Utilize smartphone as a novel detector for enzymatic urea hydrolysis in microfluidic system

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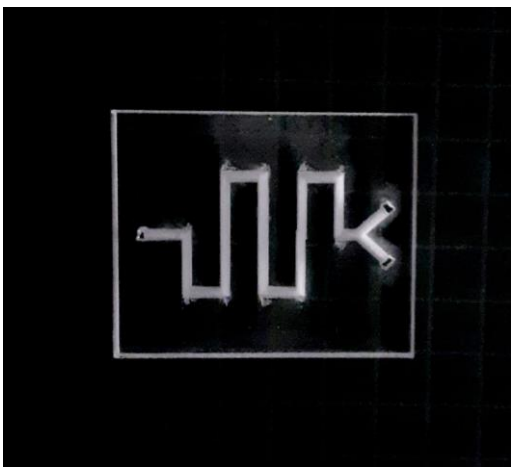
Abstract

In this work, PMMA(Poly methyl methacrylate) microfluidic system was used as a micro-reactor for urea hydrolysis by urease enzyme with use conductivity principle and utilize sound level meter(SLM) App in smartphone as a novel detector by considering the peak height in the App as an indicate for urea concentration. the advantage of use small volume and how the reaction carried on in the microfluidic system with simple and low cost are discussed, and the results were analyzed and statically determine. The linearity, detection limit (3×noise)and Correlation Coefficient, 62.5-500 ppm, 31.25 ppm and 0.992 respectively also, recovery studied were between(98.5-100.13%).

KEYWORDS: Microfluidic, Conductivity, Mobil-phone, urea, urease enzyme

1. INTRODUCTION

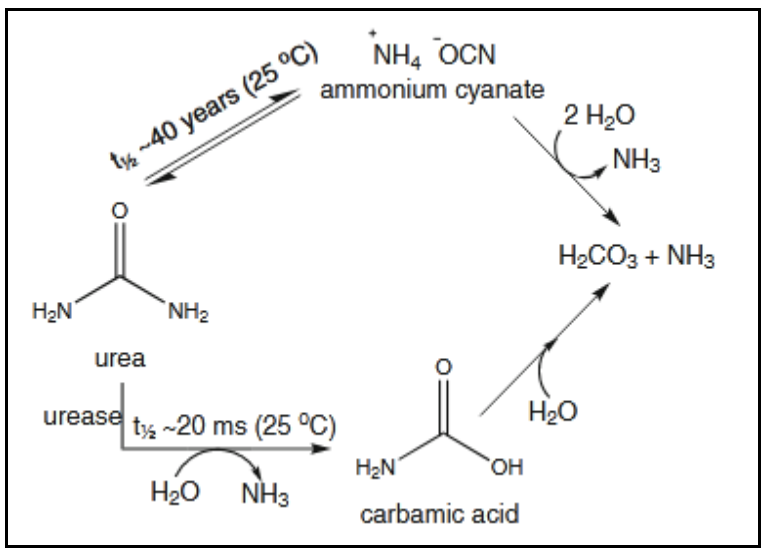
Microfluidic system is generally defined as a component that handles fluids (liquid or gas) in a small quantity (micro or Nano liter) Since most of the fluid handling applications are related to chemical and biomedical research, microfluidics have been developed for chemical and biomedical applications as miniaturized analytical technology, Conventional macroscopic equipment for wet laboratory processing can be miniaturized into microscopic equipment, One of the objectives of the development of microfluidic systems is to provide a total solution from the application of the sample to the display of analytical results, Therefore, microfluidic device is also called biochip, lab on chip (LOC), or micro total analysis system (μTAS) [1-3] figure(1.1).



Fig(1.1) An example of a microfluidic device.

The small size of the microfluidic device means that there must be at least one dimension of the channel in the micrometer scale. The behavior of fluids at microscale can differ from macroscale behavior, Factors such as energy dissipation, surface tension and fluidic resistance start to dominate the system at micro level. A microfluidic chip or system contains a network of microchannel that are connected to the outside of the channel by inputs and outputs pierced through the chip. These interconnections act as an interface between the macro- and micro world. Liquids or gas are injected and removed

from the microfluidic chip through these holes [4], [5]. Microfluidics is a multidisciplinary field that includes engineering, physical, chemical, and nanotechnology resources focused on the design, fabrication, and use of miniaturized devices. A microfluidic system involves generic components, such as: first a system to introduce reagents and samples ,second a technique for moving fluids through the chip and allowing them to be mixed ,third many other instruments, such as detectors and separation units. The recent development in microfluidics is due to the ability to combine several fluidic functions into one system [6-9]. Drug development is a complex process requiring many expensive and time-consuming steps. For a compound to be developed into a new drug and reach the market, It takes an average of 10-15 years and costs from $1.5 billion to over $1.8 billion. In order to provide successful improvement and cost reduction for the drug development process, more efficient and rapid methods to screen and validate potential drug candidates are urgently needed [10], [11]. In recent years, microfluidic chip technology has become an ideal choice for drug screening at the cellular level, Microfluidic devices are consist of microstructures, that closely resemble the extracellular environment and provide versatile drug concentration adjustment [12]. Microfluidic technologies can play an important role in the field of cancer research as well as developing personalized medicine. In the field of non-invasive liquid biopsy research, exosomes, CTCs, and circulating tumor nucleic acids are used as biomarkers of the minimum residual disease for the monitoring of disease , The method of separation and detection varies among samples due to the different sources and characteristics of these biomarkers, which in turn contributes to inherent strength and imitations . The ability of microfluidics in the identification, isolation and characterization of biomarkers has been validated by several studies. While microfluidic technology shows promise, there are still obstacles to overcome before being translated into a clinic [13-18]. Conductivity detection is perhaps the simplest of the electrochemical analysis protocols employed within microfluidic systems, However, this low cost technology can provide valuable insights and is normally adopted by using a pair of facing Pt electrodes separated by a small and well-defined distance [1], [19-21]. A polymethylmethacrylate (PMMA) chip and a pair of Pt wires with an end-to-end space of approximately 20µm conductivity measurements have been used to control the solution concentration of amino acids, proteins, peptides and oligonucleotides [1], [22], [23].



Diagram(1.1) Urea decomposition pathways

The lower pathway shows the biologically active and fast urease catalyzed the urea hydrolysis, The upper pathway shows that product is occurs spontaneously. The result of both degradation pathways is the formation of two molecules of ammonia (NH3) and one carbonic acid molecule(H2CO3) from one molecule of urea Diagram(1). In this work Designing a simple micro-reactor room used to perform basic reactions such as preparing the substance to be estimated with a

specific PH value or by heating or mixing two substances and interacting with the last one to give a specific product, using the lowest possible volumes to make such a design, that called (microfluidic chip), where all the details were drawn by a computer programing and engraving it on the acrylic polymer. Measure the concentration of urea using conductivity principle by connecting microfluidic chip with platinum electrodes that connect to the smartphone via earphone.

2. Materials and Methods

2.1 Chemicals and Materials

All chemicals were of analytical grade reagent and were used as received, we used Deionized water with conductivity less than (0.02)µS, and all measurements were carried out at temperature of approximately 27°C throughout this work.

1. 500(ppm) Urea (Thomas Baker, Mumbai, India) Stock Solution was prepared by dissolving 0.1(g) in Deionized water and Complete it to exactly 200(ml).
2. 4000(ppm) urease (Thomas Baker, Mumbai, India) was prepared by dissolving 2(g) in Deionized water and Complete it to exactly 500(ml).
3. PMMA (Poly methyl methacrylate) Microfluidic device
4. platinum electrodes, 0.2(mm) diameter and 2(cm) length
5. 3 mL syringes
6. clear packaging tape
7. Smartphone (Samsung Galaxy A10)
8. Earphones

2.2 Instrumentation

In this study ,we used PMMA microfluidic chip with integrated platinum electrodes as a reactor connected to mobile phone as novel detector for this system, this system were designed and used in advance chemistry research laboratory of Chemistry Department- College of Science Kufa University, As a semi-automated home-made system .

2.3 Fabrication and designing of PMMA(Poly methyl methacrylate) microfluidic device:

In this study a CNC(Computer numerical control) milling machine been used to designing micro-features in PMMA (Poly methyl methacrylate) microfluidic system. The design is consists of two inlets, one for urea solution and another for urease solution and one outlet for receiving the product of the reaction. by using Hamilton syringes we can drive fluid through the microfluidic channel, and with a two integrated platinum(pt) electrodes at the end of channel used as a conductivity cell, these electrodes was connected to a smartphone via earphones.

2.4 Smartphone as a novel detector for microfluidic system:

The smartphone have been used in this study was (Samsung Galaxy A10) a good platforms and Android device which are easy to work and using. it was employed as a detector by using a sound level meter(SLM) application that measured and display a graph of Sound Intensity (dB) vs. Time (s) and the intensity of peak that measured depends upon the amount of ions that release from reaction through specific time, this App was connected to an integrated platinum electrodes in microfluidic system via earphone, and the measurements of this App can be exported in an e-mail or via Google drive as a .txt attachment with comma separated values (CSV).CSV file is created and modified on the phone's internal memory. The range of sound level are accurately measured for smartphone usually no more than(80 or 90 dB) which is within the range of human voice sound intensities(40-60 dB) .

2.3 The Optimization of Experimental conditions:

A number of experiments were carried out to establish the conditions of Maximum peak production, The chemical variables such as concentration of both urea and urease solutions, and some physical variables such us cell constant conductivity ,sample volume, flow rate, shape and size of the microfluidic and different injection method were investigated . Within the optimal operating conditions, the working and the standard solutions were prepared by serial

dilution of the stock solution with various range to find the standard curve and determination the limit of detection(LOD), The relative standard deviation (RSD), reproducibility, recoveries and regression coefficient of this reaction.

2.4 Procedure:

In this technique 4000(ppm) urease and each of urea solutions were carried out and mixed together through a microfluidic channel with the volume of 0.5(ml) for both solutions and the pH of reaction are approximately equal to 7 , the solutions were injected manually via Hamilton syringes by using the sequential method, as both solutions were moved on through the channel the reaction between them can only occur where they mix, at the trailing and leading edges, the product (both of Ammonium ion NH_4^+ and Bicarbonate ion HCO_3^-) were detected when they reaching the pt electrodes and the signal appeared on a sound analyser app in a smartphone, the results were treated statistically when the signal was obtained, and then it was reading with an excel program, the height peck recorded on a smartphone screen can be related to the concentration. And due the time between injection and detection is typically under a minute, the mixing of both solutions were incomplete and equilibrium is not attained before detection.

3. Results and Discussion

3.1 Designing and fabricating of PMMA(Poly methyl methacrylate)microfluidic system:

In this project there was no microfluidic system existed in the laboratories of Chemistry department, College of Sciences ,University of Kufa, therefore, we decided to design and construct a semi-automated microfluidic system. To be used as simple and reliable system for this work . the image in figure (3.1) show The material and tools that have been used. First of all PMMA microfluidic device was fabricated by using CNC (Computer numerical control) milling machine, Milling is a subtractive manufacturing method that uses rotating cutting tools to extract material from a starting stock piece, usually referred to it as the work piece, Milling machines with CNC functionality are available with a wide range of technical requirements, including varying levels of stage precision, spindle speeds, and automation, Modern CNC mills are flexible and capable to manufacture devices with features ranging from several meters to several microns in scale, Recent advances in technological features have enabled enhanced precision and resolution down to the micron scale, leading to the use of the term micromilling to describe the manufacture of increasingly more complex microscale resolution parts[24], [25] . Micromilling can be useful in microfluidics application because of machining microchannels and features directly into the final part, that offers a key advantage in the milled of plastic work piece with a time less than 30 min[24].

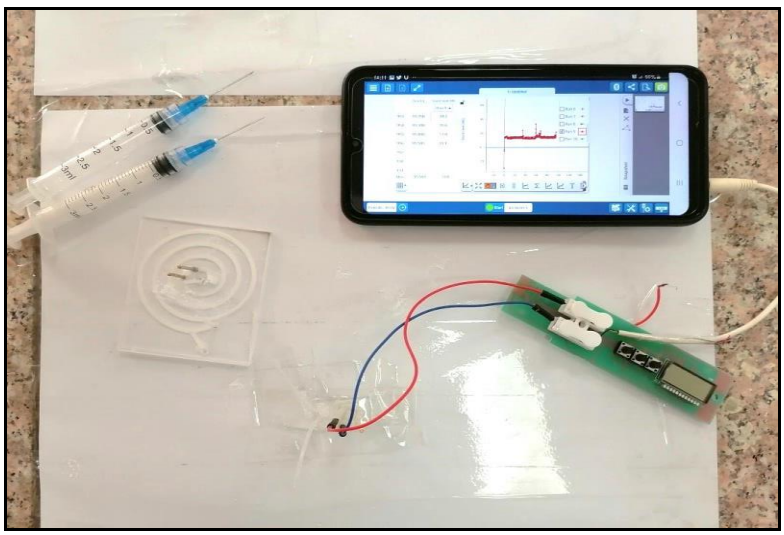
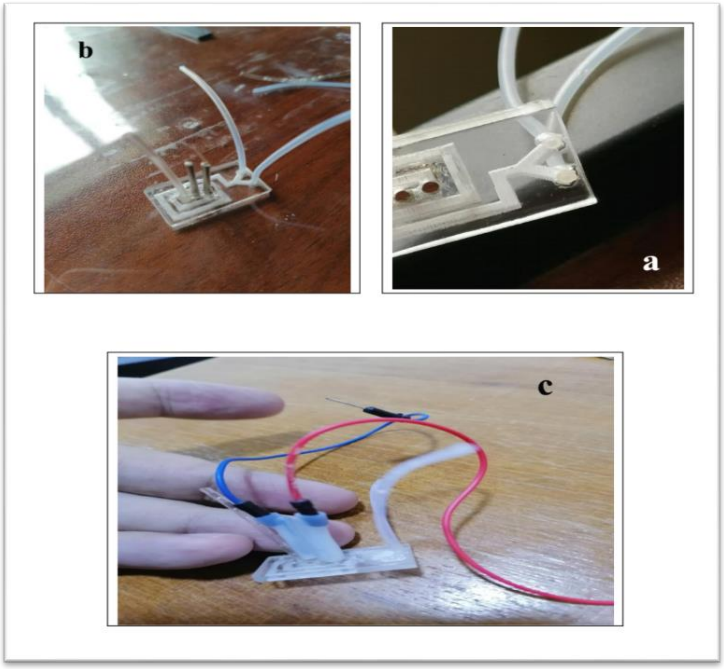


Fig (3.1) Shows the connectivity system using the microfluidic that connected to the mobile phone as a detector

The milling capabilities of the machine have then been used to designing micro-features in PMMA microfluidic system. The dimensions of design used in this study (channel width: 1.35 (mm), channel length: 80 (mm), channel volume: 0.1 (ml) and PMMA thickness: 2.41 (mm)),this design is consists of two inlets, one for urea(Carbonyl diamide) solution and another for urease(EC 3.5.1.5) solution and one outlet for receiving the product of the reaction. by using syringes we can

drive fluid through the microfluidic channel, and utilization a certain tape for sealing microfluidic device and prevent it from leaking, when the liquid flows slowly through the channel, the flow is laminar, that means the flow at the wall of channel is zero due to friction, which causes the flow in the center of the channel to be faster, so the liquid near the walls mixes with the bulk via diffusion along microfluidic channel radius. The sharp bends in and curvature of the channel create turbulence that enhances mixing, and in the end of channel a two platinum electrodes was integrated, these electrodes was connected to smartphone via earphones Figure (3.2).



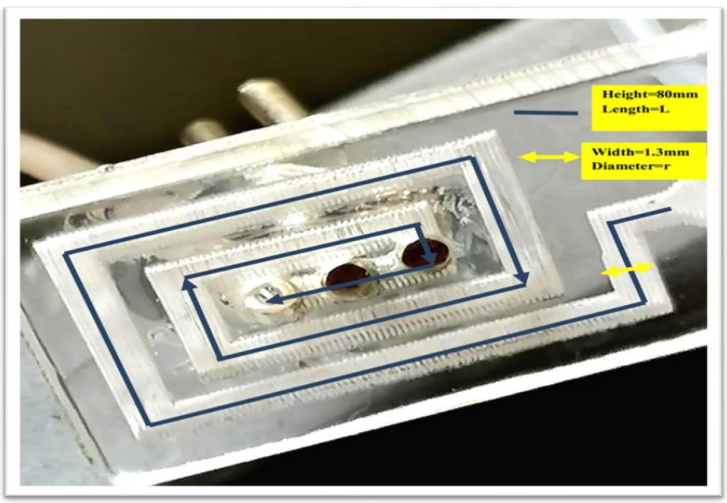
Fig(3.2) Shows the connectivity system connected to the microfluidic used in this study.

3.2 Measuring the Sample volume in the microfluidic system:

One of the most important topics in this study was the study of the sample size, which represent the measurement of the total volume within the microfluidic channel, it was measured practically by using Hamilton syringe and the result was equal to (0.1 mL) and theoretically by applying the volume of a cylinder law from knowing the length of channel and it's width on a microfluidic slide, so the result was equal to (0.106 mL) figure(3.3). From this result we observe a Similarity between the two sizes, which an indicate that the microfluidic design is accurate to give a precise practical size.

Volume of microfluidic channel $=L*(r)^2*3.14 =8cm*(0.065cm)^2*3.14$

$=0.106cm^3=0.106$ mL



Fig(3.3) Shows the calculation of sample size in a microfluidic system

3.3 Measuring the volume and constant of the conductivity cell:

The most important think in this study was the attempt to find a new designs that are more user-friendly, low-cost, small-sized, and environment friendly devices, by studying the volume and constant of the conductivity cell and Measuring it's volume according to the law that was used to calculate the channel size, figure (3.3), but by use the distance starting from the first platinum rod to the end of second one as a length.

Cell volume $=L*(r)^2*3.14 = 0.3cm*(0.065cm)^2*3.14$

$=0.00397\ cm^3 = 3.97\mu L$

L= The length

r = Channel Radius

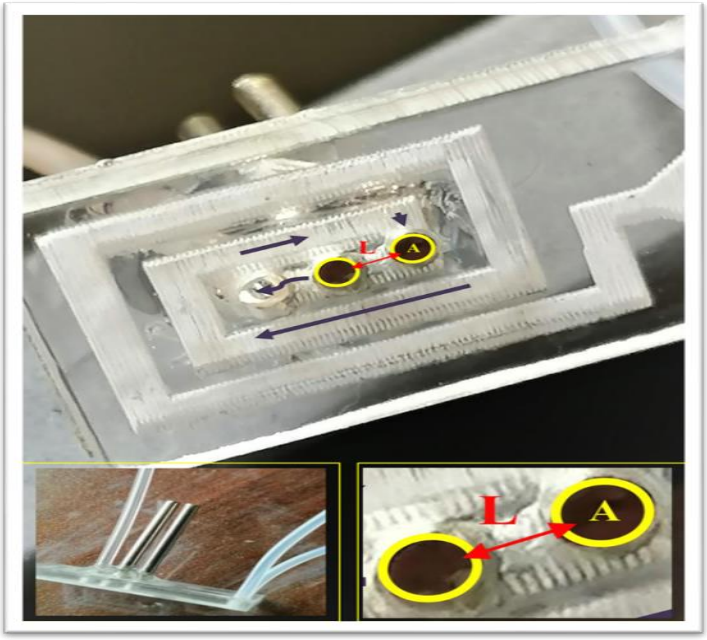
Engineering a conductivity cell required calculating factor called the cell constant, this constant is multiply by the measured conductivity value to get a Specific conductivity. The cell constant (k) is directly proportional to the distance between two electrodes and inversely to their surface area shown in figure(3.4).

Cell constant(K) $=L/A=L/(r)^2*3.14=0.1cm/(0.05cm)^2*3.14$

$=12.7388\ cm^{-1}$

L= The distance between the two electrodes.

r= Radius of platinum rod (circle shape).



Fig(3.4) It shows how to calculate the cell constant in a microfluidic system

3.4 Study the shape and size of the microfluidic chip:

More than one form of the microfluidic system Figure (3.5) have been studied using 500 ppm of urea and 4000 ppm of urease. the speed of Response and the sample size give a clear signal in mobile phone. Figure (3.6) shows the results appears in the sound level meter (SLM) app by using the mobile phone, which indicates that the design in the pictures(c) has the best and quick response, unlike the pictures (a, b) where the signal is not clear and distorted by the total size or the design of the microfluidic system.

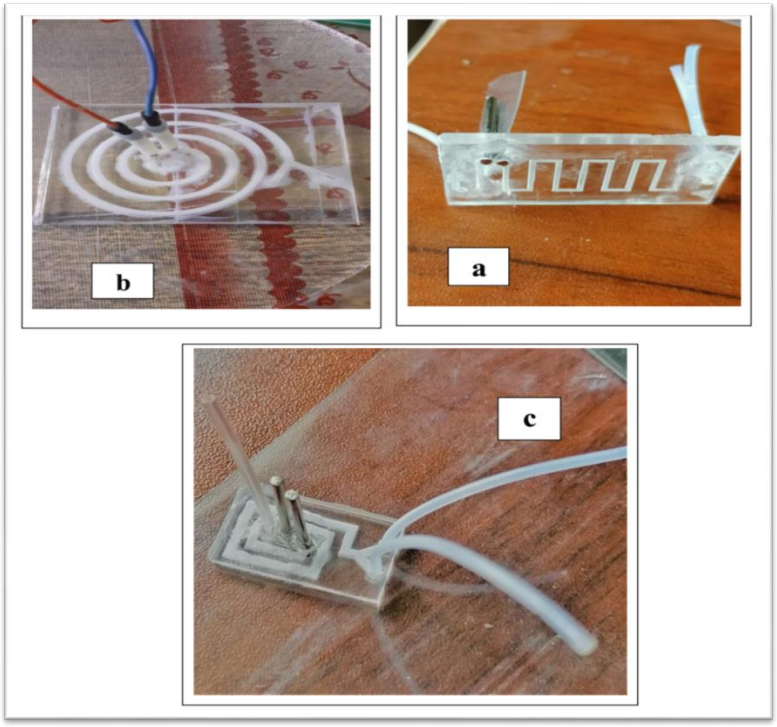


Fig (3.5) The design shapes home-made microfluidic system

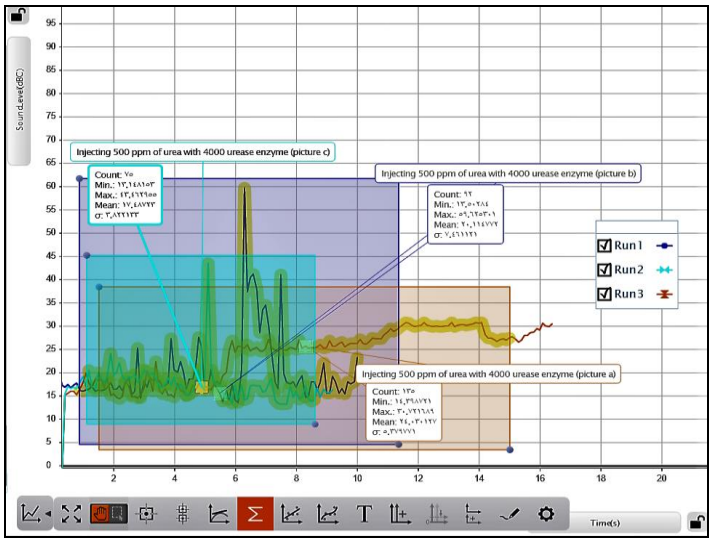


Fig (3.6) Shows the mobile phone output in studying the microfluidic system design shapes

3.5 The effect of flow rate:

It has been proven that the flow rate is a major factor in the microfluidic system, which has a lot to do with the efficiency and sensitivity of the urea- urease enzyme reaction signal. In this study the hand was used as a propulsion for the flow in both fast and slow ways. Figure (3.7) shows the effect of the flow rate on the peak height resulting from the interaction of (500 ppm) urea with (4000 ppm) urease enzyme, as the peak height increased in the slow flow because the reaction is complete and in the case of the fast flow we notice the incompleteness of the reaction and thus the reaction outputs are unclear and confused.

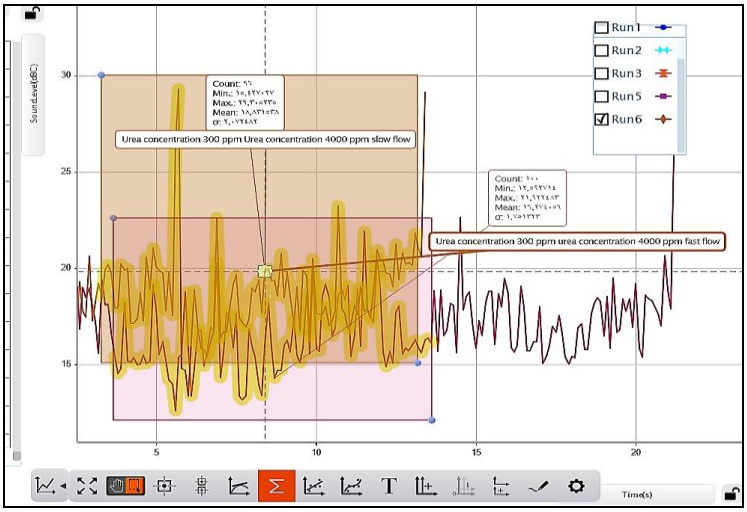


Fig (3.7) Effect of Flow rate on peak height of urea with urease in a microfluidic system.

3.6 The effect of urease concentration:

The concentration of Urease, at (pH 7) as recommended [26] plays an important role in the urea detect. Different concentrations of Urease solution have used in the range of (4000-2000 ppm). The optimum concentration was chosen depend on the maximum and reproducible peak height that present in sound level meter(SLM) App when they react with (500ppm) urea. Figure (3.8) shows that the increase in Urease concentration, leads to more intense peak height. So (4000ppm) Urease was chosen as an optimum concentration due to it good signal and the reproducibility of peak height.

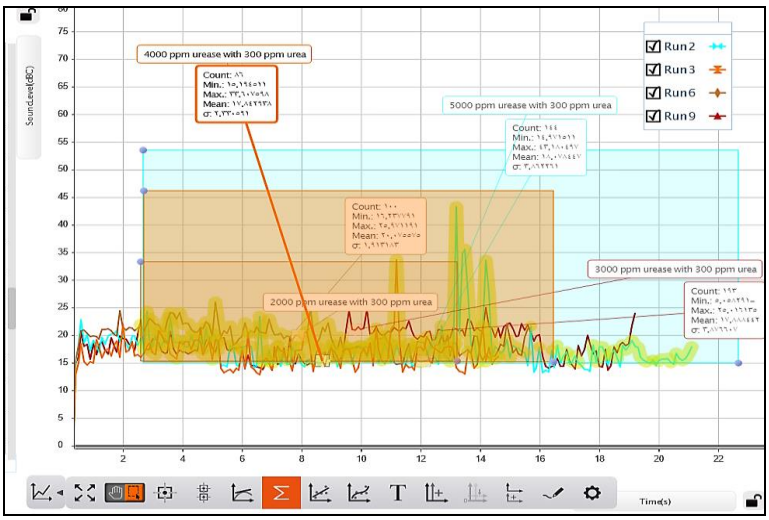
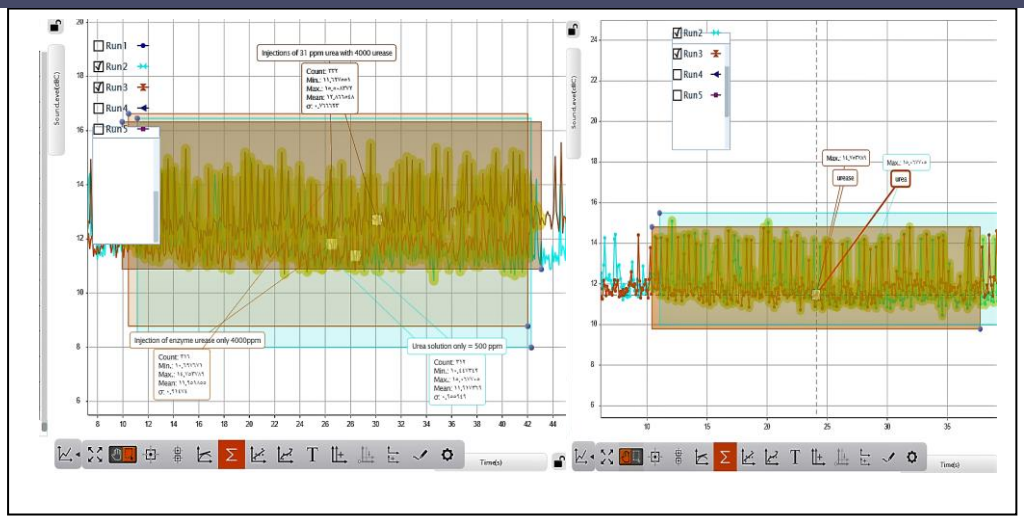


Fig (3.8) The effect of urease Conc. on peak height.

3.7 Peak evaluation and limit of detection calculations:

Figure(3.9) shows the measurement signal for both urea with a concentration of (500 ppm) and urease (4000 ppm) were injected separately, and it was found that the highest measured peak was between (14.75-15.06 dB), which represents (3×noise). When we measuring the lowest concentration of urea in this procedure (31.25 ppm), it was found that the measuring peak is (15.50 dB), which was considered the limit of the detection in this method, because it is close to the highest calculated peak for both urea and urease when they injection separately.



Fig(3.9) Detection limit of urea

3.8 Standard calibration curve of Urea:

Under the conditions established, a calibration curve of Urea was obtained. Figure (3.10) was a constructed graph between the sound intensity in dB (peak height, Y, mm)and the Urea concentration (X)in the range (62.5-500) ppm , and the results are shown a typical linear calibration curve in Table(3.1) and Figure(3.12) .The linear graph has a regression coefficient and Correlation coefficient of (r2 =0.992)for (6 point) [26]. The detection limit (3σ×noise) was 31.25 ppm and r.s.d % for ten replicate analysis of standards 125 ppm Urea was 0.65%.

Table (3.1) Optimum conditions of determination Urea by microfluidic system

Parameters	Value
Flow rate	Handing
Sample volume of microfluidic shape	0.1 mL
Cons. of Urease	4000ppm
Cell Conductivity volume	3.97µL
Cell Conductivity constant	12.7388 cm^{-1}

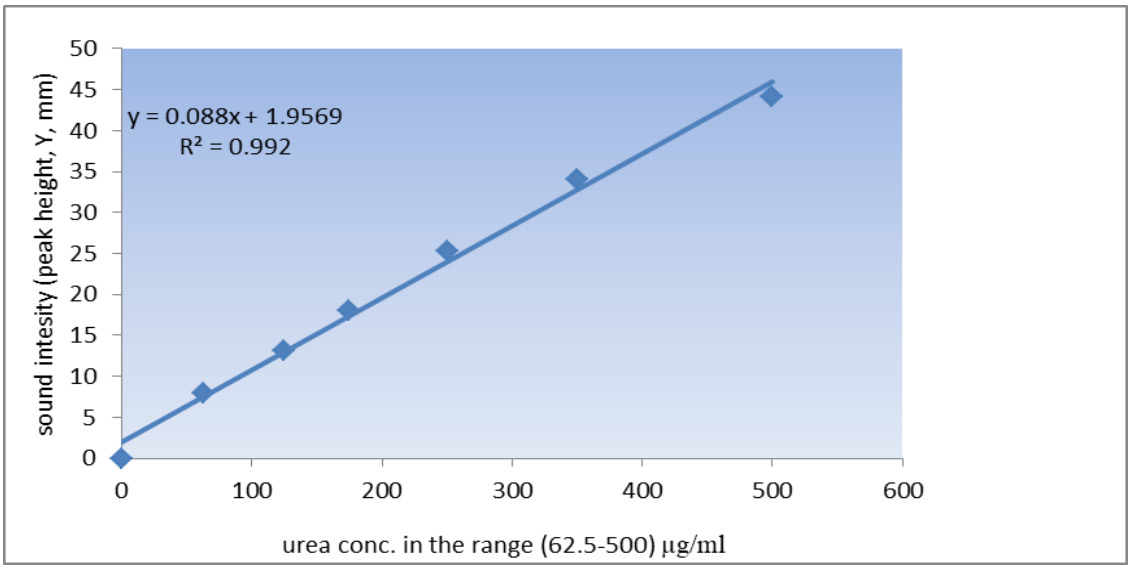


Fig (3.10) The corresponding calibration curve of Urea

3.9 Accuracy and application of the method:

In order to established the accuracy and validity of the home –made microfluidic system used to determine a several representative sample analysis Table (3.2). The batch samples were analyzed by classical [27] manual method using a standard additions method to avoid all possible interferences [28]. The average recoveries for five measurements were in the range (98.5 – 100.13 %). Good agreements between the results were obtained as shown in Table (3.2), which clearly indicated the home –made microfluidic system and the proposed method is suitable for the determination of Urea.

Table (3.2) Determination of Urea in synthetic sample

Samples	Claimed (ppm)	Determination of Urea by home –made microfluidic system *		Determination of Urea by classical Method(Conductivity)	
		Found (ppm)	Recovery % ±r.s.d%	Found (ppm)	Recovery % ±r.s.d%
1	250	254.85	98.5±0.57	250.93	99.30±0.33
2	500	500.11	100.13±0.57	499.7	100.8±0.48

Conclusion:

Semi-automated home-made Designing and constructing PMMA(Poly methyl methacrylate)Microfluidic system using CNC (Computer numerical control) milling machine offers a simple sensitive and reproducible mean for determination of urea .The mobile-phone was used as a novel detector for Microfluidic system, this detector was consider as an inexpensive and suitable tool for laborites.

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