Special Conference Edition, April, 2022
http://dx.doi.org/10.4314/bajopas.v13i1.10S

Bayero Journal of Pure and Applied Sciences, 13(1): 53 - 59
ISSN 2006 – 6996

MODIFICATION, CHARACTERIZATION AND EVALUATION OF ANTI-MICROBIAL PROPERTY OF Ag-TiO_2 NANOPARTICLES COATED TRADITIONAL LEATHER

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ABSTRACT
This study aims to modify the traditional leather for footwear products in order to confer antimicrobial property based on coating the leather with Ag-TiO_2 nanoparticles. Silver nanoparticles has been known for its antibacterial activity. Titanium dioxide is widely used in self-cleaning and self-sterilizing of surfaces. The synthesis of TiO_2 and Ag-TiO_2 nanoparticles were carried out by a hydrothermal method, which offers significant advantage in terms of short synthesis time, energy saving and low-cost. Anatase TiO_2 nanoparticles with dimension below 10 nm were obtained as studied by X-ray diffraction. In addition, the cytotoxicity of the nanoparticles were tested and evaluated against Artemia Salina and were found to be non-cytotoxic. The Fourier-transform infrared spectroscopy (FT-IR) showed that the structure of leather did not show any change upon addition of the nanoparticles. The antimicrobial activity was tested against two bacterial isolates (S. aureus and P.aeruginosa) and one fungal Isolates (C. albicans). The result showed that the leather that had been coated with Ag-TiO_2 NPs offers potent antimicrobial property In contrast to the leather coated with only TiO_2 Therefore silver is identified as the main antimicrobial agent. This study suggests that an added value to the traditional leather product was achieved.
Keywords: Traditional leather; TiO_2 and Ag-TiO_2 nanoparticles; antimicrobial property; cytotoxicity.

INTRODUCTION

Leather is a multifunctional material with unique properties: flexibility, mechanical strength, vapor permeability, air permeability and softness (Velmurugan *et al*., 2014). Due to its nature, leather served as a favorable medium for growth and proliferation of microorganisms, and therefore, it often requires treatment with substances to prevent bacterial attack. Metal nanoparticles are used to improve leather properties thereby conferring properties such as: flame retardancy, abrasion resistance, self-cleaning and antimicrobial resistance (Jiang *et al.,* 2015).

Nowadays the increasing demands and specifications from users of traditional leather products. Therefore, finding a desirable finishing agent has sparked strong interest in leather industry. In daily life, the long term contact of clothes with human body may result in rapid growth of bacteria and fungi, thus, this greatly reduces the value of leather products. To solve this problem antimicrobial coatings have been crafted on leather surface (Fernandes *et al*.,2013). Functional properties such as self-cleaning, water resistance, antibacterial properties have been studied via finished technology on leather surfaces (Gowri *et al.,* 2010)

Microbial growth is a common problem in the leather industry, which has been typically controlled through the use of antimicrobial chemicals. However, many of these chemicals, mostly volatile organic compounds (VOCs), have been recently banned worldwide due to their carcinogenic effect and environmental toxicity. As a result, phenolic and other heterocyclic compounds that are often used in the tanning industry as fungicides are susceptible to become unacceptable. Therefore, the development of new compounds with prolonged antifungal effect and no toxicity is of great importance (Kolomaznik *et al*., 2008).

Titanium dioxide nanoparticles (TiO_2-NPs) are already used in various practical applications, such as water and air purification, self-cleaning and self-sterilizing surfaces, and optical and dielectric devices (Sönmezoglu *et al*., 2013). Therefore, the combination of Ag–NPs and TiO_2-NPs will extend the applicability of both

nanoparticles as a single system with enhanced properties. TiO_2 nanoparticles have been prepared by different approaches, e.g. sol gel, hydrothermal and solvothermal methods. Therefore, in this work, a variation of the hydrothermal method for low temperatures has been developed for the synthesis of Ag–TiO_2 NPs.

MATERIALS AND METHODS

Materials

Description and preparation of the leather substrates

Leather materials (labeled 1 and 2) of goat skin origin processed as shoe lining leathers were supplied by the Nakudu Tannery, Sharada, Kano. Both leathers were cut in 4 × 1/4 pieces. Leathers were cut in circular pieces (10 mm diameter × 1.0 mm thick). Afterwards, a cleaning treatment was performed in order to remove all the impurities, which is based on the immersion of the samples in ethanol (70%, AGA), followed by water for 10 minutes. The samples were then left to dry at room temperature and stored in a desiccator before being functionalized with TiO_2 and Ag–TiO_2 nanoparticles (Carvalho *et al*., 2018).

Synthesis of TiO_2 and Ag–TiO_2 NPs

The synthesis of TiO_2 nanoparticles was carried out by a hydrothermal method, which offers significant advantages in terms of time, energy savings and low cost. A 10% (v/v) solution of titanium butoxide was prepared by the addition of 50ml distilled water without any stirring. The solution was placed in a container that can withstand temperatures up to 70°C. This container was placed in an oven for 4 h at 70°C. The mixture was filtered and residue was dried to yield a white powder.

To prepare the Ag-TiO_2 Nanoparticles, the dried TiO_2 nanoparticles (0.8 g) were dispersed in a solution of 0.018 g of $AgNO_3$ and 40 g of distilled water. Subsequently, a solution of 0.08 g of NaOH pellets and 10ml H_2O was added to the above mixture. The reaction was carried out under constant stirring at room temperature. The obtained powder of Ag–TiO_2 was filtered, washed and dried at 50^0C (Carvalho *et al*., 2018).

Coating of the leathers with the TiO_2 and the Ag–TiO_2 nanoparticles

Different pieces of leather were coated with the TiO_2 and the Ag–TiO_2 NPs. Two aqueous dispersions of 4% (w/w) of each nanoparticle were prepared, and the samples were immersed in 100 ml of each solution for a period of 24 h at room temperature. Subsequently, the leather samples were dried in the oven at 50°C. The treated leathers were washed with water to simulate the environmental conditions of operation and to prove that the nanoparticles were successfully incorporated on the leather surface. Finally, the samples were dried at room temperature (Carvalho *et al.,* 2018)

Characterization

Fourier Transform Infrared (FT-IR) Analysis

The infrared spectral analysis were recorded using a Fourier Transform Infrared spectrometer (FT-IR) on Shimadzu 8400s model within the 400-4000 cm^{-1} wavenumber range. A small amount of each sample was introduced into infrared machine and the spectra were viewed and analyzed.

Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) SEM model (FEI Nova Nano SEM 450) at 5.0 kV was used to examine the morphological structure of the samples. Each sample was placed on a double adhesive tape which was on a sample stub, having been sputter coater by a Quorum Technologies quoter with model Q150R, and leading to 5nm of gold coating on each sample. The stubs were taken to the chamber of the SEM machine where they were viewed via NaVCaM utility, with focusing and adjustment carried out as necessary, The brightness and contrasting was automatically adjusted, afterward the morphologies of different magnification were viewed and analyzed (Velmurugan *et al.,* 2014).

X-ray Diffraction

The structural properties and composition of the nanoparticles were analyzed by X-ray diffraction (XRD) using SHIMADZU-XRD 700, X-ray diffractometer, Cu kα radiation (1 = 1.154656 λ) in 2θ range from 10 – 70° and 1 sec time per step. Each sample was finely ground and homogenized. The average bulk composition was then determined. The powdered sample was prepared using the sample preparation block and compressed in the flat sample holder to create a flat, smooth surface that was later mounted on the sample stage in the XRD cabinet.

The intensity of the diffracted X-rays was continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d-spacings appropriate to diffract X-rays at that value of θ. Although each peak consists of two separate reflections (Kα1 and Kα2), at small values of 2θ the peak locations overlap with Kα2 appearing as a hump on the side of Kα1. Greater separation occurs at higher values of θ. Typically these combined peaks are treated as one. The 2λposition of the diffraction peak is typically measured as the center of the peak at 80% peak height (Velmurugan *et al.* 2014).

Antimicrobial property

The antimicrobial property of uncoated and coated leathers with both nanoparticles were tested against two bacteria strains and one fungus. The antibacterial activity were tested against Gram negative, *Pseudomonas aeruginosa* (PAO1) ATCC 15692 and a Gram positive, *Staphylococcus aureus* ATCC 6538 respectively. The result obtained were compared with the activity of Augumentin (30 µg). The fungi strain use was *Candida albicans* SC 5314 and the result obtained was compared with the activity of Ketoconozole (600 µg).

Potato Dextrose Agar and Sabouraud Dextrose Broth (SDB Merck) were used to prepare the culture media for the fungi and bacteria strains, respectively, and incubated at 37°C for 24 hrs. Zone of inhibition (ZOI) were obtained as manually measured in millimeter (mm) to determine the antimicrobial activity of the samples. (Velmurugan *et al*., 2014).

Cytotoxicity Assay (Brine Shrimp Lethality Test)

The cytotoxicity of the TiO_2 and Ag-TiO_2 NPs were evaluated because these nanoparticles can easily penetrate inside the human body.

This test was conducted to determine the level of lethal concentration of the sample, using brine shrimp eggs (*Artemia salina*) in order to ascertain the toxicity level of the leather that have been coated with the nanoparticles.

RESULTS AND DISCUSSION

FT-IR analysis

From the result given in Table 1, the peaks at 3484 cm^{-1} and 3324 cm^{-1} wavenumbers indicate the presence of O-H stretching (alcohol and phenol) functional groups in the uncoated and coated TiO_2 and Ag-TiO_2 nanoparticles, respectively, these indicate the interaction of the functional groups in the compound. 2128 cm^{-1} and 2124 cm^{-1} absorption peaks indicate the presence of nitrates (C=N) functional group in the uncoated and coated TiO_2 nanoparticles, respectively, these indicate the interaction of the functional group in the compound. 1655cm^{-1} and 1633 cm^{-1} bands indicate the presence of amine functional group in the uncoated and coated TiO_2 nanoparticles respectively which result to the interaction of the functional group in the compound. 1328 cm^{-1} and 1048 cm^{-1} bands is due to the presence of ester and ether functional group in the coated and uncoated nanoparticles, respectively, these indicate the interaction of the functional group in the compound. 799 cm^{-1} and 821 cm^{-1} indicated the presence of alkene functional group in the coated and uncoated nanoparticles, respectively, which result to the enhancement of the functional group in the compound.

Preparation of the Stock Solution

The sample (1g) was placed in a sterilized sample vial, Methanol (2ml) was added and the mixture was shaken until the test fractions dissolved in the solvent. 500 µml, 50 µml and 5 µml of the stock solution were each placed into a sample vial using micro pipette and 1000 µg/ml, 100 µg/ml and 10 µg/ml concentrations were prepared respectively. These were allowed to stand for about 24 hours in order for solvent to evaporate (Edwin *et al*; 2007)

Hatching of the Brine Shrimp Eggs

Artemiasalina (leach) eggs were added in a hatching chamber containing seawater. The hatching chamber was kept for 24 -48 hours for the eggs to hatch into shrimp larvae.

Screening of Sample

Three drops of dimethylsulphoxide (DMSO) and seawater (4ml) were added to each of the 1000µg/ml and 100µg/ml, and 10µg/ml concentrations, respectively. Each test was conducted in triplicate.

Using a dropper, 10 larvae of Artimia Selina were introduced into each of the sample vials containing the mixture above. Seawater was added to each sample vial in order to make the volume of its content exactly 5ml. Each vial was then allowed to rest for 24 hours after which the survivals were counted (Meyer *et al*; 1982).

$$\%\ Mortality = \frac{No\ of\ dead\ artemia\ salina}{Initial\ number\ of\ live\ artemia\ salina}\ x\ 100$$

SEM analysis result

Figures 1a, 1b, and 1c illustrate the SEM images of the leather samples before and after coating with the nanoparticles. The uncoated leather sample showed pristine surface (non-agglomerated). In contrast the appearance of bright agglomerates can be observed in figure 1b and c and small bright spots can also be seen which were not observed in the image of the pristine surface. The diffraction peaks in a wide range of 2θ angle are about 25.65, 30.80, 38.95, 44.09 and 45.89 correspond to the crystal planes of (101), (004), (200), (105), and (211) respectively, This could be attributed to the formation and position tetragonal anatase phase of TiO_2 nanoparticles. The main characteristic peak at 2θ is 25.65 which belonged (101) in lattice planes of face-centered cubic (FCC) structure indicating the formation of Ag–TiO_2 NPs.

The crystalline size of and were estimated to about 4.05 nm using Debye-Scherrer formula

$$D = k\lambda / \beta\cos\theta$$

Where, D = Crystalline size of nanoparticles.
λ = Wavelength of X-ray radiation source.
K = Geometric Factor.
β = Angular FWHM (Full-width at half maximum) at the diffraction.

Table: 1: FTIR Wavenumbers of the coated and uncoated leather and their corresponding functional groups

Uncoated leather	Leather Coated with TiO_2	Leather Coated with Ag-TiO_2	Bond	Functional group
3484	3324	3197	O-H	Alcohol, phenol
2128	2124	2117	C=N	Nitrites
1655	1633	1666	N-H	1°Amines
1328	1048	1056	C-O	Alcohol, Carboxyl, Ether, Ester
799	821	905	=C-H	Alkenes

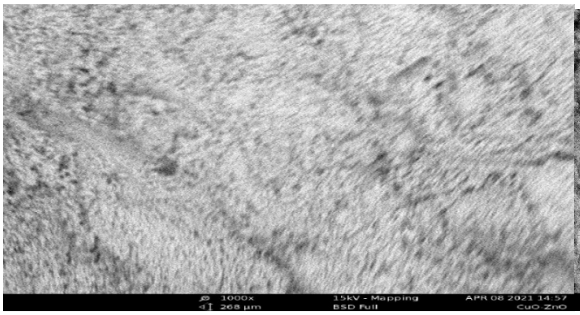

Figure 1a: SEM micrograph of the uncoated leather

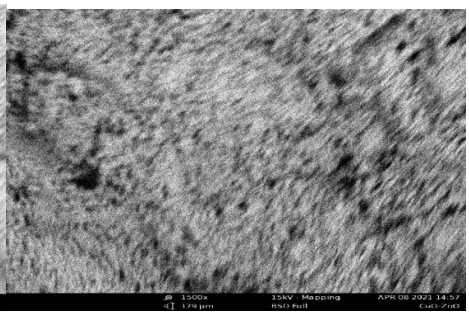

Figure 1b: SEM micrograph of the leather sample Coated with TiO_2

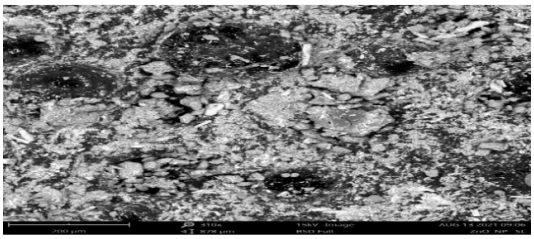

Figure 1c: SEM micrograph of the leather sample Coated with Ag TiO_2.

XRD analysis

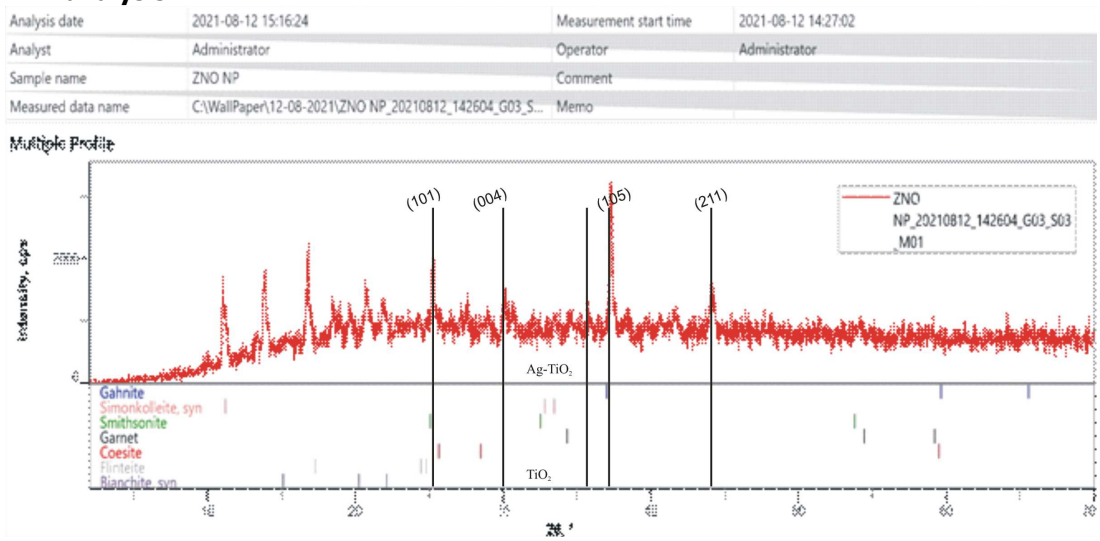

Figure 2: XRD pattern of the TiO_2 and Ag–TiO_2 nanoparticles.
The vertical lines indicate the position of anatase.

Antimicrobial Property

Table 2 shows the antibacterial activity of the uncoated leather, the leather coated with TiO_2 nanoparticles and the leather coated with Ag-TiO_2. From the results *Staphylococcus aureus* shows the lowest zone of inhibition of 173.61mm. The *Pseudomonas aeruginosa* shows the highest zone of inhibition as compared to the rest of the microorganisms in the uncoated leather. In the gram negative bacteria, the bacteria cell wall membrane has 2 to 3 nm thin layer of Peptidoglycan and 30 nm for gram positive respectively, The cell wall membrane is made up of proteins, phospholipids and lipopolysaccharides. Interaction between microorganism and nanoparticles changed the permeability and lead to cells destruction. In addition, it was observed that all the uncoated leather exhibited a little zone of inhibition, this could be due to the chemicals present in the initial treatment of the leathers, which are mostly antifungal. The possible interaction could be either due to (a) electrostatic attraction between negative charge cell wall and positive charge nanocomposite. (b) effect of concentration may affect the cell wall of bacteria.

Interaction takes place between positive ions from composite with negative bacterial cell wall enhances the antibacterial activity. However, the leather coated with Ag-TiO_2 nanoparticles show higher antimicrobial activity against all the organisms. This is because of the antimicrobial properties possess by the nanoparticles of silver and Ag-TiO_2 which enhance the antimicrobial properties of the leather coated with the nanoparticles.

Table 2 Antimicrobial activity of the coated and uncoated leathers

Organism used (ISOLATES)	ZOI of Uncoated Leather (Negative Control)	ZOI of Leather Coated with TiO_2 (mm)	ZOI of Leather Coated with Ag-TiO_2 (mm)	Standard Control (mm) (Positive Control)
Staphylococcus aureus	173.61	182	201	204.08 (Augmentin 30 µg)
Pseudomonas aeruginosa	219.3	159.24	253	277.78(Augmentin 30 µg)
Candida albican	181.82	202.43	211.	359.71 (Ketoconazole 600 µg)

KEY: ZOI Zone of inhibition

Cytotoxicity assay

The cytotoxicity of the fraction extract is based on lethal concentration at 50% (LC_{50} µ g/ml). It has been demonstrated that; early developmental stages of A*rtemia salina* are highly vulnerable to toxins (Meyer *et al.,* 1982).

The cytotoxicity was assessed in terms of LC_{50} (lethality concentration) 10 brine shrimp nauplii were taken into three replicates of each concentration of Ag-TiO_2 nanoparticles.

The LC_{50} value and the percentage of mortality after 24hours for the various test concentrations of Ag-TiO_2 NPs were determine and evaluated.

Table 3 shows the average death and LC_{50} of *Artemia salina* (brine shrimp) at different concentration of the Ag-TiO_2 nanoparticles. In the cytotoxicity test, the *Artemia salina* tested with Ag-TiO_2 NPs showed that the lethality was directly proportional to the concentration of Ag-TiO_2 NPs. Maximum mortality rate was observed at 1000µg/ml concentration which indicate that the Ag-TiO_2 nanoparticles is non-cytotoxic.

TABLE: 3: CYTOTOXICITY ASSAY

Covered with Ag-TiO_2 (µg/ml)	Control	Replica	Dead shrimps after 24 hours	LC_{50}(µg/ml)
VD_{01-01}				
1000	10	3	26	244.308
100	10	3	5	
10	10	3	2	
VD_{01-02}				
1000	10	3	13	245.033
100	10	3	9	
10	10	3	5	
VD_{01-03}				
1000	10	3	12	228.471
100	10	3	8	
10	10	3	5	
VD_{01-04}				
1000	10	3	18	258.037
100	10	3	11	
10	10	3	7	

CONCLUSION

A simple and reliable method of synthesis (hydrothermal method) has been used to prepare TiO_2 and Ag-TiO_2 nanoparticles with anatase phase.

All leather samples coated with the Ag-TiO_2 nanoparticles showed antibacterial and antifungal activity, as compared with samples coated with the TiO_2 nanoparticles alone. Therefore, silver is identified as the main antimicrobial agent. Moreover, a low toxicity of Ag-TiO_2 nanoparticles was demonstrated by cytotoxicity assays. This highlighted the potential of Ag-TiO_2 nanoparticles as an ecological alternative to volatile organic biocides and organic solvents, used nowadays. The study also added value to the local footwear and leather industries reducing the bulk chemical pollution.

ACKNOWLEDGEMENT

Virtually there is no book or any publication that is the work of its author alone, but greatly supported and contributed by others. However, I greatly offer my sincere appreciation to the people whose valuable contributions helped make this academic write-up possible. I particularly owe my very special gratitude and thanks to Professor Umar Muhammad Sani Indabawa, the Ag. Coodinator Nigeria Police Academy, Comrade Tajouddeen Bashir Baba, the ward-head of (Mai Unguwar) Yakasai "A" and Mallam Hashim Isah,for his encouragements and advices. Special thanks and prayers to my parents, for their moral and financial supports, towards my educational career. Last but not the least, my final thank you goes to my ever supportive wife, for her understanding, encouragement and the proof reading of this manuscript.

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APPENDIX

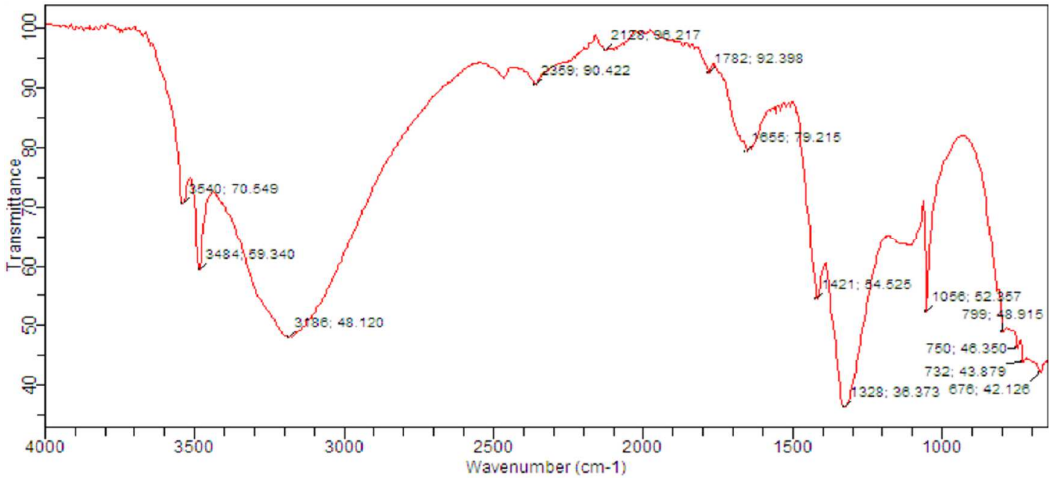

Figure 3a: FTIR Spectrum of the uncoated leather

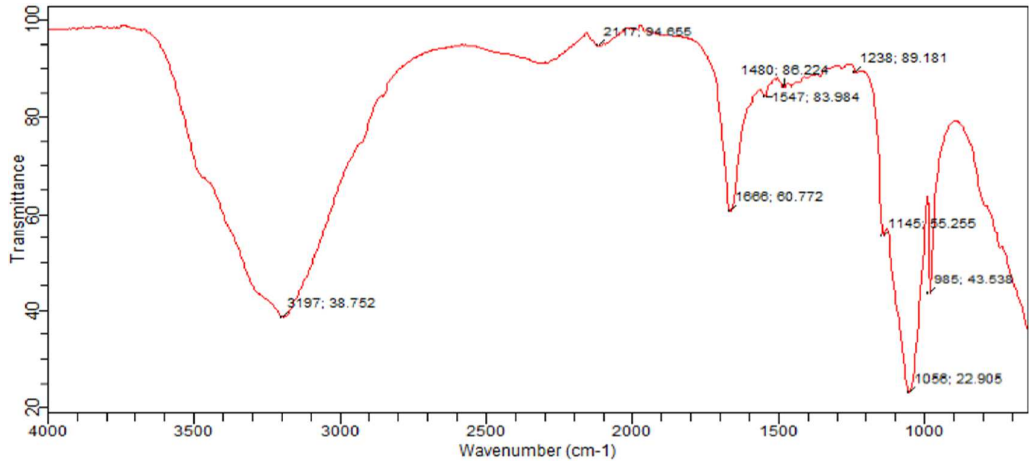

Figure 3b: FTIR spectrum of the Leather Coated with TiO_2

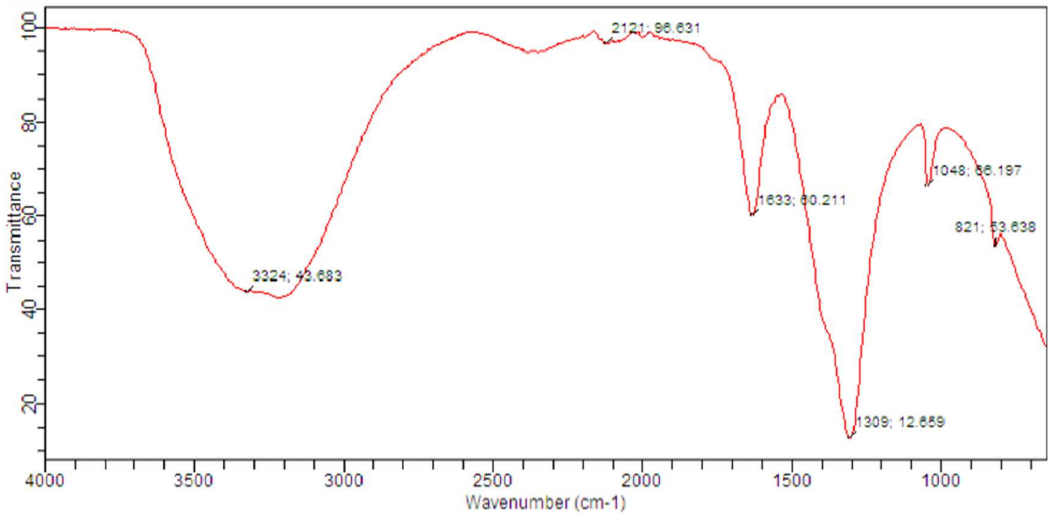

Figure 3c.: FTIR spectrum of the Leather Coated with Ag-TiO_2