

Spectrophotometric Determination of Atenolol Using Zinc Oxide Nanoparticles Prepared From Lemon Peel Fruit Extract

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Abstract: Background: A direct and inexpensive spectrophotometric approach was proposed to evaluate atenolol in its pure form and its pharmaceutical form atenolol using zinc oxide nanoparticles made from citrus peel extract. Measurements were taken at Tikrit University's College of Science between October 8, 2023, and January 18, 2024.

Methods: Determining up how much the addition of ATN to ZnONPs solution changes its wavelength is the most important objective of this research.

Results: For the combination of nano solution and medication, the results revealed the greatest absorption value at 282 nm wavelength. The linear relationship in the concentration range of 1-30 $\mu\text{g/mL}$ followed the Beer-Lambert law with an excellent correlation coefficient $R^2 = 0.9964$. The Sandell's significance value is $0.01976 \mu\text{g/cm}^2$, and the molar absorptivity value is $1.3476 \times 10^4 \text{ L/mol.cm}$. A quantitative limit value of $0.7780 \mu\text{g/ml}$ and a detection limit value of $0.2567 \mu\text{g/ml}$ were obtained from the statistical and quantitative validation of the suggested approach.

Conclusion: The movement towards green synthesis methods has gained popularity due to its ease of preparation, affordability, and lack of environmental harm. This method has been successful in creating nanomaterials from green sources. Additionally, it was discovered that the wavelength value of ZnONPs increased from 278 nm to 282 nm when the drug atenolol (ATN) was introduced to the ZnONPs solution. This indicates that the molecules were bound to the drug solution.

Key points: citrus peel extract, green synthesis, ZnONPs, atenolol.

Introduction

With the increasing demand for waste minimization and achieving sustainable development goals through the adoption of the fundamental principles of green chemistry, there is an obvious need for alternative green methods for nanoparticles synthesis(1,2). The adoption of green processes in different technologies has been increasingly widespread and is becoming necessary as a result of global environmental problems associated with harsh conventional chemical and physical processes(3). Plants are the most preferred green and facile route for the synthesis of nanoparticles as they promote largescale production of stable nanoparticles of various shapes and sizes. Using natural plant extracts is an ecofriendly, simple and cost-effective approach(4,5). Green chemistry is considered one of the modern branches concerned with preparing materials that are not harmful to the environment, and therefore it is considered an ideal way to reduce environmental pollution(6). As well as reducing the use of substances harmful to humans and the environment(7). Green nanotechnology is one subfield of green technology that makes use of the ideas of green engineering and green chemistry. By transforming them into nanomaterials with sizes ranging from 1 to 100 nanometers, green nanotechnology products, processes, and applications are anticipated to make a substantial contribution to environmental and climate protection by conserving raw

materials, energy, and water in addition to lowering greenhouse gas emissions, global warming, and hazardous waste.(8). Nanotechnology has contributed to changing the medical rules used in the prevention, diagnosis, and treatment of diseases, as it has provided new methods for nano-drug carriers inside the body that are able to target different body cells(9), and the role of phytochemicals in the overall structure, architecture, and other aspects that include attractive nanoparticles. It is very important because it produces a necessary parity between botany and nanotechnology, and this parity provides development in the field of nanotechnology(10). The main motivation for using plant extracts in the production of nanomaterials is that they are readily available, safe and effective. It does not cause any toxic damage, as in a chemical preparation that leads to the formation of the substance. Toxic chemicals are absorbed on the surface, which has a negative effect when used in the medical field(11), while the main reaction that occurs when using plant extracts is a redox reaction(12). The category of materials whose dimensions range from (1-100) nanometers, and the small size of these materials leads to their properties being different from materials found in their natural size (larger than 100 nanometers)(9) (13). Nanoparticles also contain a greater number of surface atoms than microparticles, which improves their functional capabilities, and this distinction makes them used in many applications. Fields such as medicine, catalysis, sensors, optoelectronics, food industry, textile industry, as well as in agriculture (14,15). Silver nanoparticles are of great interest due to their many unique properties (e.g. size and shape depending on optical, electrical and magnetic properties) that can be incorporated into many biological applications, biosensing materials, composite fibers, superconducting materials, cosmetic products and electronic components. Using several physicochemical methods to synthesize and stabilize silver nanoparticles(16), including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction and radiolysis(17), Nanotechnology is an emerging field of science and technology and it deals with the preparation of nanoparticles with different particle size distribution, shapes and compositions. Nowadays, nanoparticles have been synthesized in several ways, and the biological method is preferred over the physical and chemical method. In addition, metal oxide nanoparticles have important optical, electrical, and mechanical properties that depend on the shape, size, and chemical composition of the nanoparticles. Metal oxide nanoparticles of iron, titanium, silver, copper, zinc, cerium, and bismuth have been previously studied(18). Among all metal oxide nanoparticles, zinc oxide nanoparticles (ZnO NPS) have unique physical and chemical properties and are used in many biological applications especially in antimicrobial, antioxidant, antifungal, anti-inflammatory and anticancer activities. Different physical and chemical properties depending on the morphological shape such as spherical, hexagonal, ribbon-shaped, needle-like, flower-like, wire-shaped, rice-shaped and nanorods etc(19). One of the main important food applications of ZnO nanoparticles is as antimicrobial agents in food packaging materials. In addition, they improve the properties of packaging materials such as barrier qualities, mechanical strength and stability, if ZnO nanoparticles are mixed in various materials, including glass and cellulose, in different ways(20). One of the important chemical properties of (ZnONps) is its ability to continuously change its electrical and surface properties, in addition to its ability to form active oxygen species (ROS) that lead to cell death after the antioxidant activity shown by the cells is overcome(21). Zinc oxide is distinguished from other metals in that it has a wide range of electronic properties. Oxygen reacts with these electrons to form hydroxyl radicals and monovalent superoxide O_2^- . This in turn reacts with hydrogen ions H to form HO_2^- . With the transfer of electrons, monovalent hydrogen peroxide is produced, which reacts with hydrogen ions(22). Nanoparticle zinc oxide is at the forefront of reductive reactions and Ros generation in cellular environments due to its bonding sites and its high reductive potential(23). Nanoparticle zinc oxide is an inorganic compound with molecular formula ZnONPs. It is in the form of a white powder that is almost insoluble in water. It is widely used as an additive in many food materials and products(24). At a time when the industry has flourished and developed at the expense of pollution in the environment at an alarming rate, therefore, materials that are used to restore natural life in the environment must be chosen with high precision, as it has been proven that ZnO nanoparticles possess multiple use properties in different applications, except that they are non-toxic and environmentally friendly. These attractive properties of nanoparticles,

such as the ability to dopants and high chemical sensitivity, created a solution to the problem of deadly gases such as nitrogen oxides and carbon dioxide, and on natural ones and others, when used as gas sensors(25).

Atenolol is 2-{4-[2-hydroxy-3-(propan-2-yl amino) propoxy] phenyl} acetamide and its structural formula:

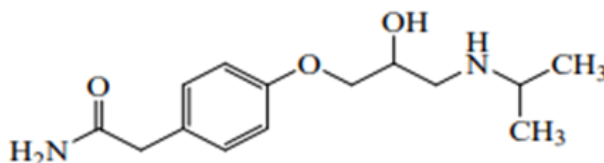


Figure .1. The structural formula of Atenolol

Its molecular formula is $C_{14}H_{22}N_2O_3$ and its molecular weight is 266.431 g/mol (26), It is a White or light yellow powder (27) that melts at 332 °C. It is freely soluble in acetic acid, and slightly soluble in water, ethanol, methanol or acetone (28).

Atenolol is a beta receptor blocker (adrenaline affects several receptors, including beta and alpha), and it mainly affects the heart (reduces its pulse rate), affects the vascular system (lowers blood pressure), and affects to a lesser extent the lungs (impairs their function) (29). ATN is used to treat chest pain (angina) and high blood pressure (hypertension). It is also used to treat high blood pressure, to reduce the severity of heart attacks, control some forms of irregular heartbeat, to manage high blood pressure, and to prevent chest pain. It can be used alone or in combination with other antihypertensive agents including thiazide diuretics, hydralazine, prazosin, and alpha-methyldopa (30,31).

Atenolol is one of the most clinically used beta-agonists and is used to treat angina pectoris due to coronary atherosclerosis and acute myocardial infarction. It is often used as a reference drug in randomized controlled trials for hypertension. However, questions have been raised about atenolol as the best reference drug for comparison with other antihypertensive drugs. Thus, our aim was to perform a systematic review of the effect of atenolol on cardiovascular morbidity and mortality in hypertensive patients (32).

Methods

Chemicals

Atenolol from Samarra Pharmaceutical Industries (SDI), The pharmaceutical preparation (Atenolol-100 mg) produced by Accord Healthcare Limited /UK, Zinc acetate dihydrate, Hydrochloric acid solution and sodium hydroxide solution were provided by BDH Company with a purity of 99.9% , Deionized water was manufactured in the Analytical Chemistry Laboratory at the College of Science, Tikrit University.

Procedure

A solution of ZnONPs was prepared by dissolving 20 gm of lemon peel powder in 200 ml of nonionic water with good stirring in a round volumetric flask, then boiling it at 100 °C for 3 hours, then we left the solution until it cooled to room temperature and filtered the mixture using filter paper, so that The solution is a transparent yellow color, left to cool and then filtered with filter paper. The resulting extract contains oil aggregates that are a very rich source of vitamin C, antioxidants, potassium, and citrus enzymes, which help reduce nano-zinc oxide (ZnONPs).

25 ml of prepared lemon peel extract was taken and added to 50 ml of zinc acetate solution. The mixture was heated and magnetically stirred for 30 minutes, setting the pH = 7.84 using sodium hydroxide solution (NaOH 0.1M). At the end of the magnetic stirring process, the color of the mixture was observed to change. To the white color with the formation of a white precipitate, the mixture was filtered with filter paper and the precipitate collected from the mixture was dried for 8

hours and at 70 C in a drying oven. After that, the precipitate was collected and 0.1g of each of the zinc oxide nanoparticles was taken and placed in a bowl with a little of nonionic water and put it in the water bath equipped with ultrasound for 30 minutes at 60°C, then complete the volume using a 100 ml volumetric bottle to the mark, thus obtaining a solution of zinc oxide nanoparticles (ZnONPs) and to be used in subsequent experiments.

When 1 ml of atenolol ATN solution with a concentration of (100 µg/ml) was added to 2 ml of ZnONPs solution and then 1 ml of the base solution (NaOH 0.1 M) was added, the absorption spectrum of the product was measured (after diluting it with nonionic water in a volumetric vial of 10 ml up to the mark) versus the mock solution (nonionic water), it was found that it gives the greatest absorption at the wavelength (maxλ) 282 nm.

Results

The reaction conditions were adjusted by conducting several spectroscopic measurements to demonstrate the effect of several factors, including the effect of the sodium hydroxide base, the best possible size of the base to be added to the solution, as well as the best size of the nanomaterial solution. These measurements were made using a 10 ml volumetric flask, and the results were as follows:

The effect of the sodium hydroxide base was clear as it led to an increase in absorbance, as shown in Figure (2):

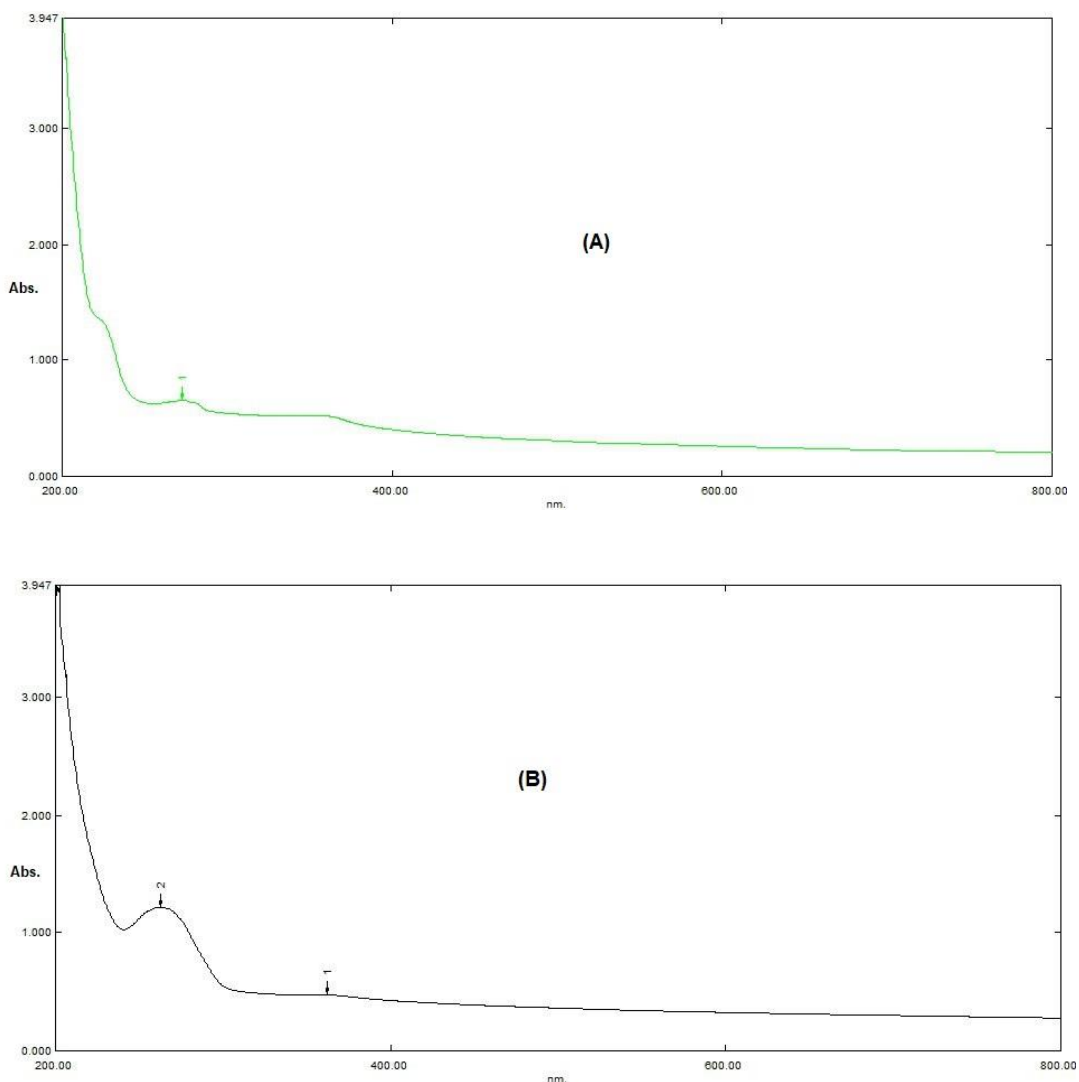


Figure .2. (A , B) ,(A) ZnONPs + ATN ,(B) ZnONPs +ATN+ NaOH .

The best volume the sodium hydroxide base that was added to the mixture of the nano-solution and the drug (mix ratio 1.5:3 mL) is 1.8 ml, as shown in Figure (3):

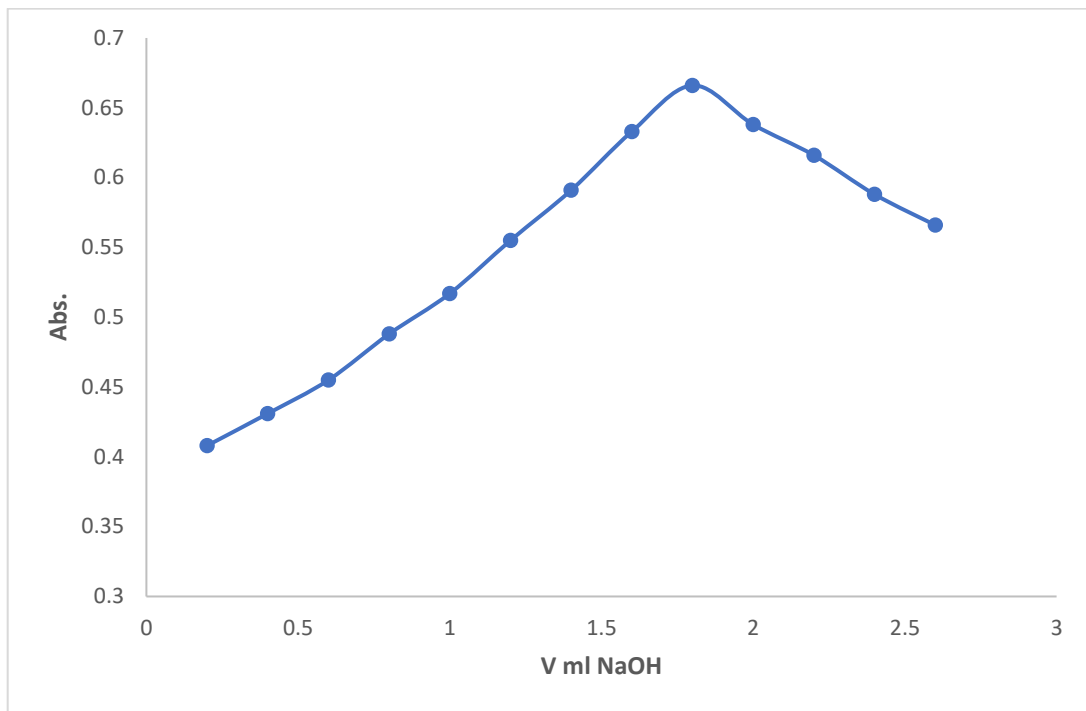


Figure .3. The optimal volume of the sodium hydroxide base (NaOH) .

And the best volume of ZnONPs solution is 2.8 mL, as shown in Figure (4):

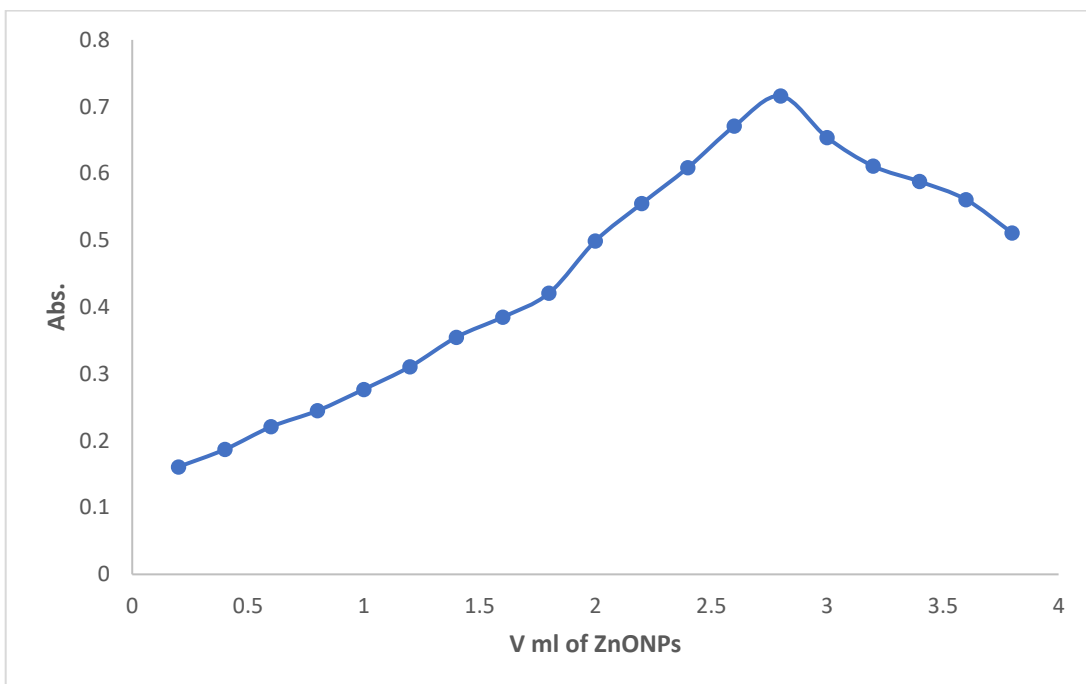


Figure .4. The optimal volume of ZnONPs

Depending on the optimal conditions for the next step experiments, a series of 10 ml vials were prepared with additional concentrations of (1-30 $\mu\text{g/ml}$) of the standard drug atenolol ATN and added to 3 ml of ZnONPs and 1 ml of 0.1 M NaOH solution at no. pH = 11.70. The anionic brand was not allowed to reach a limit in 10 ml volumetric bottles, then the absorbance of the solutions was measured against each photo at a wave weight of 282 nm and the figure (5) represents the regeneration curve that follows the Beer-Lambert law in the range of concentrations (2-30 micrograms) /ml) Classic atenolol began to deviate from the Beer-Lambert law at a concentration of more than (30 $\mu\text{g/ml}$). The molar absorption was calculated and amounted to 1.3476 It was 0.9964 .

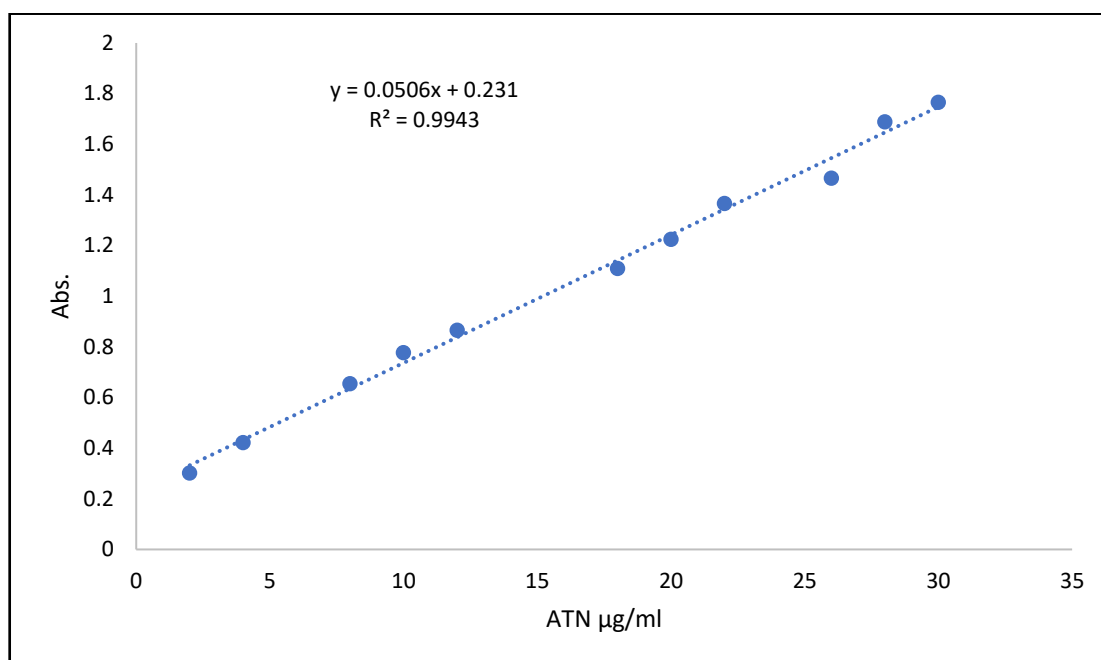


Figure .5. Calibration curve

Accuracy and Precision

Optimal conditions were used in the working method for the purpose of testing the accuracy and compatibility of the method used. Three different concentrations of the atenolol ATN solution were taken within the limits of the Beer-Lambert law in the calibration curve (at a rate of three readings for each concentration) and the recovery rate (Rec%) and the standard deviation were calculated. Relative Score (RSD%) The method was found to have high accuracy and good agreement, as shown in the table, as shown in Table (1):

Table.1. Accuracy and Precision

Taken ATN µg/mL	Found ATN µg/ml	Recovery%	R.SD%	Average of Recovery%
4	3.89	97.49	0.14	98.62
10	10.019	100.19	0.24	
20	19.64	98.20	0.53	

The limit of detection (LOD) and the limit of quantity (LOQ) were calculated based on the following mathematical relationship (33):

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where σ means the relative deviation of the intersection resulting from the straight line equation ($Y=aX+b$), i.e. the value of b , and it is calculated by repeating the calibration curve for five times. S stands for the slope value of the calibration curve and the detection limit value (LOQ) is $0.2567\mu\text{g/ml}$ and the quantitative limit value (LOD) is $0.7780\mu\text{g/ml}$.

The direct method was applied to the pharmaceutical preparation containing atenolol ATN (Atenolol 100 mg), and three different concentrations of solutions of the preparations were taken, namely (4, 6, 10, $\mu\text{g/ml}$), and the solutions were treated with the same steps followed when A calibration curve was prepared and its absorbance was measured at a rate of five readings for each concentration and at 282 nm, and the recoverability and relative standard deviation were calculated, as shown in the table. (2):

Table .2. Direct method to the pharmaceutical preparation

Preparative drug	Taken ATN $\mu\text{g/mL}$	Found ATN $\mu\text{g/ml}$	Recovery %	Av. of Rec. %	RSD%
Atenolol	4	3.87	96.75	98.32	0.65
	6	5.86	97.77		0.43
	10	10.046	100.46		0.29

Content Uniformity Assay of Tablets

The absorbance of the study solutions was used to examine the homogeneity level of the pharmaceutical drug (Atenolol-100 mg) produced by Accord Healthcare Limited /UK. Ten individual tablets were placed separately using a 100 ml volumetric vial and dissolved in deionized water to the mark (1000 $\mu\text{g/ml}$), then the 10 ml from each bottle and dilute it with non-ionic water in 100 ml bottles, so that the final concentration becomes 100 $\mu\text{g/ml}$, then 1 ml was withdrawn from the last solution and measurements were made following the steps followed in the working method under ideal conditions, and the arithmetic average was used to evaluate the level of homogeneity of Calibration curve. The results obtained as an average of recall (Rec \pm SD) were 98.95 \pm 0.85, as show in Figure(6).

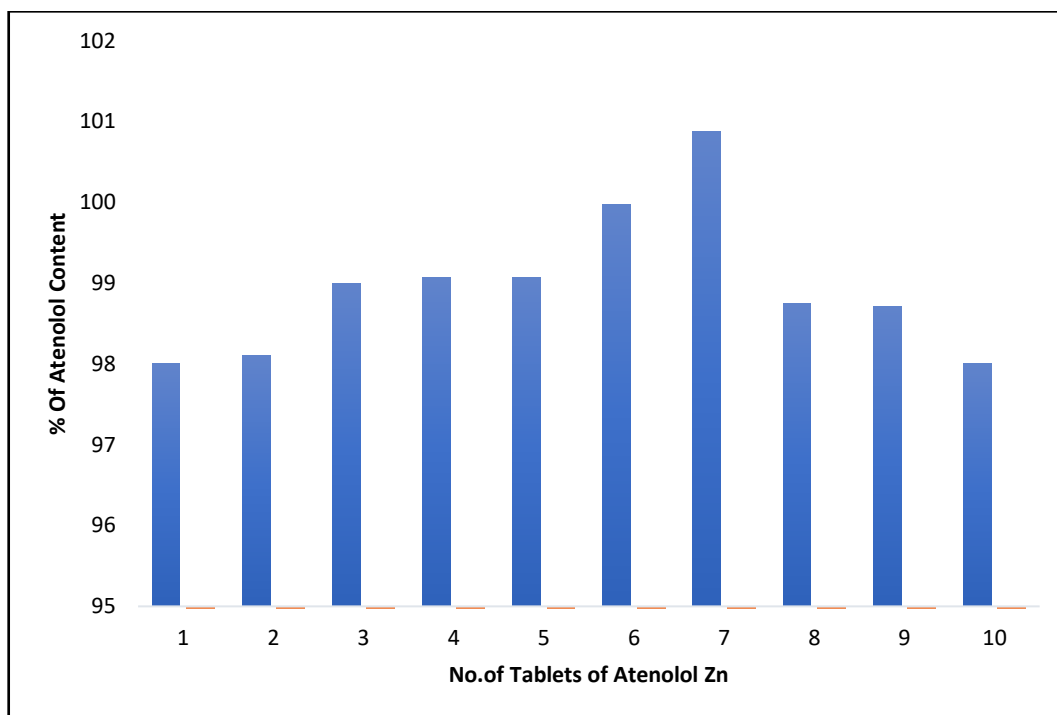


Figure (7): Content Uniformity Assay of Tablets (Novaten 100 mg)

Diagnosis of nanoparticles

UV/VIS spectroscopy is a very special method for nano-X-ray characterization that informs the connection of nanomaterials. The measurement procedures were carried out using a dual-wavelength spectrometer (200-800 nm) and a 1 cm wide quartz cell was used against the ionic aqueous photo solution. The absorption spectrum of ZnONPs depends on the size of the nanostructures, which are formed from the reduction of Zn⁰ ions to Zn⁺² and are stable on the surface of the fuel: lemon peels. The ultraviolet radiation spectra of nanozinc oxides are in the nanoscale ranges of about 262. For this reason, the highest shock absorber for ZnONPs, which were in the form of particles, appeared. Spherical at a wavelength of 262 nm, after operating the surface of ZnONPs with atenolol (ATN), the wavelength was shifted from 262 nm to 266 nm, and this will turn into a heterogeneous surface of ZnO particles as a result of the interaction of ZnONPs with atenolol, as shown in the figure (7).

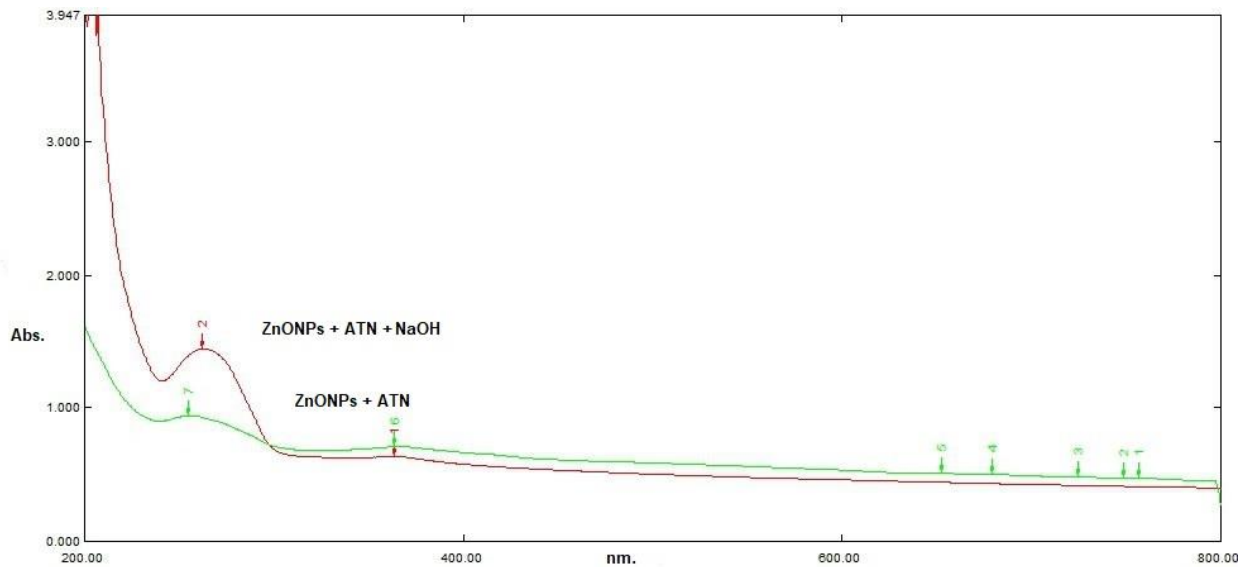


Figure .7. UV-VIS for ZnONPs+ATN & ZnONPs+ATN+NaOH

The XRD technique considers the surfaces of materials as mirrors, and thus the laws of radiation reflection are applied to them (θ incidence θ reflection = angle of incidence = angle of reflection). To understand the intensity of absorption, the concept of constructive and destructive interference is used by applying the interference criteria of Bragg's law. Radiations in the same phase are amplified. And the radiation in the opposite phase is extinguished. Thus, constructive interference results from reflection from regular crystal planes, and this leads to the reflection of X-ray beams in the same phase, which generates high-intensity beams in the diffraction spectrum. The crystal structures of the nanoprecipitate (ZnONPs) prepared using lemon peel extract were confirmed by XRD analysis as shown in Figure (8) XRD results of nanoprecipitate (ZnONPs) using lemon peel extract, which revealed many parameters and structural properties of the materials, such as the size of the particles, the width of the bands at average height, the location of the peaks, and the distances between the crystalline layers, and that D represents the particle size, and the average size of the crystals was (5.6 nm) according to Scherrer equation to calculate the size of crystals, as shown in Table (8).

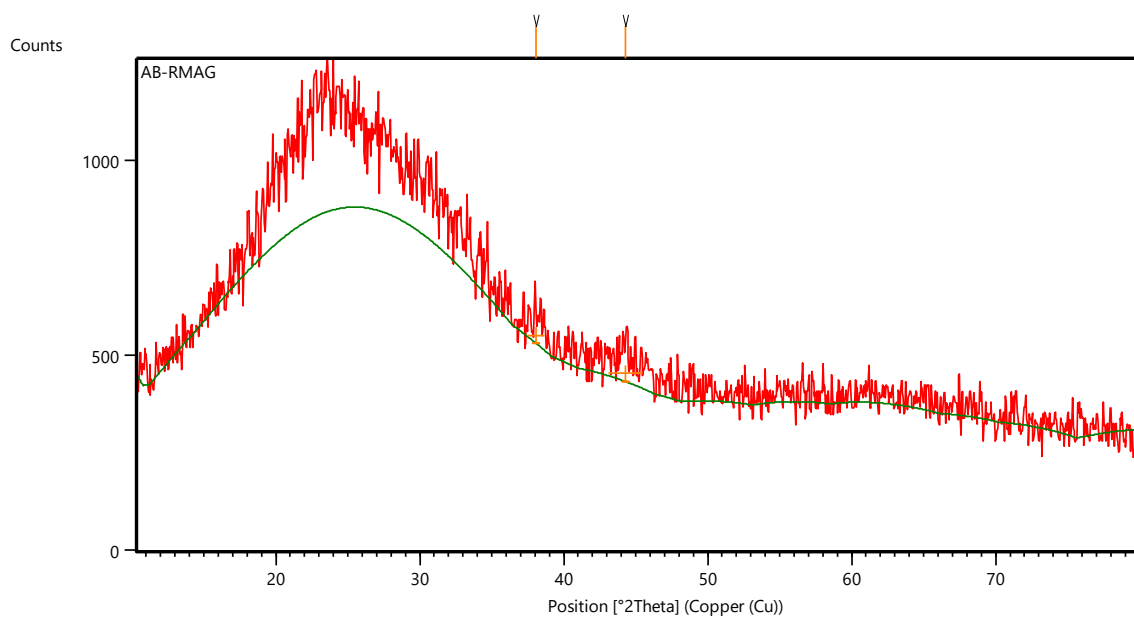


Figure .8. The imaging XRD for ZnONPs

Table .3. XRD analysis data and information obtained

Material	2 θ	FWHM [°2 θ .]	d.spacing [Å°]	Height [cts]	Rel.Int. [%]	D nm	Average
ZnONPs	38.0671	1.1808	2.36396	40.00	99.91	7.435229	5.614525
	5.614525	2.3616	2.04661	40.03	100.00	3.793821	

Discussion

Green chemistry applications are simple to use, inexpensive, and safe for researchers to experiment using because the materials are natural, free of toxins, and are not capable of blowing up or exploding., etc.

In the current study, the researchers chose silver nitrate because silver has distinctive properties. When an aqueous zinc acetate solution was added to the lemon peel extract, the extract was converted into nanoparticles. These particles have better properties than the same material in its normal size because nanoparticles contain more surface atoms and thus the ability to interact increases. The absorption spectrum of ZnONPs depends on the size of nanoparticles formed by the reduction of Zn⁰ to Zn⁺² and settled on the surface of cinnamon extract. The UV spectra of silver nanoparticles have absorption bands around 400 nm.. The absorption spectrum of ZnONPs depends on the size of the nanostructures, which are formed from the reduction of Zn⁰ ions to Zn⁺² and are stable on the surface of the fuel, lemon peels. The ultraviolet radiation spectra of nanozinc oxides are in the nanoscale ranges of about 282. Therefore, they started at the top after absorption of ZnONPs, which were in the form of particles. Spherical at a wavelength of 282 nm, after functionalizing the surface of ZnONPs with atenolol (ATN), the wavelength was shifted from 278 nm to 282 nm, and this turned into an anisotropic ZnO particle surface as a result of the reaction of ZnONPs with atenolol.

On the other hand, atenolol had a positive effect on the synthetic nanoparticles (ZnONPs), as it increased the wavelength of the nanoparticles when mixed with the drug and measured together in a UV spectrometer. Many influencing factors were studied, such as the effect of the acidic environment. It was observed that when 1 ml of HCl was added to the drug-nano mixture, a red shift occurred. On this basis, the best size of hydrochloric acid was studied. It was observed after performing several spectroscopic measurements and using different volumes of NaOH solution that it gives the highest absorption at a volume of 1.2 ml. In the same way, the optimal volume of atenolol solution was chosen, and the result was 2.8 ml. By fixing and adopting these conditions, an Atenolol calibration curve was prepared, and the results obtained were good in terms of linearity and when studying precision and accuracy. In this method, high recall rates and good relative standard deviation values were obtained. The success of the method was also confirmed by using the pharmaceutical preparation Atenolol (Atenolol 100 mg) and calculating accuracy and accuracy, and the results were very good.

Contributions of the Author

Each author took part in both the theoretical and practical portions of the scientific investigation, gave an analytical analysis of the data they collected, and collectively helped to provide the overall findings of the study.

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