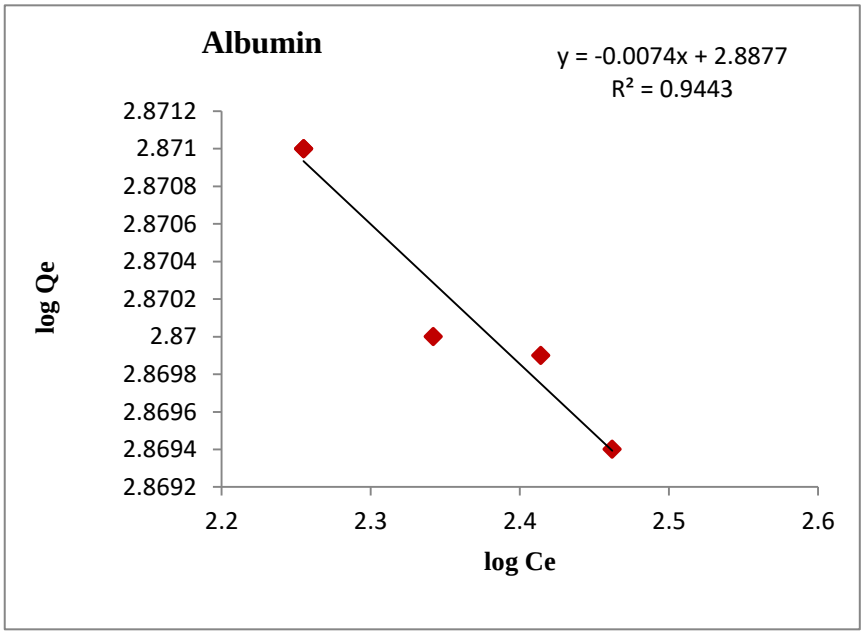


Figure 3-19: Freundlich isotherm for adsorption of Albumin on ZnO NPs.

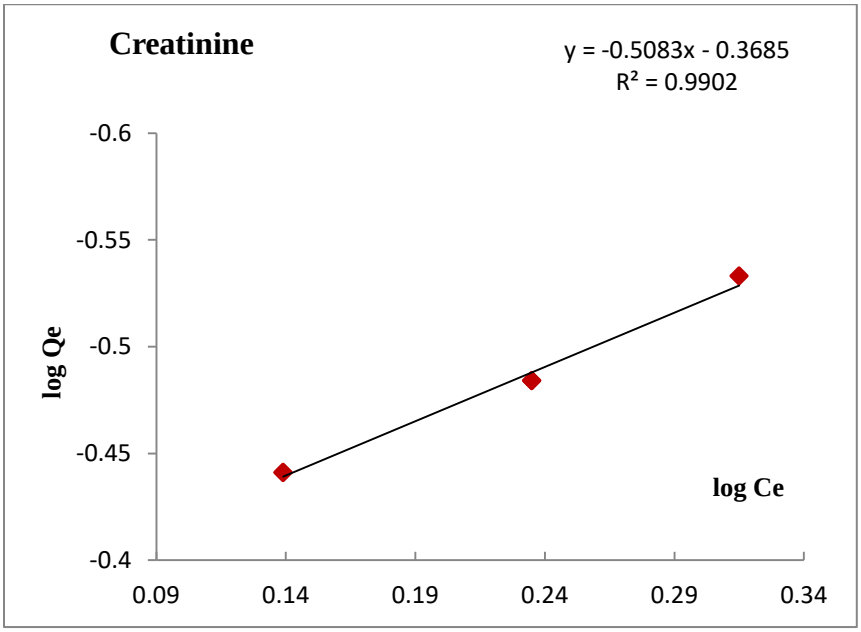


Figure 3-20: Freundlich isotherm for adsorption of Creatinine on ZnO NPs.

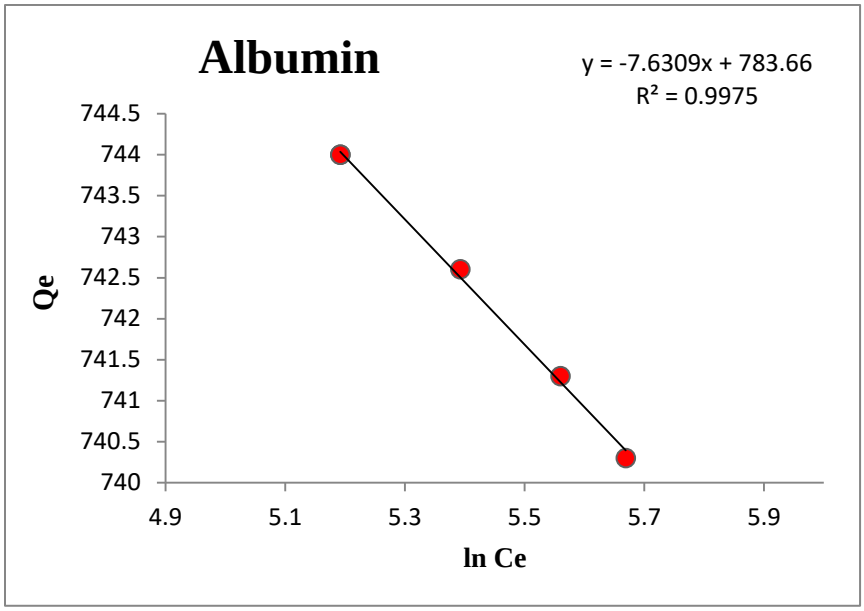


Figure 3-21: Temkin isotherm for adsorption of Albumin on ZnO NPs.

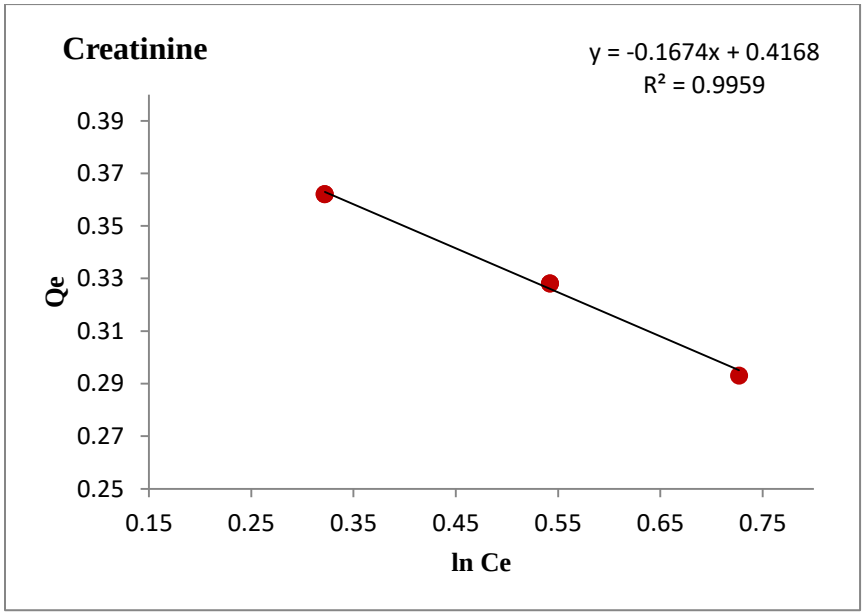


Figure 3-22: Temkin isotherm for adsorption of Creatinine on ZnO NPs.

3.10 Thermodynamic parameters of adsorption on ZnO NPs:

We determined the thermodynamic parameters (free energy change ΔG, enthalpy change ΔH and entropy change ΔS) by studying the effect of temperature. (ΔH) was calculated by drawing Vant - Hoff - Arrhenius equation for adsorption of albumin and creatinine on ZnO NPs, as shown in Figure(3-23) and (3-24).

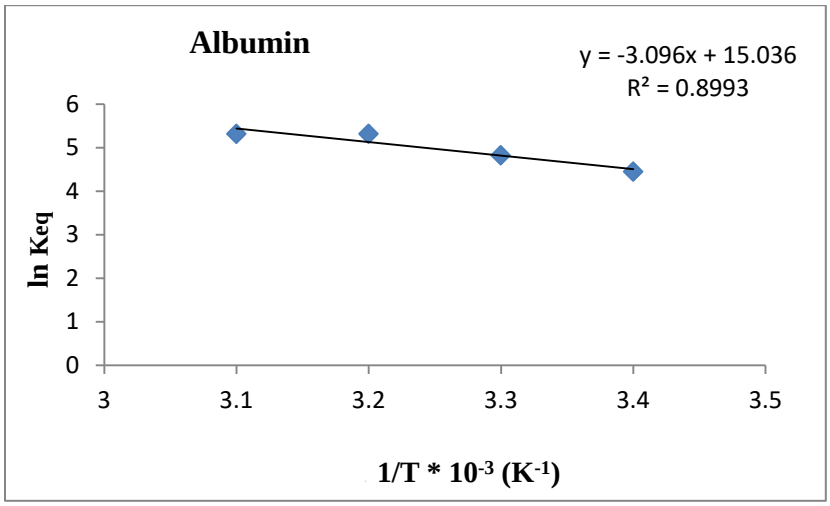


Figure 3-23: The relationship between ln Keq and 1/T for adsorption of Albumin on ZnO NPs.

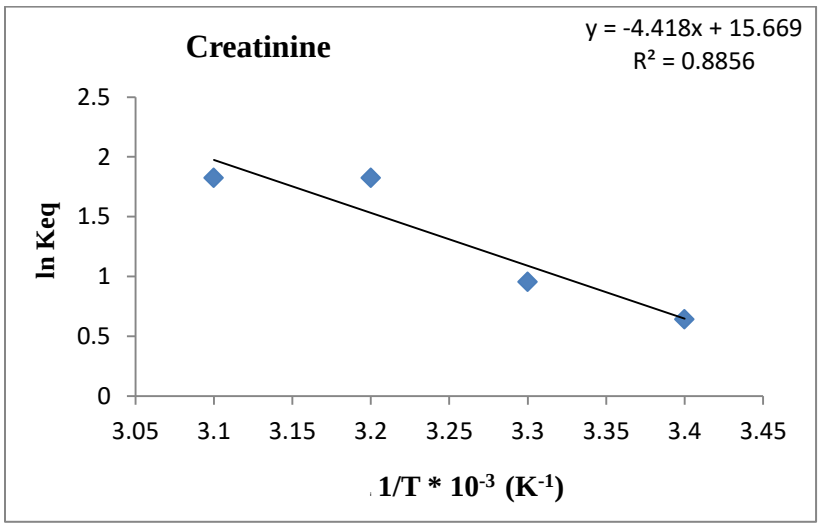


Figure 3-24: The relationship between ln Keq and 1/T for adsorption of Creatinine on ZnO NPs.

The results of thermodynamic parameters are shown in table (3-4), positive values of (ΔH) indicate that the process of adsorption of albumin and creatinine on the surface of zinc oxide NPs is endothermic process and this indicates the increase of adsorption efficiency with increased of temperatures. Negative values of (ΔG) indicate that the process of adsorption on the surface of ZnO NPs is spontaneous. Positive values of (ΔS) indicate that the system is irregular. The values of (ΔH) also show that the adsorption process was physical suggesting that it is multilayered, also the type of bonding forces is Vander Waals force.

Table (3-5): Thermodynamic parameters of adsorption on ZnO NPs.

	T (k°)	**ΔG (KJ/mol.K)**	**ΔH (kJ/mol)**	**ΔS (KJ/mol)**
Albumin	288	-10.65	25.740	0.126
	298	-11.94		0.126
	308	-13.61		0.127
	318	-14.05		0.125
Creatinine	288	-1.53	36.731	0.132
	298	-2.36		0.131
	308	-4.67		0.134
	318	-4.82		0.130

3.11 The activation energy

We calculated the activation energy by studying the effect of temperature and the amount of surface coverage and by drawing the equation (2-9) as shown in Figure (3-25), (3-26) for both albumin and creatinine, the results showed that the activation energy is equal to (0.0148) KJ/mol for albumin and (-0.0116) KJ/mol for creatinine.

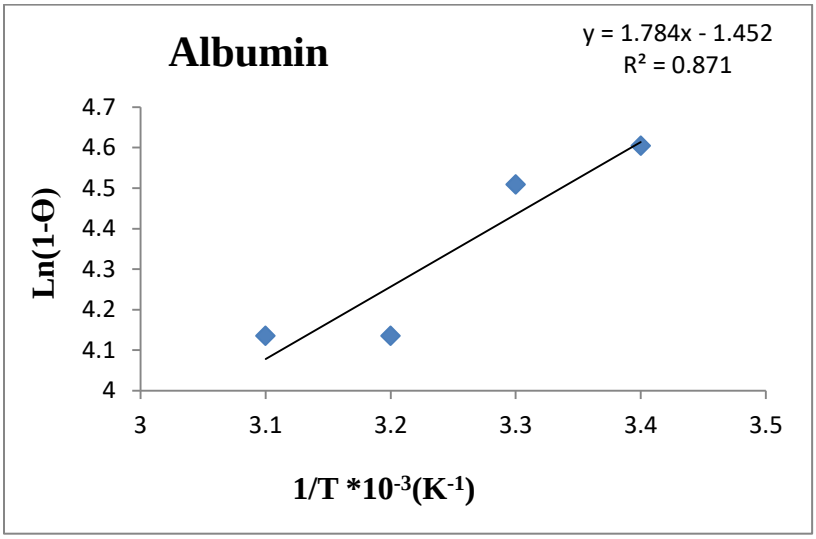


Figure 3-25: The relationship between ln (1-Θ) and 1/T for adsorption of Albumin on ZnO NPs.

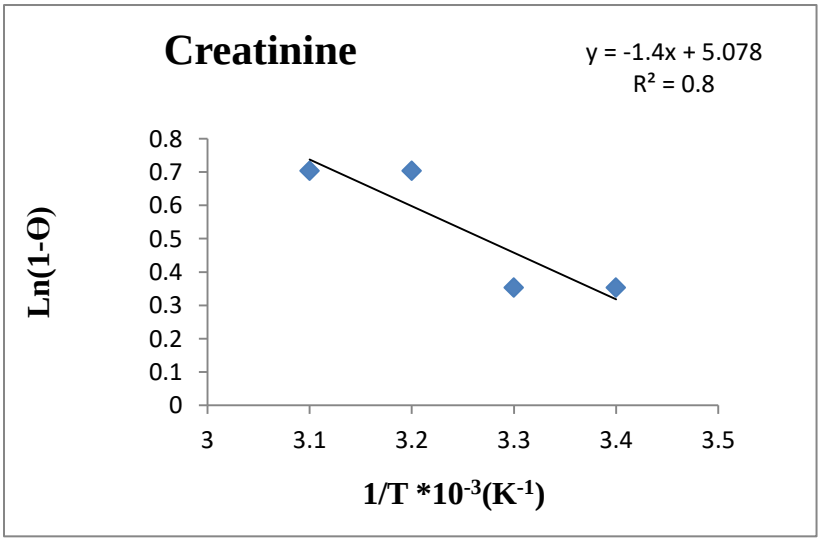


Figure 3-26: The relationship between ln (1-Θ) and 1/T for adsorption of Creatinine on ZnO NPs.

3.12 Conclusion:

Through this work and from the results obtained we find the following:

1. By studying different influences, the optimum conditions for albumin adsorption were at 30min, 2.25 g/dl and 30mg of ZnO NPs while creatinine at 10min, $0.5x10^{-3}$ g/dl and 10mg of ZnO NPs. At 25°C and pH= 7 for both vehicles.

2. The adsorption results revealed that albumin adsorption was higher than creatinine adsorption.

3. It was also found that the adsorption of albumin corresponds to the Langmuir model while creatinine adsorption was consistent with the Temkin model. Also, negative values of 1/n confirmed the incompatibility of the adsorption of both albumin and creatinine with the Freundlich model.

4. Through thermodynamic results it was revealed that the process of adsorption is spontaneous, endothermic and the system is also irregular.

3.13 Recommendation:

- Due to the large use of nanomaterials in medicine, it would be useful to conduct further studies on their interaction with other types of proteins. as well as with the various types of non-protein compounds found in the body.

- Zinc oxide can be manufactured in the same way and prepared for medical use to be used for other purposes such as detoxification and harmful substances in the body or to detain medical drugs and direct them to the required place as an effective adsorption agent.

- It is useful to conduct extensive studies on the methods of preparation of nanoparticles and its use for the removal of pollutants due to the high percentage of pollution and significantly.
- Also can be studied the interaction of proteins with another type of nanoparticle with studying the adsorption kinetics of this reaction.

References

References:

1- Hansda, C., Maiti, P., Singha, T., Pal, M., Hussain, S. A., Paul, S., & Paul, P. K. (**2018**). ***Photophysical study of the interaction between ZnO nanoparticles and globular protein bovine serum albumin in solution and in a layer-by-layer self-assembled film***. Journal of Physics and Chemistry of Solids, 121, 110-120.

2- Saptarshi, S. R., Duschl, A., & Lopata, A. L. (**2013**). ***Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle***. Journal of nanobiotechnology, 11(1), 26.

3- Cedervall, T., Lynch, I., Foy, M., Berggård, T., Donnelly, S. C., Cagney, G., ... & Dawson, K. A. (**2007**). ***Detailed identification of plasma proteins adsorbed on copolymer nanoparticles***. Angewandte Chemie International Edition, 46(30), 5754-5756.

4- Monopoli, M. P., Walczyk, D., Campbell, A., Elia, G., Lynch, I., Baldelli Bombelli, F., & Dawson, K. A. (**2011**). ***Physical− chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles***. Journal of the American Chemical Society, 133(8), 2525-2534.

5- Monopoli, M. P., Åberg, C., Salvati, A., & Dawson, K. A. (2012). ***Biomolecular coronas provide the biological identity of nanosized materials***. Nature nanotechnology, 7(12), 779.

6- Gunalan, S., Sivaraj, R., & Rajendran, V. (**2012**). ***Green synthesized ZnO nanoparticles against bacterial and fungal pathogens***. Progress in Natural Science: Materials International, 22(6), 693-700.

7- John D. (**2008**). ***Oxford Dictionary of Chemistry***,6th ed. Oxford: Oxford University Press.

8- Ozin, G. A. (**1992**). ***Nanochemistry: synthesis in diminishing dimensions***. Advanced Materials, 4(10), 612-649.

9- Abdin, A. R., El Bakery, A. R., & Mohamed, M. A. (**2017**). ***The role of nanotechnology in improving the efficiency of energy use with a special reference to glass treated with nanotechnology in office buildings***. Ain Shams Engineering Journal, 9, 2671–2682.

10- Keçili, R., Büyüktiryaki, S., & Hussain, C. M. (**2018**). ***Advancement In Bioanalytical Science Through Nanotechnology Past, Present And Future***. TrAC Trends in Analytical Chemistry, 110, 259-276.

11- Poole Jr, C. P., & Owens, F. J. (**2003**). ***Introduction to nanotechnology***. John Wiley & Sons.

12- Vinson, J. R., & Sierakowski, R. L. (**2006**). ***The behavior of structures composed of composite materials,*** 2nd ed. Kluwer Academic Publishers.

13- Sanjay, S. S., & Pandey, A. C. (**2017**). ***A brief manifestation of nanotechnology***. Springer, New Delhi. In EMR/ESR/EPR Spectroscopy for Characterization of Nanomaterials, Advanced Structured Materials 62, 47-63.

14- Wolfram, S. (**2002**). ***A new kind of science*** (Vol. 5, p. 130). Champaign, IL: Wolfram media.

15- Klabunde, K. J., & Sergeev, G. B. (**2013**). ***Nanochemistry***, 2nd ed. Newnes.

16- Yadav, T. P., Yadav, R. M., & Singh, D. P. (**2012**). ***Mechanical milling: a top down approach for the synthesis of nanomaterials and nanocomposites***. Nanoscience and Nanotechnology, 2(3), 22-48.

17- Poinern, G. E. J. (**2014**). ***A laboratory course in nanoscience and nanotechnology***. CRC Press.

18- Chen, X., & Mao, S. S. (**2007**). ***Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications***. Chemical reviews, 107(7), 2891-2959.

19- Fierro J. L. G. (**2006**). ***Metal Oxides: Chemistry & Applications***. CRC Press.

20- Özgür, Ü., Alivov, Y. I., Liu, C., Teke, A., Reshchikov, M., Doğan, S., ... & Morkoç, H. (**2005**). ***A comprehensive review of ZnO materials and devices***. Journal of applied physics, 98(4), 11.

21- Wahab, R., Hwang, I. H., Shin, H. S., Kim, Y. S., Musarrat, J., & Siddiqui, M. A. (**2012**). ***Zinc Oxide Nanostructures and their Applications.*** Intelligent Nanomaterials: Processes, Properties, and Applications, 183-212.

22- Kołodziejczak-Radzimska, A., & Jesionowski, T. (**2014**). ***Zinc oxide—from synthesis to application: a review***. Materials, 7(4), 2833-2881.

23- Hahn, Y. B. (**2011**). ***Zinc oxide nanostructures and their applications***. Korean Journal of Chemical Engineering, 28(9), 1797.

24- Banerjee, D., Lao, J. Y., Wang, D. Z., Huang, J. Y., Ren, Z. F., Steeves, D., ... & Sennett, M. (**2003**). ***Large-quantity free-standing ZnO nanowires***. Applied Physics Letters, 83(10), 2061-2063.

25- Frade, T., Jorge, M. M., & Gomes, A. (**2012**). ***One-dimensional ZnO nanostructured films: Effect of oxide nanoparticles***. Materials Letters, 82, 13-15.

26- Li, J. Y., Chen, X. L., Li, H., He, M., & Qiao, Z. Y. (**2001**). ***Fabrication of zinc oxide nanorods.*** Journal of crystal growth, 233(1-2), 5-7.

27- Pan, Z. W., Dai, Z. R., & Wang, Z. L. (**2001**). ***Nanobelts of semiconducting oxides***. Science, 291(5510), 1947-1949.

28- Kong, X. Y., Ding, Y., Yang, R., & Wang, Z. L. (**2004**). ***Single-crystal nanorings formed by epitaxial self-coiling of polar nanobelts***. Science, 303(5662), 1348-1351.

29- Wahab, R., Ansari, S. G., Kim, Y. S., Seo, H. K., & Shin, H. S. (**2007**). ***Room temperature synthesis of needle-shaped ZnO nanorods via sonochemical method.*** Applied Surface Science, 253(18), 7622-7626.

30- Huang, M. H., Mao, S., Feick, H., Yan, H., Wu, Y., Kind, H., ... & Yang, P. (**2001**). ***Room-temperature ultraviolet nanowire nanolasers***. science, 292(5523), 1897-1899.

31- Wu, J. J., Liu, S. C., Wu, C. T., Chen, K. H., & Chen, L. C. (**2002**). ***Heterostructures of ZnO–Zn coaxial nanocables and ZnO nanotubes***. Applied Physics Letters, 81(7), 1312-1314.

32- Chen, W. J., Liu, W. L., Hsieh, S. H., & Tsai, T. K. (**2007**). ***Preparation of nanosized ZnO using α brass***. Applied Surface Science, 253(16), 6749-6753.

33- Liu, J., Huang, X., Duan, J., Ai, H., & Tu, P. (**2005**). ***A low-temperature synthesis of multiwhisker-based zinc oxide micron crystals***. Materials Letters, 59(28), 3710-3714.

34- Huang, Y., He, J., Zhang, Y., Dai, Y., Gu, Y., Wang, S., & Zhou, C. (**2006**). ***Morphology, structures and properties of ZnO nanobelts fabricated by Zn-powder evaporation without catalyst at lower temperature***. Journal of materials science, 41(10), 3057-3062.

35- Wang, Z. L., Kong, X. Y., & Zuo, J. M. (**2003**). ***Induced growth of asymmetric nanocantilever arrays on polar surfaces***. Physical Review Letters, 91(18), 185502.

36- Xu, T., Ji, P., He, M., & Li, J. (**2012**). ***Growth and structure of pure ZnO micro/nanocombs***. Journal of Nanomaterials, 2012.

37- Yang, S., Wang, Y., Wang, L., Zhang, G., Vazinishayan, A., & Duongthipthewa, A. (**2016**). ***Growth and characterization of ultra-long ZnO nanocombs***. AIP Advances, 6(6), 065209.

38- Chiu, W. S., Khiew, P. S., Cloke, M., Isa, D., Tan, T. K., Radiman, S., ... & Chia, C. H. (**2010**). ***Photocatalytic study of two-dimensional ZnO nanopellets in the decomposition of methylene blue.*** Chemical Engineering Journal, 158(2), 345-352.

39- Polshettiwar, V., Baruwati, B., & Varma, R. S. (**2009**). ***Self-assembly of metal oxides into three-dimensional nanostructures: synthesis and application in catalysis***. ACS nano, 3(3), 728-736.

40- Xie, Q., Dai, Z., Liang, J., Xu, L., Yu, W., & Qian, Y. (**2005**). ***Synthesis of ZnO three-dimensional architectures and their optical properties.*** Solid state communications, 136(5), 304-307.

41- Liu, J., Huang, X., Li, Y., Sulieman, K. M., Sun, F., & He, X. (**2006*). Selective growth and properties of zinc oxide nanostructures.*** Scripta materialia, 55(9), 795-798.

42- Suwanboon, S., Amornpitoksuk, P., Haidoux, A., & Tedenac, J. C. (**2008**). ***Structural and optical properties of undoped and aluminium doped zinc oxide nanoparticles via precipitation method at low temperature***. Journal of Alloys and Compounds, 462(1-2), 335-339.

43- Sakai, K., Oyama, S., Noguchi, K., Fukuyama, A., Ikari, T., & Okada, T. (**2008*). Optical properties of nanostructured ZnO crystal synthesized by pulsed-laser ablation***. Physica E: Low-dimensional Systems and Nanostructures, 40(7), 2489-2493.

44- Salavati-Niasari, M., Mir, N., & Davar, F. (**2009**). ***ZnO nanotriangles: Synthesis, characterization and optical properties***. Journal of Alloys and Compounds, 476(1-2), 908-912.

45- Tonto, P., Mekasuwandumrong, O., Phatanasri, S., Pavarajarn, V., & Praserthdam, P. (**2008**). ***Preparation of ZnO nanorod by solvothermal reaction of zinc acetate in various alcohols. Ceramics International***, 34(1), 57-62.

46- Dai, Z. R., Pan, Z. W., & Wang, Z. L. (**2003**). ***Novel nanostructures of functional oxides synthesized by thermal evaporation***. Advanced Functional Materials, 13(1), 9-24.

47- Bahl, A., Bahl, B. S., Tuli, G. D. (**2000**). ***Essentials of physical chemistry***. S. Chand & company ltd.

48- Dąbrowski, A. (**2001**). ***Adsorption—from theory to practice***. Advances in colloid and interface science, 93(1-3), 135-224.

49- Shaw, D. J. (**1980**). ***Introduction to colloid and surface chemistry***, 3rd Ed. Butterworths.

50- جلال محمد صالح. (**1980**). ***كيمياء السطح والعوامل المساعدة***. الطبعة الأولى, جامعة بغداد.

51-Adamson, A. (**1976**). ***Physical chemistry of surfaces***, 3rd Ed. Wiley Inter Science.

52- Hayward, D. O., Trapnell, B. M. (**1964**). ***Chmisorption***, 3rd Ed. Butter Worths.

53- Tompkins, F. C. (**1978**). ***Chmisorption of gases on metals***. Academic press.

54- Madan, R. L., Tuli, G. D. (**2000**). ***Physical Chemistry***. S. Chand & Company, ltd.

55- Mohammad, W. N. (**2009**). ***Thermodynamic and Kinetic Study Of Adsorption (Cr+3, Fe+3, Co+2, Ni+2) Ions From Aqueous Solutions On Titanium Phosphate***, M.Sc. Thesis, Kerbala University,.

56- Murtadha, D. M. (**2009**). ***Thermodynamic Study of the Adsorption of the Ligand (2-[(2- Thiazolyl)azo]-4-benzyl phenol) some complex on nuclei dates coal surface***, M.Sc. Thesis, Kufa University.

57- Oscik, J., Cooper I. L. (**1982**). ***Adsorption***. John Willy and Sons.

58- Langmuir, I. (**1918*). The adsorption of gases on plane surfaces of glass, mica and platinum***. Journal of the American Chemical society, 40(9), 1361-1403.

59- El-Khaiary, M. I. (**2008**). ***Least-squares regression of adsorption equilibrium data: comparing the options***. Journal of Hazardous Materials, 158(1), 73-87.

60- Frenudlich, H. (**1906**). J. Phys. chem.

61- Temkin, M., Pyzhev, V. (**1940**). Acta Physicochim. URSS.

62- Hu, X. J., Wang, J. S., Liu, Y. G., Li, X., Zeng, G. M., Bao, Z. L., ... & Long, F. (**2011**). ***Adsorption of chromium (VI) by ethylene di amine-modified cross-linked magnetic chitosan resin: isotherms, kinetics and thermodynamics***. Journal of hazardous materials, 185(1), 306-314.

63- Champe, P. C. Harvey, R. A. Ferrier, D. R. (**2008**). ***Lippincott's Illustrated reviews: Biochemistry***, 4th Ed, Lippincott Williams & wilkins.

64- Gromiha, M. M. (**2010**). ***Protein bioinformatics: from sequence to function***. Academic Press.

65- Ritter, P. (**1996**). ***Biochemistry: A foundation***. Brooks/Cole publishing company.

66- Nelson, D. L., Cox, M. M., & Cox, M. M. (**2005**). ***Lehninger principles of biochemistry***. W.H. Freeman.

67- Quinlan, G. J., Martin, G. S., & Evans, T. W. (**2005**). ***Albumin: biochemical properties and therapeutic potential***. Hepatology, 41(6), 1211-1219.

68- Farrugia, A. (**2010**). ***Albumin usage in clinical medicine: tradition or therapeutic?***. Transfusion medicine reviews, 24(1), 53-63.

69- Caraceni, P., Tufoni, M., & Bonavita, M. E. (**2013**). ***Clinical use of albumin***. Blood transfusion, 11(Suppl 4), s18–s25.

70- Sano, K., Haneda, K., Tamura, G., & Shirato, K. (**1999**). ***Ovalbumin (OVA) and Mycobacterium tuberculosis bacilli cooperatively polarize anti-OVA T-helper (Th) cells toward a Th1-dominant phenotype and ameliorate murine tracheal eosinophilia***. American journal of respiratory cell and molecular biology, 20(6), 1260-1267.

71- Breathnach, R., Mandel, J. L., & Chambon, P. (**1977**). ***Ovalbumin gene is split in chicken DNA.*** Nature, 270(5635), 314.

72- Nisbet, A. D., SAUNDRY, R. H., Moir, A. J., Fothergill, L. A., & Fothergill, J. E. (**1981**). ***The complete amino-acid sequence of hen ovalbumin. European Journal of Biochemistry***, 115(2), 335-345.

73- Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., & Winiwarter, W. (**2008**). ***How a century of ammonia synthesis changed the world***. Nature Geoscience, 1(10), 636.

74-Tombach, B., Schneider, J., Matzkies, F., Schaefer, R. M., & Chemnitius, G. C. (**2001**). ***Ampero metric creatinine biosensor for hemodialysis patients***. Clinica chimica acta, 312(1-2), 129-134.

75- Shemesh, O., Golbetz, H., KRIss, J. P., & Myers, B. D. (**1985**). ***Limitations of creatinine as a filtration marker in glomerulopathic patients.*** Kidney international, 28(5), 830-838.

76- جابر سالم موسى القحطاني. (**2011**) ***الطب البديل مكمل للطب الحديث***، الطبعة الأولى، مكتبة العبيكان.

77- Parhi, P., Golas, A., Barnthip, N., Noh, H., & Vogler, E. A. (**2009**). ***Volumetric interpretation of protein adsorption: capacity scaling with adsorbate molecular weight and adsorbent surface energy***. Biomaterials, 30(36), 6814-6824.

78- Chen, H., Yuan, L., Song, W., Wu, Z., & Li, D. (**2008**). **Biocompatible polymer materials: role of protein–surface interactions**. progress in polymer science, 33(11), 1059-1087.

79- Koegler, P., Clayton, A., Thissen, H., Santos, G. N. C., & Kingshott, P. (**2012**). ***The influence of nanostructured materials on bio interfacial interactions***. Advanced drug delivery reviews, 64(15), 1820-1839.

80- Lins, L., Thomas, A., & Brasseur, R. (**2003**). ***Analysis of accessible surface of residues in proteins***. Protein science, 12(7), 1406-1417.

81- Lee, E. S., Gao, Z., & Bae, Y. H. (**2008**). ***Recent progress in tumor pH targeting nanotechnology***. Journal of Controlled Release, 132(3), 164-170.

82- Emanuele Scopelliti, P., Bongiorno, G., & Milani, P. (**2011**). ***High throughput tools for the study of protein-nanostructured surface interaction***. Combinatorial chemistry & high throughput screening, 14(3), 206-216.

83- Verma, A., & Stellacci, F. (**2010**). ***Effect of surface properties on nanoparticle–cell interactions***. Small, 6(1), 12-21.

84- Worrall, J. W., Verma, A., Yan, H., & Rotello, V. M. (**2006**). ***"Cleaning" of nanoparticle inhibitors via proteolysis of adsorbed proteins***. Chemical Communications, (22), 2338-2340.

85- Roach, P., Farrar, D., & Perry, C. C. (**2006**). ***Surface tailoring for controlled protein adsorption: effect of topography at the nanometer scale and chemistry***. Journal of the American Chemical Society, 128(12), 3939-3945.

86- Cedervall, T., Lynch, I., Foy, M., Berggård, T., Donnelly, S. C., Cagney, G., ... & Dawson, K. A. (**2007**). ***Detailed identification of plasma proteins adsorbed on copolymer nanoparticles***. Angewandte Chemie International Edition, 46(30), 5754-5756.

87- García-Álvarez, R., Hadjidemetriou, M., Sánchez-Iglesias, A., Liz-Marzán, L. M., & Kostarelos, K. (**2018**). ***In vivo formation of protein corona on gold nanoparticles. The effect of their size and shape***. Nanoscale, 10(3), 1256-1264.

88- Chakraborti, S., Chatterjee, T., Joshi, P., Poddar, A., Bhattacharyya, B., Singh, S. P., ... & Chakrabarti, P. (**2009**). ***Structure and activity of lysozyme on binding to ZnO nanoparticles***. Langmuir, 26(5), 3506-3513.

89- Deng, Z. J., Mortimer, G., Schiller, T., Musumeci, A., Martin, D., & Minchin, R. F. (**2009**). ***Differential plasma protein binding to metal oxide nanoparticles***. Nanotechnology, 20(45), 455101.

90- Bardhan, M., Mandal, G., & Ganguly, T. (**2009**). ***Steady state, time resolved, and circular dichroism spectroscopic studies to reveal the nature of interactions of zinc oxide nanoparticles with transport protein bovine serum albumin and to monitor the possible protein conformational changes.*** Journal of Applied Physics, 106(3), 034701.

91- Chatterjee, T., Chakraborti, S., Joshi, P., Singh, S. P., Gupta, V., & Chakrabarti, P. (**2010**). ***The effect of zinc oxide nanoparticles on the structure of the periplasmic domain of the Vibrio cholerae ToxR protein***. The FEBS journal, 277(20), 4184-4194.

92- Chakraborti, S., Joshi, P., Chakravarty, D., Shanker, V., Ansari, Z. A., Singh, S. P., & Chakrabarti, P. (2012). ***Interaction of polyethyleneimine-functionalized ZnO nanoparticles with bovine serum albumin***. Langmuir, 28(30), 11142-11152.

93- Bhogale, A., Patel, N., Sarpotdar, P., Mariam, J., Dongre, P. M., Miotello, A., & Kothari, D. C. (**2013**). ***Systematic investigation on the interaction of bovine serum albumin with ZnO nanoparticles using fluorescence spectroscopy***. Colloids and Surfaces B: Biointerfaces, 102, 257-264.

94- Simón-Vázquez, R., Lozano-Fernández, T., Peleteiro-Olmedo, M., & González-Fernández, Á. (**2014**). ***Conformational changes in human plasma proteins induced by metal oxide nanoparticles***. Colloids and Surfaces B: Biointerfaces, 113, 198-206.

95- Choi, S. J., & Choy, J. H. (**2014**). ***Biokinetics of zinc oxide nanoparticles: toxicokinetics, biological fates, and protein interaction***. International journal of nanomedicine, 9(Suppl 2), 261-269.

96- Satzer, P., Svec, F., Sekot, G., & Jungbauer, A. (**2016**). ***Protein adsorption onto nanoparticles induces conformational changes: particle size dependency, kinetics, and mechanisms***. Engineering in life sciences, 16(3), 238-246.

97- Sharma, D., Sharma, S., Kaith, B. S., Rajput, J., & Kaur, M. (**2011**). ***Synthesis of ZnO nanoparticles using surfactant free in-air and microwave method.*** Applied Surface Science, 257(22), 9661-9672.

98- Baseri, J. R., Palanisamy, P. N., & Kumar, P. S. (**2012**). ***Adsorption of basic dyes from synthetic textile effluent by activated carbon prepared from Thevetia peruviana***. Indian journal of chemical technology, 19, 311-321.

99- Kleinwechter, H., Janzen, C., Knipping, J., Wiggers, H., & Roth, P. (**2002**). ***Formation and properties of ZnO nano-particles from gas phase synthesis processes***. Journal of materials science, 37(20), 4349-4360.95-

100- Bhunia, A. K., Kamilya, T., & Saha, S. (**2016**). ***Synthesis, characterization of ZnO nanorods and its interaction with albumin protein***. Materials Today: Proceedings, 3(2), 592-597.

101- Singh, N., Mehra, R. M., Kapoor, A., & Soga, T. (**2012**). ***ZnO based quantum dot sensitized solar cell using CdS quantum dots.*** Journal of renewable and sustainable energy, 4(1), 013110.

102- Monshi, A., Foroughi, M. R., & Monshi, M. R. (**2012**). ***Modified Scherrer equation to estimate more accurately nano-crystallite size using XRD***. World journal of nano science and engineering, 2(3), 154-160.

103- Kadhim, Y. H., Ameer, N. A., & Latteef, A. A. (**2017**). ***Synthesis and Characterization of ZnO and Ag Nanoparticles***. Journal of University of Babylon, 25(3), 1010-1017.

104- Indira, K. (**2013**). ***Removal of methylene blue dye from aqueous solutions by neem leaf and orange peel powder***. International Journal of ChemTech Research, 5(2), 572-577.

الخلاصة

موضوع هذه الاطروحة يتعلق بامتزاز الالبومين والكرياتينين على سطح اوكسيد الزنك النانوي.

في البداية حضر السطح وشخص باستخدام; طيف الاشعة تحت الحمراء (FT-IR), مجهر القوة الذرية (AFM) ,المجهر الالكتروني الماسح (SEM), وطيف حيود الاشعة السينية(XRD).

اجريت سلسلة من تجارب الامتزاز تحت ظروف مختلفة : وقت التوازن, كمية المادة المازة, تأثير الدالة الحامضية(pH), درجة الحرارة, تركيز الالبومين و الكرياتينين , وسرعة التحريك.

اظهرت النتائج ان الكرياتينين يتطلب وقت توازن اقصر (2.5 min) بينما الالبومين لديه (30 min) للوصول الى وقت التوازن , ووجد ان نسبة امتزاز الالبومين اعلى من الكرياتينين بنسبة 28%.

بعد تحسين المتغيرات , العلاقة بين تركيز الالبومين والكرياتينين على اوكسيد الزنك النانوي تم تقييمها باستخدام نماذج ايزوثيرمات امتزاز مختلفة مثل لانكماير ,فرندلش وتمكن.

اظهرت قيم معامل الارتباط (R^2) ان معادلة لانكماير وتمكن تبدو مناسبة لبيانات التوازن بشكل افضل لامتزاز الالبومين والكرياتينين مقارنة مع معادلة فرندلش .

الدوال الثرموديناميكية مثل الانثالبيΔH, الانتروبي ΔS وطاقة كبس الحرة (ΔG) تم حسابها ايضا. ووجد ان عملية امتزاز الالبومين والكرياتينين على سطح ZnO النانوي تلقائية, ماصة للحرارة ,زيادة في عشوائية النظام وامتزاز فيزيائي.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية التربية للعلوم الصرفة
قسم الكيمياء

دراسة الامتزاز للتفاعل بين اوكسيد الزنك النانوي مع الالبومين والكرياتينين

رسالة مقدمة الى

مجلس كلية التربية للعلوم الصرفة – جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

تقدمت بها

ميس احمد كاظم

بإشراف

أ.د. حميدة عيدان سلمان

أ.د. هناء عداي علي

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