Development of Chitosan-Metal Based Nanoparticle Chemosensor for Detection of Tramadol in Urine (Ex Situ)

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Abstract

Drug tests provide chemical analysis of a specific drug in the body system in other to aid management of drug use and can be carried out in human by analyzing biological specimen for the drug or its metabolites. Chitosan Functionalized Methyl green was prepared and used as the starting material in the preparation of a chemosensor based on cerium nanoparticles for the detection of Tramadol in human urine (*es-situ*). The absorption of the chemosensor in urine with Tramadol was observed using the UV-Visible and FT-IR Spectrophotometer. The pH test shows an increase in the alkalinity of the Tramadol in urine sample as the days increases. The kinetics study revealed a linear and perfect graph with an R^2 value above 0.5. The FTIR spectra shows the formation of new functional groups on the chemosensors prepared. An hypsochromic shift was observed on formation of Chitosan functionalized methyl green when compared to methyl green alone due to the interaction of chitosan with the former. The cerium nanoparticles gave a plasmon resonance band at 392.5 nm due to the formation of nanoparticle. The FTIR spectra of methyl green, Chitosan Functionalized methyl green, Cerium chitosan functionalized methyl green nanoparticles and cerium chitosan functionalized methyl greeen nanonparticles containing tramadol in urine all have an aliphatic primary amine NH stretching absorption bands at 3486 cm^{-1}, 3498 cm^{-1}, 3452 cm^{-1}, 3478 cm^{-1} respectively. There was also an alkenyl C=C str in all. However, there was an appearance of a sulfonate at 1117 cm^{-1} in the Cerium chitosan functionalized methyl green nanoparticles. This was also observed at 1155 cm^{-1} in cerium chitosan functionalized methyl green nanoparticles containing tramadol in urine. The chemosensor developed was sensitive to the tramadol drug.

Keywords: tramadol, chitosan, urine, kinetics, drug abuse.

1. Introduction

Drug of abuse, both misused prescription and illicit drugs, have become a serious health issue and global problem during the past decade (Maisto *et al.,* 2015). Some of these drugs are controlled because their abuse could lead to a higher disadvantage and social problem compared to other drugs. These substances could be prescriptions for pain management and other legitimate indications but found to be taken incorrectly sometimes purposefully to get results like getting high and other illicit use. Most controlled drugs are highly addictive and could lead to mental problems.

Periodic drug test is required to monitor the use of drugs based on prescription and not for illegitimate use. A drug test is required to determine the presence of these drugs in the body. The test is meant to provide a chemical analysis of a specific drug (most importantly drugs that have been listed as illegal) in the body system in other to aid in management of drug use. This can be carried out in human by analyzing biological specimen (urine, hair, blood, breath, sweat and saliva) in order to determine the

presence of specified parent drugs or their metabolites. (Moeller *et al.,* 2008). These tests are usually qualitative tests. Since abuse of prescriptions drugs have become a major problem, there is a need for the drug tests in pre-employment screening. Drug testing is often carried out by Employers dealing with positions where public safety is of utmost importance for example the Airline industries, railways and hospitals. Drug screening tests may involve immunoassay techniques and chromatographic techniques. Positive results from an immunoassay test should be followed by confirmatory testing using GC/MS or high-performance liquid chromatography (Standridge *et al.,* 2010). The confirmatory tests are more expensive and time consuming, but are more accurate than immunoassay tests which may give false-positive results which lead to serious medical or social consequences (Moeller *et al.,* 2008). Complaints have been made about the relatively high rates of false positives using this test. (Abadinsky, 2014). Even GC-MS can fail to identify a positive specimen if the column is designed to detect only certain substances (Fenton, 2002).

The concept of a chemical sensor is one in which a material is used as a sensing agent and exhibits a selective interaction with a target species or analyte (Diamond *et al.*, 2008; Bobacka *et al.,* 2003). The specific interaction between the sensor and the analyte produces a signal, which can then be observed via an appropriate detection scheme (Singh *et al.,* 2008, Schmittel *et al.,* 2007). They may also be considered as analytical devices which are used in various fields such as medicine and chemistry. Chemosensors of interest should not only possess high surface area and large ordered pores but must also be entrenched with good stability (Gao *et al.,* 2007). The sensor requires a receptor which is capable of selectively binding to an analyte. It also requires a site with some tunable molecular resolution as well as a transduction mechanism that diverts the recognition into a modification of the tunable property. They monitor ion flux concentrations, cations and anions transportation within cells. They detect analytes in the solution.

Chitosan is a linear polysaccharide composed of glucosamine and N-acetyl glucosamine units linked by β 1-4 D glycosidic bonds. It is a white to light red solid powder, insoluble in water but soluble in organic acids (Pockel *et al.,* 2015). It is considered as the second most naturally occurring polymer after fiber (Zhai *et al.,* 2004). Chitin is a non-starch organic material which has similar structure to cellulose (Chen *et al.,* 2003). The difference in the structures is the replacement of hydroxyl group at the C-2 position of cellulose by an acetamide group in chitin. The reaction of Chitosan is more versatile than cellulose due to the presence of $–NH_2$ groups (Dutta *et al.,* 2004). Chitosan is a linear polyamine and has reactive amine groups (Dutta *et al.,* 2004). The objective of this research is the developing a less expensive and effective material for rapid detection of the controlled drug, tramadol in human urine.

2.0 Materials and Methods

2.1 Materials

Chitosan powder (low molecular weight) was purchased from Sigma Aldrich Company in India. Solvents were obtained from the Chemistry laboratory of the Federal University of Agriculture Abeokuta, Ogun State, Nigeria. The solvents were of analytical grades. Tramadol drug was obtained from a local pharmacy outlet in Abeokuta, Ogun State, Nigeria.

The pH analysis were carried out using Ezodo pH instrument (pH5011). The Absorption rate for the

samples were studied using a T92+ PG UV Visible spectrophotometer at Chemistry Laboratory, Federal University of Agriculture, Abeokuta. The FTIR analysis of the samples were read on a Shinadzu FT-IR Spectrophotometer at the Central Research Laboratory, University of Ibadan, Ibadan.

Other equipment and apparatus used include the Analytical weighing balance, Magnetic Stirrer, Filter paper, Conical flask, beaker, Measuring cylinder, Petri dish, spatula and glass rod.

2.2 Synthesis of Chitosan Functionalized Methyl Green (CFMG)

Chitosan was weighed (1.0 g) and dissolved in 200 ml of ethanol containing 1 % glacial acetic acid solution. It was stirred continuously for 30 minutes using a Magnetic stirrer at a temperature of 60 ^{0}C to form an homogenous solution. An ethanol solution of 50 ml was utilized to dissolve 1 g of Methyl green after which 50 ml of water was then added to the mixture. In a ratio of 1:1, 100 ml of the functionalizing agent (MG) was added to 100 ml of the chitosan solution. The mixture was placed on the magnetic stirrer and stirred for 50 minutes at room temperature (Ejeromedoghene *et al*. 2018).

2.3 Synthesis of the Chitosan metal based nanoparticles chemosensor

Solutions of the metal salt (Cerium salt, Nickel salt, Zinc Salt, Cobalt salt, $FeSO_4.7H_2O$ and $CuSO_4.5H_2O$) (0.01 M) were prepared by dissolving the known amount of the metal salt in 50 mL distilled water (Scheme 1). In a ratio of 1: 1, the Chitosan functionalized methyl green was added to the metal salt solution and stirred at 80 ^{o}C for 15 minutes to form the chitosan-metal complex. M^{n+} ions were then further reduced to M^o by adding 10 mL of 1 M $NaBH_4$ slowly into the mixture with vigorous stirring for about 45 minutes at 80 ^{o}C (Ejeromedoghene *et al*., 2018). The residue was then collected by filtration.

2.4 Collection of Urine Sample

Urine samples were collected for five consecutive days. The first urine excreted from the body at day break was collected in a transparent container. pH of the urine sample was taken for each day. Urine samples of previous days were tested again to note change in result.

To 100 ml of the urine sample, 50 mg Tramadol was added while another 100 ml of urine was left as the blank. 5 ml of the urine sample was placed in a sample bottle and then 1 ml of chemosensor was added to check for colour change. This same process was carried out for other urine with drug samples. The urine samples were tested using the Ultra-violet spectrometer and also the Infra-red spectrometer. The peaks and functional groups present were noted.

The pH of the urine sample was checked and likewise the pH of the sample of urine with drug. This was done for 5 consecutive days. The change in the pH for each day was also noted.

3.0 Results and Discussion

3.1 Naked Eye Test

The visual observation of the metal nanoparticles on the analyte was compared as shown in the Table 1. The original colour of the Chitosan-Cerium nanoparticles chemosensor was blue and still retained its colour upon reacting with urine and Tramadol in urine sample. The Chitosan-nickel nanoparticles chemosensor its original colour as green while it gave a light shade of green upon

reaction with Urine and Tramadol in urine sample (Fig 1).

The Chitosan-copper nanoparticles chemosensor gave a blue colour while on reaction with urine and Tramadol in urine, they gave a light blue colour. The Chitosan-zinc Nanoparticle chemosensor which was originally yellow retained its yellow colour upon interaction with the chemosensor. The chitosan-iron nanoparticles chemosensor was initially blue but gave a light blue colour upon reaction with urine and the Tramadol in urine sample. Lastly for the Chitosan-cobalt nanoparticles chemosensor, the blue colour was retained in the urine and in the Tramadol in urine sample (Fig 2).

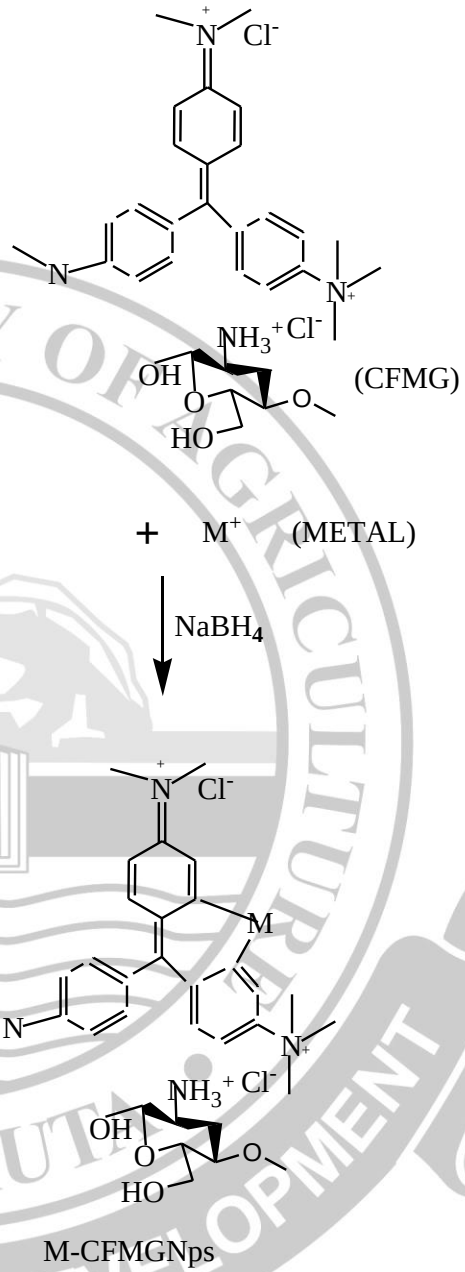
3.2 pH CHANGE

The change in the pH of the analyte: urine alone and Tramadol in urine were recorded with respect to time (5 days' interval) in Table 2.

The pH of the urine was between 5.9-6.0. Upon addition of drug, the pH remains the same but after a couple of hours, there was a rise in the pH making it more alkaline. However, as the number of days' increases, the pH of the urine sample becomes more basic. The pH of urine sample with drug is less basic in comparison to the pH of urine sample without drug.

3.3 Absorption Kinetics

The absorption kinetics of the urine with Tramadol sample and its interaction with the Cerium based nanoparticles Chemosensor was monitored using a UV-Vis Spectrometer (Table 3). The result was also represented graphically in Fig 3.



Scheme 1: Preparation of the Chitosan Metal Based Nanoparticles Chemosensor (M-CFMGNps)

The absorbance of the urine and drug sample can be said to have a minimal increase after which it becomes constant as the time is increased. However the absorption of the chemosensor with the urine with drug sample is slightly constant with respect to time. Based on the graphical representation of the data above, The R^2 indicates

that the graph is linear and perfect since its value for both Tramadol in urine and Tramadol in urine with chemosensor is above 0.5.

3.4 FTIR Spectrophotometeric Result

The FTIR spectrum of Methyl green, Chitosan functionalized methyl green, Chitosan-cerium nanoparticles chemosensor and Chitosan-cerium nanoparticles chemosensor with urine and drug sample were compared as shown in Fig 4.

Methyl Green, Chitosan functionalized methyl green, Chitosan-cerium nanoparticles chemosensor and Chitosan-cerium nanoparticles chemosensor containing urine with Tramadol all have an aliphatic primary amine NH stretching absorption bands at 3486 cm^{-1}, 3498 cm^{-1},3452 cm^{-1}, 3478 cm^{-1} respectively. A conjugated c=c str occurs in all at 1641 cm^{-1},1660 cm^{-1}, 1662 cm^{-1} and 1648 cm^{-1} respectively. However, there is an appearance of a sulfonate at 1117cm^{-1} in Chitosan-cerium nanoparticles chemosensor which was absent in methyl green and Chitosan functionalized methyl green. The sulfonate was also observed at a peak of 1166cm^{-1}in Chitosan-cerium nanoparticles chemosensor containing urine with tramadol drug. The sulfonate is due to the cerium (iv) sulphate salt used in the preparation of the nanoparticles.

The FTIR result of Tramadol, Tramadol in urine, Chitosan-cerium nanoparticles chemosensor and Tramadol in urine with Chitosan-cerium nanoparticles chemosensor were compared in Fig 5. From Fig 5, there is a presence of an amine (NH) stretch in Tramadol, Tramdol in urine and the Tramadol in urine with Chitosan-cerium nanoparticles chemosensor at 3380 cm^{-1}, 3426 cm^{-1} and 3394 cm^{-1} respectively. Methoxy group was found in tramadol at 2864 cm^{-1} which is absent in the other compounds. Isothiocyanate which is absent in tramadol was observed in the other three compounds at 2091 cm^{-1}, 2068 cm^{-1} and 2089 cm^{-1} respectively. However, the Tramadol aid the reduction of the absorption band for the Isothiocyanate in urine at 2091 $cm^{-1}$to 2068 cm^{-1} and this increased to 2089 cm^{-1} on addition of the Chitosan-cerium nanoparticles chemosensor There is a presence of an amide band at 1642 cm^{-1} for urine while a conjugated C=C stretch was found in Tramadol, Tramadol in urine, Tramadol in urine with Chitosan-cerium nanoparticles chemosensor at 1610 cm^{-1}, 1642 cm^{-1} and 1620 cm^{-1}. A carbonate ion was observed at 1455 cm^{-1},1469 cm^{-1} and 1455 cm^{-1} for the three compounds (Urine, Tramadol and Urine with drug) which is absent in Tramadol in urine Chitosan-cerium nanoparticles chemosensor. Hence, the cerium metal is said to have an effect that led to the disappearance of the carbonate ion. Aliphatic fluoro compound, chloro compound and bromo compound was found in Tramadol at 1058 cm^{-1}, 777 cm^{-1} and 714 cm^{-1} which is absent on addition of the urine, drug and Chitosan-cerium nanoparticles chemosensor.

Table 1: Naked Eye Test

M-CFMG-N	COLOUR	M-CFMG-N + URINE	M-CFMG-N +URINE + DRUG
Ce Nano chemosensor	Blue	Blue	Blue
Ni Nano chemosensor	Green	light green	light green
Cu Nano chemosensor	Blue	Light Blue	Light blue
Zn Nano chemosensor	Yellow	Yellow	Yellow
Fe Nano chemosensor	Yellow	Yellow	Yellow
Co Nano chemosensor	Blue	Faint blue	Faint blue

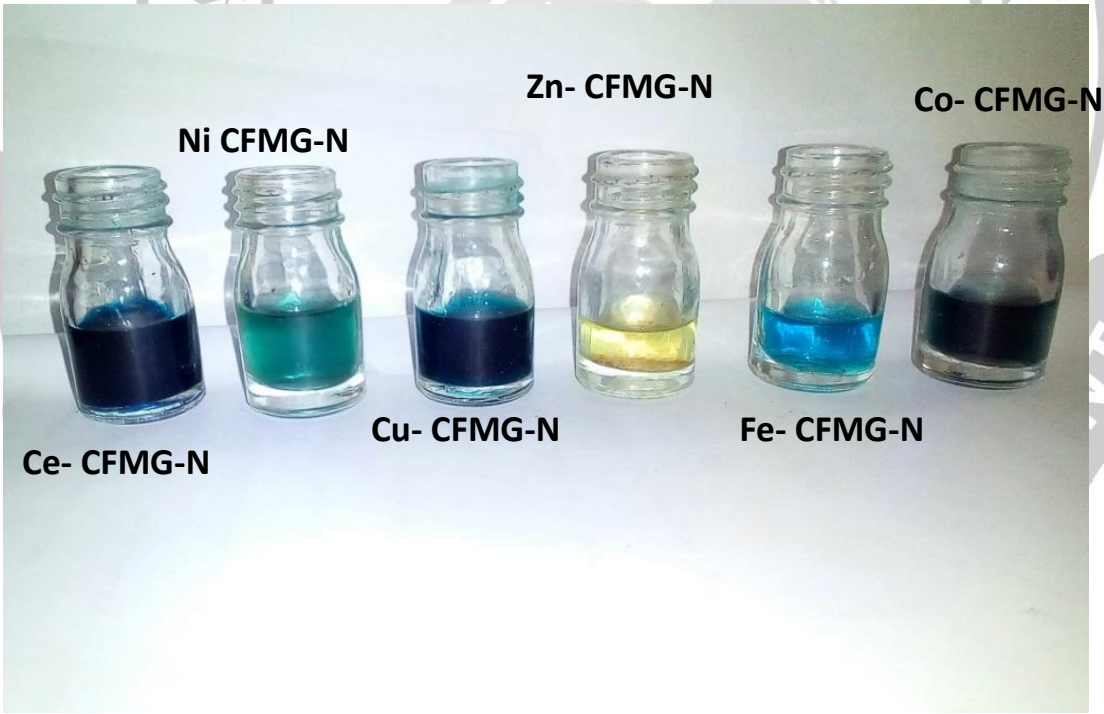


Fig 1 : Prepared Chitosan Metal-Based Nanoparticle Chemosensors

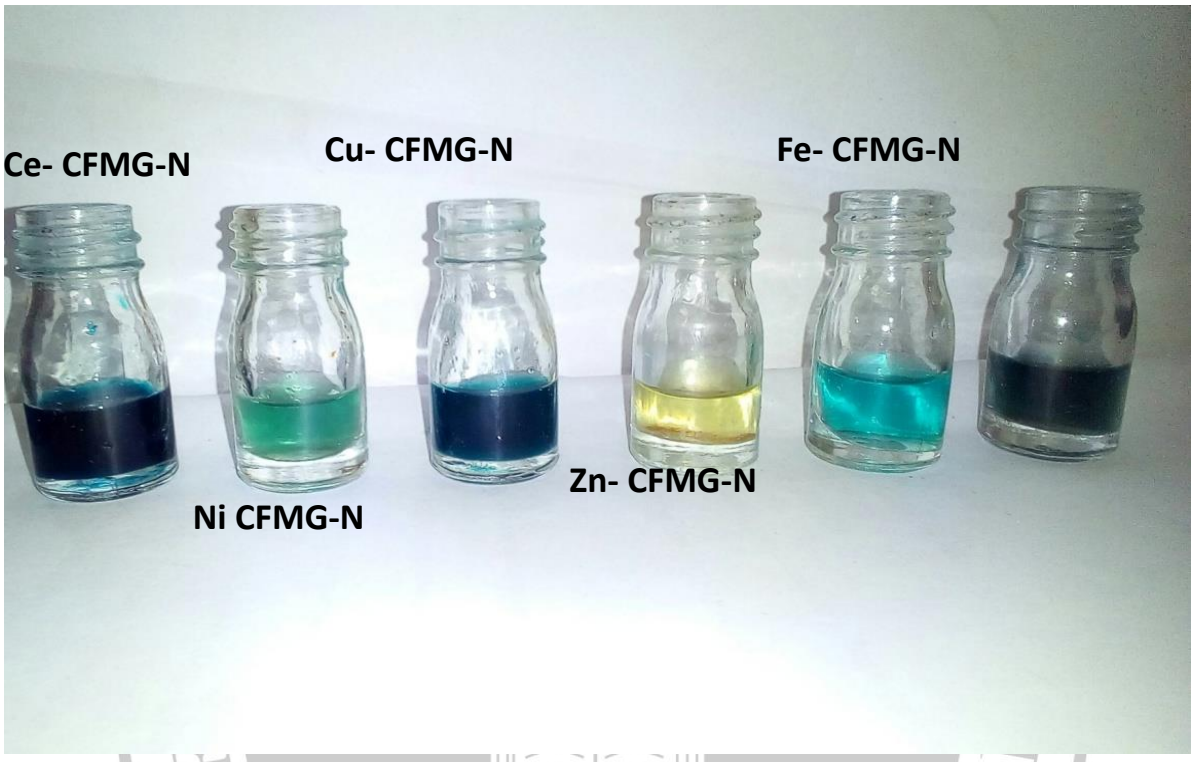


Fig 2: Reaction of the Chitosan Metal Based Nanoparticles with Tramadol in Urine Sample.

Table 2: pH change in urine sample and tramadol in urine samples for 5 days

	SMP 1 (WOD)	SMP2 (WD)	SMP3 (WOD)	SMP4 (WD)	SMP5 (WOD)	SMP6 (WD)	SMP7 (WOD)	SMP8 (WD)	SMP9 (WOD)	SMP10 (WD)
DAY1	5.9	5.9								
DAY2	8.8	8.4	6.0	6.0						
DAY3	9.1	9.2	9.0	8.9	5.9	5.9				
DAY4	9.1	9.1	9.0	8.9	8.4	8.3	5.9	5.9		
DAY5	9.0	9.0	9.0	9.0	9.0	9.0	8.8	8.2	5.9	5.9

Table 3: Tramadol absorption in urine and in chemosensor

TIME	ABSORPTION URINE AND DRUG	URINE, DRUG AND CHEMOSENSOR
0.00 HR	**0.385**	**1.659**
1.00 HR	**0.390**	**1.659**
2.00 HR	**0.389**	**1.670**
3.00 HR	**0.409**	**1.670**
5.00 HR	**0.409**	**1.670**

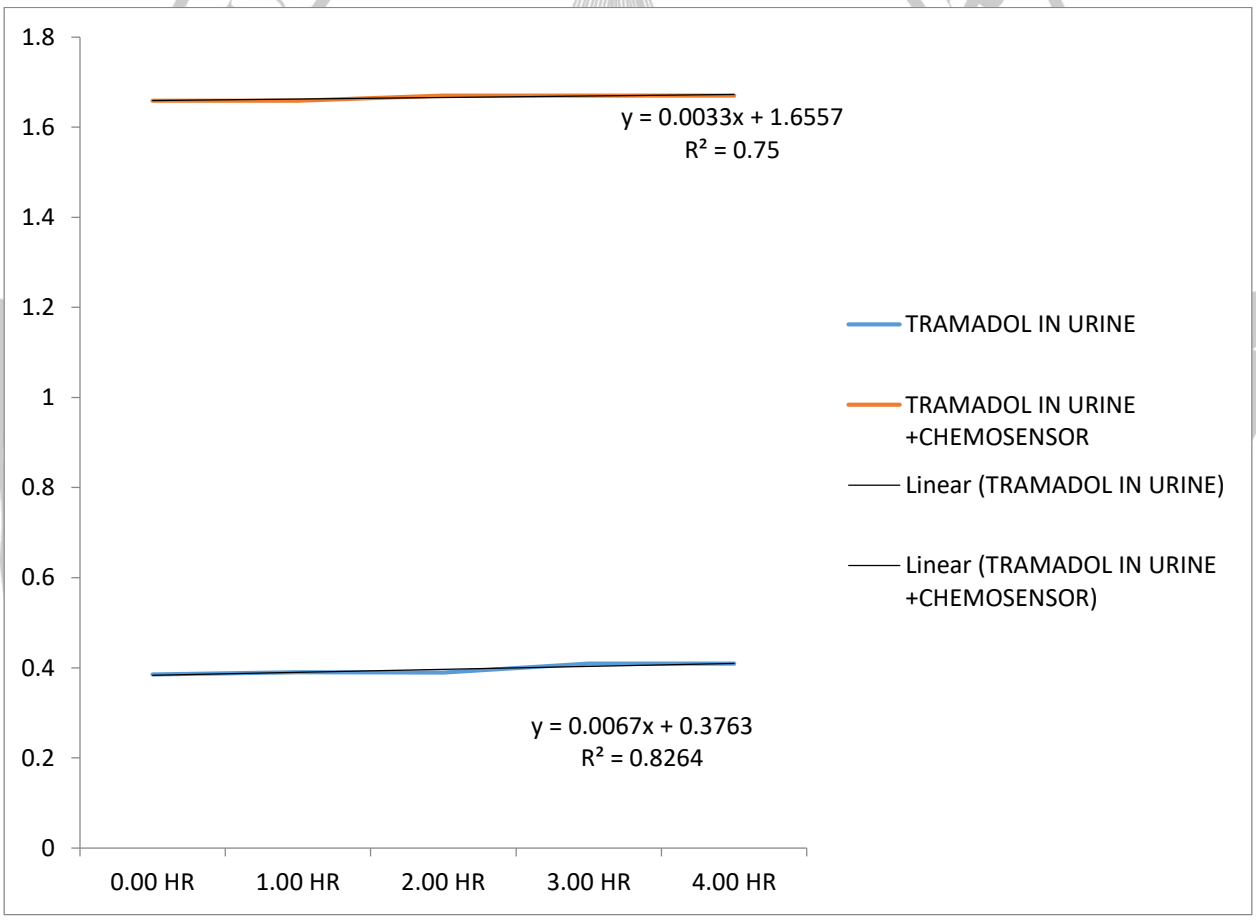


Fig 3: Graphical representation of absorption of Tramadol in urine and Tramadol in urine on reaction with Chitosan-cerium nanoparticles chemosensor

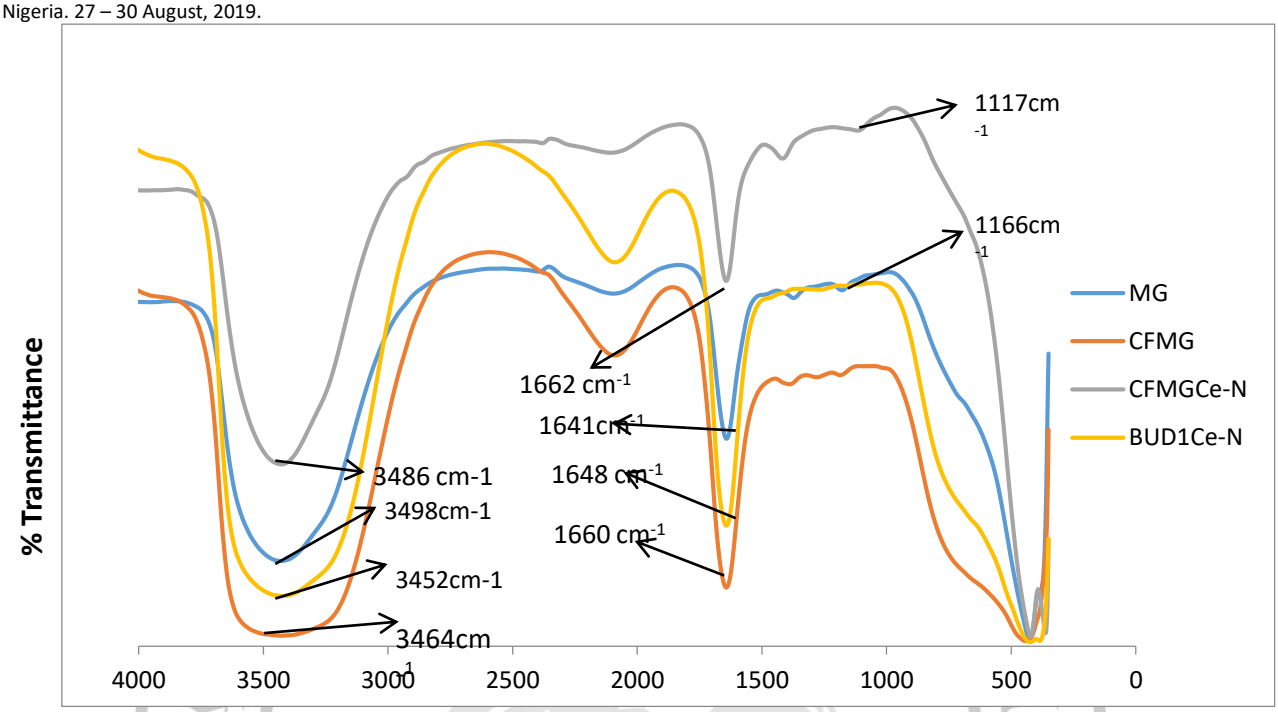


Fig 4: COMPARSON OF FTIR RESULTS OF MG, CFMG, CFMGCe-N and UDCe-N

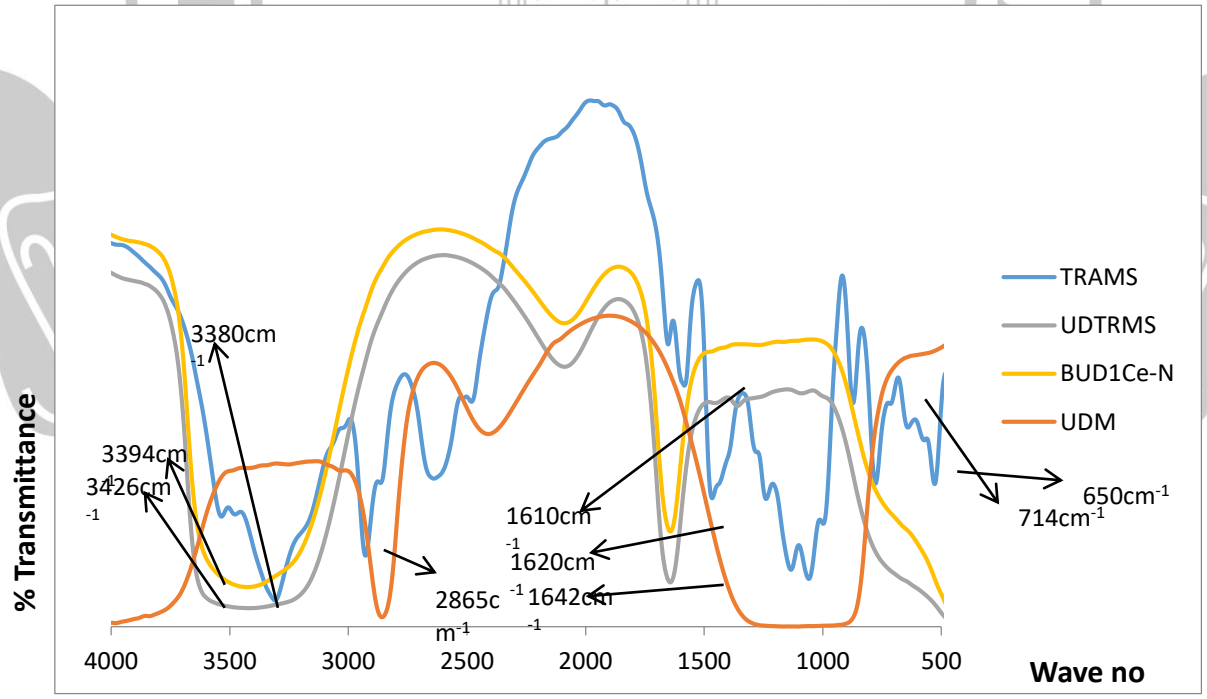


Fig 5: Comparison of FTIR spectra for Tramadol, Urine Alone, Tramadol in urine and Chitsoan- Cerium Nanoparticles Chemosensor.

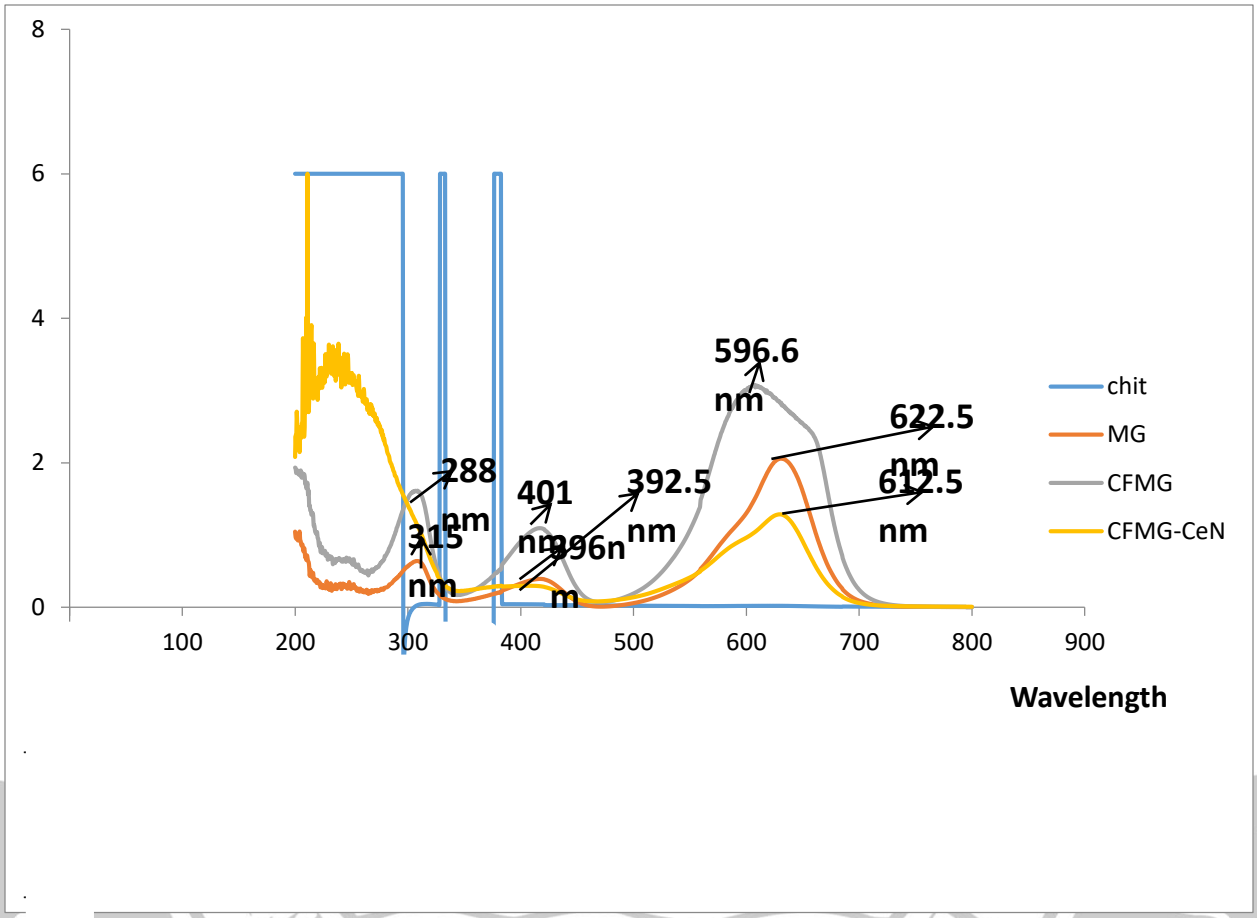


Fig 6: Absorption spectra for Chitosan, Methyl Green, Chitosan Functionalized Methyl Green and Chitosan-cerium nanoparticles chemosensor

4.5 UV result

The electronic properties of Chitosan, Methyl green, Chitosan functionalized methyl green and the Chitosan-cerium nanoparticles chemosensor were monitored using the UV-VIS absorption spectrometry as shown in Fig 6 below:

There was no peak for the observed for chitosan. It is established from this result that methyl green absorbs in the UV-VIS spectrum. Methyl green (mg) absorbs at 315nm, 396nm and has its peak at 622.5nm. This can be attributed to the extended electronic conjugation of the compound (methyl green). The Chitosan functionalized methyl green absorbs at 288nm, 401.5nm and 596.6nm. There is an hyperchromic shift for the Chitosan functionalized methyl green when compared to methyl green probably due to the reaction of chitosan with the former (causing a change in its environment). The Chitosan-cerium nanoparticles chemosensor have a weak absorption band at 392.5nm but has its absorption maxima at 612.5nm.

An hypochromic shift was observed on reacting Chitosan functionalized methyl green with cerium metals and reduction to form Chitosan-cerium nanoparticles chemosensor.

4.0 Conclusion

Based on analysis of the metal nanoparticles using the UV- Spectrometry, Chitosan-cerium nanoparticles chemosensor showed the best peak when analyzed with the UV-spectrophotometer and was used as the major chemosensor for further analysis. There was a higher absorption of the Tramadol in urine sample on reaction with the chemosensor. As the number of days increases, the Tramadol in urine sample was seen to show a higher level of alkalinity.

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