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Detection of Acetone using nanostructured WO_3 for Diabetes Mellitus Monitoring Applications

by

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Dedications

This thesis is dedicated to every young black child, “you can achieve anything you’ve ever dreamed of as long as you put your mind into it”.

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List of Publications, Presentations, and Awards

Article in Peer-Reviewed Journals

1. **V Saasa,** T Malwela, M Beukes, M Mokgotho, CP Liu, B Mwakikunga. Sensing Technologies for Detection of Acetone in Human Breath for Diabetes Diagnosis and Monitoring. Diagnostics 2018, 8 (1), 12.

2. V Saasa, M Beukes, Y Lemmer, and B Mwakikunga. Blood ketone bodies and breath acetone analysis and their correlations in type 2 diabetes mellitus. Diagnostics 2019, (9), 224.

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1. **Valentine Saasa**, Thomas Malwela, Yolandy Lemmer, Mervyn Beukes, Bonex Mwakikunga. Detection of acetone in diabetes mellitus using gas-chromatography mass spectrometry and tungsten oxide-based sensor. The 7th CSIR Emerging Young Researchers Symposium, CSIR, 25th -28th June 2018. Oral Presentation.

2. **Valentine Saasa**, Thomas Malwela, Yolandy Lemmer, Mervyn Beukes, Bonex Mwakikunga. Detection of breath acetone using gas-chromatography mass spectrometry and tungsten oxide-based sensor as a potential replacement of blood glucose reader for diabetes mellitus monitoring. 1st International Conference on Nanotechnology: Theory & Applications. Cairo, Egypt, 10th – 13th Dec 2018. Oral presentation.

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using Gas Chromatography-Mass Spectrometry as a reference. Biochemistry symposium. University of Pretoria, Oct 2018. Poster presentations.

4. **Valentine Saasa,** Thomas Malwela, Mervyn Beukes, Bonex Mwakikunga. Detection of acetone in diabetes mellitus using gas-chromatography mass spectrometry & WO_3 based sensor. Nanotechnology Innovation Centres Symposium. CSIR, Nov 2018. Poster presentations.

5. **Valentine Saasa,** Thomas Malwela, Mervyn Beukes, Bonex Mwakikunga. Detection of acetone in diabetes mellitus using gas-chromatography mass spectrometry and tungsten oxide. International Conference on Recent Advances in Medical and Health Sciences (ICRAMHS). Cape Town, 28th-29th July 2017.

Ph.D. Awards

1. Outstanding woman in Science Communication 2016, **British Council**
2. Best South African Women in Science-Doctoral fellowship 2017, **Department of Science and Technology.**
3. 1st Prize in Science, Technology, and Innovation (South African Youth Awards 2018, **National Youth Development Agency)**
4. South African Young Scientist to attend Lindau Nobel Laurette Meeting**,** Germany 2019. **Academy of Science of South Afica.**

Abstract

Diabetes mellitus which is characterized by a high levels of blood glucose is a major source of mortality, morbidity and health costs worldwide. Major gaps exist in efforts to comprehend the burden nationally and globally, especially in developing nations, due to a lack of accurate, cheap and non-invasive data and devices for monitoring and surveillance. In Africa, type 2 diabetes mellitus represents 90% of diabetes cases. The disease mainly relies on management and monitoring. Although reliable blood glucose monitoring techniques and devices exist worldwide, the challenge is with the cost, invasiveness, and long sample preparation. Herein this study, the challenge was addressed by synthesizing WO_3 materials for the detection of acetone in a simulated human breath. Acetone has been reliably confirmed to be the biomarker of diabetes mellitus. The Gas Chromatography-Mass Spectrometry (GC-MS) was employed to quantify acetone in type 2 diabetes mellitus. A statistically significant correlation (R=0.756) between blood glucose and breath acetone was observed, between blood acetoacetate and breath acetone (R=0,897), and between beta-hydroxybutyrate and breath acetone (R=0,821). Furthermore, we used semiconducting metal oxide (WO_3) to investigate its selectivity, sensitivity, and response towards acetone. Semiconducting metal oxides sensor has the potential to detect volatile organic compound (VOCs) at low concentrations as low as 0.1 ppb. Other advantages of semiconducting metal oxides sensors include, facile and cheap device fabrication, portability, real-time analysis, and facile operating principle. We used two synthesis methods for fabrication of acetone sensors namely solvothermal method whereby solvent ratios were varied, and the sol-gel method where carbon nanospheres were used as a template and cobalt as a dopant. The sensor fabricated with 51:49 water: ethanol is found to demonstrate high response and good selectivity to 2 ppm level of acetone when compared with the one fabricated with pure ethanol, 18:92 (ethanol: water) and 92% water. Furthermore, the sensor could respond to low concentrations of acetone ranging from 0.5 to 4.5 ppm of acetone at 100 °C. For the sol-gel method, the 0.6 % Co-doped WO_3 showed higher response and selectivity towards acetone gas from as low as 0.5 ppm at a very low operating temperature of 50 °C. Contrary, there was a very low response from other gases including toluene, NO_2, NH_3, CH_4 and H_2S operating at a similar temperature. This highlights the acetone selectivity of our 0.6 % Co-doped WO_3 sample. Based on the two methods used for the synthesis of the acetone sensor, we can conclude that the Co-doped sensor shows

better performance as compared to the as-prepared WO_3. This is from the findings that the Co-doped WO_3 can respond and select acetone concentration at 50 ◦C, which is a very low temperature in comparison to other platforms described in literature. An envisioned portable point of care diabetic device could therefore be operated at 50 ◦C in any point of care setting.

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CHAPTER 1

INTRODUCTION

1.1 General Overview and Rationale of the Study

The detection of acetone in diabetes-affected breath strengthens the possibility for successful treatment and also maintains the demand for cheap, non-invasive and quantitative diagnosis of diabetes mellitus [1]. People living with type 1 and type 2 diabetes mellitus are required to monitor their disease daily; at least twice a day in order to manage and monitor the blood glucose level. This is done by drawing blood from a patient and using a typical glucometer like an Accucheck™ (Roche, Switzerland, Indiana glucometer). This glucometer measurement involves pricking the skin to obtain a drop of blood and placing such a drop on a sensitive area of a strip which is pre-inserted in an electronic reading instrument. This method is painful, invasive and can be unsafe. Therefore, it does not suit everyone, especially in the case where the patient needs several samplings each day. Moreover, the finger-pricking test, over time, is expensive with the bulk of the cost per patient coming from the disposable strips rather than the electronic reader (Table 1.1). Finger-pricking can also be a source of what is called "finger sepsis" which is an effect of continuous pricking evidenced by swelling, incorporation of opportunistic infections and subsequent amputation of the finger. This effect is further compounded by the fact that diabetic patients suffer from delayed wound healing [2].

Table 1.1 Pricing of glucometers for monitoring blood glucose level [3]

Model (Company		Technology	Description	Cost of monitoring
Accuchek Active		Invasive	Prick tip of the finger to draw blood, then drop the blood to a chemically active disposable test-strip.	R580 device R185 strips per month R117 needle P/M Total= R4202 Per Year
On call plus glucose meter		Invasive	Prick tip of the finger to draw blood, then drop the blood to a chemically active disposable test-strip.	R2000 kit per month Total= R24 000 per year
Acetone Nanosensor		Non-invasive	You simulate breath acetone gas. Expose the WO3 nanosensor to acetone gas, the sensor will change the resistance upon the exposure to acetone.	Very cheap to fabricate

The non-invasive technologies, more especially the nanosensor breathe technologies which are portable, cheap to manufacture, highly sensitive and easy to use, have potential in diabetes monitoring. According to the International Diabetes Federation (IDF), diabetes will be the seventh leading cause of death in 2030 and the number of diabetes patients will remarkably increase by more than 50% worldwide by 2030 when compared to the number in 2011. Therefore, there is a high demand for a better and pain-free method of monitoring both type 1 and type 2 diabetes mellitus. Managing and monitoring blood glucose reduces the risk of diabetic coma and ultimately the death rate.

Human exhaled breath contains thousands of different volatile organic compounds (VOCs) derived from the body’s metabolic processes. In patients with diabetes mellitus, the body produces excess amounts of ketones such as acetone because the body uses fats rather than glucose to produce energy, which are then exhaled during respiration [4]. This also happens during , exercise, fasting and or diabetes mellitus, the liver produces ketone bodies to act as an additional energy source, which are later metabolized into acetone and other ketone bodies (Figure 1.1). The acetone that is produced travels through the blood and is excreted through urine or exhaled breath. Breath acetone has been shown to have strong correlation with blood glucose levels [5-13]. Acetone has been successfully used as a biomarker for diabetes mellitus, especially in type 1 diabetes mellitus [14]. Thus, measurement of acetone from breath gives better diagnostic control of a patient's diabetic condition, rather than through the use of blood glucose measurements alone as they are easier to use, give quick results and non-invasive

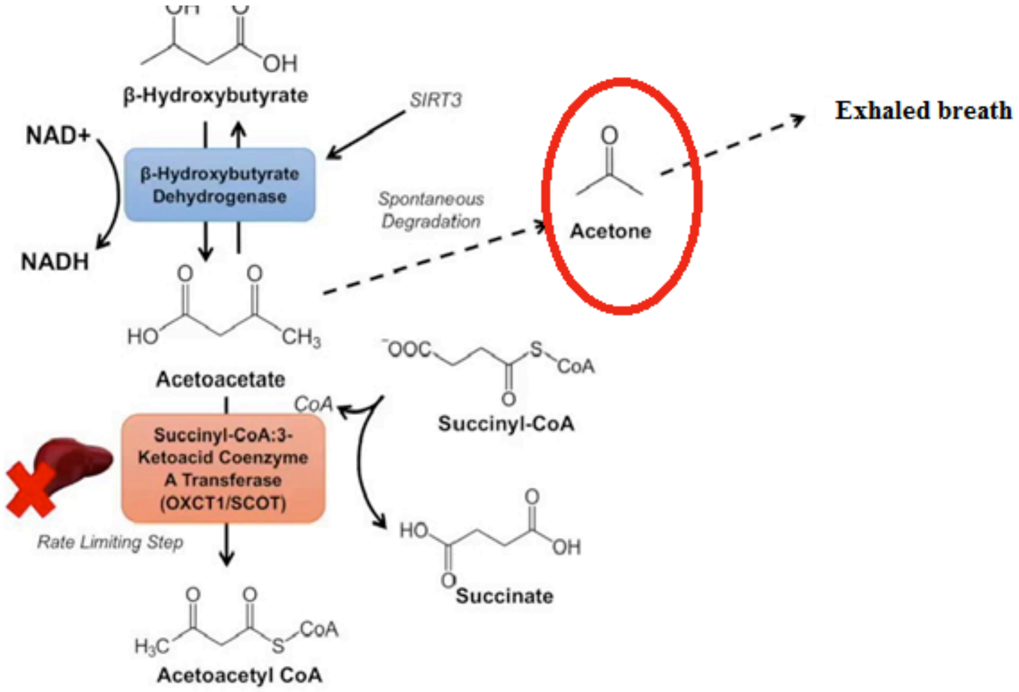

Figure 1.1 Acetone metabolism pathways [15]

Many studies have reported on non-invasive analysis of breath for detection of diseases using techniques such as gas chromatography with mass spectrometry (GC–MS), proton transfer reaction with mass spectrometry (PTR–MS) and many other sensitive techniques [16-20]. Although these techniques are sensitive and reliable, they are not portable for daily monitoring. On the contrary, semiconducting metal oxides (SMO) have attracted attention in the field of breath analysis for disease detection due to their ability to detect low ppb to ppm of volatile organic compounds. More interestingly, they are easy and cheap to fabricate, have potential of miniaturization, simplicity of their use, and compatibility with electronics. Among semiconducting metal oxides, tungsten oxide (WO_x) has attracted much attention in the field of gas sensors due to its excellent physical and chemical properties. Tungsten oxide exists in different phases found at different temperatures such as R phase and ε phase. The γ-WO_3 is the most studied phase as it can be found at room (RT) and higher temperature at which most devices are designed to operate. It has demonstrated excellent gas sensing responses to H_2S, NH_3 [21] and acetone [22, 23], etc.

The general mechanism of the semiconducting metal gas sensors is based on the changes in electrical properties before and after exposure to the target gases or vapours. However, there are still major draw-backs with the performance of these sensors, such as poor performance at room temperature, most materials perform better at a high temperature which is not practical for diabetes monitoring devices which is supposed to operate at a low temperature [22, 24, and 25]. Furthermore, other shortcomings include poor selectivity, which is the inability to select one gas, to avoid false diagnosis or false positive, and last but not least poor recovery which is the inability to recover back to their initial electronic state [26].

Therefore, this thesis will be investigating the level of breath acetone in both diabetic and non-diabetic patients using the well-known Gas Chromatography Mass Spectrometry as a reference and further explore different tungsten oxides by:

- Varying solvent ratios in order to obtain desired acetone sensing properties
- Adding the noble material onto the tungsten oxide to further enhance acetone sensing properties for low detection of acetone for application in diabetes monitoring and diagnosis.

1.2 Aims of the project

The ultimate aim of the study is to provide an acetone sensor for non-invasive monitoring of diabetes mellitus using human breath. For this study; breath acetone in diabetic and non-diabetic patients were quantified using Gas Chromatography Mass Spectrometry. Tungsten trioxide properties were investigated for response, recovery, selectivity and low temperature operation.

In chapter 2 different technologies for detection and monitoring of diseases using human breath's biomarkers will be reviewed.

In chapter 3 breath acetone, blood glucose, blood acetoacetate and betahydroxybutyrate have been quantified in both diabetic and non-diabetic patients. The correlation between blood ketone and breath acetone was determined, as well as correlation between blood acetone and breathe acetone.

Chapter 4 shows how a low acetone sensor were synthesized and characterized for their ability to detect acetone at low operating temperature for the first time as compared with reports available in literature.

Chapter 5 describes how the Co-doped-WO was synthesized and optimized for low acetone detection. The cross selectivity of acetone, response and recovery time of the sensor and how it was characterized using different instruments to check the sensor's properties were also done.

The impact of the results in this study on non-invasive monitoring of diabetes mellitus in future has been discussed in chapter 6.

CHAPTER 2

LITERATURE REVIEW

Valentine Saasa[1, 2], Thomas Malwela[1], Mervyn Beukes[2], Chaun-Pu Liu, Bonex Mwakikunga[1]

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2.1 Introduction

This chapter provides details on the history of human breath analysis and techniques used to detect breath biomarkers for disease diagnosis and monitoring as the core subject being investigated in this study. Human breath has been used as the biomarker for different diseases such as toluene for cancer and ammonia for liver failure. This study will focus on breath acetone as a biomarker for diabetes mellitus. Different technologies for detection of breath acetone have also been reviewed in detail. The metal oxides chemoristive sensor as the potential technique for breath acetone detection in this study have also been reviewed in detail and identified the gap which includes the sensor's selectivity, low temperature operating sensor, etc. Most of this chapter has been published as a review paper in the **diagnostic journal, volume 8**; however we have included few sections of importance which were not included in the review paper. The section includes but is not limited to the history of breath analysis, tungsten oxides and their properties.

2.2 Historical roots of Human Breath and Development

The use of human breath to assess the physiological state of patients can be dated back to the times of Hippocrates, "the father of clinical medicine". He was the first person to use human breath for disease detection [27]. Early medical practitioners relied on the odor of human breath to make a diagnosis. They have associated certain breath odor with specific diseases, such as a fruity like smell with diabetes mellitus; musty fish smell was linked to liver failure, newly-moun clover was associated with fetor hepaticus, and many more [28]. Several years later, between 1882/83, there was a report on quantitative analysis of CO_2 in the breath of guinea pics by Lavoisier. The study has shown that breath CO_2 is the resultant of combustion in the body [29]. The modern human breath analysis emerged in between the late 1960's and early 1970's, same time with modern analytical chemistry. This was when Pauling used a liquid partition to show that human breath contains over 200 volatile organic compounds [30] . Ever since then, they has been several studies and reviews on breath as a non-invasive potential tool for disease diagnosis, monitoring and environmental exposure assessment [31-33].

2.3 Human Breath for Diagnosis of Diseases

Human breath is composed of nitrogen (78%), oxygen (15-18%), carbon dioxide (4-6%), volatile organic compounds and water. The volatile organic compounds, described fully in the section below, are derived in the body or from the environment, and make up the rest of the breath component [34]. Human breath has been used as a potential tool for the diagnosis and study of diseases. Pauling first analyzed the human breath in 1971 [35] using gas chromatography, where around 250 different gases were identified. Currently, scientists are able to detect more than 300 different volatile organic compounds and other particles in breath [36]. Thus, human breath analysis is also crucial for delivering real-time and rapid screening, non-invasive, and diagnosis of complex diseases such as cancers [37]. Furthermore, it is not only non-invasive, but also has several advantages as compared to traditional diagnostic techniques, which include painless procedures and sampling that does not require skilled medical staff [38].

The human breath contains several hundred VOCs with concentrations ranging from part-per-trillion (ppt) to part-per-million (ppm). The cellular and biochemical origin of many of these VOCs has not been determined and some of them might be of exogenous origin. Breath acetone concentration ranges from 300 to 900 ppb in healthy people to more than 1800 ppb in individuals with diabetes mellitus [8]. Therefore, acetone can act as a biomarker for metabolic (diabetes) conditions in the bloodstream. In certain cases such as fasting, exercising and being diabetic, the liver produces ketones to act as an additional energy source, which is then metabolized into acetone and other ketone bodies. Using breath analysis techniques, acetone concentrations in the exhaled breath have been shown to correlate with the acetone concentrations in the blood as well as with other ketones such as beta-hydroxybutyrate. In addition, it is also found that the level of blood glucose can be correlated to the volatile organic compound levels such as acetone. Measurement of acetone from breath can allow a better diagnostic control of a patient’s diabetic condition than through the use of blood glucose measurements alone [39].

2.3.1 Volatile Organic Compounds (VOCs)

Volatile organic compounds (VOCs) are either subtracted from inspired air (by degradation and/or excretion in the body or added to alveolar breath as a result of metabolic processes [40]. The compounds exchange across the alveolar blood capillary membrane into exhaled breath [37]. There are many other sources of VOCs in human breath, including airway surfaces, inhaled atmospheric air, blood and peripheral tissues throughout the body. Some of these volatile organic compounds are the end-products of biochemical reactions, whilst others might be from the body’s physiological activities such as cell-to-cell signaling. There are different classes of VOCs that can be measured in the human breath. These include saturated hydrocarbons, unsaturated hydrocarbons, and oxygen-containing, sulfur-containing and nitrogen-containing compounds [34]. The saturated hydrocarbons include pentane aldehydes and ethane, which are formed by the lipid peroxidation of fatty acid components of cell membranes triggered by reactive oxygen species, while the unsaturated hydrocarbons include isoprene, and are produced by the Melavalic pathway of acetoacetate from lipid peroxidation [41]. The oxygen-containing compounds, which include acetone, are produced from decarboxylation of acetoacetate from lipid peroxidation [40]. The sulfur-containing compounds include ethyl mercaptane and dimethyl sulfide, and they are all produced from the incomplete metabolism of methionine [42]. Last but not least, the nitrogen-containing compounds are produced during liver impairment and uremia [43]. So, the analysis of VOCs in inspired air and alveolar breath is a useful research tool with crucial applications in clinical medicine [44]. As already mentioned in the text, different VOCs rather than acetone for diabetes are used as the biomarkers for different diseases in medicine. We are going to outline how other researchers have been able to diagnose diseases such as cancer, liver disease, and kidney failure and so on using the human breath. Phillips and coworkers' research on VOCs showed that 22 VOCs are breath markers of lung cancer [45]. Methylated alkanes and selected alkanes have been shown to be able to distinguish lung cancer patients from healthy controls [46].

The rule of the lung cancer breath diagnosis is to check if a person's breath contains more than 1 of the 11 VOCs with a concentration that is higher than the breath diagnostic cut-off. If the peak area of the 11 VOCs is >200, the patient is considered as a lung cancer patient [47]. Liver disease is one of the most prominent extra-oral causes of bad breath. It was found that , acetone, dimethyl sulfide, 2-butanone, 2-pentanone, were significantly higher in the alveolar air of liver disease patients [48]. Impairment of the liver function increases the concentrations of these compounds, which have a characteristic smell of a rotten cabbage [49]. Other researchers used gas chromatography to demonstrate that the levels of all sulfur-containing molecules were elevated in the breath of patients with cirrhosis, even outside the liver coma [48]. The human nose, whereby physicians were sniffing the breath, could only detect the characteristic sulfur odor. The alveolar concentration of dimethyl sulfide is more than 30 nmol/mol or ppbv, which was observed in 25 of the 52 liver cirrhosis patients. Furthermore, hydrogen sulfide and methyl mercaptane have also been reported as possible mediators. However, in vitro experiments showed that the free -SH group of methyl mercaptane reacts with blood, which results in an irreversible binding and oxidation. In contrast, dimethyl sulfide is a neutral, stable molecule that can be transported from the blood into alveolar air and be expired [48].

2.3.2 Acetone Metabolism

Acetone is an exhaled volatile organic compound that has been used as a biomarker for diabetes mellitus, especially in type 1 diabetes mellitus [14]. It is an end-product of the non-esterified fatty acids oxidation, which results in acetyl-CoA and ultimately acetoacetate through spontaneous decarboxylation as depicted in Figure 2 [50]. The acetone that is produced travels through the blood and is excreted through urine, sweat and/or exhaled breath. For the exhaled breath, it has been found that the partition coefficient is 330 parts in the blood for every part that leaves with expired air [51]. It has been found that quantification of acetone concentration in human breath, using breath analysis techniques, correlates strongly with acetone concentration in the blood and other ketone bodies such as beta-hydroxybutyrate. Furthermore, another study by Worrall et al. [45] has found the correlation between blood glucose and volatile organic compounds. Thus, measurements of acetone from breath pose a better diagnostic control of a patient’s diabetic condition.

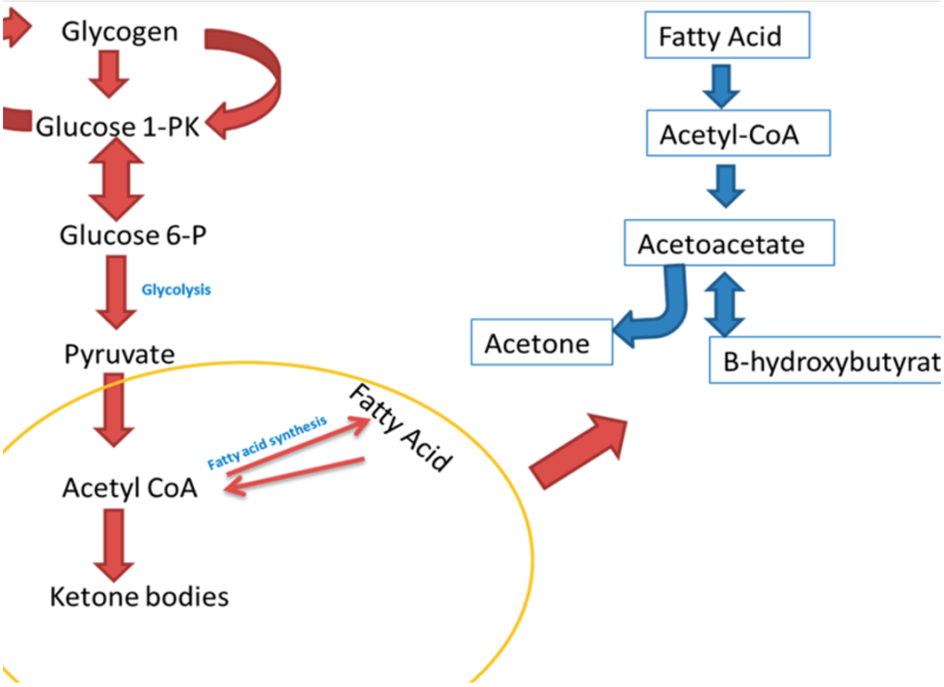

Figure 2.1 Ketone bodies synthesis (ketogenesis): in-depth step-by-step pathway analysis of the formation of ketone bodies from fatty acid mentalism.[52]

Acetone levels in the breath have also been used for the detection and monitoring of abnormal concentrations of blood beta-hydroxybutyrate levels in diabetes mellitus [53]. It is reported that acetone is also emitted from the skin and its concentrations correlate with breath acetone and blood beta-hydroxybutyrate [54]. The odor of acetone in diabetic patients is used as a symptom of diabetic ketoacidosis because acetone is produced as a direct byproduct of the spontaneous decomposition of acetoacetic acid. However, mild acidosis may be due to prolonged fasting or when the individual is on a ketogenic diet or a very-low-calorie diet [55]. Medical researchers have shown that the acetone concentration in exhaled breath from a normal individual is lower than 0.8 ppm, whilst that for a diabetic patient is higher than 1.8 ppm in seven diabetic and seven non-diabetic patients [56]. However, Anderson's review has shown that different ranges of breath acetone can be found in different conditions. For example, adults on ketogenic diets can have elevated levels of up to 40 ppm. Children with epilepsy are normally given the ketogenic diets to minimise the occurance of seizures, which leads to a high breath acetone concentration of 360 ppm [57].

2.4 Various Techniques Used in Diabetes Monitoring and Diagnosis

The monitoring of numerous volatile compounds in-breath is a promising and expanding field. Techniques such as selected ion flow tube mass spectrometry (SIFT–MS), gas chromatography coupled to mass spectrometry (GC–MS), solid-phase microextraction (SPME), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC–MS) have provided highly selective analysis of VOCs in breath [58]. Although the GC and other mentioned analytical methods are highly sensitive and selective for diagnosis of diabetes mellitus, they are expensive and the issue of portability is particularly important considering that diabetes mellitus should be monitored and diagnosed in real-time for daily healthcare purposes [56].

Gas Chromatography-Mass Spectrometry (GC–MS) is a hyphenated technique, which combines the separating power of gas chromatography with the detection power of mass spectrometry. It plays a fundamental role in determining how many components exist in a mixture and their respective proportions. The mass spectrometric detector is commonly used to obtain the “fingerprint” spectrum of the molecule. Mass spectra provide information on the molecular weight, elemental composition, functional groups present, and in some cases, the geometry and spatial isomerism of the molecule [59]. With GC–MS, analysis of breath acetone requires sample preparation which includes acetone standard preparation and derivatization of acetone on the solid-phase microextraction (SPME) needle. The SPME derivatization is mostly used because the mass of acetone is too small and acetone is volatile [60]. Preparation of acetone standards takes about 20 min and having to derivatize it on the SPME takes 20 min [61]. The other disadvantage with sample preparation is the collection of breath from patients, which requires expensive Tedlar bags. The Tedlar bags can only store the human breath for not more than 4 h [60, 62]. The GC–MS also suffers from temporal resolution. It takes considerable time, at least minutes if not tens of minutes, to separate the constituents of a gas mixture fully on a capillary column [51]. To analyze the acetone level in human breath using GC–MS, one needs to get extensive training from the manufacturer of the instrument or someone who has knowledge of GC–MS. Diabetes monitoring needs a self-monitoring device that can be used in real-time for daily healthcare purposes.

Proton-transfer reaction mass spectrometry (PTR-MS) is a technique developed specifically for the detection of gaseous organic compounds in the air [63]. In a PTR-MS system, primary ions, H_3O^+, react with neutrals under well-defined conditions. This is assured by allowing the H_3O^+ reactions to proceed within a flow-drift tube [64]. Compared to GC–MS, PTR-MS delivers relative VOC concentrations with high sensitivity (down to parts per trillion) without sample preconcentration. Such preconcentration steps are time-consuming as already mentioned before. In addition, PTR-MS allows online measurements (over a full night, for example). The drawbacks of PTR-MS, on the other hand, include its inability to distinguish between substances having the same molecular weight.

All substances identified by their product ions with particular mass-to-charge ratios (m/z) must therefore be considered as possible contributors [65]. It requires a trained operator, or rather, a person who is scientifically literate. It does not suit illiterate people who are diabetic. Last but not least, PTR-MS is bulky; it is not suitable for daily monitoring of diabetes mellitus [66].

Selected ion flow tube mass spectrometry (SIFT–MS) quantifies trace amount of gases in air and breath. It works by chemical ionization of the small gas molecules in air or breath samples injected into the helium which is a carrier gas using H_3O+, NO+ and O_2^+ precursor ions. The precursor ions and trace gas molecules react for a given time, thus the precursor and product ions will be detected and counted by a mass spectrometer, thus effecting quantification. The advantage of SIFT-MS is that, the trace gases can be detected down to ppb level without the need of sample collection [67]. The shortfall of SIFT-MS as a potential instrument for monitoring diabetes is its inability to identify compounds in a mixture of gases. SIFT–MS, like GC–MS and PTR-MS, also requires a trained operator, and as already mentioned, diabetes affects old people who perhaps cannot read and write, so such instruments are not suitable for personalized monitoring devices. The other major and common drawback is portability.

Quantum cascade lasers (QCLs) are semiconductor lasers using transitions within the conduction band. Hence, the wavelength can be tailored by the thickness of the layer rather than being determined by the band-gap [68]. They can be fabricated to operate at any of a very wide range of wavelengths from ~3 m to ~24 m. They represent very promising sources for gas-sensing applications [69]. Systems based on widely tunable QCLs can be used to measure multiple gas species, and narrowly targeted systems can detect and measure gas concentrations in the parts-per-trillion range [70]. The principle of laser spectroscopy is based on the laser light being absorbed in the frequencies corresponding to the spectral absorption lines of the specific gases [71]. These absorption lines originate from the electronic, rotational or vibrational states of the molecules. The electronic-state transitions are much more energetic compared to the other two, and therefore are easy to detect using visible or near-UV wavelengths. The rotational and vibrational states can be probed using near- and mid-infrared light sources, due to much lower transition energies. The transitions occurring in the mid-infrared region, however, are much stronger, thus make low detection limits possible. QCL-based are emerging in the field of medical diagnostics. Such an application requires extremely fast sampling times, relatively small size and accurate results in order to avoid misdiagnosis; QCLs can fulfill these criteria [72]. However, there are some limitations to QCLs in diagnosis and monitoring of diabetes mellitus, currently limited by available technology to reach sufficient specificity which includes selectivity required for practical use.

Light-addressable potentiometric sensors (LAPSs) are part of the field-effect-based biochemical sensors. They enable the monitoring of analyte concentrations in a spatially resolved manner on the sensor surface [73]. The LAPS principle is an invention by Hafemann et al. 1988., and which is based on the scanned light pulse technique (SLPT) (engestrom) with the intention to measure in aqueous solutions [74]. The advantage of LAPS is its light-addressability, which makes the high-integrated level easily obtainable. LAPS are able to obtain larger amounts of information than sensor arrays. Their large continuous surface can be used to determine the direction of gas as additional information for gas recognition. Image detection of respiratory gas based on LAPS is proposed as a development in the diagnosis of diabetes [75]. However, the drawbacks of LAPS in the diagnosis of diabetes mellitus as compared to Chemoresistive sensors are as follows; it is bulky, thus not suitable for real-time point of care, and it requires a trained operator. Diabetes monitoring requires a self-manageable device. For all the mentioned reasons, a portable diagnostic device with real-time monitoring as well as outstanding acetone sensitivity and selectivity should be developed. In this regard, semiconductive metal oxides (SMOs) are promising for diagnosis and monitoring diabetes mellitus non-invasively. This is due to their potential in real-time analysis, facile operating principle (resistivity change upon exposure of acetone to the SMO's surface layers), simple device fabrication and ready miniaturization [76]. The basic operating principles, as well as strengths and weaknesses of each method, are summarized in Table 2.1.

Table 2.1 Comparison of breath acetone analysis techniques

Technique	Principle	Detection Limit	Advantages	Disadvantages
GC-MS	Separate and analyze compounds by MS using chromatographic column (polar or non-polar)	ppb and ppt levels	Highly selective and sensitive	Preconcentration steps, bulky, long sampling time, need for standards and require a trained operator.
PTR-MS	Analysis of ionized molecules of target analytes by reaction with H_3O^+ MS.	Low ppb levels	Real-time analysis	Lack of specificity, Narrow range of detectable compounds, bulky and require trained operator
SIFT-MS	Analysis of ions produced by the reaction analytes and precursor ions (H_3O+, NO+ or O_2+) by quadrupole MS.	Low ppb and ppt levels	Real-time, the capability of ppt detection, a broad range of detection.	Cannot identify compounds, bulky and require a trained operator.
QCL	Electrons are recycled from period to period, containing each time to the gain and the photon emission.	Low ppb levels	Real-time analysis, the potential for portability and miniaturization	Selectivity required for practical use and currently limited by available technology to reach sufficient specificity.
LPAS	Analysis of trace gases. It uses photoacoustic effect whereby the conversion of light to sound in all materials (solid, liquids and gases).	ppt-ppb levels	Real-time analysis	Bulky, require trained operator.
SMOS-based chemoresistive sensors	Measures resistivity changes based on the thinning or thickening the depletion layer of n-type SMOs and hole accumulation layer of p-type SMOs around the surface when exposed to oxidizing or reducing gas ambient.	ppm, ppb and ppt levels	Real-time analysis, portable, inexpensive and miniaturization	Relatively low sensitivity and less selectivity.

As already mentioned, several online detection methods have been developed for the analysis of breath acetone samples using mass spectrometry, such as proton-transfer-reaction mass spectrometry (PTR-MS), selected ion flow tube mass spectrometry (SIFT–MS) and gas chromatography–ion mobility spectrometry (GC–IMS) [66]. Among the developed analytical methods for VOCs, in particular, gas chromatography-mass spectrometry (GC–MS) is one of the most promising techniques. Due to the high sensitivity of typical VOCs, GC–MS has been widely employed in breath analysis. However, the concentration of VOCs in the typical breath sample is quite low, and thus a kind of sample preconcentration process, such as a cold-trap or adsorption trap method, is still necessary before analysis for the sensitive and accurate determination in most of the cases [20, 60]. These methods provide high sensitivity; however, the techniques often require time-consuming processes. SPME is one of the most advanced sampling techniques for the GC analysis and has some advantages over conventional extraction methods, such as simple operation, being solventless and having easy automation [77, 78]. Acetone breath concentrations taken from diabetic individuals using various analytical techniques are shown in Table 2.2.

Table 2.2 Techniques and its detection limits for breath acetone concentration

Technique	Acetone Concentration	Reference
GC-MS	0.049 ppb	[79]
	0.22-3.73 ppb	[80]
	06.95-145.99 ppb	[81]
	0.195-0.659 ppm	[82]
PTR-MS	0.19-1.3 ppm	[79]
	50 ppb	[83]
	200-2000 ppb	[84]
SIT-MS	1-20 ppm	[85]
	293-870 ppb	[84]

2.5 Nanomaterial-Based Approaches for the Detection of Acetone

The growing interest in nanotechnology has resulted in the identification of many unique physical and chemical properties associated with nanomaterials. The advances in nanotechnology are so profound that their impact echoes into society far beyond the boundaries of ordinary science [86]. The implementation of nanotechnology in the field of breath analysis with the Chemoresistive sensors has been increased in recent years. The nanoscale size of these building blocks provides them with several qualities, such as large surface-to-volume ratios and unique chemical, optical and electrical properties. The large surface area of the nanomaterials provides highly active interfaces, thus increasing sensitivity and lowering the response and recovery times. Different nanomaterials have been exploited for VOC-sensing elements, including nanoparticles and nanowires of different materials. Furthermore, the dynamic range, as well as the selectivity of the nanomaterial-based gas sensors, can be tailored to accurately detect specific breath VOCs of a given disease [48, 87].

Chemoresistive sensors based on semiconducting metal oxides have attracted attention for their potential for miniaturization and their low cost, simple fabrication, ease of use, good compatibility with microelectronic processes and reliability [88-95]. As already mentioned, several methods such as GC–MS, IMS, and SIFT–MS have been utilized to analyze trace compounds in the human breath. The major advantages of the mentioned techniques are high sensitivity, selectivity and low limit of detection. However, they are of high cost and not portable for personal diagnostic tools [96]. Chemoresistive sensors have been used mostly in the past years to detect sub-ppm acetone in human breath. It was found that a nanosized gamma iron sesquioxide (-Fe_2O_3) sensor is able to detect 1 ppm of acetone in the background of VOCs present in normal breath [97]. The screen-printed titanium dioxide (TiO_2) sensor showed sensitivity for type 1 diabetes diagnosis [98]. Furthermore, pristine and Pt-functionalized tungsten trioxide (pt-WO_3) hemitubes demonstrated very high sensitivity and response to H_2S and acetone gas [99]. WO_3, particularly in its "monoclinic phase”, is promising for its selective and quantitative detection of acetone in the ppb concentration range. This is attributed to the spontaneous electric dipole moment of the "-phase, which increases the interaction with analytes having high dipole moment (e.g., acetone) [51]. A portable diagnostic device with real-time monitoring, highly sensitive with potential for miniaturization and pain-free implementation should be developed. Currently, Chemoresistive SMOs have been receiving a great deal of attention for exhaled breath sensing in portable and real-time diabetes mellitus diagnosis. We have gathered recent publications in Table 4 on diabetes diagnosis by detection of acetone using SMO sensors. In addition, noble metal catalysts such as Pd [100], Pt [98, 101] and Au [102], as well as graphene-based catalytic materials and grapheme oxide, have been integrated with nanostructured SMOs for selective acetone detection [103, 104].

Table 2.3 Recent publications on chemo-resistive SMO-based exhaled breath sensors for potential use in the diagnosis of diabetes mellitus using acetone as a biomarker

Material	**Sensitivity (response) (ppm)**	**Detection limit (ppm)**	**Response/ recovery time (S)**	**Operating temperature (°C)**	**Reference**
ZnO:Pt	188	1000	45	400	[105]
ZnO:Nb	224.0	1000	56	400	[105]
$PrFeO_3$	234.4	500	6.1	180	[106]
$CdNb_2O_6$	2	10	9	600	[107]
In/WO_3-SnO_2	66.5	50	2.12	200	[108]
2D C_3N_4-SnO_2	11	67	7	380	[109]
TiO_2	15.24	500	9.19	270	[110]
2D ZnO nanosheets	106.1	500	-	300	[111]
$W0_3$ decorated with Au and Pd	-	1000	6	300	[112]
In_2O_3 nanoparticle	21.5	1000	2	250	[113]

The rationale of using SMOs either as composites such as ZnO: Pt, ZnO: Nb, In/WO_3 -SnO_2 or 2D C3 N4 -SnO_2, doped such as WO_3 decorated with Au and Pd, or pure metal oxides such as TiO_2, 2D ZnO nanosheets or In_2 O_3 nanoparticles, is that they offer a variety of advantages. These include low cost and facile fabrication [114, 115]. Most reports on metal oxides for acetone sensing strengthen the emphasis on the facile synthesis of the SMOs [116-118]. Ai et al. used a microwave-assisted method, which is normally a cheap and fast method for material synthesis, to produce Fe_3O_4 nano-roses for gas-sensing applications [119]. The other interesting qualities of SMOs include their stability at high working temperature, and last but not least, their abundance in nature.

The solid-state sensors are based on the adsorption/desorption or chemical reaction on the surface of the thin-film material. This leads to a change in the physical structure that is detected by the sensor device. The physical structure includes changes in temperature, refractive index, mass and more [51]. Considering the influencing factors on gas-sensing properties of metal oxides, it is necessary to reveal the sensing mechanism of the metal oxide gas sensor. The exact fundamental mechanisms that cause a gas sensor response are still controversial, but essentially, trapping of electrons at adsorbed molecules and band bending induced by these charged molecules are responsible for a change in conductivity [120]. The negative charge trapped in these oxygen species causes an upward band bending and thus a reduced conductivity compared to the flat band situation. When O_2 molecules are adsorbed on the surface of metal oxides, they would extract electrons from the conduction band and trap the electrons at the surface in the form of ions. This will lead to a band bending and an electron-depleted region [92, 121]. The reaction of these oxygen species with reducing gases or competitive adsorption and replacement of the adsorbed oxygen by other molecules decreases and can reverse the band bending, resulting in an increased conductivity [122-124]. O^- is believed to be dominant at the operating temperature of 300-450 °C, [125], which is the operating temperature for most metal oxide gas sensors.

Different factors influence the sensitivity of the metal oxides. These include chemical composition, surface modification by noble metal particles, microstructure, humidity and temperature [92]. In addition to chemical composition, sensors based on the two mixed components tend to be more sensitive than the individual components. Kim et al. [76] in 2016 found a sensitive and selective acetone sensor using a WO_3 nanofiber functionalized by Rh_2O_3 [56]. Furthermore, the conductivity response is also influenced by the efficiency of catalytic reactions with detected gas participation, taking place at the surface of the gas-sensing material. Therefore, control of the catalytic activity of the gas sensor material is one of the most commonly used means to enhance the performance of gas sensors. However, in practice, the widely used gas-sensing metal oxide materials, such as TiO_2, ZnO, SnO_2, Cu_2O, Ga_2O_3 and Fe_2O_3, are the least-active from a catalytic point of view [92]. Moreover, as we continue with the factors that influence the sensor sensitivity, microstructure plays a crucial role. For example, a sensor's sensitivity can be significantly increased by using materials with very small grain sizes, and this simulated result agrees well with the experimental observation. Lu et al. [126] have indicated that the SnO_2-based sensor response to 500 ppm CO increases drastically if the particle diameter becomes smaller than 10 nm.

2.5.1 Limitations of Semiconducting Metal Oxides (SMOs)

Generally, the major limitations of SMOs include the following:

- Sensitivity, a change of measured signal per analyte unit, that is, the slope of a calibration graph;
- Selectivity, a characteristic that determines whether a sensor can respond selectively to a single analyte;
- Stability, the ability of a sensor to provide reproducible results for a certain is. This includes retaining the sensitivity, selectivity, response and recovery time;
- Durability, the ability to withstand damage due to temperature, chemical addition and so on;
- Response time, the time required for the sensor to respond to a stepped concentration change from zero to a certain concentration value;
- Recovery time, the time it takes for the sensor signal to return to its initial value after a stepped concentration change from a certain value to zero
- Room temperature operation and so on, the ability to detect gases at room temperature.

Nevertheless, there has been extensive research in trying to overcome the limitations of the SMOs in order to get good sensors. It was found that controlling the particle size and porosity of the material can enhance the sensitivity of the material. Numerous reports on SMOs reveal a high sensitivity when the average grain size was reduced to several nanometers [127-129]. SnO_2 and TiO_2 mesoporous powders fabricated using self-assembly of a surfactant followed by treatment with phosphoric acid as well as conventional tin oxide powders with surfaces modified by mesoporous SnO_2 show higher sensor performance than corresponding metal oxide powder materials, which have lower specific surface area [130, 131]. Doping is also one way of improving the sensitivity of an SMO sensor that has been used for a long time. The sensitivity of metal oxide gas sensors can be greatly improved by dispersing a low concentration of additives, such as Au [132], Ag [133], Cu [134], Co, Pd [135, 136], Pt [137] and F [66] on the oxide surface.

Two approaches have been developed to address the problem of selectivity. The first one is to prepare a material that is strictly sensitive to one analyte and has a low or zero cross-sensitivity to other analytes that are present in the working system. This is done by firstly optimizing the working temperature, doping elements and their concentrations [130]. The second approach is based on the preparation of materials for discrimination between several analytes in a mixture. It is impossible to do this by using one sensor signal; therefore, it is usually done either by modulation of sensor temperature [138] or by using sensor arrays [139, 140]. The issue of low stability can lead to problems of false alarms, uncertain results and the need to replace the sensor. Metal oxides with small grains, nanorods, nanotubes, nanowires and so on can be easily degraded due to their high reactivity. However, stability can be increased by calcination and annealing as the post-processing treatment and also reducing the working temperature of the sensor element.

Synthesis of mixed oxides and doping metal oxides with carbon nanotubes has been reported to increase the stability of the sensor [130]. To overcome the response/recovery time of the sensor and room temperature operation, a patent has been filed that has a sensor with UV light to clean up the gas molecules so that the gas sensor is not saturated with the breath [141]. The search for non-invasive diagnosis and monitoring of diabetes mellitus remains a worldwide goal. There are other complementary technologies being developed to help healthcare in monitoring diabetes mellitus non-invasively [142]. These include glucose-sensing technologies which are selective for glucose with a fast, predictable response to changing glucose concentrations [119]. This follows the non-invasive blood-glucose-sensing technique which measures in vivo blood glucose concentrations without collecting a blood sample. It uses near-infrared spectroscopy for accomplishing such a measurement. The concept is to pass a band of near-infrared radiation through a vascular region of the body and then to extract the corresponding blood glucose concentration from the resulting spectral information [143]. Different points of care testing (POCT) devices are available which are based on electrochemical or optical detection for measuring blood glucose [144].

2.5.2 Tungsten Oxide as the Material of interest in this study.

Tungsten oxide (WO_x) is a transition metal oxide with a band gap ranging from 2.6 to 3.6 Ev [145]. Due to the emerging field of nanotechnology, research on the synthesis and characterization of tungsten oxide nanostructures has been increasing. Thus, the technology allows manipulating the tungsten material to afford desired properties that do not exist in its bulk form [146]. Those properties include; increased surface energies, quantum confinement effects, electronic band structure, and optical properties. All these properties are essential in acetone detection.

There has been a great interest in the nanostructured WO_x due to its versatility and unique characteristics. Recently it's been widely investigated metal oxide in much research from solid-state chemistry, condensed-matter physics and nanomedicine. In contrary to other metal oxides like ZnO, SnO_2, NiO, TiO_2, the study of acetone sensing is more advanced [147]. In this section, we are going to detail the WO_X crystal structures, electronic band structure, and electrical conductivity and sensing mechanism.

2.5.2.1 Crystal Structures and Phase Transition

Tungsten Oxides (WO_x) structures are described as a three-dimensional network of corner-sharing WO_6 octahedra, with several crystal phases [148]. These include monoclinic II (ε-WO_3, triclinic (δ-WO_3), monoclinic I (γ-WO_3), orthorhombic (β-WO_3) and tetragonal phase (α-WO_3). These phases are named in the order of an increase in temperature. For example, you get the monoclinic phase at <−43 °C, triclinic at −43 °C to 17 °C, orthorhombic at 330 °C to 740 °C, and tetragonal phase at >740 °C [149]. Figure 3 shows the polyhedral representations of the six crystal structures.

How the phase is classified is based on the tilting angles and rotation direction of WO_6 octahedra with reference to the "ideal" cubic structure. Additionally, Table 4 below presents the lattice constant data for WO_3 crystal phases [150]. Interestingly, the nanoscale WO_3's phase transitions are much lower than the WO_3 bulk and the phase transition is irreversible due to large surface-to-volume ratio, small grain size and very high surface energy [147].

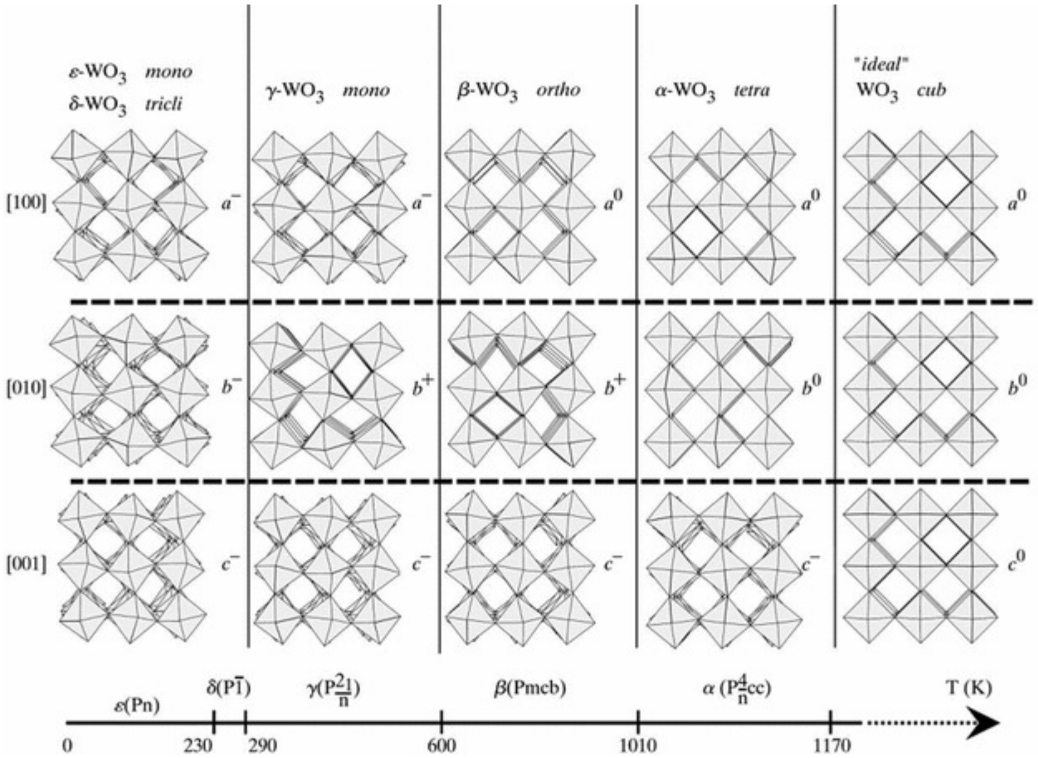

Figure 2.2 Tilt patterns and stability temperature domains of the different polymorphs of WO_3

Table 2.4 Lattice constant data for different WO_3 crystal phases.

Lattice constant (Å)	ε-WO_3	δ-WO_3	γ-WO_3	β-WO_3	α-WO_3	Ideal Cubic WO_3
a	7.378	7.309	7.306	7.384	5.25	3.84
b	7.378	7.522	7.540	7.512	N/A	N/A
c	7.664	7.686	7.692	7.846	3.91	N/A

Among the above, mentioned phases, γ-WO_3 is considered a promising phase for the detection of acetone, while ε-WO_3 has high selectivity to acetone [22, 23, 151]. Although ε-WO_3 has been reported to have high selectivity [152-154], it is also said not to be stable at high temperatures [155], and it usually becomes stable after doping with Cr or incorporating Si into the lattice of WO_3 [156].

2.5.2.2 Structures of Nonstoichiometric WO_x

We have shown in section 2.5.2.1 that WO_3 is a transition metal oxide, in this case, the metal oxide is composed of the perovskite units. Additionally, it is also widely known for its nonstoichiometric characteristics, thus the lattice is able to withstand a substantial amount of oxygen deficiency [157]. A slight reduction in its oxygen content drastically changes the electronic band gap and ultimately increases the conductivity [158]. The decrease in WO_3 oxygen content normally leads to a structural change. The few known nonstoichiometric WO_x compositions include $W_{18}O_{49}$, $W_{20}O_{58}$, and $W_{24}O_{68}$. These oxides are formed by corner-sharing WO_6, which alternate with octahedra that are partially established by edge-sharing, figure 2.3 [147].

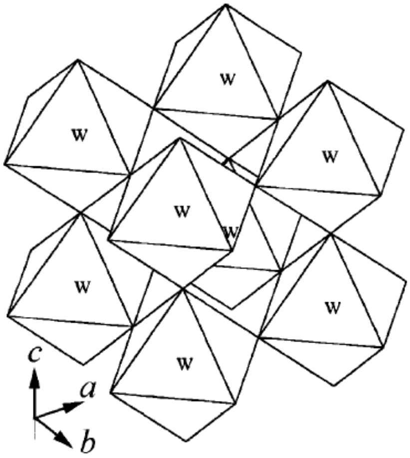

Figure 2.3 Arrangement of [W-O_6] octahedra in the structure of monoclinic WO_3.

2.5.2.3 Electronic Band Structure

WO_3 is an n-type semiconductor, with an electronic band gap equivalent to the difference between the energy levels of the valence band, which is formed by filled O 2p orbitals and the conduction band formed by empty W 5d orbitals [159]. The monoclinic phase demonstrates a fundamental absorption edge, E_g, of 2.6 eV [160]. The distortion of the cubic structure in the monoclinic phase results in an increase in bandgap and W 5d level occupation [161]. This has been confirmed experimentally by absorption/emission spectroscopy, and X-ray photoelectron spectroscopy (XPS) [162].

The nanoscaled WO_3 bandgap increases with the reduction in grain size [159]. This is often observed experimentally and is called a blue shift of the optical absorption band-edge. It is due to a decrease in nanostructure dimensions, and the blue shift is normally attributed to the quantum confinement effect. The QC effect is divided into two regimes, which include strong and weak [163]. The strong quantum confinement effect takes place when the size of the crystal is reduced too much smaller than the Bohr radius for the material (≈ 3 nm for WO_3). On the other hand, the weak quantum effect takes place when the crystal size is larger than the Bohr radius [164].

The properties such as electrical conductivity make this material an ideal candidate for conductance-type gas sensor devices for the detection of acetone [24, 152]. The gas sensing properties are due to the change in electrical conductivity of tungsten oxide material upon exposure to the target gas. The detailed mechanism of gas detection will be discussed later in this chapter.

2.5.2.4 Electrical Conductivity

As WO_3 is an n-type semiconductor, its electrical conduction heavily depends on the free electron concentration which is in their conduction bands (50). The concentration of free-electron is solely determined by the concentration of stoichiometric defects, such as oxygen vacancies [50]. The electrical conductivity of a material can be influenced by structural factors such as grain size, grain boundary, crystal phase and dopants [51]. Thus, the synthesis method has a major effect on the electrical properties of the material. This was evidenced in the report of Valentine *et al.*, when they observed high carrier concentration (5 × 10 19 cm^{-3}) and electron mobility (6.5 $cm^2\ V^{-1}\ s^{-1}$) during the synthesis of WO_3 by sputtering and thermal evaporation at a high substrate temperature [52].

Increasing the roughness of the structure, however, increases resistivity. The conductivity was reported to decrease within the WO_3 nanoparticles, due to an increasing volume of the grain boundaries, which contributes to more trapping and scattering of free charge carriers. [55] If the size of the crystallites is smaller than the electron mean free path, grain boundary scattering dominates and hence the electrical conductivity decreases. In addition, lattice strain and crystal distortions can also affect the motion of charge, causing a decrease in conductivity. [56, 57].

2.5.2.5 The sensing mechanism of WOx

The metal oxide gas sensor is based on the principle of chemoresistance which is the change in electrical conductivity/resistivity of the sensor material upon the exposure to a target gas. This means that the gas molecules that interact with the metal oxides will either act as a donor or acceptor of charge thus changing the resistivity of the metal oxide. Depending on the type of majority carriers in the semiconducting material and the type of gas (oxidizing or reducing), there will be an increase or decrease in the senor’s resistance [165].

For an n-type semiconductor such as WO_3-based sensors, the mechanism has been intensively studied and reported in the literature [23, 25, 166-168]. The gas sensing mechanism of WO_3 can be described as follows:

The change in resistance of tungsten oxide sensors is dependent on the adsorbed oxygen species (O^{2-}, O^{-}) on the surface. Upon their exposure to air, O^{2-} gets adsorbed onto the surface which later adsorbs electrons from the conduction band. This decreases the concentration of electrons in the conduction band which ultimately decreases the electrical conductance of the material, Figure 2.4 [169]. This mechanism is expressed as follows:

$$O_2(ads) + e^{-} \rightarrow O^{2-}(ads); \qquad (1)$$

$$O^{2-}(ads) + e^{-} \rightarrow 2O^{-}(ads); \qquad (2)$$

In the case where the sensor is exposed to a reducing gas such as acetone, the chemisorbed oxygen species ($O_2^{-}{}_{(ads)}$, $O^{2-}{}_{(ads)}$, $O^{-}{}_{(ads)}$) react with acetone vapor and releases electrons back to the conduction band and as a result, electrical conductance increases. The reaction mechanism is expressed as follows:

$$CH_3COCH_3\ (gas) + O^{2-} \rightarrow CH_3C{+}O + CH_3O^{-} + 2e^{-}; \qquad (4)$$

$$CH_3C^{+}O \rightarrow C^{+}H_3 + CO; \qquad (5)$$

$$CO + O^{2-} \rightarrow CO_2 + 2e^{-}. \qquad (6)$$

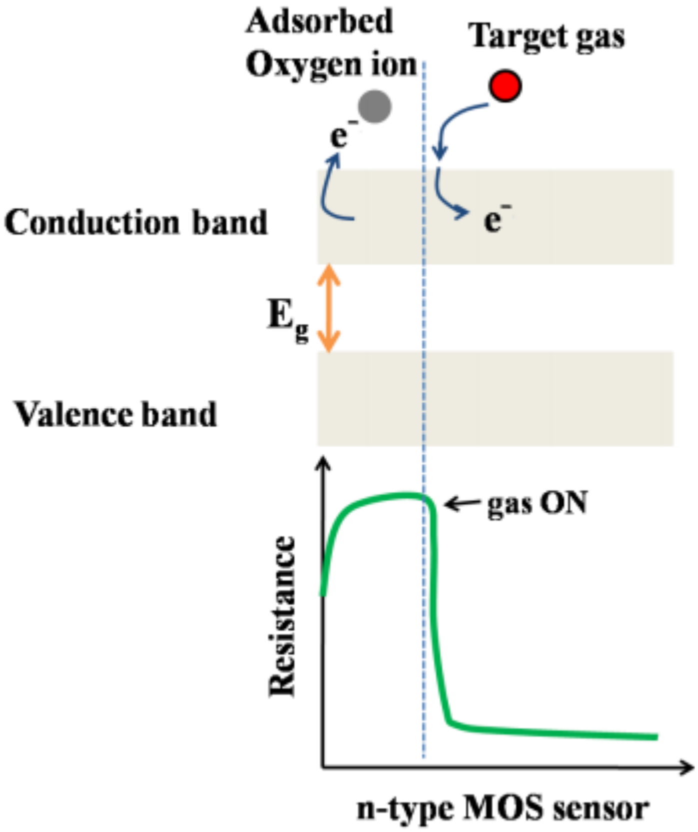

Figure 2.3 Schematic representation of the change in the sensor's resistance on n-type MOS upon exposure to a reducing gas [170].

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CHAPTER 3

Analysis of ketone bodies in the breath and blood of type 2 diabetic patients

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3.1 Introduction

Ketone bodies such as beta-hydroxybutyrate, acetoacetate and acetone are mostly found in human blood, urine, and breath. Mesasuring altered concentrations of ketones in the blood, urine, and breath is important for the diagnosis and treatment of diabetic ketosis. Studies have suggested that breath gases, including acetone, may be related to simultaneous blood glucose (BG) and blood ketone levels in type 1 diabetes [1-3]. However, from our knowledge based on literature, they have never been a study on analysis or correlation of ketone bodies and breath acetone on type 2 diabetes. In our previous study, Saasa et al. MSc thesis analyzed ketone bodies in both breath and blood of type 2 diabetic and non-diabetic patients. In this chapter, we are going to assess the relationship between blood ketone bodies and breath acetone, along with blood glucose and breath acetone of type 2 diabetes and determine whether breath acetone could be used as a biomarker for diabetes mellitus.

The study assesses the efficacy of a simple breath test as a non-invasive means of diabetes monitoring in type 2 diabetes mellitus. Human breath samples were collected in Tedlar™ bags and analyzed by solid-phase microextraction and gas chromatography-mass spectrometry (SPME-GC-MS). The measurements were compared with capillary fasting blood glucose (BG) and ketone levels taken at the same time on a single visit to a routine hospital clinic in 60 subjects with type 2 diabetes and control volunteers. Ketone bodies of diabetic subjects showed a significant increase when compared to the control subjects. A statistically significant relationship was found between breath acetone and blood acetoacetate (R = 0.897) and between breath acetone and β-hydroxybutyrate (R = 0.821).

3.2 Research questions

- Does type 2 diabetics also produce ketone bodies?
- Is there any correlation between acetoacetate and breath acetone in type 2 diabetes?
- Is there any correlation between beta-hydroxybutyrate and breath acetone in type 2 diabetes mellitus?
- Is there a correlation between blood glucose and breath acetone in type 2 diabetes?
- Can breathe acetone be used independently as the biomarker for diagnosis and monitoring of diabetes mellitus?

3.3 Hypothesis

Low insulin, insulin resistance, and no insulin inhibits glycolysis and promotes beta-oxidation which produces ketone bodies namely; acetoacetate, beta-hydroxybutyrate, and acetone. The acetone formed by the beta-oxidation could be detected in the patient’s breath.

3.4 Aims of the study

- Determination of blood ketone bodies (acetoacetate and beta-hydroxybutyrate) and breath acetone in type 2 diabetes mellitus patients.
- Correlation analysis of blood acetoacetate and breath acetone.
- Correlation analysis of blood beta-hydroxybutyrate and breath acetone.
- Correlation studies on blood glucose and breath acetone.
- Assessment of the efficacy of the acetone breath test as a means for non-invasive diabetes monitoring.

3.5 Materials and Methods

3.5.1 Materials

Acetone	Sigma Aldrich, Germany
Acetoacetate Assay Buffer	Abcam®, United Kingdom
Acetoacetate Standard	Abcam®, United Kingdom
Acetoacetate Substrate	Abcam®, United Kingdom
β-HB Assay Buffer	Abcam®, United Kingdom
β-HB Enzyme Mix	Abcam®, United Kingdom
β-HB Substrate Mix	Abcam®, United Kingdom
β-HB Standard	Abcam®, United Kingdom
Pentafluorobenzylhydroxilamine-hydrochloride	Sigma, Germany
Polydimethylsiloxane/divinylbenzene	Sigma, Germany
Tedlar bags	Keinkeventures, USA
Vials	Perkin Elmer, Waltham, U.S

3.5.2 Instrumentation

For the blood ketone bodies and glucose measurements, Biotek 96 well plate reader, Agilent, U.S.A, was used. For breath acetone quantification, an Agilent Technologies model 6890N Gas Chromatography (GC) interfaced with Agilent Technologies model 6890N Mass Selective Detector (MS) coupled with Solid Phase Microextraction (SPME) was used for analysis.

3.5.3 Study Population

A total of sixty participants aged between 18 to 60 years were recruited for this study based on the prevalence of diabetes in this locality. This comprises of thirty (30) type 2 diabetes mellitus patients and thirty (30) non-diabetes individuals as controls. Inclusion criteria included being diabetic while an exclusion criterion was non-diabetes. Blood from fasting individuals was used. A routine glucose test was also performed for participants to confirm the diabetic state. Informed consent (attached as an appendix) was obtained from participants as well as ethical clearance (Ref: 118/2015, attached as an appendix) from the ethics committee of Council for Scientific and Industrial Research (CSIR).

3.5.4 Collection of samples

Blood samples were collected by standard vein puncture into the plain tube. The blood was centrifuged at 3000 rpm for 10 minutes. The plasma was separated into a separate tube and kept in -80 C freezer until analysis. The breath samples were also collected simultaneously with blood samples using the Tedlar™ bags and analyzed immediately using the Gas Chromatography-Mass Spectrometry.

3.5.5 Biochemical Analysis

Ketone bodies (acetoacetate (AcAc) and beta-hydroxybutyrate β-OHB), were analyzed using Abcam's Acetoacetate (ab180875) and Beta-hydroxybutyrate (ab83390) respectively. The Acetoacetate kits quantifies AcAc in human blood. The AcAc reacts with a substrate to generate a colored product that can be measured at 550 nm. The reaction is only specific for AcAc and does not detect Beta-hydroxybutyrate. The Beta-hydroxybutyrate kits utilize beta HB Dehydrogenase to generate a product that reacts with the colorimetric probe with an absorbance band at 450 nm.

The plasma blood from 30 diabetics and 30 non-diabetic patients were analyzed using Abcam's glucose assay kit to quantify the amount of glucose in the plasma. In this assay, glucose oxidase specifically oxidizes glucose to generate a product that reacts with a dye to generate color (570nm). The generated color is proportional to the glucose amount.

3.5.6 Breathe acetone analysis using SPME-GC-MS

In this study, we used the Solid-Phase Microextraction coupled with Gas-Chromatography (HS-GC/MS) to quantify acetone in diabetic and non-diabetic mellitus patients. The method is very simple, fast and sensitive for optimization and accurate quantification of acetone in human breath. Acetone gas standards in the concentration range of 0.073, 0.59, 1.66, 3.32 and 6.64 ppmV were prepared. An SPME fiber pre-coated with 20 mg/ml of O-2, 3; 4; 5; 6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) was exposed inside the 3L Tedlar bag at 40 ◦C containing the standards and human breath for 10 minutes. Acetone present in the breath samples underwent on-fiber SPME derivatization to form the stable oxime (Figure 3.1).

An Agilent Technologies model 6890N Gas Chromatography interfaced with Agilent Technologies model 6890N Mass Selective Detector was used for analysis. A 30 m x 0.25 mm with 0.25 µm RXi®-5 SilMS (Restek, U.S.A) was used as the analytical column. The injection port temperature which was also the desorption temperature for the SPME fiber was 250 ◦C, and the desorption time was 2 min. The GC split valve was set to open after the 2 min desorption time. The GC injector liner was a quartz SPME liner with an internal diameter of 0.75 mm (Supelco Inc. Bellefonte, PA). The column temperature was regulated through a gas chromatography program, an initial temperature of 60 ◦C, an increase to 150 ◦C at 10 ◦C/min, and then an increase to 300 ºC (held for 1 min). Total ion current was monitored using electron-impact ionization mode (70 EV). Quantification was performed using characteristic mass. The peak at m/z 181 was used for the quantification of the acetone-PFBHA derivative.

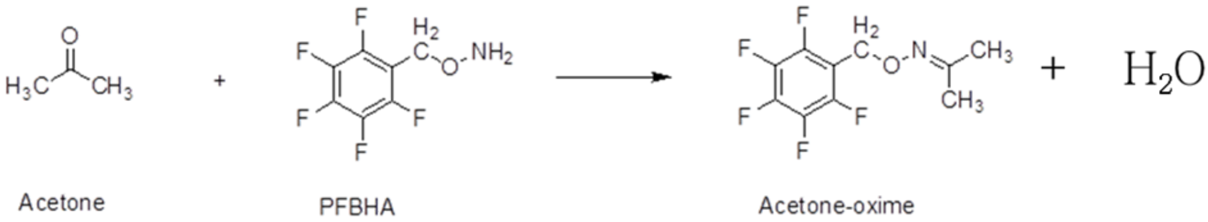

Figure 3.1 Schematic representation of the reaction between breath acetone and the derivatizing agent (PFBHA) reacting to the SPME fiber.

3.6 Results and Discussion

3.6.1 Biochemical analysis

Assessment of ketone bodies is an important practice more especially in clinical institutions to check and monitor for diabetic ketoacidosis (DKA) and to evaluate if acetone can be used in monitoring and controlling the disease. The high amount of ketone bodies in the blood is usually an indicator that the catabolism of fatty acid is greater than the one for carbohydrates [4, 5]. In this study, fasting 30 diabetic and 30 non-diabetic patient's plasma blood were analyzed for ketone bodies (acetoacetate and beta-hydroxybutyrate) using the Abcam®'s acetoacetate and beta-hydroxybutyrate Assay kits. Other metabolic parameters which include fasting blood glucose, high-density cholesterol (HDL), low-density cholesterol (LDL), total cholesterol, triglycerides and breath acetone were also measured (Table 3.1).

Generally, plasma blood glucose, acetoacetate, beta-hydroxybutyrate and acetone levels differ from individual to individual and it also varies from day to day, even for the same individual as can be seen in figure 3.2. It depends on the everyday diet, medications, stress and physical activities [6]. In this study different diabetic and non-diabetic patients showed the above-mentioned characteristics, thus all the non-diabetic patients showed different plasma metabolites levels. The plasma glucose mean value of 8.6 mmol/l in diabetic and 5.7 mmol/l in non-diabetic patients were observed.

Table 3.1 Clinical data on type 2 diabetes mellitus and non-diabetes mellitus.

	Type 2 DM (*n*=30*)*	**Non-diabetes (*n*=30*)***	**P-value**
Age	47±10	41±10	<0.001
Gender	13/17	11/19	0.10
BMI (kg m^{-2})	28.4±4.5	25.4±4.0	0.47
Plasma glucose (mmol/l)	8.6±2.43	5.7±1.44	0.007
HB1Ac (%)	10.3±2.57	-	-
Total cholesterol (mmol/l)	5.10±1.40	4.5±1.42	0.17
Triglycerides (mmol/l)	1.57±1.3	1.04±1	0.01
HDL cholesterol (mmol/l)	1.15±0.27	1.33±0.47	0.34
LDL cholesterol (mmol/l)	2.56±1.32	2.43±0.97	0.52
B-hydroxybutyrate (mmol/l)	0.46±0.02	0.44±0.41	0.55
Acetoacetate (mmol/l)	0.09±0.02	0.05±0.03	0.47

NB: Data in mean ± SD, BMI (body mass index), HDL (high-density lipoprotein), and LDL (low-density lipoprotein)

Some diabetic patients showed a very good plasma glucose level even if they were diagnosed with diabetes mellitus. Thus they control their diet, medication, and exercise. Whilst, in other diabetic patients the uncontrolled plasma blood glucose (11.30, 28.30, 10.30, 10.80, 10.00 mmol/l) was observed. Some patients showed a very low plasma glucose level (3.90 mmol/l) which indicates the state of hypoglycemia that is usually observed in type 1 diabetes mellitus patients. The plasma glucose confirms that the patients are diabetic and some patients can monitor their glucose level.

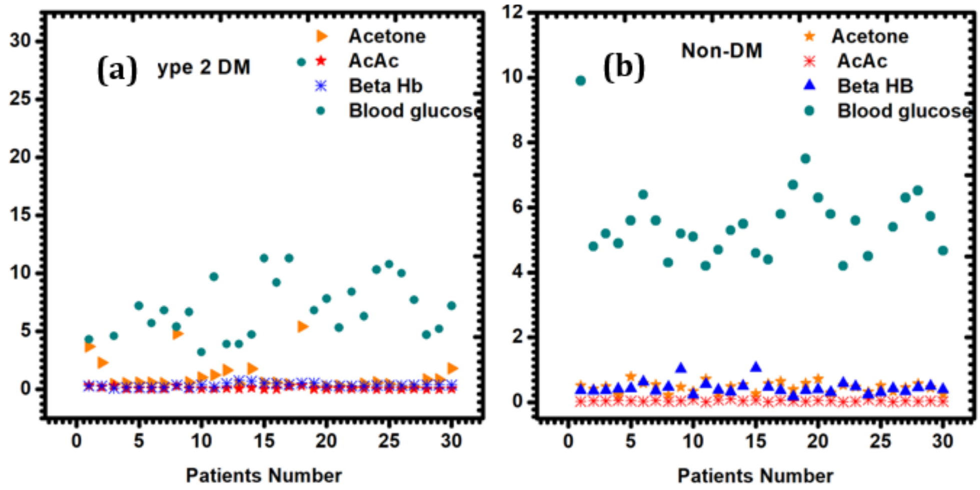

Figure 3.2. Scatter plot for plasma blood glucose, acetoacetate, beta-hydroxybutyrate in (a) type 2 diabetic and (b) non-diabetic patients.

Monitoring blood glucose alone does not give a clear state of diabetic danger. Hyperketonemia in diabetes is due to insufficient insulin action. It has also been observed in other endocrine-related diseases where excess hormone secretion antagonizes insulin action [7]. Using the Abcam's acetoacetate and beta-hydroxybutyrate assay kits on 30 diabetes and 30 non-diabetes patients, we observed a physiological amount of ketones bodies with the mean value of 0.08 & 0.46 for diabetes and 0.05 & 0.44 for non-diabetes patients. Furthermore, beta-hydroxybutyrate and acetoacetate concentration might offer more insights on the severity of ketoacidosis, if it is related to diabetes, alcohol, or starvation [8]. A blood ketone level less than 0.5 mmol/l is considered to be physiological, whereas hyperketonemia is defined by a value greater than 1 mmol/l and ketoacidosis is considered to be probable above 3 mmol/l [6, 9]. We did not observe hyperketonemia in this study group. However, this does not mean that type 2 diabetes does not undergo hyperketonemia but simply implies that the patients control the disease. It has been reported that ketone bodies were higher in insulin-dependent patients than non-insulin dependent. However, Yamane et al. [5] found a good correlation of ketone bodies and skin acetone even in controlled diabetes. Our study also found a good correlation in controlled type 2 diabetes which will be discussed in the next section.

3.6.2 Breath acetone analysis

Quantifying breath acetone is of importance to this study, as we hope to find a significant correlation between plasma ketone and breathe acetone. Thus, it will strengthen the movement of finding a portable chemoresistive acetone sensor devise that will be able to detect acetone from the human breath from as little as 0.1 ppm. Using the Solid Phase Microextraction (SPME) procedure and GC-MS analysis, we successfully managed to quantify the acetone level in the breath of both diabetic and non-diabetic patients. The reaction of acetone in the breath with a derivatizing agent (PFBHA) forms a very stable acetone-oxime. Fig.3.3 shows the mass spectrum of the acetone oxime with a base peak at m/z 181. Acetone concentration higher than 1.8 ppmv was found in diabetic breath (figure 3.2 (a)). For non-diabetic breath, acetone concentrations lower than 0.8 ppmv was observed (figure 3.2 (b)). This study is consistent with the literature [10-13].

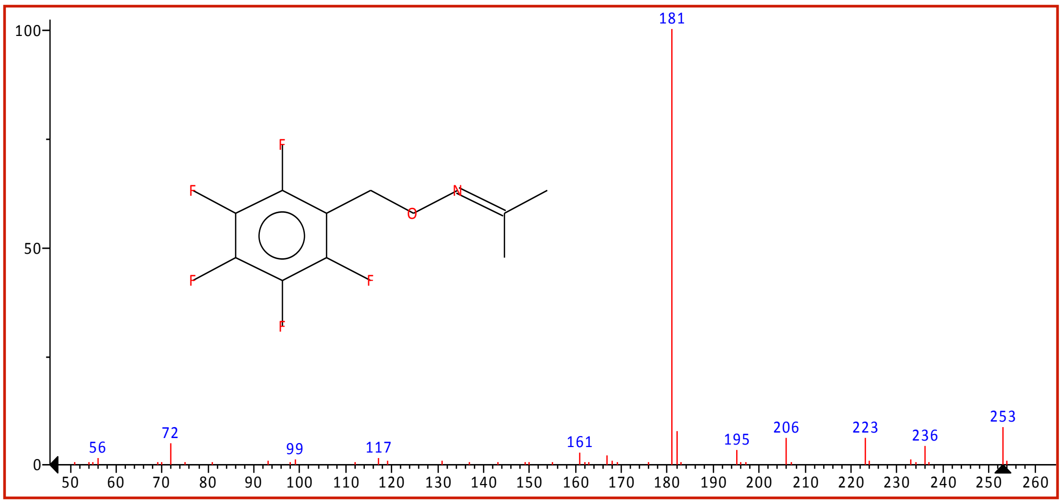

Figure 3.3 The GC-MS mass spectrum of acetone-oxime.

3.6.2.1 Correlations between breath acetone and plasma Acetoacetate, breath acetone and beta-hydroxybutyrate

After successfully determining the plasma concentration of acetoacetate, β-hydroxybutyrate and breath acetone, we found it necessary to check the correlation of the blood ketones with breath acetone. The diversity of ketone bodies among 30 diabetes patients appeared at baseline (figure 3.4). Significant positive correlations between breath acetone and plasma AcAc and between breath acetone and plasma β-OHB were observed at baseline (R = 0.897 and R = 0.821). This shows a positive indicator of using acetone as a non-invasive biomarker of diabetes mellitus. Other studies have found the correlation of R=0.856 between exhaled acetone and blood acetoacetate and R=0.709 for exhaled acetone and β-OHB in type 1 diabetes mellitus [14]. There are many hypotheses to explain the relationship. One reason being, acetone is a metabolite produced after enzymatic decarboxylation of AcAc, which is in equilibrium with β-OHB via an enzymatic controlled process by β-OHB dehydrogenase [15]. Although the exponential relationship between acetone and β-OHB, and acetone and AcAc were observed, acetone reflected overall ketone metabolite concentrations in diabetic patients. This is due to the fact that acetone presents positive deviations from well-known gas/liquid partition laws, such as Henry's law or Raoul's law which states that that the solubility of a gas in a liquid is directly proportional to the partial pressure of the gas above the liquid[16]

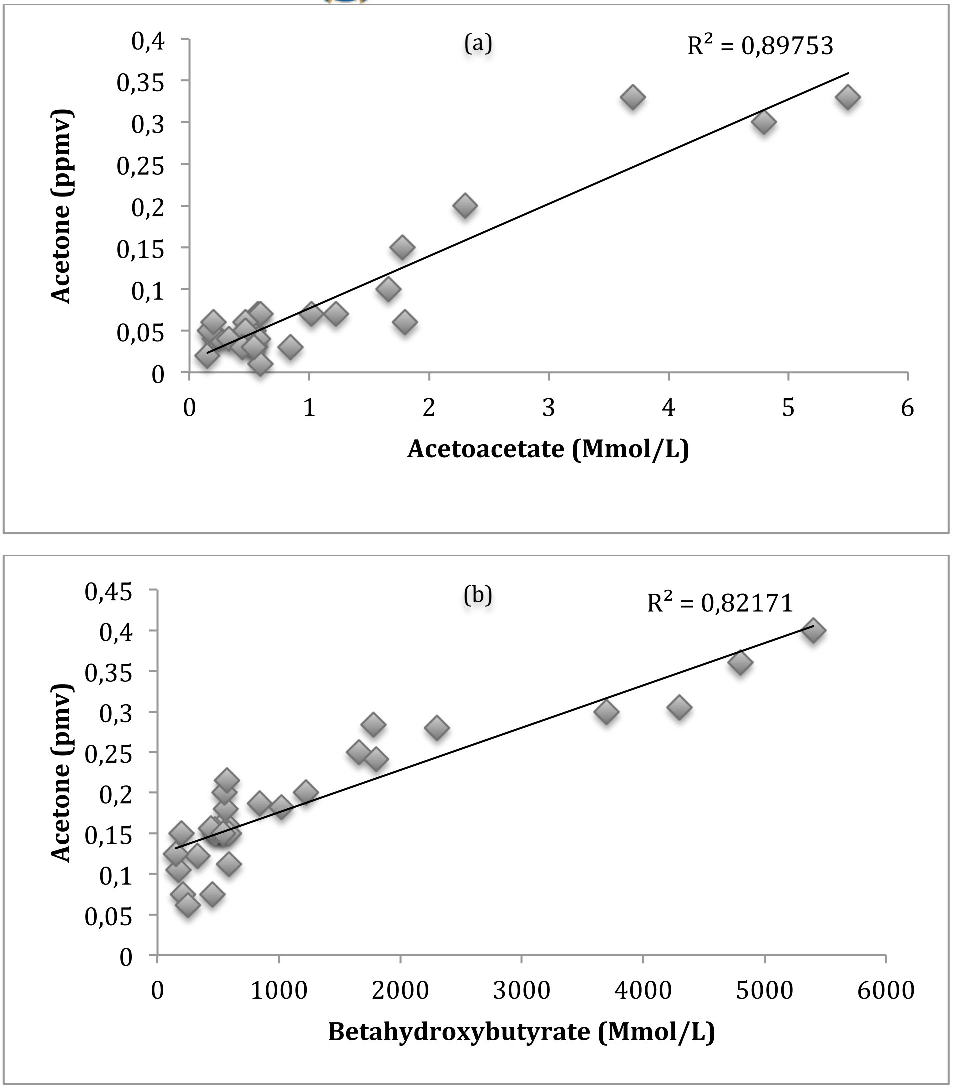

Figure 3.4.: (**a**) Correlation between breath acetone and acetoacetate; (**b**) Correlation between breath acetone and beta-hydroxybutyrate. The correlations were calculated using linear regression.

3.7 Conclusion

The high amount of ketone bodies (acetone, acetoacetate, and beta-hydroxybutyrate) were observed in diabetic patients as opposed to non-diabetic patients. Breath acetone levels were found to increase with blood β-hydroxybutyrate and blood acetoacetate levels. This might suggest a potential to develop breath gas analysis to provide an alternative to blood testing for both type 1 and type 2 diabetes monitoring, and to assist with the prevention of diabetic ketoacidosis.

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CHAPTER 4

Evaluating the effect of varying ethanol and water compositions on the acetone sensing properties of WO_3 for diabetes mellitus diagnosis

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4.1 Introduction

Tungsten oxide-based gas sensors have attracted a lot of attention in breath acetone analysis due to their potential in the clinical diagnosis of diabetes. In nanomedicine, early diagnosis and monitoring of the diseases is considered a need in the world; especially for diabetes mellitus due to continuous painful pricking. The exhaled human breath has been widely used as a potential alternative for the study and diagnosis of medical conditions. Different analytical instruments have been successfully used to detect and quantify Volatile Organic Compounds (VOCs) in human breath. This includes techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) [1], Shift-MS [2], PTR-MS [3], etc. The drawbacks of this method are portability, which requires a trained operator, sample preparation and collection.

Semiconducting metal oxides sensor, on the other hand, has the potential to detect VOCs at low concentrations as low as 0.1 ppb. Other advantages of semiconducting metal oxides sensors include, facile and cheap device fabrication, portability, real-time analysis, facile operating principle (resistivity change upon exposure of acetone to the SMO's surface layers). In this chapter, we used tungsten trioxide (WO_3) as a material of interest to investigate its selectivity and sensitivity towards low acetone in a simulated human gas condition. Many studies have been conducted on acetone selectivity and sensitivity, however, most reports were conducted at a very high operating temperature [4-9] and other added either noble metal on the surface or a surfactant[10]. All the mentioned great works are however not good enough for potential use in diabetic diagnosis as they report low sensitivity for acetone detection and high working temperatures.

Herein, we report the gas sensing performance of WO_3 materials which were synthesized by varying water and ethanol ratios using a facile solvothermal method for low ppm acetone detection at a low operating temperature.

4.2 Research questions

- Can the as-prepared WO_3 detect low ppm acetone concentration of acetone?
- Can the as-prepared WO_3 selectively detect acetone gas over other tested gases?
- Can the as-prepared WO_3 detect and select acetone at a low operating temperature?

4.3 Hypothesis

The solvothermal method allows for a change in crystalline phases and control of the morphology of WO_3 such as high aspect ratio structure and large surface area which plays a crucial role in achieving desired acetone sensing properties.

4.4 Aims of the study

- To Synthesize Tungsten Trioxide (WO_3) using the facile solvothermal method for acetone sensing screening.
- To optimize the acetone sensor produced with varying solvent ratios.
- To investigate the sensitivity, selectivity and low-temperature acetone sensing properties of the as-prepared WO_3

4.5 Materials and Methods

4.5.1 Materials

Tungsten hexachloride (WCL6)	Sigma Aldrich, Germany
90% ethanol	Sigma Aldrich, Germany

4.5.2 Instrumentation

To ascertain the tungsten oxides phases, X-ray diffraction (XRD) (Panalytical X'pert PRO PW 3040/60) equipped with a Cu-Kα (λ = 0.15418 nm) monochromatized radiation source was used. Scanning electron microscopy (SEM) studied by Auriga Zeiss field emission scanning electron microscopy and transmission electron microscopy (TEM) JOEL 2100 was used for the morphology of the samples. The surface area and surface diameters of tungsten oxides were characterized by BET Micrometirics TRISTAR 3000.

4.5.3 Synthesis of nanostructured tungsten oxides.

This work reports a facile method for the synthesis of tungsten oxide nanostructures with varying morphologies such as nanoparticles, nanocubes, and nanorods. The process also enables for the change in the crystalline phases, which includes hexagonal WO_3, monoclinic $W_{18}O_{49}$, and monoclinic WO_3, by varying the solvent composition. An amount of 4.05 g of tungsten hexachloride was dissolved in 100 mL of ethanol as a stock solution. To prepare the tungsten trioxides nanostructures, an amount of 10 ml of the stock solution was mixed with the following ratios of ethanol and water. Sample 1, named S1 was based on as-purchased ethanol (90% ethanol: 10% water), sample 2 (S2) was 1:9 of water: 90% ethanol translating to ~18 water content, sample 3 (S3) was 51:49 of water: ethanol and sample 4 (S4) was based on pure distillled water translating to 92% water content. The solution was then transferred to a 100 mL Teflon-lined acid digestion bombs. The reaction was run at 200 °C for 10 h using an electric oven. After, the as-prepared were collected and washed with ethanol by centrifugation (Pro-Analytical C2004) at a relative centrifugal force (rcf) of 1500 rcf for every 15 minutes until the solution is clear to remove any impurities.

4.5.4 Fabrication of sensors and Sensing Measurements.

The sensor device was prepared using the alumina interdigitated electrodes (2mm x 2mm) (sustruto sensors) with one-side consisting of a pt-electrode and the other side having a micro-heater that is used for gas sensing measurements. The as-prepared nanostructured powders were dissolved in ethanol to form a paste and drop cast the paste uniformly onto the pt-electrodes of the alumina substrate. The impregnated substrate was then heated at 300 ˚C for 1 hour to remove the solvent and for the adhesion of the paste. The gas sensing measurement was conducted using a gas sensing station KSGAS6S (KENOSISTEC, Italy). The measurements were tested at 50 °C, 75 °C, 100 °C, 150 °C and 200 °C by changing the voltage. Synthetic air with a constant flow rate of 0.5 l/min was used as a carrier gas. The acetone gas was tested at varying humidity ranging from 0, 10, 20, 40, 60, 80 %RH to best suit application in breath acetone detection. Other gases such as C_7H_8, NO_2, NH_3, H_2S, and CH_4 were tested with dry air. The response of the sensor was determined using R_a/R_g where R_a is the resistance of the sensor in dry air while R_g is the resistance of the sensor in the target gas.

4.6 Results and Discussion

4.6.1 X-ray diffraction

The effect of varying solvent mixing ratios during the synthesis of WO_3 samples was examined by X-ray diffraction (XRD). Figure 4.1 depicts the XRD patterns of the as-prepared tungsten oxides, which show that phase composition and intensities of the reflections from different planes depended on the solvent mixing ratio. The diffraction peaks of the sample (S1) prepared with ethanol could be indexed to the standard hexagonal WO_3 (JCPDS: 33-1387), and the one (S2) prepared with 82:18 EOH: H2O could be indexed to the standard W18O49, (JCPDS: 05-0392). When the water content was increased in the remaining two samples (S3 and S4), the monoclinic WO_3 (JCDPS: 00-0043-1035) was observed. The results observed here are in agreement with the HRTEM study which is discussed later in the paper. Using the Bragg's equation $n\lambda = 2dsin\theta$, where d is the inter-planar-spacing observed on HRTEM images, we found that the 2θ correlate well on all the samples.

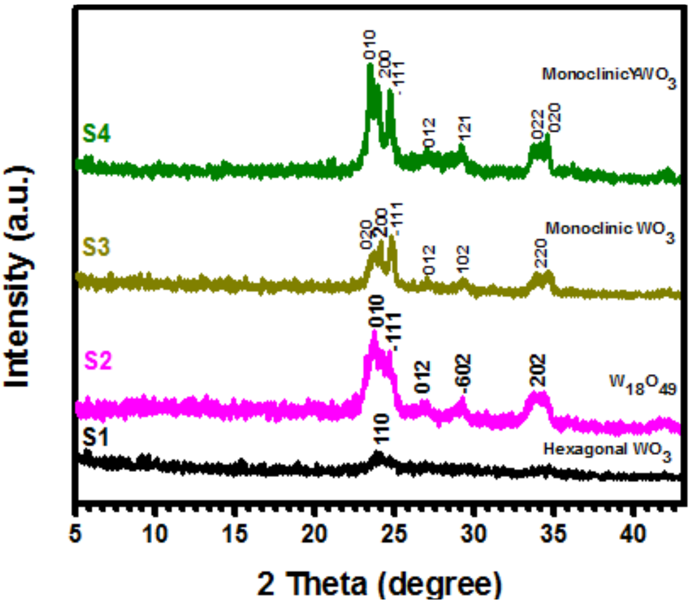

Figure 4.1 The XRD patterns of the as-prepared WO_3 nanostructures obtained after varying solvent content from pure ethanol.

The diffraction peaks of sample (S1) prepared with ethanol only compared with the standard hexagonal WO_3 (JCPD: 33-1387), the S2 prepared with 9:1 EOH: H2O compared with standard W18O49, (JCPDS: 05-0392) and S3 (1:1 EOH: H2O) and S4

(H2O-only) with increased water content compared to monoclinic WO_3 (JCDPS: 00-0043-1035).

The predominance of (010) plane shows that the WO_3 nanorods grew along the [010] direction. Furthermore, it can be seen that the (010) plane is parallel with the (020) which corresponds with the layered rods on the scanning electron microscopy micrograph (Fig 4.2). When the water content increases, we observed the monoclinic WO_3 (JCDPS: 00-0043-1035) in both samples with 51:49 and 18:82 (ethanol: water) ratios (see figure 4.1). The results confirm that the amount of water in the solvent can control the crystallinity of tungsten oxide. It was worth noting that the oxygen which was in water contributed to the formation of tungsten oxides crystalline phases. Furthermore, the crystallite size D of the dominating peak (010) was calculated using Scherer's equation:

$$D = \frac{0.9\lambda}{\beta COS\theta} \tag{1}$$

where λ and β are the wavelengths of CuKα radiation and the FWHM of the (010) peak.

It was noted that the crystallite size D of the material decreases with an increase in water (see Table 4.1). Trinh et al. [11] highlighted that the metal oxides sensor's response is mainly dependent on the crystallite size of the material of interest being tested. This indicates that as the grain size decreases, there will be an improvement of the gas sensor response to gases. When the grain size is comparable to 2L (L being the thickness of the layer), D=2L a space charge develop in the region of crystallite and maximum response will be achieved.

4.6.2 Morphological Characterization

The effect of varying solvent mixing ratios on the morphology and microstructure of WO_3 was further examined using SEM and HRTEM, respectively. The wet chemical synthesis enables the control of the particle size, shape, and the possibility of large-scale production as compared to physical synthesis. Tungsten oxide nanostructures having different morphologies of one-dimensional nanostructures, such as nanoparticles mixed with nanorods, nanorods mixed with nanoplates and nanorods only, were produced by the solvothermal process. Figure 4.2 shows the SEM morphologies of WO_3 obtained from samples synthesized by varying the solvent contents. As can be seen from Figure 4.2 that as the content of water in solvent is increased, the shape of the particles changed from the mixture of nanoparticle and rod-like structures in Figure 4.2(a), nano rod-like structures in Figure 4.2(b), a mixture of nanoparticles and plate-like structures in Figure 4.2(c) to a mixture of larger nanorods and nanoparticles in Figure 4.2(d).

Thus, samples prepared using high ethanol content produced nanoparticles and nano rod-like structures while the ones prepared using high water content formed plate-like structures, nanorods, and nanoparticles. As seen from XRD results, SEM observations also confirm that the morphology of WO_3 was controlled by varying the compositions of water and ethanol

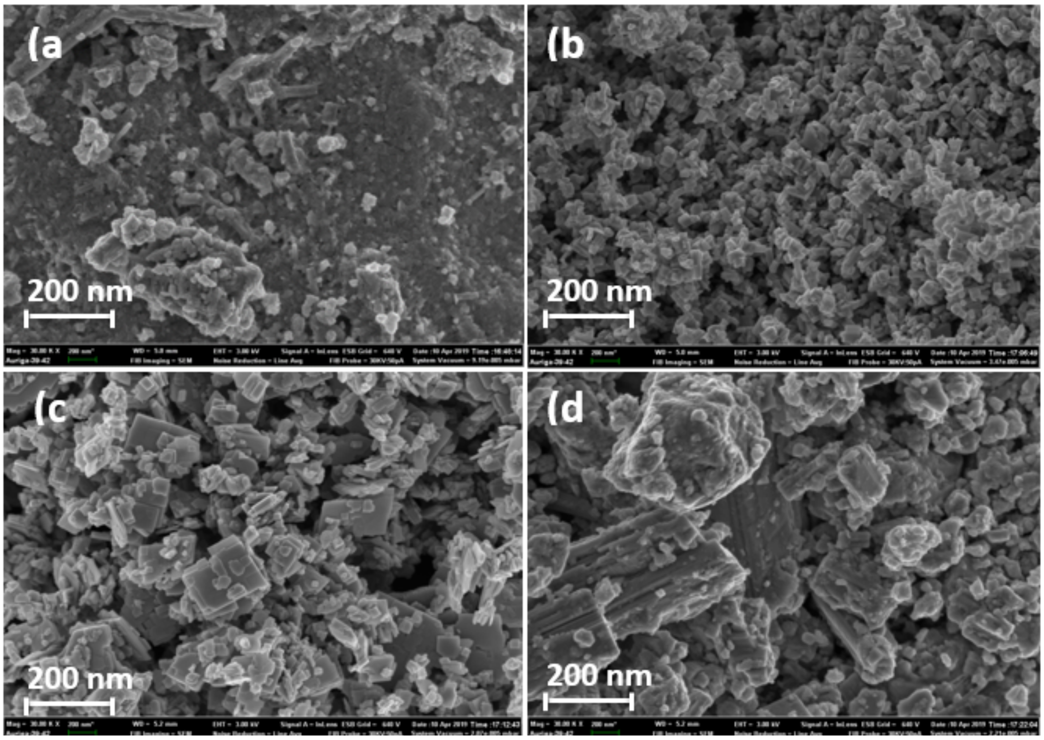

Figure 4.2 SEM morphologies of the as-prepared WO_3 nanostructures obtained after varying solvent from (a) pure ethanol to (b) 1:9 (ethanol: water), (c) 1:1 (ethanol: water) and (d) pure water.

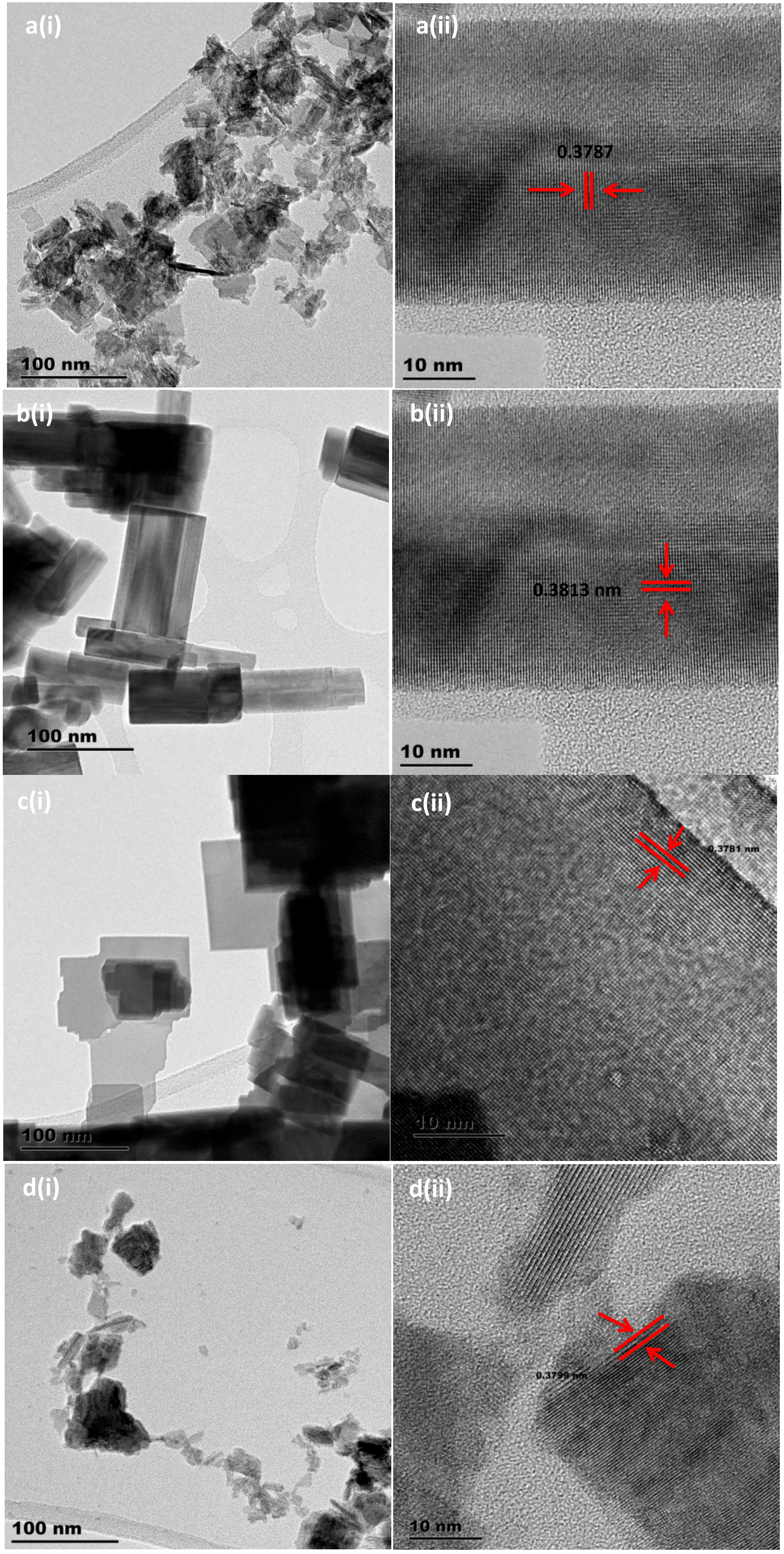

Figure 4.3 HRTEM morphologies of the as-prepared WO_3 nanostructures obtained after varying solvent from (a) pure ethanol to (b) 1:1 (ethanol: water), (c) 1:9 (ethanol: water) and (d) pure water.

HRTEM morphologies in Figure 4.3 (a-d) are in good agreement with SEM in Figure 4. 2 (a-d) that when the solvent composition changes, the morphology also changes. Interestingly, the HRTEM micrograph in Figure 4.2(b), shows that the nanorods grew along the [010] direction, which is also in agreement with the XRD. Moreover, these results show that the composition of the solvents contribute significantly to the formation of crystalline phase and morphology of tungsten oxide, like nanorods WO_3, nanorods $W_{18}O_{49}$, and hexagonal nanoparticle mixed with rods-like WO_3 [12, 13].

HRTEM study also confirmed the crystallite size and different phases as observed with XRD. Using Bragg's equation, phases were confirmed and the XRD results are found to be in agreement with the HRTEM. The crystallite size of S1, S2, S3, S4 were found to be ~11.3, 8.0, 7.5 and 7.7, respectively. The crystallite estimation is in accordance with the size calculated using Scherer's equation. As the ratio of water and ethanol is nearly equal, crystallite size decreases and increases when there is nearly pure ethanol and water as a solvent. This trend is also observed in the surface area and XRD analysis. Therefore, the sample produced with a nearly equal ratio of water to ethanol is expected to have the highest response to acetone gas as compared to other samples.

4.6.3 Proposed growth mechanism - Wang et al. model.

From the SEM and HRTEM observations, we have learned that the water in the reaction controls the phase and morphologies of the as-prepared tungsten oxide nanostructures. This is consistent with the mechanism proposed by Wang et al. [14] wherein the formation of WO_3 takes place, firstly by nucleation and secondly by subsequent growth. For nucleation, the samples prepared in the non-aqueous system, ethanol slowly decomposes as illustrated in reaction (1) with simultaneous release of water molecules, which coordinates the W^{6+} cations to form tungsten oxy/hydroxychloride complexes ($[W(OH)_xCln]^{6-(n+x)}$ or $[WO_yCln]^{6-(n+2y)}$). Afterward, WO_3 nuclei are produced along with the condensation/ polymerization of the complexes (reaction (1)).

$$CH_3CH_2OH \rightarrow CH_2CH_2 + H_2O \qquad (1)$$

$WCl_6 + 3H_2O \rightarrow [W(OH)_xCln]^{6-(n+x)}$

Or (2)

$[WO_yCln]^{6-(n+2y)} \rightarrow WO_3 + 6HCl$

The addition of 51 % water in sample S3, induced hydrolysis of WCl_6, the Cl^- ions were replaced by OH^- ions and produce W–OH. Below is the reaction scheme for the formation of WO_3 nuclei [15].

$WCl_6 + 6H_2O \rightarrow W^{6+} + 6Cl^- + 6H+ + 6OH^- \rightarrow (W^{6+} + 6OH-) + (6H^+ + 6Cl^-)$ (3)

Or (4)

$(W^{6+} + 6OH-) \rightarrow W(OH)_6 \rightarrow WO_3 + 3H2O$

4.6.4 Proposed growth mechanism- Current model.

The Wang et al. model is good for the reaction of WCl_6 with H_2O in the absence of ethanol. Also, the reaction of WCl_6 directly with H_2O is energetically expensive. For instance, it requires a lot more energy (ΔH 0f (298K) = -1693 kJ/mol) to dissociate water into H^+ and OH^- than dissociate ethanol (ΔH 0f (298K) -178kJ/mol) into $CH_3CH_2O^- + H^+$. In accordance with the Kirchhoff's law applied to chemical reactions, one would expect WCl6 to react more with ethanol than with water. Based on a simple current-divider model where chemical potential powering a reaction has two pathways running in parallel with enthalpies (or activation energies) of such pathways represented as resistances, it can be shown that one can calculate the relative probabilities of WCl_6 reacting with ethanol or water to be 88% or 12% respectively.

This calculation shows that although ethanol is more likely to take charge in the reactions, the WCl_6 reaction cannot be entirely trivialized as there is still a 12% chance. This analysis explains why the final WO_3 produces from the ethanol-water solvent will have phases, composition (stoichiometry) and morphology depends on the composition of ethanol and water in the said solvent.

4.6.5 Predicting the properties of ethanol-water mixture/solvent and its effects on the final WO_3 morphology

We refer to a classic theory called effective medium theory [16] for us to calculate the properties of the ethanol-water mixture of various compositions of ethanol and water which is presented by equation (5). For instance, we can estimate the water-composition dependency of the dielectric constant of the ethanol-water mixture as follows. The dielectric constants are presented in figure 4.6 against the BET surface and water-ethanol ratios. More details on the effect of the solvent ratio on the sensing properties are discussed in section 4.6.7.

$$\varepsilon_{eff} = \varepsilon_{ethanol} + 3p_{water}\varepsilon_{ethanol}\frac{\varepsilon_{water} - \varepsilon_{ethanol}}{\varepsilon_{water} + 2\varepsilon_{ethanol}} \tag{5}$$

Table 4.1 Summary of water content, surface area, pore diameter, crystallite size

Sensor	p_{water}	Surface area	Pore Diameter	Crystallite Size
S1	10	73.16	4.47	11.36
S2	18	12.35	3.61	8.02
S3	51	14.77	11.63	7.51
S4	92	60.82	4.05	7.74

4.6.6 Brunauer Emmett Teller (BET) analysis

The BET analyses were conducted to give more insight into the porous nature of WO_3 structures and the effect of varying solvent mixing ratios. The characteristics such as the surface area, volume and pore size of WO_3 nanostructures are summarized in Table 4.2. It has been observed that the surface area increased for sample S1 prepared by nearly pure ethanol and decreased for samples S2 and S3 prepared by 51:49 of ethanol: water ratios, and later increased for sample S4 where nearly only water was used as a solvent. This observation is consistent with the crystallite size observed with XRD and HRTEM. The pore volume decreased with the addition of water, however, a high pore diameter of about 10.54 nm was observed at a solvent ratio of 51:49 (ethanol: water) as compared to other samples, i.e., sample S3 having the largest value of DP (pore diameter). It may not have the largest SBET (surface area per unit mass) and it may also not have the largest VP (the volume of the pore per unit mass) but the fact that it has the largest Dp suggests that Dp gives it the biggest advantage to sense acetone over the other samples.

Table 4.2 Summary of the BET specific surface area, pore volume (Vpore) and pore diameter.

Sample name	**Morphology**	**S_{BET} (m^2/g)**	**D_p (nm)**	**V_p (cm^3/g)**
S1	Nanoparticles, Nanorod-like	73.16	4.4771	0.072526
S2	Nanorod-like	12.35	3.6157	0.005885
S3	Nanoparticles, Sheet-like	14.77	11.6315	0.043437
S4	Larger Nanorods, Nanoparticles	70.35	4.0528	0.051541

Furthermore, the porous structure of the as-synthesized tungsten oxides indicates that there was a removal of the solvents and interspaces among the assembled nanorods and particles. These WO_3 nanostructures with higher surface areas and porosity play a significant role in the performance of gas sensing materials. The sample with higher pore diameter showed a higher response and selectivity to acetone as compared to the sample with the higher surface area. As it was also observed and mentioned by Tshabalala et al.[17], a large surface area alone does not influence the good sensing response, it is however influenced by the porous surface which ultimately allows the gas to be adsorbed into the whole sensing layer, enhancing the response of the sensor.

4.6.7 Gas sensing properties of WOx sensors

The sensing performances of WO_3 samples synthesized by varying solvent mixing ratios to acetone concentration ranging from 0.5 to 4.5 ppm were investigated at 50, 75, 100, 150 and 200 °C, respectively. Figure 4.4(a-e) shows the dynamic response curves of the sensors. All the sensors demonstrated an increase in sensors response upon an increase in acetone concentration. This behavior is common for the semiconducting metal oxides including WO_3. It can be described by mechanics and kinetics of gas adsorption and desorption [18]. Additionally, the n-type semiconductor metal oxides have been proven to have great sensing properties for reducing gases [17]. The gas sensing response (R) is defined as:

$R = Ra/Rg$ (6)

Where Ra and Rg represent the resistance of the sensor in dry air and the target gas, respectively.

Sensor S3 showed the highest response to acetone in all temperatures as compared to other sensors. For example, the response of 2 ppm acetone at 50 °C was 1.9, 2.4, 5.3, and 3.42 for S1, S2, S3, and S4, respectively, the response at 100 °C was 172.82, 278.53, 534.93 and 321.05, respectively. In the two mentioned temperatures, the response of S3 was almost double compared to all the other samples. This was also observed in the two remaining temperatures (150 and 200 °C). This could be attributed to the high pore diameter and monoclinic phase. The high pore diameter, in this case, plays a crucial role as it offers more sites for surface adsorption and desorption of acetone. Interestingly, the results obtained here are not entirely surprising that the high surface area does not always contribute to an excellent sensing response. Tomer et al [19] and Tshabalala et al [17] also observed the same findings. Sample S3 has less surface area as compared to other samples but showed high response in all temperatures.

We observed an increase in sensor response from 50 ◦C, 75 ◦C, 100 ◦C and with the highest respond at 100 ◦C, and a decrease from 150 ◦C and 200 ◦C as shown in Figure 4.5. When the operating temperature is low, the chemical activation of nanoparticles, nanorods and nanoplates can be low and consequently result in a low response. However, increasing the temperature usually leads to a positive chemical activation, with the gas sensor demonstrating maximum response at the operating temperature of 100 °C. This temperature was therefore selected as the operating temperature for further investigation in this study. More interestingly, all the sensors could respond to even a low concentration (0.5 ppm). This gives an advantage to the current sensors in an application that requires low concentration detection of acetone especially for diabetes mellitus monitoring and management.

As most good performing acetone sensors are achieved at high ppm concentration, for example, this study [20], the Y-doped SnO_2 showed good acetone performance at 50 ppm.

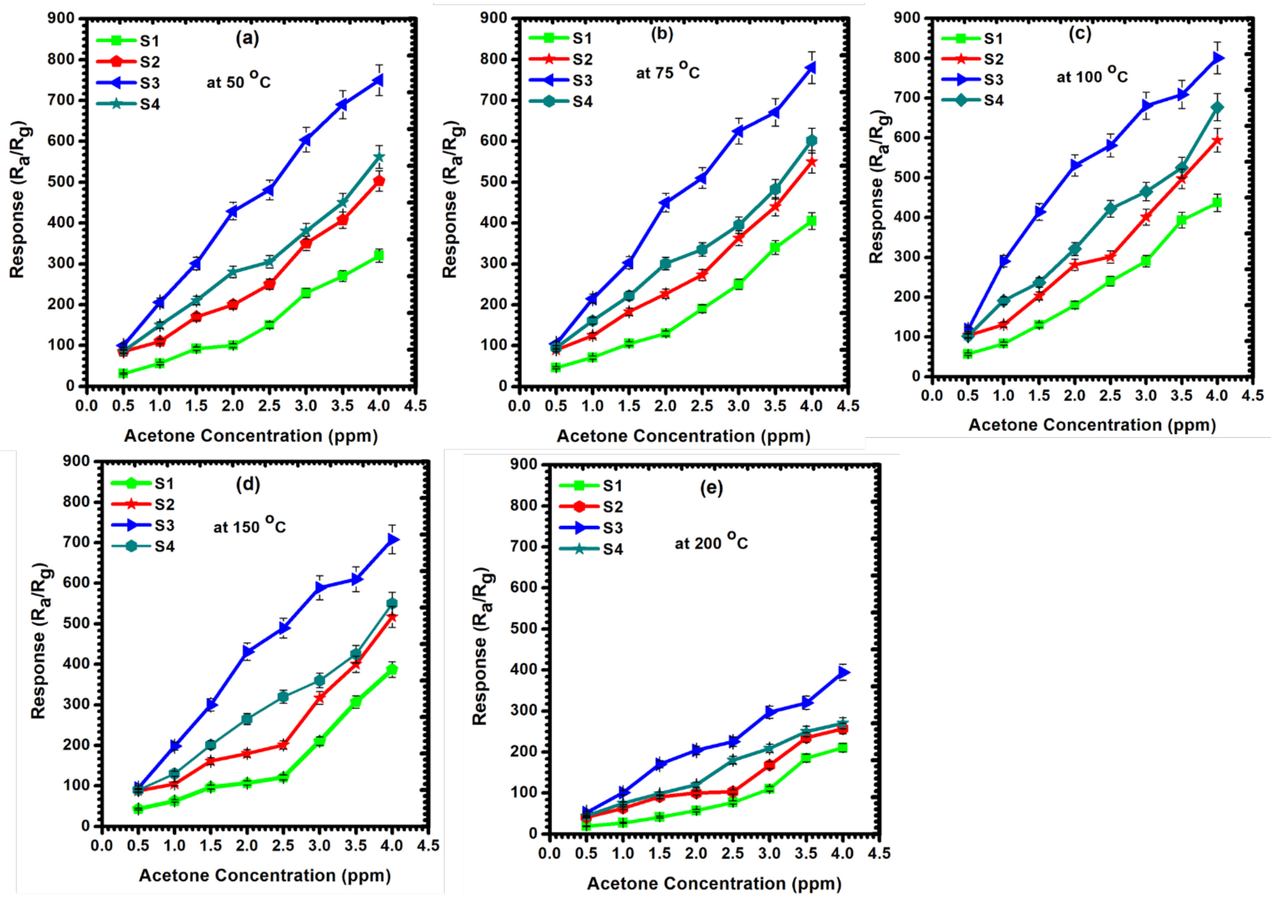

Figure 4.4 Response versus acetone concentration for samples synthesized with varying solvent composition at (a) 50 °C, (b) 75 °C (c) 100 °C, (d) 150 °C and (e) 200 °C.

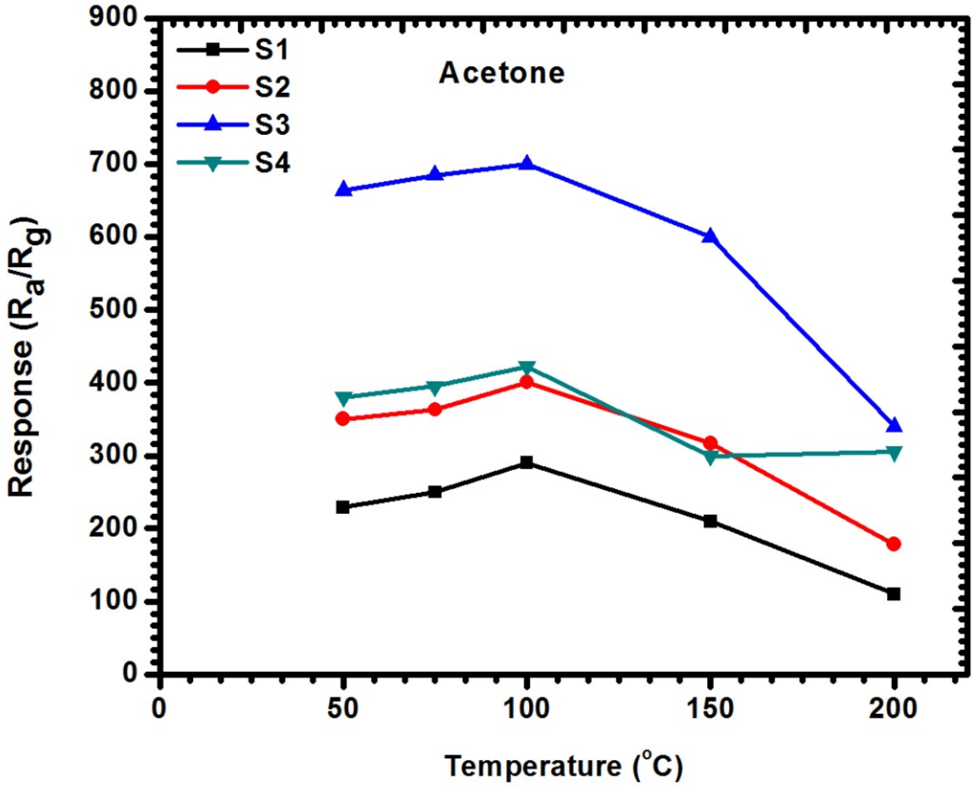

Figure 4.5 Response of sensor synthesized with varying solvent composition to 2 ppm of acetone at different operating temperatures (50-200˚C)

Figure 4.6 depicts that when the S3 sensor is exposed to dry acetone, the response increases with increasing acetone concentration. However, the response to acetone decreased with an increasing %RH. This is due to the gas sensing sites of the sensor mostly occupied by a H_2O vapours with an increase of %RH. Therefore, the concentration of chemisorbed oxygen on the sensor will decrease and results in the weakened acetone response. Furthermore, this plot can also clearly distinguish a diabetic patient region and a healthy patient region. The response to dry acetone is 1.3 and for acetone at 60% RH (ambient relative humidity in the human breath) is 0.64 both tested at 1 ppm. The difference in sensor response is 0.66; this makes it possible for the diagnosis and monitoring of diabetes mellitus by using breath acetone.

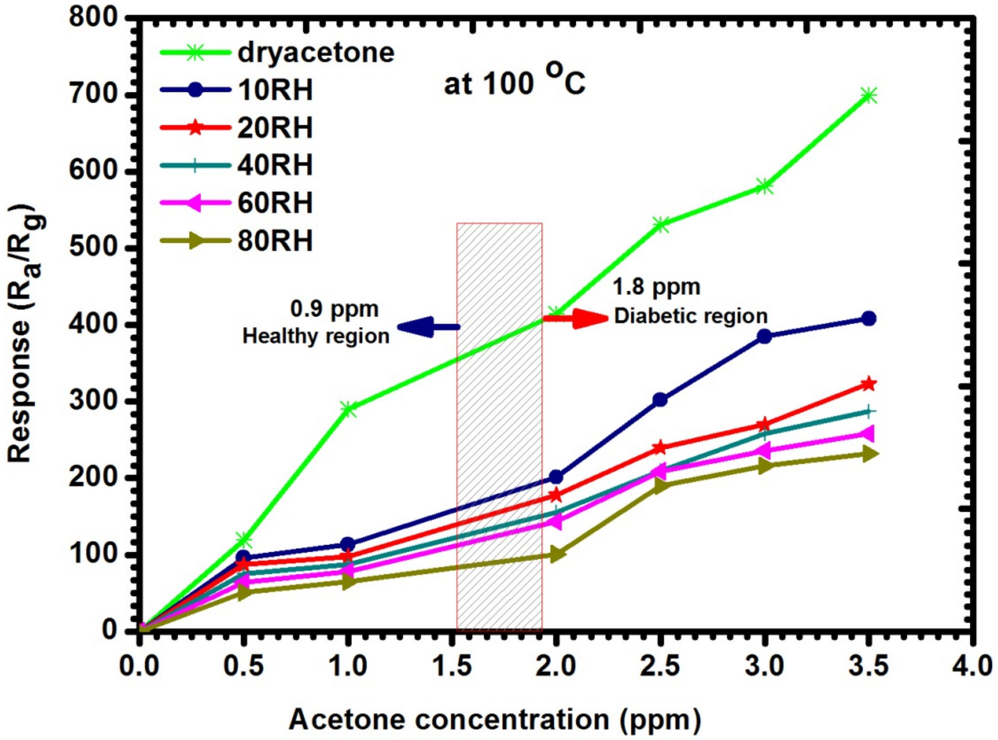

Figure 4.6 Sensor (S3) responses to acetone concentration (0 - 4 ppm) at varying RH from 0 to 80% at 100 °C. Diabetic patients (> 1.8) can be clearly distinguished from healthy patients (<0.9) by, at least, 67% difference in sensor response.

In gas sensors, selectivity is one of the key parameter more especially gas sensors for disease detection to ensure correct diagnosis and avoid false-positive results. To perform the selectivity test, we used the selectivity (σ) equation [21]:

$$\sigma = \frac{R_{gas}}{R_{gas1} + \ R_{gas2} + R_{gas3} + R_{gas4} + \cdots + R_{gasN}} \times 100$$

where R_{gas}, is the gas of interest and $R_{gas1} + R_{gas2} + \cdots + R_{gasN}$, is the sum of all gases.

The values for selectivity of the gases against the sensors were calculated from the responses obtained from 0.5 to 4, at 60% RH (ambient) ppm at 100 °C are presented in Table 4.3. The results show that all the sensors select acetone better than any other gases, however, sample S3 showed the highest selectivity. The high sensitivity and response of S3 could be influenced by high pore diameter, small crystallite size, and plate-like morphology. It has been reported that the pore diameter can positively influence the sensitivity of the sensor.

Table 4.3. The response, sensitivity (σ), WO_3 tested at various gases at 100 C, at 0.5 to 4 ppm

ppm	Sensor	Response (%)						Selectivity					
		Acetone	Toluene	NO_2	NH_3	H_2S	CH_4	Acetone	Toluene	NO_2	NH_3	H_2S	CH_4
0.5	S1	57	11.48	9.34	15.4	10.36	21.11	84,2*	10.14	8.09	14.09	9.06	16.92
	S2	104	31.48	17.04	28.1	18.90	38.51	77,59*	15.24	7.71	13.38	16.41	19.3
	S3	119	37.03	19.5	32.16	21.63	44.07	77,07*	15.66	7.68	13.33	8.650	19.21
	S4	102	31.85	16.72	14.36	18.54	37.77	85,54*	16.81	8.71	6.94	76.55	20.58
1	S1	83	20.74	13.6	22.13	15.09	30.74	81,13*	12.6	7.92	13.56	13.56	19.88
	S2	131	40.74	21.47	35.4	23.81	48.51	77,09*	25.42	15.65	13.33	9	19.21
	S3	290	76.29	47.54	78.37	52.72	107.4	80,04*	13.24	7.86	13.65	8.79	19.7
	S4	191	55.55	31.37	51.62	34.72	70.74	78,27*	14.64	7.77	13.46	8.67	19.42
1.5	S1	130	34.07	21.31	35.13	23.63	48.14	80,1*	13.19	7.86	13.66	8.79	19.71
	S2	203	62.96	33.27	54.86	36.90	75.18	77,13*	15.61	7.68	13.33	9	19.22
	S3	414	111.48	67.86	116.9	75.27	153.33	78,88*	13.47	7.79	14.22	8.71	19.52
	S4	237	77.77	38.85	64.05	43.09	87.77	76,07*	16.52	7.62	13.22	8.52	19.04
2	S1	180	37.03	29.5	48.64	32.72	66.66	83,89*	10.35	8.08	14.01	17.99	20.33
	S2	281	74.07	46.06	75.94	51.09	104.07	80,01*	13.27	7.85	26.57	8.79	19.7
	S3	531	158.88	87.86	143.5	96.54	196.66	77,69*	15.05	7.79	13.41	8.63	19.32
	S4	321	103.7	52.62	86.75	62.94	118.8	75,56*	16.15	7.59	13.16	9.21	18.94
2.5	S1	240	55.55	39.34	64.86	43.61	88.88	82,12*	11.65	7.98	13.87	8.92	20.04
	S2	301	76.6	49.35	81.35	54.72	111.48	80,58*	12.81	7.89	13.71	17.16	19.81
	S3	581	178.14	95.24	157	105.63	215.18	77,34*	15.43	7.69	13.36	8.61	19.26
	S4	422	112.96	63.6	104.9	70.50	143.7	85,14*	14.03	7.44	12.91	8.32	18.56
3	S1	290	84.81	47.54	78.37	52.72	107.4	78,21*	14.72	7.75	13.45	8.66	19.41
	S2	401	129.62	65.73	108.4	72.90	148.51	76,36*	16.27	7.63	13.25	8.55	19.09
	S3	681	245.92	114.75	189.2	127.27	259.25	72,72*	17.93	7.63	12.56	8.54	19.08
	S4	465	140.74	69.18	114.1	76.72	156.29	83,48*	15.97	7.26	12.56	8.11	18.12
3.5	S1	437	100	64.42	106.2	71.45	145.55	89,61*	13.15	7.48	12.97	8.37	18.68
	S2	594	150.74	81.47	134.3	90.36	184.07	92,67*	13.91	7.06	12.21	7.89	17.51
	S3	801	265.92	114.26	188.4	126.72	258.14	84,04*	17.86	6.96	12.02	7.78	17.25
	S4	677	155.5	82.13	135.4	91.09	185.55	100,42*	13.27	6.59	11.36	7.37	16.26
4	S1	320	118.51	71.63	118.1	79.45	161.85	58,23*	15.78	15.71	10.05	10.05	22.87
	S2	503	186.29	97.37	160.5	108.26	220.31	65,99*	17.09	8.26	14.39	9.27	20.87
	S3	750	277.77	131.31	216.5	145.63	296.65	70,23*	18.03	13.51	8.71	8.73	19.51
	S4	562	208.14	110.98	183	123.09	250.41	22,31*	7.24	14.58	9.36	9.45	21.09

When the values of the morphological properties in Table 4.1 of the final WO_3 samples are plotted against the corresponding dielectric constants of the ethanol-water solvents and the water content in the mixture thereof, the profiles as shown in Figure 4.7 are obtained. These profiles suggest that both specific surface area and specific pore volume have similar trajectories with a minimum value between sample 2 and sample 3 whereas specific pore diameter has a definite maximum in sample 3 where the solvent had a water content of 51% and an effective dielectric constant of 42.

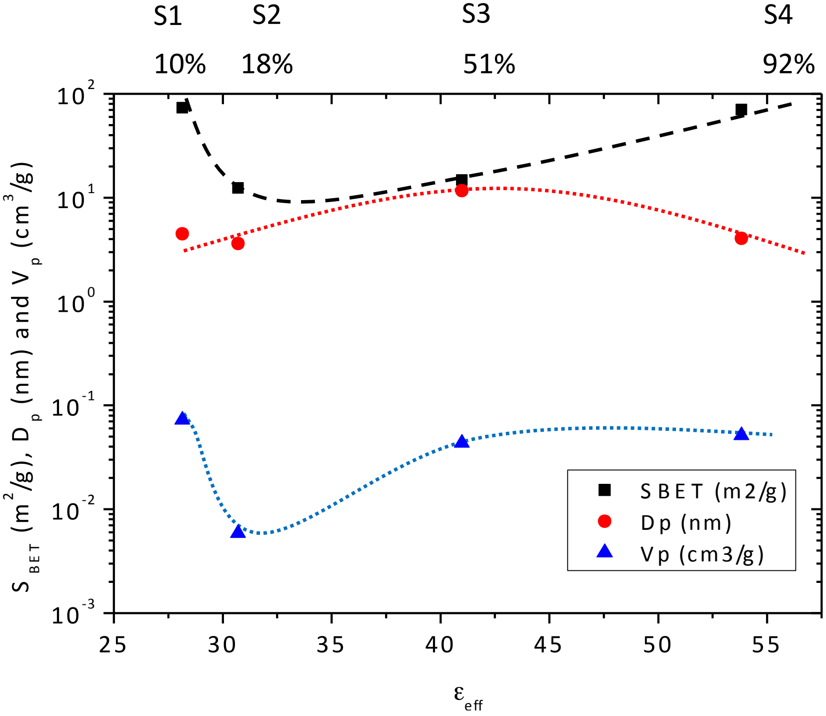

Figure 4.7 Surface properties of S1, S2, S3, and S4 against the corresponding dielectric constants of the ethanol-water solvents and the water content in the mixture against the BET surface area.

We further used selectivity data in Table 4.3 and plotted against all the gases at 2 ppm and 100 °C. As can be seen from figure 4.8, we observed that acetone shows higher selectivity towards acetone in all the sensors as compared to other gases. An ideal sensor should have a selectivity of 100% to one gas and 0% to other gases; however, there is a significant difference between an acetone gas and other tested gases. The high response, good selectivity, ability to respond well at varying humidity of the sample S3 which is produced by a very simple and cheap method opens an opportunity for further research investigations into the use of these sensors for application in breath acetone detection for diabetes mellitus monitoring.

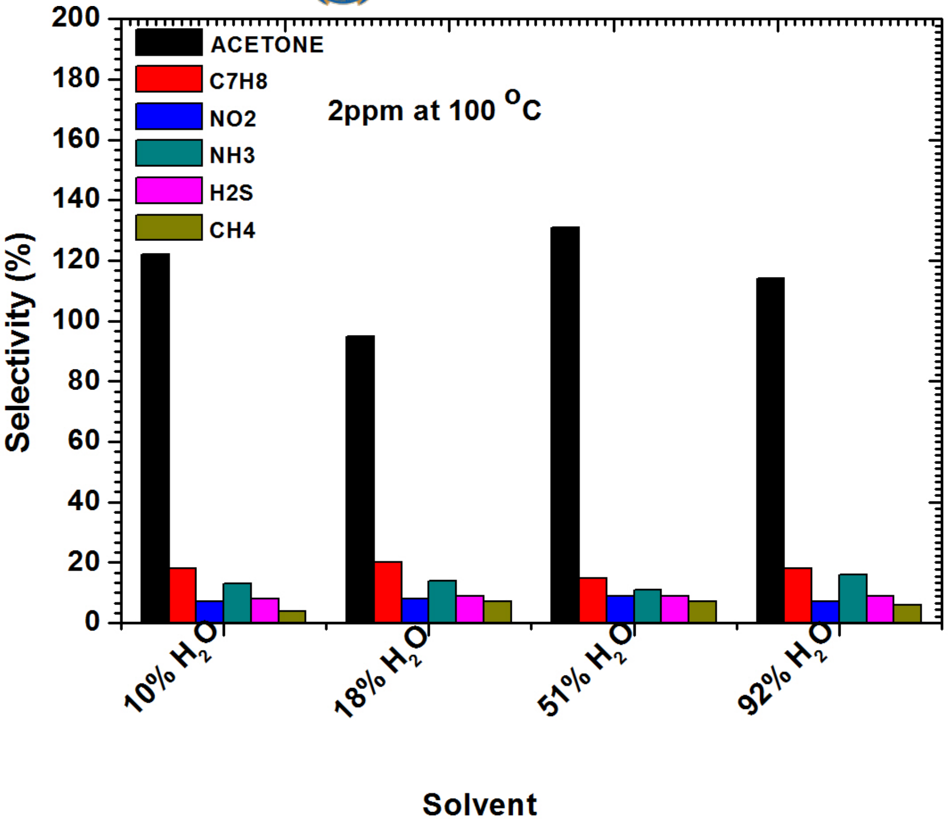

Figure 4.8 Selectivity values of sensor S3 produce with varying solvent content against varying gases at 100 C at 2 ppm concentration.

We have gathered the gas sensing performances of materials reported in the literature and compared it with this present work, the summary is shown in Table 4.3. Among these materials, it can be seen that WO_3 (S3 sensor) showed a good response at low operating temperature, without doping or functionalization. Furthermore, this also opens more research scope on a non-invasive way of monitoring diabetes mellitus using the breath acetone. We have further compared the selectivity of various materials in literature with our present work in Table 4.4. Quite interestingly, the current work demonstrates high selectivity towards acetone at low ppm of acetone at low operating temperatures. This makes it a promising material for the development of sensors for the detection of acetone.

Table 4.4 Gas responses of acetone in the present study and those reported in the literature

Material	**Fabrication method**	**Acetone concentration (ppm)**	**Operating temperature (°C**	**Rg/Ra**	**Ref**
WO_3-nanoplates	Topochemical conversation	1000	350	9	(9)
WO_3-nanoplates	Hydrothermal	2000	307	10	(10)
Cr-WO_3	Flame spray pyrolysis	1	400	2.9	(16)
WO_3-nanotubes	Electrospinning	100	250	40	(25)
Cu-WO_3	Electrospinning	20	300	6.4	(26)
WO_3	Solvothermal	**1**	100	**131**	**This work**

Table 4.5 Comparison of other materials from literature to the present WO_3's selectivity to acetone

Material	**Acetone concentration (ppm)**	**Operating temperature (°C**	**σ (%)**	**Ref**
$In/WO_3/SnO_2$	50	200	95	[19]
Co-WO_3	50	350	117	[25]
Pt-WO_3	2	350	78	[26]
ZnO	100	230	76	[27]
PdO/CO_3O_4	1	350	22	[28]
Co-ZnO	100	360	2	[29]
WO_3	**1**	**100**	**121**	**This work**

The mechanism of WO_3-based gas sensors has been intensively studied and reported in the literature [6, 30-33]. The gas sensing mechanism of WO_3 nanoparticles, nanocubes and nanorods sensors are described as follows:

The change in resistance of tungsten oxide sensors is dependent on the adsorbed oxygen species (O^{2-}, O^{-}) on the surface. Upon their exposure to air, O^{2-} gets adsorbed onto the surface which later adsorbs electrons from the conduction band. This decreases the concentration of electrons in the conduction band which ultimately decreases the electrical conductance of the material [34]. This mechanism is expressed as follows:

$O_2(ads) + e^- \rightarrow O^{2-}(ads);$ (7)

$O^{2-}(ads) + e^- \rightarrow 2O^-(ads);$ (8)

$O^-(ads) + e^- \rightarrow O2-(ads).$ (9)

In the case where the sensor is exposed to acetone gas, the chemisorbed oxygen species ($O_2^-{}_{(ads)}$, $O^{2-}{}_{(ads)}$, $O^-{}_{(ads)}$) react with acetone vapor and releases electrons back to the conduction band and as a result, electrical conductance increases. The reaction mechanism is expressed as follows:

$CH_3COCH_3\ (gas) + O^{2-} \rightarrow CH_3C+O + CH_3O^- + 2e^-;$ (10)

$CH_3C^+O \rightarrow C^+H_3 + CO;$ (11)

$CO + O^{2-} \rightarrow CO_2 + 2e^-.$ (12)

In the current study, the enhanced acetone performance of WO_3 indicates that the following parameters played a major role; large pore diameter, high crystallinity, and expedite diffusion channels of WO_3 nanorods. WO_3 nanorods having a pore diameter of about 10 nm and high crystallinity were found to have excellent acetone-sensing properties than the WO_3 nanoparticles. Since the pore diameter of both nanorods and nanoparticles are mesoporous, gas transport is thought to have occurred through molecular diffusion. This observation is in agreement with the study of Sakai et al., which revealed that gas transport in SnO_2 with larger pores occurred mainly through molecular diffusion whereas surface diffusion dominated in micropores. Surface diffusion will have less effect on smaller mesopores [31, 32].

4.7 Conclusions

The as-synthesized WO_3 composed of nanoparticles, plate-like structures and nanorods were successfully synthesized by varying solvent content using the solvothermal method. The sensor fabricated with 51:49 water: ethanol is found to demonstrate high response and good selectivity to 2 ppm level of acetone when compared with the one fabricated with pure ethanol, 18:92 (ethanol: water) and 92% water. However, the sensor exhibited low response under increased relative humidity at 100 °C. Furthermore, the sensor could respond to low concentrations of acetone ranging from 0.5 to 4.5 ppm of acetone at 100 °C. More interestingly, the sensor could distinguish the healthy region and diabetic region even under high relative humidity. These characteristics could be attributed to their high pore diameter, crystallite phase which consequently played a major role in the adsorption of oxygen species and enhanced the interactions of acetone and WO_3 on the surface. The gas-sensing performance of WO_3 indicates that it has a potential application in low concentration and fast response of acetone detection. These properties are suitable for sensors that can be used for the detection of exhaled acetone from human breath, more specifically in diabetic patients.

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CHAPTER 5

Co-doped WO_3 hierarchical nanostructures with carbon nanospheres template and their improved acetone sensing performance

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5.1 Introduction

The diagnosis and monitoring of diseases through the use of volatile organic compounds in human breath has gained much attention due to its non-invasiveness and early disease diagnosis [1-3]. Detecting VOCs in human breath is not new as the ancient physicians knew that the smell of fruity odor of acetone was associated with diabetes mellitus, the fish-like smell was linked to liver disease, urine-like-smell in the breath was associated with kidney failure [4]-[5]. The modern breath analysis emerged around the 1970s with scientists using gas chromatography (GC) identifying more than 200 components in human breath [6, 7]. Thus far, techniques such as PTR-MS, GC-MS, SIFT-MS and many more have been successful in detecting different VOCs in the breath. However, the biggest drawbacks are sample collection, vapor desorption and lack of portability, remaining key problems in making breath analysis economical and practical; in its use [8].

Semiconducting metal-oxide gas sensors, as opposed to the latter, have been given a lot interest in the field of breath analysis due to their captivating properties which indludes low-cost, ease of use, portability and low cost of manufacturing [9]. Furthermore, they are best suited for detecting volatile organic gases at low concentration levels in view of sensitivity, stability and robustness [10]. Thus making them an ideal candidate for a monitoring trace amount of gases such as acetone for disease detections.

Herein, we present Co-doped WO_3 with carbon as a template that has been successfully synthesized through the facile sol-gel method. The synthesized Co-doped-WO_3 was characterized by X-ray diffraction, Scanning electron microscope, Transmission electron microscope, energy dispersive X-ray spectrometry, and Brunauer–Emmett–Teller and X-ray photoelectron spectroscopy. The gas sensing properties of WO_3 doped with Co from 0 to 0.8 wt. % was also investigated on VOCs. The fabricated sensor based on 0.6 wt.% Co-doped WO_3 with carbon as a template showed good sensitivity, selectivity, fast response and recovery time towards 1.5 ppm of acetone at 50 °C under 90% relative humidity. The excellent gas sensing properties could be attributed to high surface area and small crystallite size of the WO_3 and Co catalysis effect which promotes gas adsorption.

5.2 Research questions

- Can Co doping on WO_3 improve the sensitivity and selectivity of the material to acetone gas?
- Can the carbon as a template help increase the surface area of the material and thus improve sensitivity and selectivity?
- Can the Co-doped WO_3 detect and select acetone at a low operating temperature?

5.3 Hypothesis

Cobalt doped-WO_3 nanoporous metal oxides with high surface area are ideal materials for improving gas sensing performances by enhancing sensing sites and total exposures to target gases.

5.4 Aims of the study

- To Synthesize Cobalt doped Tungsten Trioxide (WO_3) using carbon as a template for acetone sensing screening.
- To optimize the acetone sensor produced at varying cobalt dopant concentrations.
- To investigate the sensitivity, selectivity and low-temperature acetone sensing properties of the Co-doped WO_3.

5.5 Materials and Methods

5.5.1 Materials

Cobalt	Sigma Aldrich, Germany
Dimethylformamide	Sigma Aldrich, Germany
Glucose	Sigma Aldrich, Germany
90% ethanol	Sigma Aldrich, Germany
Tungsten hexachloride (WCL6)	Sigma Aldrich, Germany
Acetone gas	NMISA, South Africa
Toluene	NMISA, South Africa
Methane	Air Liquid, South Africa
Hydrogen Sulphide	Air Liquid, South Africa
Nitrogen Dioxide	Air Liquid, South Africa

5.5.2 Instrumentation

To determine the Co-doped WO_3 phases, X-ray diffraction (XRD) (Panalytical X'pert PRO PW 3040/60) equipped with a Cu-Kα (λ = 0.15418 nm) monochromatized radiation source was used. Scanning electron microscopy (SEM) studied by Auriga Zeiss field emission scanning electron microscopy and transmission electron microscopy (TEM) JOEL 2100 was used for morphology. The surface area and surface diameters Co-doped WO_3 were characterized by BET Micrometrics TRISTAR 3000. The crystalline phases Co-doped WO_3 were analyzed with the PANalytical X'Pert High Score Plus program. The elementary composition, X-ray photoelectron spectroscopy (XPS) measurements were carried out at room temperature.

5.5.3 Synthesis of Co-doped WO_3

Carbon nanospheres were synthesized by the Solvothermal method according to the literature method [11]. The Co-doped WO_3 nanoporous flower-like was prepared by the Sol-gel method. To prepare the Co-doped WO_3 nanostructures, an amount of 1g tungsten hexachloride was added into a 0.18 g carbon spheres suspension solution, followed by addition of 30 ml dimethylformamide (DMF). The solution was stirred at room temperature for 24 hours. After, the as-prepared were collected and washed by centrifugation (Pro-Analytical C2004) at a relative centrifugal force (rcf) of

1500 rcf for every 15 minutes until the solution is clear to remove any impurities, and dried at 60 °C for 12h. The dried precursors were further calcined at 400 °C in air for 2 h using a glass tube furnace (Thermolyne 2110, ATS scientific).

5.5.4 Fabrication of sensors and Sensing Measurements.

The sensor device was prepared using the alumina interdigitated electrodes (2mm x 2mm) (sustruto sensors) with one-side having a pt-electrode and the other side having a micro-heater that is used for gas sensing measurements. The as-prepared nanoparticles mixed with nanorods, nanorods, nanoplates mixed with nanorods and large nanorods powders were suspended in ethanol to form a paste and drop cast the paste uniformly onto the pt-electrodes of alumina substrate. The impregnated substrate was then heated at 300 ˚C for 1 hour to remove the solvent and for the adhesion of the paste. The gas sensing measurement was conducted using a gas sensing station KSGAS6S (KENOSISTEC, Italy). The measurements were tested at 50 ◦C, 75 °C, 100 °C, 150 °C and 200 °C by changing the voltage. Synthetic air (99,999 purity, Air ProductS, South Africa) with a constant flow rate of 0.5 l/min was used as a carrier gas. The acetone gas was tested at varying humidity ranging from 0, 10, 20, 40, 60, and 80 %RH to best suit applications in breath acetone detection. Other gases such as C_7H_8, NO_2, NH_3, H_2S and CH_4 were tested with dry air. The sensor response was defined as Ra/Rg where Ra is the resistance of the sensor when exposed to dry air and Rg is the resistance of the sensor when exposed to the target gas.

5.6 Results and Discussion

5.6.1 X-ray diffraction

Figure 5.1 illustrations the XRD patterns of tungsten trioxide doped with cobalt from 0 to 0.8 wt% cobalt with carbon spheres as a template. As can be seen, the samples are very crystalline and match well with monoclinic WO_3 (Ɛ-WO_3) from JCPDS file no. 04-007-2597. With the addition of cobalt and carbon, the 013 and 1115 peaks shifted a little bit from the pure WO_3. The sample which had no carbon (0.8% cobalt) showed to be more crystalline than the ones with carbon, indicating that the carbon was incorporated in the WO_3 lattice phase, stabilizing the Ɛ-WO_3 phase. This was also reconfirmed in HRTEM (Fig 5.4), where the samples showed a polycrystalline nature. There were no cobalt peaks detected, this indicated that the addition of Co might not have an effect on the crystallinity of WO_3. This might be from the fact that the Co concentration is too low and the radius of Co^{3+} (0.64A) and of W^{6+} (0.62A) are almost the same.

The grain size of the pure and Co-doped WO_3 was further investigated by the Scherer equation ($D=K\lambda/\beta\cos\theta$), where D is the average grain size; K is the shape factor (0.9), β is the broadening at half maximum and λ is the wavelength of the X-ray sources (0.154 nm for cu kα). The average grain size of peak 013 from pure to 0.8wt% Co-doped WO_3 with carbon nanospheres as a template was found to be 8.73, 4.6, 4.8, 3.77 and 6. 13 nm respectively. This result indicates that a sample 0.6% Co-doped has the potential to exhibit good sensing performance because small grain size can give high sensing properties [13].

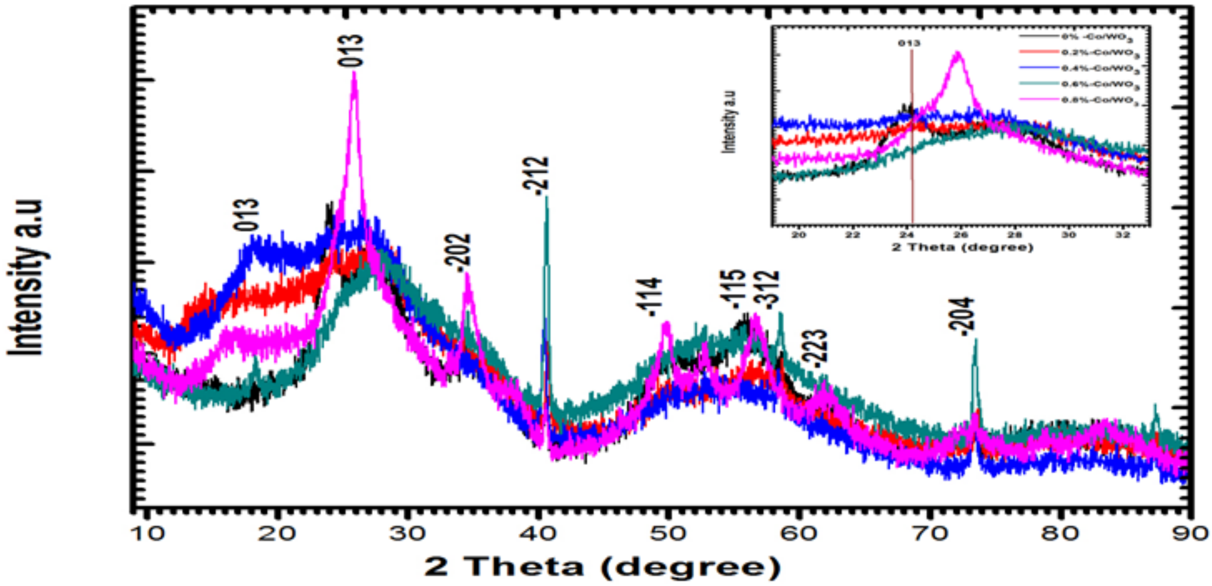

Figure 5.1 The X-Ray Diffraction pattern of WO_3 doped with Co at varying weight percentages.

5.6.2 Surface chemical composition

To confirm the addition of cobalt and carbon nanospheres on WO_3, the X-ray photoelectron spectroscopy (XPS) was employed. Figure 5.2(a) shows the full spectrum of pure and all Co-doped WO_3 samples. All the samples contain C, W, O and Co elements and their matched photoelectro peaks of C1, w4f, O1s, and Cop2 respectively. Figure 5.2(b) shows the W4f core level XPS spectrum of the pure WO_3 and all Co-doped WO_3 samples. Two peaks were observed corresponding to W4F1/2 and W4F5/2 with the values 35.9 eV and 38.0 eV for pure WO_3 and shifted with the addition of cobalt in the WO_3 lattice. Furthermore, figure 5.2(c) depicts the O1s core level binding of pure and all Co-doped WO_3.

The deconvoluted spectrum gives one peak with a binding energy of 53.1 eV. This peak can be assigned to the oxygen that bonds to tungsten. The high-resolution C1s spectrum in figure 5.2(d) is deconvoluted using four individual components which represent carbon atoms positioned at 280.2 eV, 283 eV, 284.8, 288 eV respectively. The 284.8 in all materials could be attributed to the presence of elemental carbon present in all air-exposed material while the peak at 288 could be attributed to C atom with a single bond to oxygen (C-0) [14]. We have assumed from the noisy spectrum that the major peaks are from 796.6 eV and 780.6 eV which are of $Co3^+$ 2p and 802.4 and 786.5 are of $Co2^+$ 2p respectively.

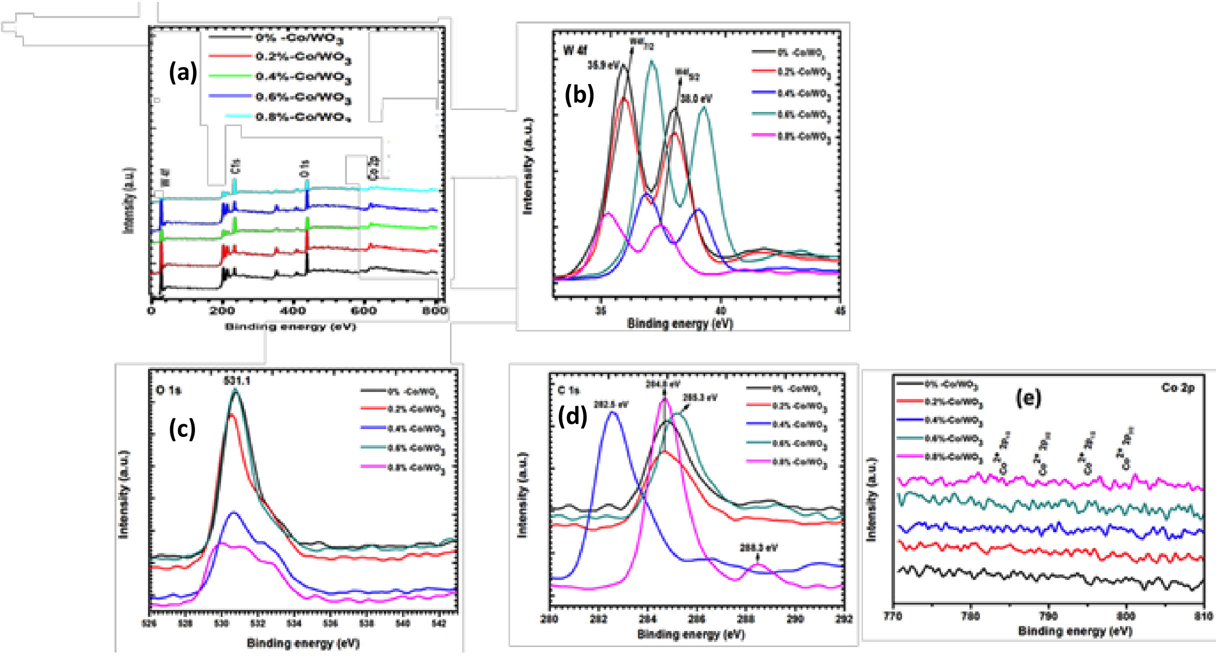

Figure 5.2 XPS spectra of Co-WO_3 (a) survey samples, (b) W 4f core-level XPS spectrum, (c) 0 1s core-level XPS spectrum and (d) C 1s core-level XPS spectrum

5.6.3 Morphological characterization of Co-doped WO_3 nanostructures

The Scanning Electron (SEM) and Energy Dispernsive X-rays (EDX) Micrographs of the sample prepared at varying weight percent of cobalt as dopant and carbon spheres as a template of tungsten trioxide are shown in figure 5.3 below. Samples with carbon as a template (a-d) showed a flowerlike hierarchical morphology which is composed of nanorods, this morphology was confirmed with TEM (figure 5.4) and the one without carbon had clustered particles.

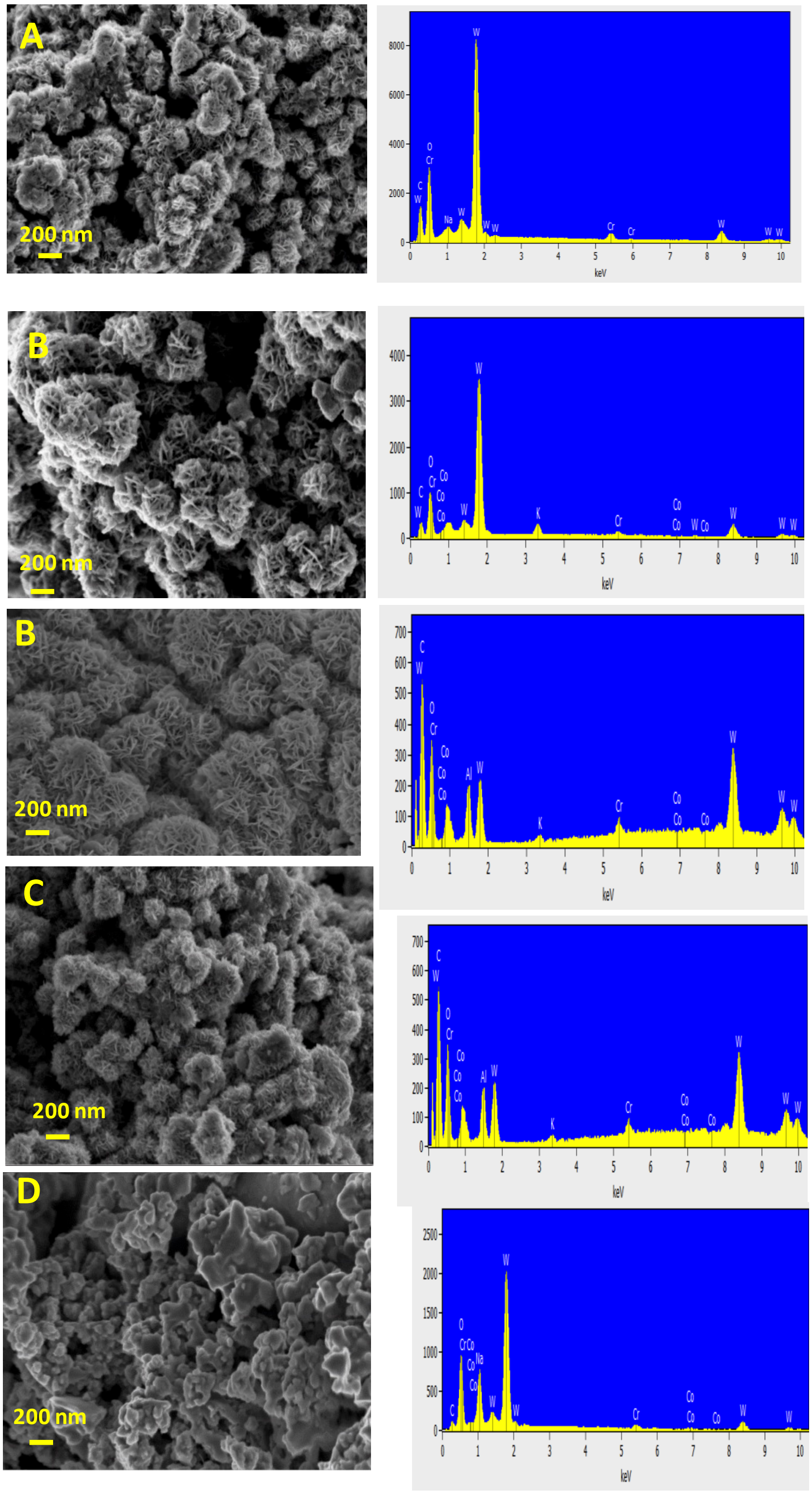

Figure 5.3 (a-e). SEM images of the (a) pure, (b) 0.2 wt. % Co/WO_3, 0.4 wt. % Co/WO_3, 0.6 wt. % Co/WO_3 with carbon as a template and 0.8 wt. % Co/WO_3 without carbon, coupled with EDS analysis

Figure 5.4 (a-b) confirms the flower-like morphology compost of nanorods synthesized using carbon spheres as templates as opposed to figure 5.4 (c-e). The samples with no carbon as the template shows arranged nanoparticles with low surface area, which will also be confirmed later by the N_2 adsorption-desorption technique. Figure 5.4 (a-e) also present typical bending fringes to determine the defects of lattice, which is predominantly caused by Co as a dopant. The lattice distance of 0.388 nm is in good agreement with the (020) lattice plane of monoclinic WO_3. The selected area electron diffraction in figure 5.4 (d &h) further ascertains that the samples are crystalline with the one with no carbon being more crystalline than the one with carbon spheres as a template which was also observed with XRD.

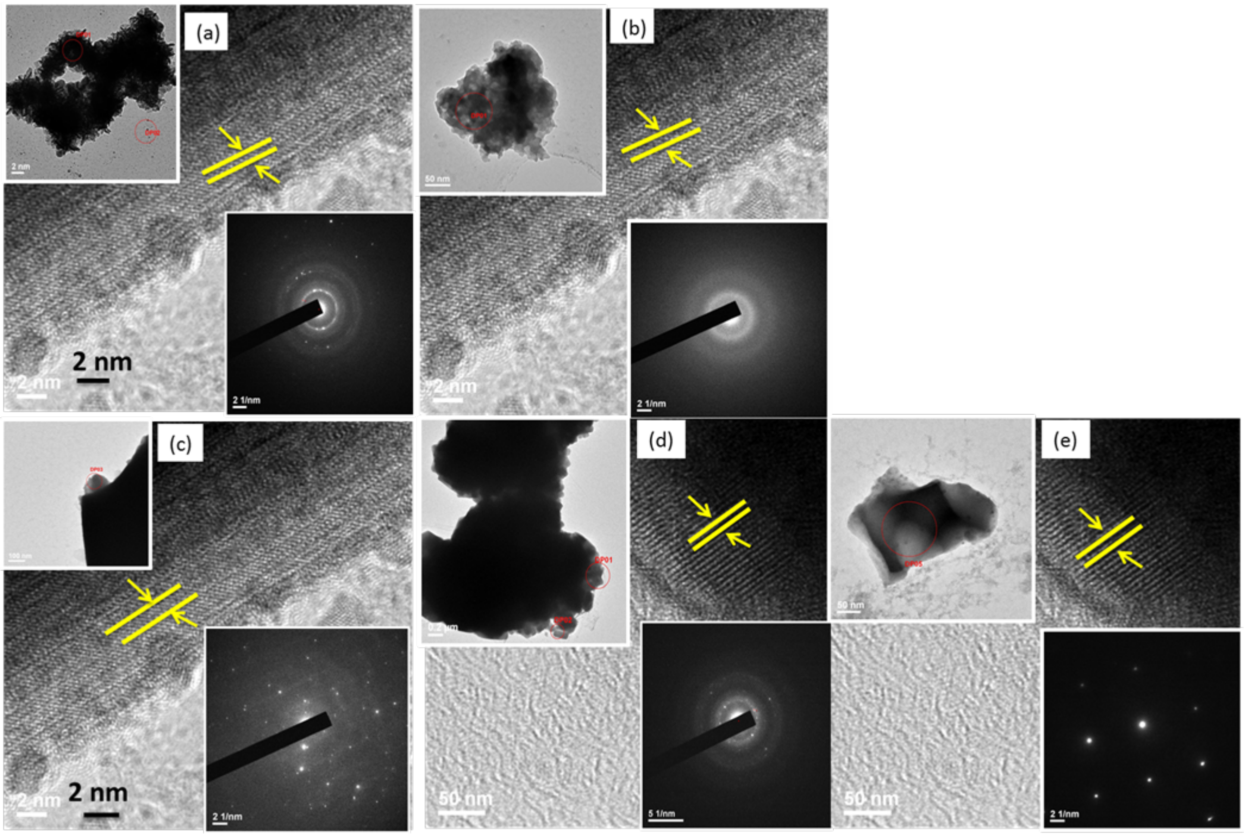

Figure 5.4 (a-e) TEM images of the (a-d) pure WO_3, 0.2, 0.4, 0.6 wt. % doped Co with carbon nanospheres and (e) 0.8 wt. % doped Co without carbon nanospheres as a template.

The N_2 adsorption/desorption isotherms and pore size distributions of pure and Co-doped WO_3 carbon as a template was further investigated to gain more insights on surface properties. As can be seen from figure 5.5(a and b), according to IUPAC classification, the adsorption\desorption isotherm belongs to type IV isotherm [15]. The hysteresis loop is observed from 0.8 to 1 (P\P0). This is H3 hysteresis loop corresponds to the capillary condensation of nitrogen in the mesoporous structure. Additionally, this type of curve is related to the asymmetrical pores and large interparticle porosity of gas bubbles. Moreover, fig 5.5(b) shows the pore diameter distribution. The average pore diameter, as can be seen, is 10. 5 nm.

The overall surface parameters including surface areas, pore diameter and pore volume of pure and Co-doped WO_3 is presented in Table 5.1 below. We have observed the decrease in pore diameter as the cobalt dopant increases. This is due to the blockage of pores by dopants. The carbon nanospheres have boosted the WO_3 surface areas. The surface area of pure WO_3 is consistent with the literature[16], which indicates that the increase in the surface is truely due to dopant and carbon as a template. Sample with 0.6 wt% Co-doped WO_3 and carbon nanospheres shows great potential for a maximum response due to its magnificent surface properties such high surface areas, large pore diameter and also small average grain size (Table 5.1).

Table 5.1 Summary of the BET specific surface area, pore volume (V_{pore}) and pore diameter and response values.

Sample	S_{BET} (m^2/g)	D_p (nm)	V_p (cm^3/g)	Response @ 1.5 ppm acetone
0% Co/WO_3	14.5	32.4	0.02	0.51
0.2%Co/W_3	68.2	15.3	0.05	0.57
0.4%Co/W_3	148.7	8.9	0.015	0.65
0.6%Co/W_3	158.4	8.2	0.07	1.54
0.8%Co/W_3	58.0	3.7	0.06	0.74

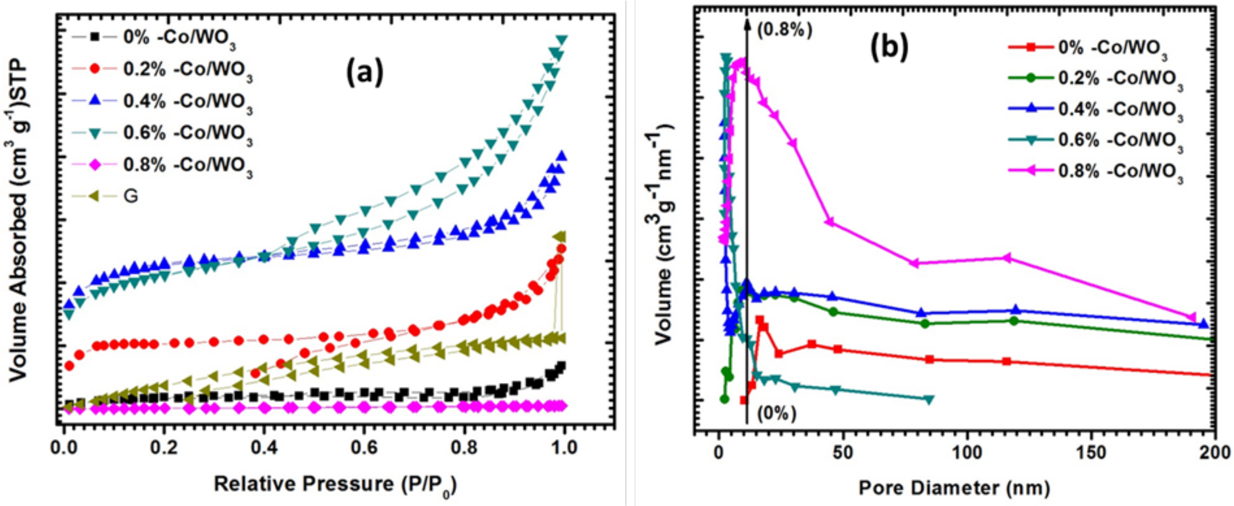

Figure 5.5 (a) N_2 adsorption-desorption isotherms and (b) pore size distributions curves.

5.6.4 Acetone sensing properties

5.6.4.1 <u>Response and recovery properties</u>

The n-type SMOs have shown to exhibit outstanding gas sensing properties when exposed to reducing gases such as acetone [17-19], H_2S [20-22], toluene [23-25] and many more. When the n-type is in contact with a reducing gas the resistivity decreases due to the electron transfer into the conduction band, and with the p-type semiconductor, the opposite occurs. The acetone sensing properties of pure WO_3 and all Co-doped WO_3 was investigated from 0.5 to 4.5 ppm of acetone under the highly humid environment of 90% RH which is similar to the humidity in the human breath, at a different operating temperature ranging from 50, 75, 150 and 200 °C, respectively. The operating temperatures were varied in order to determine the optimum operating temperature with maximum response. As can be seen from figure 5.6 (a-e), the sensor with 0.6 wt. % cobalt showed maximum response in all the temperatures. It can be agreed that the doping of Co, with the 0.6 wt% content has a great significance on the acetone sensing response.

The high acetone response at 0.6 wt. % could be attributed to the highest surface area properly caused by carbon nanospheres, small grain size, the presence of Ɛ-WO_3 phase which facilitate porous structure and stabilize Ɛ- WO_3, a phase which is best in detecting [26] and selecting acetone, and the addition of a noble metal on the surface of the WO_3. The sensor response of the n-type semiconductor to a reducing gas is generally defined as R_a/R_g, where R_a is the resistance of the material when there is air and R_g is the resistance when the target gas is present whereas the response to an oxidizing gas is defined as R_g/R_a. For a p-type semiconductor gas sensor, the definitions are reversed. More importantly, it can be seen that all the sensors can respond very well at a low acetone concentration of 0.5 ppm, this is a good properties that is required for breath acetone sensor applications.

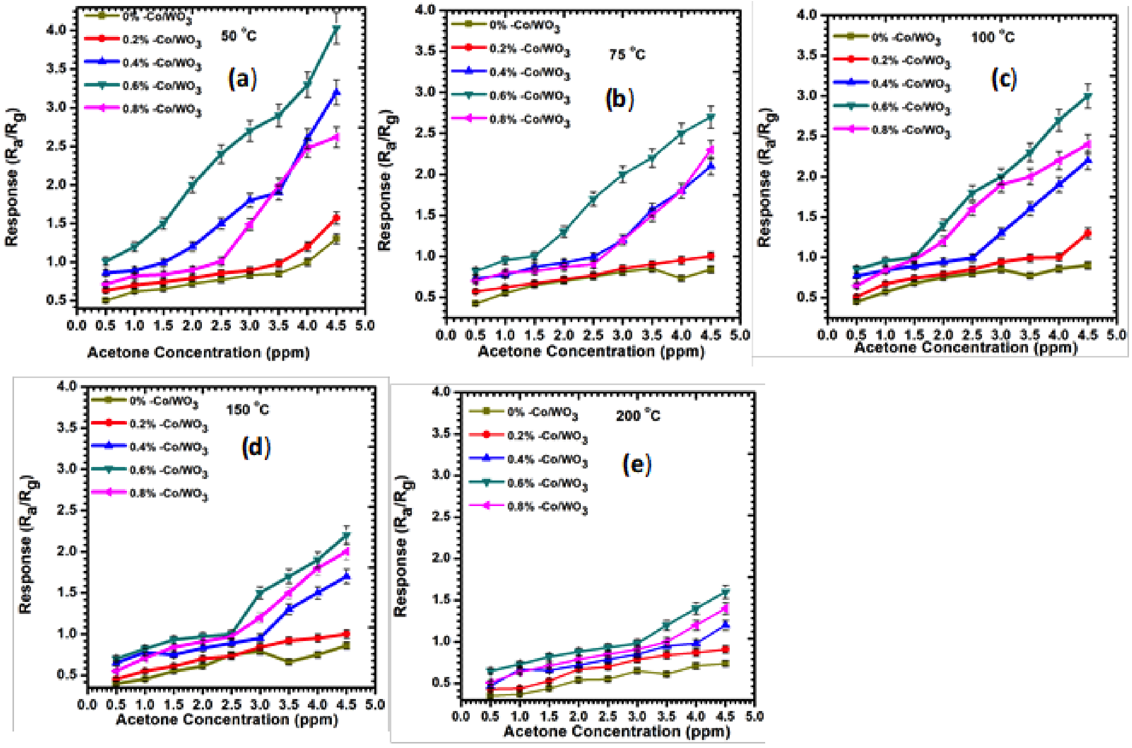

Figure 5.6 Dynamic acetone sensing response/recovery curves under highly humid condition (90% RH) at 50 ◦C (a), 75 ◦C (b), 100 ◦C (c), 150 ◦C (d), 200 ◦C(e).

Additionally, the maximum response was observed at 50 degrees (Figure 5.7) in all samples with the response values of 200, 140, 130, 97 and 88 for 50, 75, 100, 150 and 200 °C respectively at 1.5 ppm acetone concentration. This indicates a decrease in sensor response with an increase in operating temperature. The decrease in response with an increase in operating temperature may be due to the weak adsorption, low diffusion depth at high temperatures and band gap narrowing as well as a new intragap band caused by carbon nanospheres as templates and cobalt doping [27].

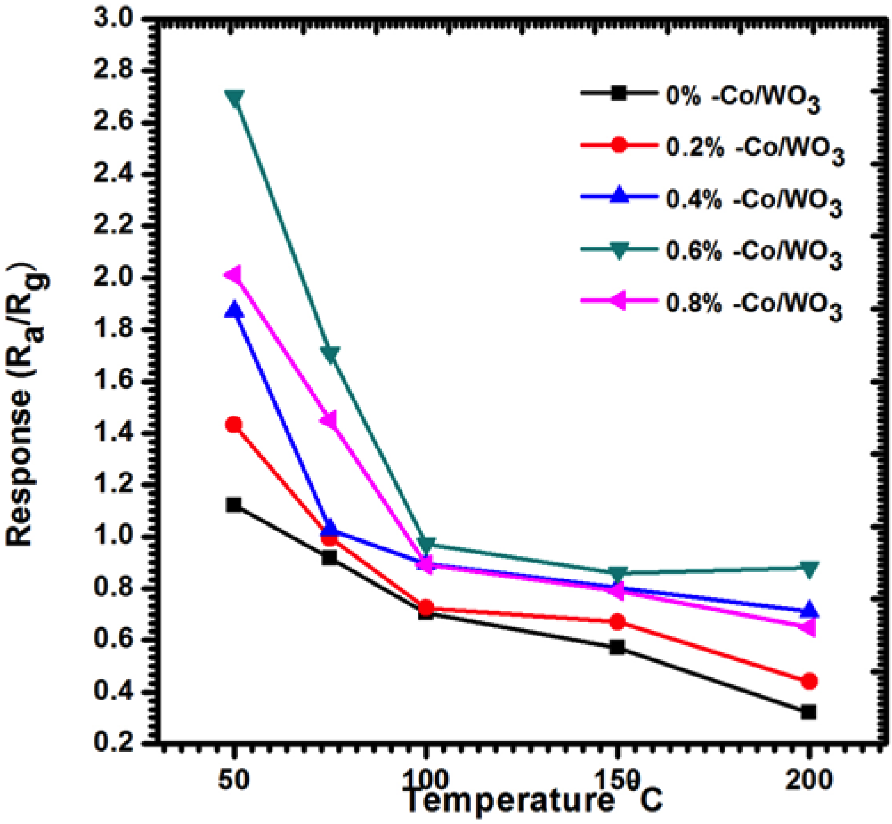

Figure 5.7 Responses of pure and Co-doped WO_3 to1.5 ppm acetone under 90% RH at different operating temperatures.

5.6.4.2 Selectivity

Selectivity is one of the most crucial properties that gas sensors must possess particularly in breath analysis. We further investigated the selectivity of pure WO_3 and Co-doped WO_3 towards 1.5 ppm of acetone, in consideration that acetone in diabetic breath ranges from 0.8 to 1.8 ppm [28]. The selectivity is defined as the cross-sensitivity to other gases

Figure 5.8 show that all the sensors select acetone better than any other gases, however, the sample doped with 0.6 wt% cobalt showed the highest selectivity. The high sensitivity and response of 0.6 wt% Co-WO_3 could be attributed to the small crystallite size, high surface area, porous morphology and stabilization of Ɛ-WO_3 monoclinic phase which has strong interaction force between electric dipole of ε-WO_3 and the largest dipole moment of acetone [31, 32]. It has been reported that the surface area can positively influence the sensitivity of the sensor [13, 33, and

34].

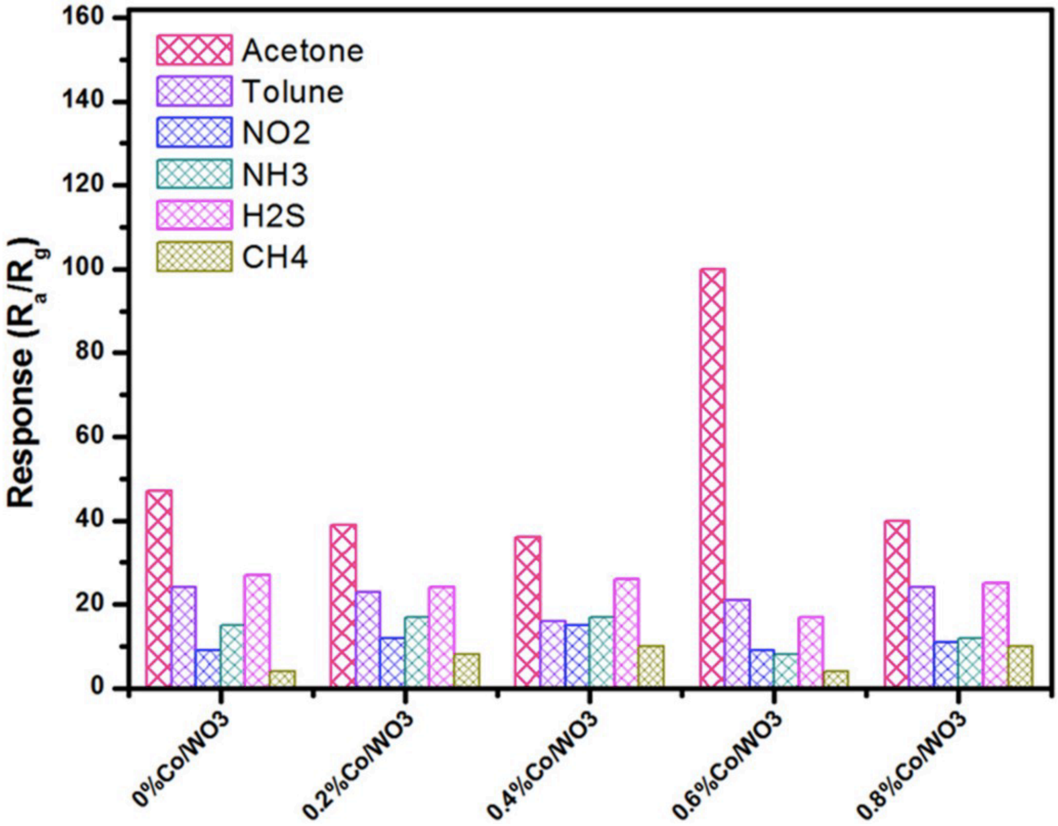

Figure 5.8 Selectivity values of pure and Co-doped WO_3 against varying gases at 50 °C at 1.5 ppm concentration.

5.6.4.3 Response and Recovery times

The response and recovery times of metal oxides sensors are yet another important factor for breath analysis, thus the response and recovery times were investigated in this study. Table 5.3 below, shows the response and recovery times of different gasses which include acetone, toluene, methanol, methane, hydrogen Sulphide, and nitrogen dioxide at 0.5 ppm concentration. The response time is usually defined as the time taken by the sensor to reach is 90% of equilibrium after the introduction of the gas of interest whilst recovery time is the time taken by the sensor to reach 90% of its original resistance in the absence of the gas. As it is presented in Table 5.3 below, sample 0.6 wt. % Co-WO_3 demonstrates fast response and short recovery times to acetone as compared to the remaining samples in this study. Figure 5.9 shows the response and recovery time of 0.5 ppm of acetone. Additionally, sample

0.6 wt. % Co-WO_3 shows the fastest response and short recovery time to acetone over toluene.

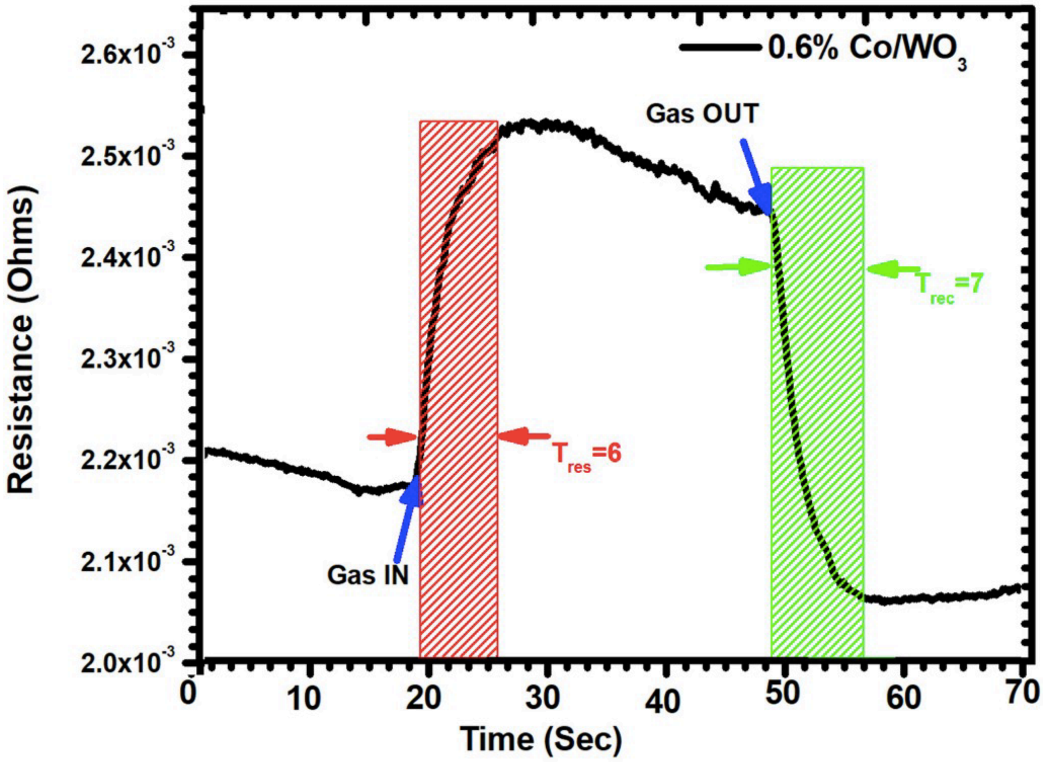

Figure 5.9 A typical response and recovery times of pure and Co-doped WO_3 to 0.5 ppm acetone under 90% RH at 50 °C.

Table 5.2 Response and recovery times of pure and Co-doped-WO_3 evaluated at 0.5 ppm acetone and toluene concentrations

Sample	**0.5 ppm Acetone**		**0.5 ppm Toluene**	
	Response time	**Recovery time**	**Response time**	**Response time**
0%Co/WO_3	17	17	13	21
0.2%Co/WO_3	19	23	23	36
0.4%Co/WO_3	27	33	32	37
0.6%Co/WO_3	6	7	15	18
0.8%Co/WO_3	30	33	35	40

5.6.4.4 Acetone Sensing Mechanism

To highlight the novelty of our work, we gathered the acetone sensing properties of semiconducting metal oxides on literature, represented in table 5.2. As far as our literature search is concerned, there are no reports on acetone sensitivity and selectivity at operating temperature lower than 100 °C up-to-date. As can be seen our current work demonstrates high response and lowest operating temperature at a very low acetone ppm under high relative humidity. The works [9, 10, 16, 25 and 26) reported in literature does not compete with the current work based on their high concentration of acetone and highest operating temperature. Our work exhibits excellent response at close to room temperate and low acetone concentration. This makes it a promising material towards the development of sensors for the detection of acetone

Table 5.2 Comparison of other materials from literature to the present WO3's selectivity to acetone

Material	**Fabrication method**	**Acetone concentration (ppm)**	**Operating temperature (°C)**	**Rg/Ra**	**Ref**
$W0_3$-nanoplates	Topochemical coversation	1000	350	9	(9)
WO_3-nanoplates	Hydrothermal	2000	307	10	(10)
Cr-Wo_3	Flame spray pyrolysis	1	400	2.9	(16)
WO_3-nanotubes	Electrospinning	100	250	40	(25)
Cu-WO_3	Electrospinning	20	300	6.4	(26)
Co-WO_3	Sol-gel	**1**	50	**2.67**	**This work**

5.7 Conclusion

The pure and Co-doped WO_3 has been successfully prepared by the sol-gel method using the carbon nanospheres as a template. The XRD pattern confirm the Ɛ-WO_3 monoclinic phase stabilized by the carbon. This phase has been largely reported to be good for acetone sensing. The XPS further confirms the presence of carbon, and cobalt species. Furthermore, the shift in W4f peaks confirms that the cobalt and carbon were doped into the WO_3 lattice. The 0.6 % Co-doped WO_3 showed the highest response and selectivity towards acetone gas from as low as 0.5 ppm at a very low operating temperature of 50 °C. Contrary, there was a very low response from other gases including toluene, NO_2, NH_3, CH_4 and H_2S operating at similar temperatures. This highlights the acetone selectivity of our 0.6 % Co-doped WO_3 sample. The obtained results from this work indicate a promising acetone detecting sensor that can be used for diabetic monitoring.

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CHAPTER 6

Concluding remarks and future recommendations

6.1 Conclusion

There has been a rapid development of molecular biological and biochemical diagnostic techniques used in medicine in recent decades, the greast focus being on blood and urine analyses. These methods require sample collection, which mostly is invasive and has an uncomfortable odor. Nonetheless, these methods are very crucial for medical diagnosis. With blood analysis, the non-volatile compounds with a large molecular weights such as ions and proteins are used during blood sample collection and analysis. Volatile compounds can be found in human breath at trace levels in the parts-per-million (ppm) and parts-per-billion (ppb) levels. According to Phillips et al, the volatile compounds in the breath have been found to have valuable information on the metabolic state and can distinguish between healthy and diseased states using different analytical instruments such as GC-MS, PTR-MS, SIFT-MS, etc.

For example, an acetone level of more than 1.8 ppm has been associated with diabetes mellitus. The drawbacks of these analytical techniques are lack of portability, requirement of a trained operator, not being point of care and challenges in sample collection. In contrast, semiconducting metal oxides as sensors have a great potential in breath diagnostics due to portability, ease of use, simple device fabrication and possible use at point of care diagnostic settings. These sensor according to Kim et al, use the principle of chemiresistance; described as the change in electrical conductivity or resistivity of thin films on exposure to a target gas. In other words, gas molecules interacting with the metal oxides either act as a donor or acceptor of charge carriers or alters the resistivity of the metal oxide which could be measured in real time.

In this research study, we have analyzed type 2 diabetic and non-diabetic breath in order to perform a correlation study between breath acetone, blood glucose and blood ketone bodies. The high amounts of ketone bodies (acetone, acetoacetate and beta-hydroxybutyrate) were observed in diabetic patients as opposed to non-diabetic patients. Breath acetone levels were found to increase with blood β-hydroxybutyrate and blood acetoacetate levels. These results suggest the potential to develop breath gas sensor analysis to provide an alternative to blood testing for both type 1 and type 2 diabetes monitoring, and to assist with the prevention of diabetic ketoacidosis.

We then further synthesized the WO_3 sensor to investigate its acetone sensing properties and its potential for diabetes monitoring. The as-synthesized WO_3 composed of nanoparticles, plate-like structures and nanorods were successfully synthesized by varying solvent content using the solvothermal method. The sensor fabricated with 51:49 water: ethanol was found to exhibit high response and good selectivity towards a 2 ppm level of acetone when compared with the one fabricated with pure ethanol, 18:92 (ethanol: water) and 92% water. However, the sensor exhibited low response under increased relative humidity at 100 °C. Furthermore, the sensor could respond to a low concentrations of acetone ranging from 0.5 to 4.5 ppm of acetone at 100 °C. More interestingly, the sensor could distinguish the healthy region and diabetic region even under high relative humidity. The gas-sensing performance of WO_3 indicates that it has a potential application in low concentration and fast response of acetone detection. These properties are suitable for sensors that can be used for the detection of exhaled acetone from human breath, more specifically in diabetic patients.

It is said that in gas sensors, the conductivity response is determined by the efficiency of catalytic reactions with detected gas participation, taking place at the surface of the gas-sensing material. Therefore, control of the catalytic activity of the gas sensor material is one of the most commonly used means to enhance the performances of gas sensors. In this study, chapter 5 used cobalt as a noble material to try to enhance the performance of WO_3 to acetone gas. The pure and Co-doped WO_3 were prepared by the sol-gel method using the carbon nanospheres as a template. The XRD pattern confirmed the Ɛ-WO_3 monoclinic phase stabilized by the carbon. The phase has been largely reported to be good in acetone sensing. The XPS further confirmed the presence of carbon and, cobalt species. Furthermore, the shift in W4f peaks confirmed that the cobalt and carbon were doped into the WO_3 lattice.

The 0.6 % Co-doped WO_3 showed the highest response and selectivity towards acetone gas from as low as 0.5 ppm at a very low operating temperature of 50 °C. Contrary, there was a very low response from other gases including toluene, NO_2, NH_3, CH_4 and H_2S operating at a similar temperature. This highlights the acetone selectivity of our 0.6 % Co-doped WO_3 sample. The obtained results from this work indicate a promising acetone detection sensor that can be used for diabetic monitoring.

Based on the two methods used for the synthesis of the acetone sensor, we can conclude that the Co-doped sensor shows better performance as compared to the as-prepared WO_3. This is from the findings that the Co-doped WO_3 can respond and select acetone concentration at 50 ◦C, which is the temperature that is close to room temperature. The portable point of care diabetic devices can best work at 50 ◦C anywhere in the world.

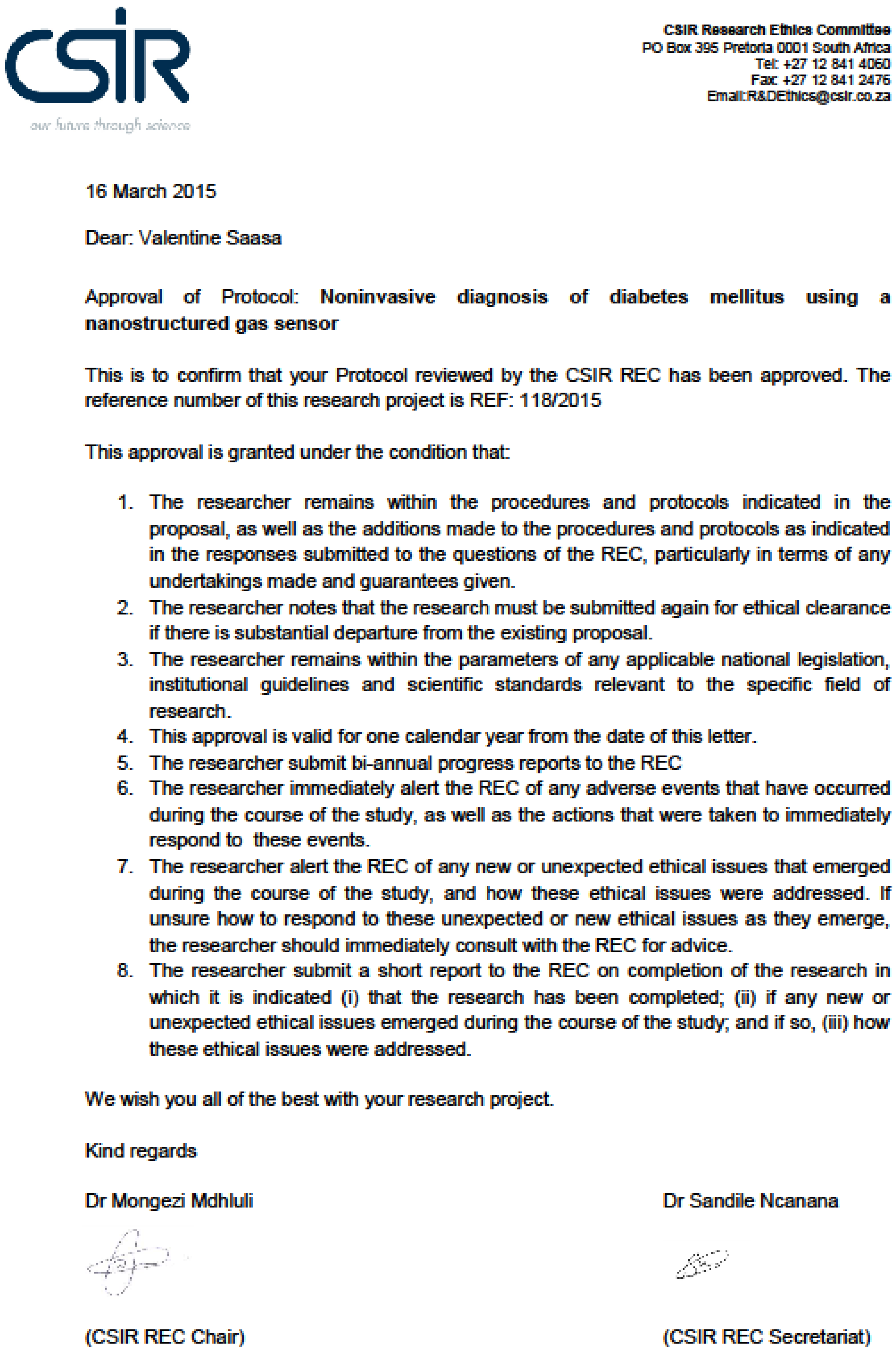
6.2 Future work and recommendations

Despite the good correlation coefficient between breath acetone and blood ketone bodies and between breath acetone and blood glucose, there is a need for improvement as both are short of the R = 97% dictated for approval into medical devices by the Foods and Drugs Association (FDA) of the USA as well as the South African Health Product Regulary Authority (SAHPRA) of South Africa. This can be achieved by daily monitoring of fasting blood glucose, ketone bodies and breath acetone over a long period of days.

Regardless of the exceptional and remarkable acetone sensing characteristics of $W0_3$ nanostructures observed in this thesis, it has been observed that the type of sensor fabrication and contacts influence the sensing performance. Nanomaterials have a high surface to volume ratio, this makes it difficult to isolate individual particles in order to contact single nanostructures for gas sensing device fabrication. Therefore, making nano-contacts to individual nanostructures using the e-beam contacting instrument will be of a great benefit in this field of research.

The gas sensing instrument was used to simulate human breath and successfully analyzed gases to check acetone performance, however, there will be a great need to test the sensor in a real-life diabetic and non-diabetic environments.

Appendix i Ethics Approval



CSIR Research Ethics Committee
PO Box 395 Pretoria 0001 South Africa
Tel: +27 12 841 4060
Fax: +27 12 841 2476
Email:R&DEthics@csir.co.za

16 March 2015

Dear: Valentine Saasa

Approval of Protocol: **Noninvasive diagnosis of diabetes mellitus using a nanostructured gas sensor**

This is to confirm that your Protocol reviewed by the CSIR REC has been approved. The reference number of this research project is REF: 118/2015

This approval is granted under the condition that:

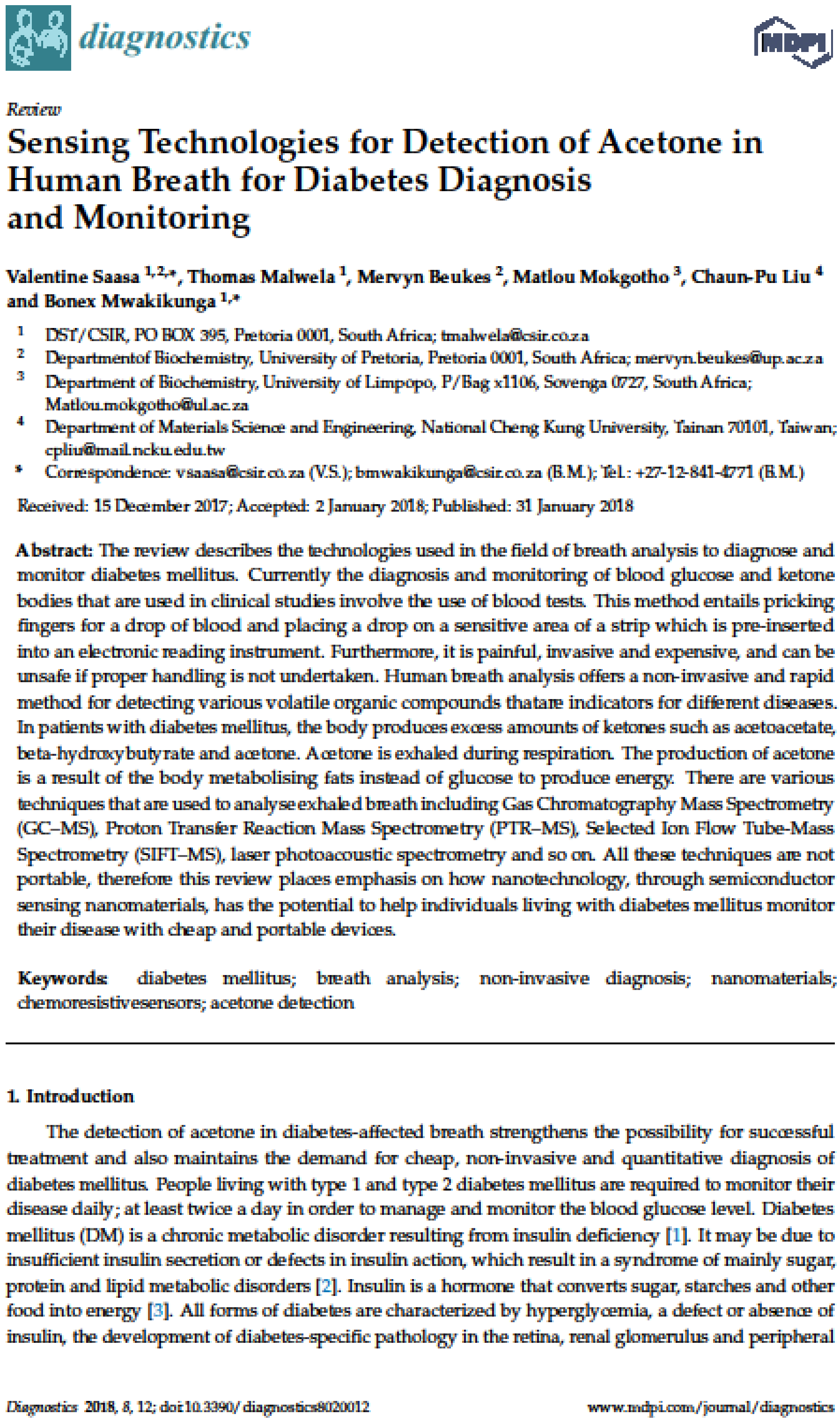
1. The researcher remains within the procedures and protocols indicated in the proposal, as well as the additions made to the procedures and protocols as indicated in the responses submitted to the questions of the REC, particularly in terms of any undertakings made and guarantees given.
2. The researcher notes that the research must be submitted again for ethical clearance if there is substantial departure from the existing proposal.
3. The researcher remains within the parameters of any applicable national legislation, institutional guidelines and scientific standards relevant to the specific field of research.
4. This approval is valid for one calendar year from the date of this letter.
5. The researcher submit bi-annual progress reports to the REC
6. The researcher immediately alert the REC of any adverse events that have occurred during the course of the study, as well as the actions that were taken to immediately respond to these events.
7. The researcher alert the REC of any new or unexpected ethical issues that emerged during the course of the study, and how these ethical issues were addressed. If unsure how to respond to these unexpected or new ethical issues as they emerge, the researcher should immediately consult with the REC for advice.
8. The researcher submit a short report to the REC on completion of the research in which it is indicated (i) that the research has been completed; (ii) if any new or unexpected ethical issues emerged during the course of the study; and if so, (iii) how these ethical issues were addressed.

We wish you all of the best with your research project.

Kind regards

Dr Mongezi Mdhluli	Dr Sandile Ncanana
(CSIR REC Chair)	(CSIR REC Secretariat)

Appendix ii Puplication #1



diagnostics

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Review

Sensing Technologies for Detection of Acetone in Human Breath for Diabetes Diagnosis and Monitoring

Valentine Saasa [1,2,*], Thomas Malwela [1], Mervyn Beukes [2], Matlou Mokgotho [3], Chaun-Pu Liu [4] and Bonex Mwakikunga [1,*]

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Abstract: The review describes the technologies used in the field of breath analysis to diagnose and monitor diabetes mellitus. Currently the diagnosis and monitoring of blood glucose and ketone bodies that are used in clinical studies involve the use of blood tests. This method entails pricking fingers for a drop of blood and placing a drop on a sensitive area of a strip which is pre-inserted into an electronic reading instrument. Furthermore, it is painful, invasive and expensive, and can be unsafe if proper handling is not undertaken. Human breath analysis offers a non-invasive and rapid method for detecting various volatile organic compounds thatare indicators for different diseases. In patients with diabetes mellitus, the body produces excess amounts of ketones such as acetoacetate, beta-hydroxybutyrate and acetone. Acetone is exhaled during respiration. The production of acetone is a result of the body metabolising fats instead of glucose to produce energy. There are various techniques that are used to analyseexhaled breath including Gas Chromatography Mass Spectrometry (GC–MS), Proton Transfer Reaction Mass Spectrometry (PTR–MS), Selected Ion Flow Tube-Mass Spectrometry (SIFT–MS), laser photoacoustic spectrometry and so on. All these techniques are not portable, therefore this review places emphasis on how nanotechnology, through semiconductor sensing nanomaterials, has the potential to help individuals living with diabetes mellitus monitor their disease with cheap and portable devices.

Keywords: diabetes mellitus; breath analysis; non-invasive diagnosis; nanomaterials; chemoresistivesensors; acetone detection

1. Introduction

The detection of acetone in diabetes-affected breath strengthens the possibility for successful treatment and also maintains the demand for cheap, non-invasive and quantitative diagnosis of diabetes mellitus. People living with type 1 and type 2 diabetes mellitus are required to monitor their disease daily; at least twice a day in order to manage and monitor the blood glucose level. Diabetes mellitus (DM) is a chronic metabolic disorder resulting from insulin deficiency [1]. It may be due to insufficient insulin secretion or defects in insulin action, which result in a syndrome of mainly sugar, protein and lipid metabolic disorders [2]. Insulin is a hormone that converts sugar, starches and other food into energy [3]. All forms of diabetes are characterized by hyperglycemia, a defect or absence of insulin, the development of diabetes-specific pathology in the retina, renal glomerulus and peripheral

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Appendix iii Publication #2

diagnostics

Article

Blood Ketone Bodies and Breath Acetone Analysis and Their Correlations in Type 2 Diabetes Mellitus

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Received: 4 November 2019; Accepted: 7 December 2019; Published: 17 December 2019

Abstract: Analysis of volatile organic compounds in the breath for disease detection and monitoring has gained momentum and clinical significance due to its rapid test results and non-invasiveness, especially for diabetes mellitus (DM). Studies have suggested that breath gases, including acetone, may be related to simultaneous blood glucose (BG) and blood ketone levels in adults with types 2 and 1 diabetes. Detecting altered concentrations of ketones in the breath, blood and urine may be crucial for the diagnosis and monitoring of diabetes mellitus. This study assesses the efficacy of a simple breath test as a non-invasive means of diabetes monitoring in adults with type 2 diabetes mellitus. Human breath samples were collected in Tedlar™ bags and analyzed by headspace solid-phase microextraction and gas chromatography-mass spectrometry (HS-SPME/GC-MS). The measurements were compared with capillary BG and blood ketone levels (β-hydroxybutyrate and acetoacetate) taken at the same time on a single visit to a routine hospital clinic in 30 subjects with type 2 diabetes and 28 control volunteers. Ketone bodies of diabetic subjects showed a significant increase when compared to the control subjects; however, the ketone levels were was controlled in both diabetic and non-diabetic volunteers. Worthy of note, a statistically significant relationship was found between breath acetone and blood acetoacetate ($R = 0.89$) and between breath acetone and β-hydroxybutyrate ($R = 0.82$).

Keywords: diabetes mellitus; ketone bodies; human breath; acetone; beta-hydroxybutyrate; acetoacetate; gas chromatography-mass spectrometry (GC-MS)

1. Introduction

Human biological samples such as breath, blood and urine contain several volatile organic compounds (VOCs). These VOCs are associated with specific metabolic pathways, and are useful as biomarkers reflecting the disease and physiological state of a human that cause changes in their metabolism [1]. Particularly, analysis of breath has been receiving more attention because of its potential as a non-invasive method for disease diagnosis and metabolic status monitoring [2]. Among thousands of VOCs, acetone is the second to highest in abundance in normal human breath gases, which has been extensively studied as a breath biomarker of diabetes or as a high abundant breath VOC in various physiological cases since the 1950s [1,3]. The studies which showed a strong link between breath acetone and plasma glucose are mostly for type 1 diabetes, but no such observation has been obtained so far from adequately controlled type 2 diabetes mellitus patients [4–7].

Diagnostics 2019, 9, 224; doi:10.3390/diagnostics9040224 www.mdpi.com/journal/diagnostics

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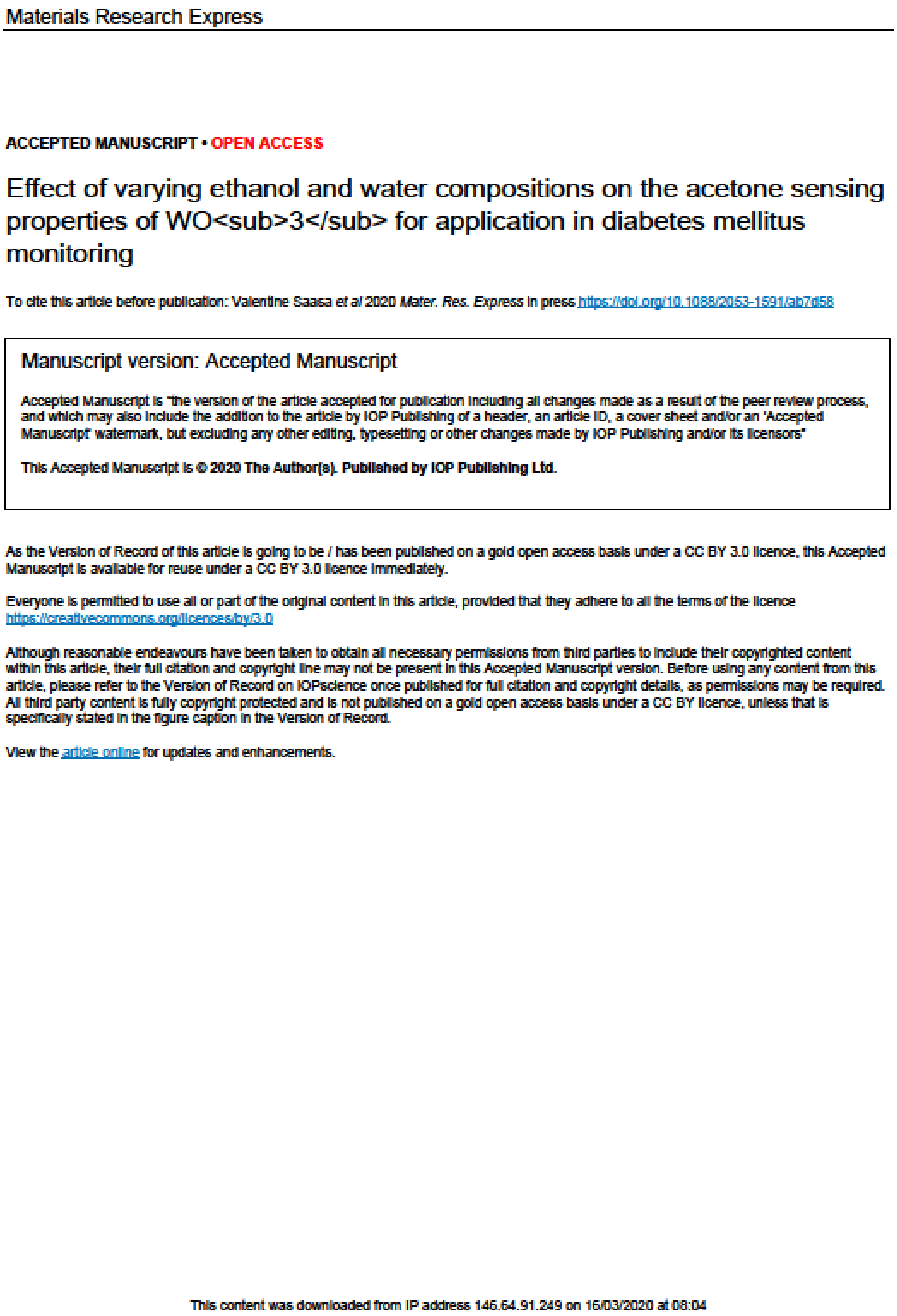
Appendix iv Publication #3



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Effect of varying ethanol and water compositions on the acetone sensing properties of WO₃ for application in diabetes mellitus monitoring

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Appendix iv Publication #4

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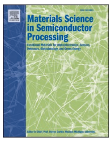

The hierarchical nanostructured Co-doped WO_3/carbon and their improved acetone sensing perfomance

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ABSTRACT

Hierarchical nanostructured Co-doped WO_3 with carbon as template has been successfully synthesised through facile sol-gel method. The synthesised Co-doped WO_3 was characterized by X-ray diffraction, Scanning electron microscopy, Transmission electron microscopy, Energy dispersive X-ray spectrometry, and Brunauer-Emmett-Teller and X-ray photoelectron spectroscopy. The gas sensing properties of WO_3 doped with Co from 0 to 0.8 wt % were also investigated on various VOCs. The fabricated sensor based on 0.6 wt% Co-doped WO_3 with carbon as a template showed good sensitivity, selectivity, fast response and recovery time towards 1.5 ppm of acetone at 50 °C under 90% relative humidity. The excellent gas sensing properties could be attributed to high surface area, small crystallite size, defect of WO_3 and Co catalysis effect which promotes gas adsorption and most importantly the stabilized monoclinic phase of WO_3, which accounts for the good selectivity.

1. Introduction

Semiconducting metal-oxides gas sensors have attracted considerable scientific interest in the field of gas sensing due to their fascinating properties such as low-cost, ease of use, and portability [1,2]. To name the few, gas sensors such as ZnO, SnO_2, In_2O_3, and WO_3 show a significant resistance change upon exposure to a trace concentration of reducing or oxidizing gases [3,4]. Furthermore, they are best suited for detecting volatile organic gases at low concentration levels in view of sensitivity, stability, robustness and so on [5]. Thus makes them an ideal candidate for monitoring trace amount of gases such as acetone in human breath, toxic gases in the environment and indoors [6].

More importantly, nanoporous semiconducting metal oxides with large specific surface area are ideal materials for improving gas sensing performances by enhancing sensing sites and total exposures to target gases [7,8]. Although many oxides have been investigated for acetone sensor development, there are still a few problems regarding selectivity towards a single gas. This is followed by a selective sensor that could operate at low or room temperature condition. The addition of noble metals onto the metal oxides surface has been mostly applied as a way to solve selectivity problems of the gas sensors [9]. This approach has shown improvement towards the selectivity of the sensor, particularly for acetone detection. Qi et al., 2008, reported a good selectivity of acetone over other gases, although the acetone concentration was very high (500 ppm) including very high operating temperature (350 C). In a study conducted by Koo et al., 2017 [10], the authors reported 1 ppm acetone selectivity using Pd functionalised CO_3O_4 at 350 C. A rapid acetone selectivity of a WO_3 nanotube sensor to 100 ppm was reported by Chi et al., 2015 [11], however the operating temperature was very high for gas sensor devices. Many other reported acetone selectivity includes [12–15], 100 ppm at 360 C, 50 ppm at 350, 50 ppm at 200 C, 2 ppm at 300 C and 0.9 ppm at 450 C, respectively.

Based on the reported literature, there is a great need to develop a gas sensor material which can detect low ppm volatile organic compounds, more specifically acetone gas [16]. There is a high demand of breath acetone sensor application in the market. Hence the current study seeks to develop a gas sensor based on Co-doped WO_3 with carbon nanospheres as a template to selectively detect low ppm of acetone at lower operating temperatures. As we might know, some diseases are associated with VOCs such as an increase in breath acetone (0.8 ppm and

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