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Thin film preparation of polyphenol oxidase enzyme (PPO) and investigation of its organic vapor interaction mechanism

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Abstract

This study investigated spin coated thin films of polyphenol oxidase (PPO) enzyme as vapor sensor to detect chloroform, acetone, ethyl acetate, isopropyl alcohol and toluene. Thin film of enzyme was produced onto a bio-composite (gelatine chitosan) first layer via 5000 rpm spin speed. The density and the viscosity of the enzyme were 1.2 g ml^{-1} and 68 mPa.s respectively. UV–visible spectroscopy and quartz crystal microbalance (QCM) measurements were carried out to analyze the reproducibility of PPO spun film. It was found that the PPO enzyme can be transferred onto a solid substrate as a solid state thin film form. The sensor films of PPO enzyme were exposed to various volatile organic compounds (VOCs) (chloroform, acetone, ethyl acetate, isopropyl alcohol and toluene) with different fixed concentrations. The sensing responses of PPO thin films versus five vapors were investigated using QCM as the time dependence frequency recording method. The PPO sensor films exhibited high sensitivity and fast responses against all VOCs. But the response rate and magnitude were changed depending on the chemical structure and the molecular size of the analyte vapor. Recorded frequency changes as monitored by QCM technique have been integrated with the Fick's second law of diffusion to determine the diffusion coefficients of analyte vapors. The results showed that the interaction characteristics between PPO and the analytes can be considered in terms of two main processes which are surface interaction and diffusion. And it was concluded that the formation of these two processes during the interaction depend on the molecular size and functional group of the analytes. These results showed that enzymes can be integrated into vapor sensor system as active layer and are promising for further sensor studies.

Keywords: polyphenol oxidase, spin-coating, quartz crystal microbalance, VOCs

(Some figures may appear in colour only in the online journal)

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1. Introduction

Monitoring the air quality has become an increasingly critical issue during the last decades as a result of necessity of awareness of the dangers of indoor and outdoor air pollution [1, 2]. Therefore gas sensors play an increasingly important role in monitoring the danger of pollution level [3], and the research in the field of gas sensing has become popular for latest researches [4, 5].

VOCs (volatile organic compounds) are one of the groups of common air pollutants which are extensively used in daily essentials such as paints, cleaning solvents, plastics, cosmetics and wood preservatives. Therefore, the probability of encountering this group of pollutant vapors in daily life is very high. The vapors of VOCs are highly toxic and need to be detected before they reach dangerous levels for human health. And in recent years there is an increasing demand to developing new VOC sensors with increasing the sensing abilities [6–9]. The intentions for developing new sensor devices are focused on high sensitivity, precise selectivity and fully reversibility with ideally at lower cost. Considering these demands, the essential concern of sensor studies is to develop a better sensing material. Because the ideal sensor features like sensitivity and selectivity are directly related to the interaction between vapor molecule and sensing material [10, 11]. With this purpose in mind, the interaction mechanism is the key feature and much of the reported research is to develop high sensitivity sensing material with clarifying the interaction mechanism [12, 13].

There are same basic features to consider when choosing a gas sensor material, which are low cost and practical ease of handling for making thin films using different techniques. Due to the existence of these features in organic materials, they are attractive as gas sensor materials. There are wide ranges of specific subsections of organic materials such as polymers [14], calixarenes [15], porphyrins [16] and phthalocyanines [17] and all have been widely used as sensing material within sensor studies. Because of the solid form of the selected sensor material is required in the organic sensor studies, organic materials must be transferred onto a solid substrate as a thin film form. There are a wide range of thin film techniques which have been used to prepare solid state films of organic materials such as Langmuir–Blodgett [18], Langmuir–Schaefer [11], spin coating [19, 20] and self-assembly [21]. Different techniques have different advantages and disadvantages and the properties of selected organic material must be considered before selecting a transfer method. Spin coating method is one of the most common coating processes for organic thin film preparation. Its effective for deposition of various organic materials without the need of any complicated preparation procedure. This technique can exhibit a fast and high precision deposition and produce ultrathin organic films. Besides the organic materials, enzymes are also organic substances and have been used generally in biosensor applications [18, 22, 23]. They catalyze most of the metabolic reactions but at the end of the reaction, they return to their original state. Owing to this ability, enzymes can enter in the unlimited number of reactions. Also enzymes are reaction selective which they only interact with specific materials. These two unique properties of enzymes make them attractive for sensor studies as a sensitive layer. In recent years, different enzymes have been tried to be integrated into sensor systems to take the advantages of enzymes in sensor studies [22–25]. Even though many enzyme based biosensors were already reported in literature [26, 27], enzymes are not used as a sensitive layer in gas sensor studies.

This work investigates the sensing capability of enzyme based vapor sensor against VOCs. Here polyphenol oxidase (PPO) enzyme was employed as sensing material. PPO was purified from banana and procedures are presented. Spin coating technique was used to transfer PPO onto a solid substrate as thin film form. In order to increase the thin film stability, chitosan-gelatine bio-composite was coated onto substrate before enzyme layer. The main challenge of this work lies in the fabrication of thin films of PPO enzyme for to use a sensitive layer to detect VOCs. Characterization of the enzyme films was performed by UV–visible spectroscopy and quartz crystal microbalance (QCM). The reproducibility of the transfer process was confirmed by both UV–vis and QCM measurements. The sensing characteristics of enzyme film were investigated using QCM kinetic measurements upon exposure to chloroform, acetone, ethyl acetate, toluene and isopropyl alcohol. The diffusion characteristics into film structure with regard to the physical properties of the examined VOCs have been emphasized. The response data have been analyzed using the Fick's second law of diffusion in order to clarify the interaction characteristics of analyte vapors [28, 29]. Kinetic graphs were explicated with the interaction mechanism between PPO and VOC and the results demonstrate that the enzymes are promising materials against VOCs detection.

2. Experimental details

2.1. Materials and film preparation

In this study, PPO was selected as active layer for VOC sensor. PPO has been purified from banana according to the technique published elsewhere [30]. In order to increase the PPO thin film stability onto a solid substrate, a bio-composite layer coated as a first layer. That bio-composite consists of chitosan and gelatine mixture in acetic acid and preparation procedure has been explained in literature [30].

Thin films of PPO were prepared via spin coating technique using an under layer of bio-composite. The 50 μl of bio-composite solution was dispensed onto a rotating substrate at spin speed of 5000 rpm. The rotation of substrate was continued for 60 s after dispense to allow the evaporation of the solvent and achieve a uniform thin film. In addition to that, bio-composite thin film was allowed for 10 min to obtain completely dry layer before PPO transfer process. Afterwards 50 μl of PPO solution was dispensed onto the rotating substrate under the same transfer conditions and rotation was continued for 60 s. Spun films were transferred onto pre-cleaned 76×26 mm (thickness is 1–1.2 mm) microscope glass slides for UV–vis measurements and 10 MHz quartz crystals for QCM technique. Cleaning processes for both substrates
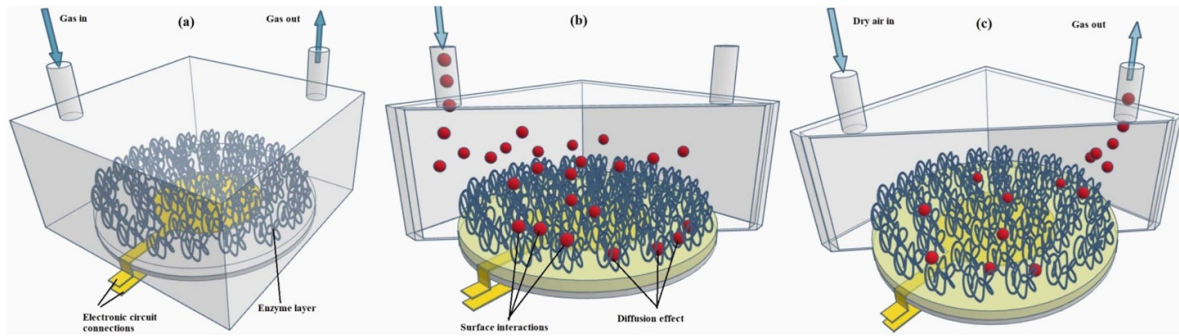
Figure 1. (a) Schematic representation of QCM gas cell, (b) vapor, thin film interaction illustration, (c) recovery process with dry air.

were performed in several steps by using chloroform, isopropyl alcohol and ultrasonic cleaner.

UV–vis measurements were performed using a PerkinElmer Lambda spectrophotometer in a range of 350–800 nm. The deposition controls of bio-composite enzyme thin film were carried out via comparison spectra of solutions and thin films in absorbance mode.

In this study, 10 MHz quartz crystal which was sandwiched between two gold coated electrodes was used for QCM measurements. It is well known that the quartz in the QCM system has a resonance frequency the ability of the system is to keep that frequency stable. The frequency is only proportional to the mass which is accumulated onto quartz crystal. Sauerbrey was expressed the relation between the frequency and the accumulated mass by the Sauerbrey equation [31]. Owing to the mass sensitivity, QCM system can be used for different characterization studies which are related to mass change and in this work deposition process and reproducibility of thin films were also investigated by QCM.

2.2. Vapor sensing measurements

The investigation of the kinetic vapor sensing of PPO thin films involved recording the dynamic response upon exposure to the VOCs. Several VOCs (chloroform, acetone, ethyl acetate, isopropyl alcohol and toluene) were chosen as analytes because they permit a comparison of the sensing response measured using different molecular groups. Therefore the influence of PPO on sensor studies can be investigated by considering these molecules physical properties. The VOCs were obtained from Sigma-Aldrich and dry air was used as recovery gas.

The experimental setup used to record kinetic vapor sensing characteristics of PPO thin films has been schematically described in figure 1. Vapor sensing properties were investigated using QCM system with a purpose built gas chamber by recording the frequency change against time. As seen from the figure 1, gas chamber has a quartz crystal holder which is directly connected to the QCM measurements system. It is completely sealed and equipped with gas inlet and outlet holes thus exposure and recovery of the sensor can be

performed without causing any frequency noise. The kinetic measurement process consists of consecutive 3 min response and 3 min recovery cycles and the concentration of the analyte vapor was calculated using the gas law [28, 32],

$$c = \frac{22.4 \rho T V_s}{273 MV} \times 10^3 \qquad (1)$$

where c is the concentration (ppm), ρ is the density of the liquid sample (g ml^{-1}), T is the temperature of container (K), V_s is the volume of the analyte solution (μl), M is the molecular weight of analyte (g) and V is the container volume.

Precise amounts of analyte solutions were measured using a microsyringe and transferred into a glass bottle. Temperature of the glass bottle was controlled and gentle heated to allow the solution to evaporate. In order to obtain intended ppm values, 50 ml syringe was used for further dilution and final injection into gas chamber. Recovery of the PPO thin films was performed by injecting fresh air into the gas chamber.

3. Results and discussion

3.1. Characterization of spin coated films

Figure 2 shows the UV–vis spectra of the PPO solution at the range between 350 and 850 nm. Proteins have maximum absorption spectra in the UV region and show specific absorption at 275–280 nm due to the tyrosine and tryptophan containing [33–35]. PPO solution exhibits a main band which is figured lower region of 550 nm continues towards 350 nm and below. And it has two weaker and broader bands between 590–770 nm and 770–850 nm.

UV–vis absorption spectra of bio-composite/PPO spun film are also given in figure 2. In order to monitor the bio-composite effect on the UV-spectra, it has been recorded after bio-composite coating and then enzyme layer was deposited. Absorption of bio-composite layer can be ignored because of its low density and imperceptible peak regions, thus it can be said final absorption spectra is entirely related to PPO film layer. UV region of spectra can not be observed clearly because of the optical noise of glass slide substrate. However, since the main absorption band proceeds above 350 nm,
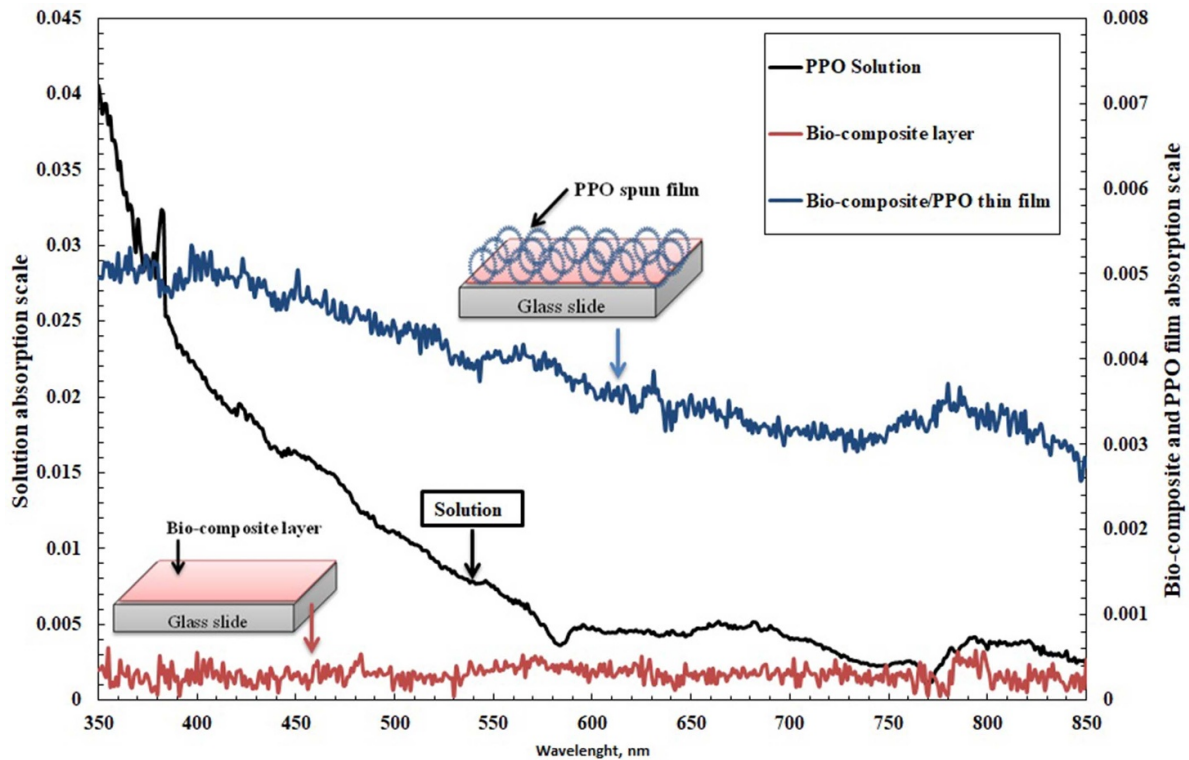
Figure 2. UV–vis absorption spectra of PPO solution and thin film layers (bio-composite-PPO).

spun film absorption can be clearly observed and compared with the solution. The solution spectra are taken in a cuvette with a 1 cm path length therefore the number of organic molecules available to absorb light is significantly less in the films and the total absorbance are much smaller resulting in a poorer signal to noise ratio for these measurements. Considering these, the UV spectra of the thin film is observed as a band spectra rather than a peak because of the weaker absorption.

The aim of measuring UV–vis spectra is to focus on the absorbance change after coating PPO layer and confirm the PPO layer transfer process onto bio-composite. Because the PPO film is the sensing layer and confirmation of that layer is much more important. According to figure 2, it can be clearly seen that the absorbance changes remarkably after coating PPO layer onto bio-composite. Furthermore, the absorbance curve of PPO layer exhibits similar characteristics with solution. But the absorption bands of thin film are in a wider band range where the individual molecules are present. Removal of the solvent, aggregation of molecules in thin film structure and/or increasing intermolecular interactions may cause some shift and broadening of absorption band [12, 28, 36].

If we examine the solution spectra as three different regions, we expect similar characteristics to be found in the PPO spectra. In the solution spectra, the first two regions starting at 350 nm and ending at 770 nm were observed as a single broadened band in the PPO spectra. The peak formed in the solution after 770 nm is also observed for the PPO layer with a very similar characteristic. As a result it can be said that the change in the spectrum after the PPO deposition was due to

the PPO molecules, and this demonstrates that the thin film of PPO is successfully deposited.

In an attempt to determine the transfer process of bio-composite and PPO thin films, the mass change on quartz crystal is investigated by QCM system. The resonance frequency, Δf, is extremely sensitive to a small mass change that described in detail by Sauerbrey [31] as given by:

$$\Delta f = \frac{-2f_o^2}{\sqrt{\rho_q}\sqrt{\mu_q}A}\Delta m \qquad (2)$$

where Δf is frequency change (Hz), f_o is the resonance frequency of quartz crystal (Hz), Δm is the mass change (g), A is the area (cm^2), ρ_q is the density of quartz (2.648 g cm^{-3}), μ_q is the shear modulus of quartz (2.947 $\times$ 10^{11} g cm^{-1} s^{-2}).

Table 1 gives the detailed information about the transfer process of each layer on quartz crystal and their recorded frequency changes. Typical frequency changes of about 58.4 HZ for bio-composite layer and 239 Hz for PPO layer are obtained from QCM results. The mass deposited onto quartz crystal is estimated as 391 ng for bio-composite and 1603 ng for PPO spun film using the equation (2). The thickness of spun film layers can be calculated by the help of weight difference method that described in the following equation [37–39]:
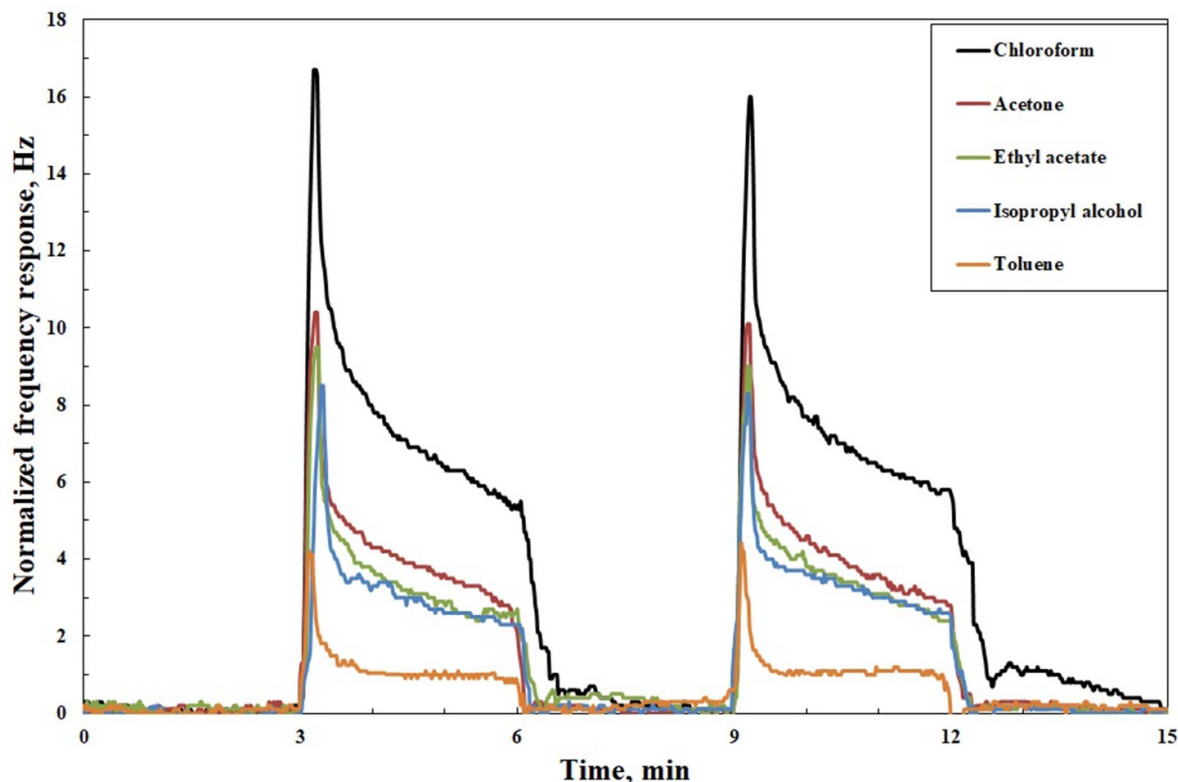
$$d = \frac{\Delta m}{\rho \cdot A} \qquad (3)$$

where Δm is the deposited mass, A is the area of quartz crystal, ρ is the density.

Table 1. Quartz crystal microbalance results of spin coated PPO thin films.

	Frequency change (Δf, Hz)	Deposited mass (Δm, ng)	Thickness (d, nm)
Bio-composite	58 ± 7.3	391 ± 48.9	3 ± 0.32
Polyphenol oxidase	239 ± 21.0	1603 ± 140.9	9 ± 0.7

Figure 3. Normalized kinetic QCM response of PPO spun film against five different vapors (chloroform, acetone, ethyl acetate, isopropyl alcohol and toluene).

Using the equation (3), the thicknesses of spun films are estimated as ∼3 nm for bio-composite layer and ∼9 nm for PPO layer. Considering these, QCM results indicate that the transfer processes of both layers are carried out successfully. Inaccuracies of the measurements are also given in the table 1 according to the measurement uncertainty. Detailed information about the measurement uncertainty of open-source QCM systems can be found in the literature for thin film measurements [40, 41].

3.2. Vapor sensing properties

Recently, organic sensor materials have new sensing elements based on enzyme platform that intended to increase sensitivity and selectivity by modifying the enzymes [22, 42, 43]. PPO is also used as an sensitive layer in biosensor systems [18, 25]. In order to examine the PPO thin film as a vapor sensor, it was exposed to five VOCs (chloroform, acetone, ethyl acetate, isopropyl alcohol and toluene). Figure 3 displays the kinetic measurement of PPO spun film, where normalized frequency change is plotted as a function of time.

The responses of PPO film were recorded upon exposure to 800 ppm of VOCs which are also labeled in the inset of figure. During the first 3 min period sensor film was allowed for the dry air and frequency change was kept at a certain baseline value. At the end of this period VOC molecules were introduced into the gas chamber. On injection of each vapor, PPO film exhibited sharp frequency change and then decreased exponentially until the end of 3 min period. It is clear that the PPO film has a fast and reversible response against all analyte VOCs. As it can be seen from the rapid change at the 3rd min, the response times are about a few seconds. After 3 min exposure period, dry air was sent back into chamber for recovery control and PPO spun films was fully recovered for all analyte vapors. To ensure the response stability, exposure and recovery cycles repeated several times and it is clear that the responses of PPO film are fast and reversible against all VOCs for every cycle.

The strongest response was recorded against chloroform followed by three analyte (acetone, ethyl acetate and isopropyl alcohol). Acetone, ethyl acetate and isopropyl alcohol produced very close frequency changes and it cannot

Table 2. Physical properties of analyte VOCs (at 20 °C).

VOCs	Density (g ml^{-1})	Vapor pressure (kPa)	Dipole moment (D)	Molar mass (g mol^{-1})
Chloroform	1.489	21.1	1.15	119.37
Acetone	0.784	24.4	2.91	58.08
Ethyl acetate	0.902	9.7	1.78	88.10
Isopropyl alcohol	0.786	5.3	1.66	60.09
Toluene	0.866	2.9	0.36	92.14

be said there is a remarkable selectivity between these vapors. Apart from these, toluene exhibited a different response characteristic and it can be recognizable with its much lower response.

QCM sensing mechanism detects very small mass changes and the response is directly related with the interaction of the film with vapor molecules. Therefore the level of response depends on the amount of the vapor molecules trapped into thin film matrix. Physical properties and molecular size of the analyte vapor can mainly affect the amount of interaction with the thin film. Table 2 gives some physical properties of the analytes used in this study.

Dipole moment of vapor is one of the main parameter which affects the interaction level and rate. The vapor with highest dipole moment can easily interact with the possible sites of thin film molecules. When the vapor is sent into a gas chamber, its vapor pressure value leads the dynamics in that closed volume. If these two parameters are considered, acetone has the highest dipole moment and it has close vapor pressure value with chloroform. Taking these in account, acetone would be expected to produce more response than chloroform. However, QCM system is based on to detect mass change rather than the amount of interacting vapor molecules. In this case, molar mass and density of analyte vapor should be considered as well. Besides, these parameters can even be more effective on total mass change than others. As seen in response results, the highest response is produced by the chloroform interaction and this shows that the highest adsorbed mass belongs to chloroform vapor. There is not a much difference in density and molar mass between acetone, ethyl acetate and isoprophyl alcohol. Considering this group, acetone has slightly higher response, yet the response levels are very close to each other. This could be attributed to the higher dipole moment and vapor pressure of acetone which can increase the interaction amount of vapor molecules and increase the absorbed vapor mass. The lowest response arises from toluene vapor. As seen in table 2, toluene has lowest dipole moment and vapor pressure, although it has closer mass values to other VOCs. This could be the main reason for lowest mass change due to the lower interacting vapor molecules. The other reason could be the size of the toluene molecule which has a chain structure. Gas sensor studies revealed two different mechanisms while examining interaction between vapor molecules and sensor material [12, 28]. One is surface interaction, where the vapor molecules directly interact with the surface of sensor material. If the vapor molecules start to diffuse into thin film structure, this produces a different mechanism called diffusion

effect. Thus, higher sensor response can arise with diffusion of vapor molecules. In such a case, the size of the vapor molecule becomes important so that the diffusion can take place. But the size of vapor molecule is not only the parameter; it can exclusively increase the accessible interaction sites for the analyte. Therefore, toluene and thin film interaction may have been limited to surface only. Thus the response against toluene is remarkable lower than the other VOCs.

As it was mentioned above, physical properties of VOCs are directly effective on the sensor response. But the chloroform vapor has a typical interaction behavior with thin film which gives that vapor more sensitivity. Chloroform proved to be a potent anesthetic agent [44] and the fundamental interactions of anesthetics with proteins have been considered in some detail. It has been shown that these types of anesthetic volatiles can specifically bind to discrete sites in proteins [45]. In addition, it has been observed that the volatile anesthetic vapors promote the binding interaction by enhancing the hydrophobic interaction among side-chains and by rearranging the hydrogen bonds in peptide backbone [46]. Owing to these specific interactions, chloroform molecules can be captured by PPO thin film easier than the other VOCs. Thus the response against chloroform is remarkably higher than the others.

The measurements indicate that the PPO thin film sensor is suitable for more than one use against all VOCs with a fast response time. Injection of vapor starts the interaction simultaneously and the rate of the interaction can be observed on the response chart. The response time is relatively fast for all vapors and this is a key feature for a sensor device. The other mechanism of the interaction is removing the vapor molecules from thin film matrix for the next measurement preparation. In figure 3, at the time of 6th min the dry air starts to remove vapor molecules and recovery also starts at same time. It can be clearly seen that the response for all vapors are completely reversible which indicates that all interacted sides of PPO molecules are ready for another interaction. Removing the vapor molecules take a few seconds except chloroform which has a quite longer recovery times than other vapors. Although chloroform vapor produces completely reversible response on PPO sensor, recovery time for takes almost 30 s for fully come back to its initial frequency value. As it was mentioned above, chloroform has a distinctive interaction characteristic with proteins. Considering this interaction, removing chloroform molecules from active binding sites within PPO film is possible but due to the strength of the interaction it takes more time than other VOCs. The other reason could be the amount of interacted chloroform molecule since they diffuse into PPO
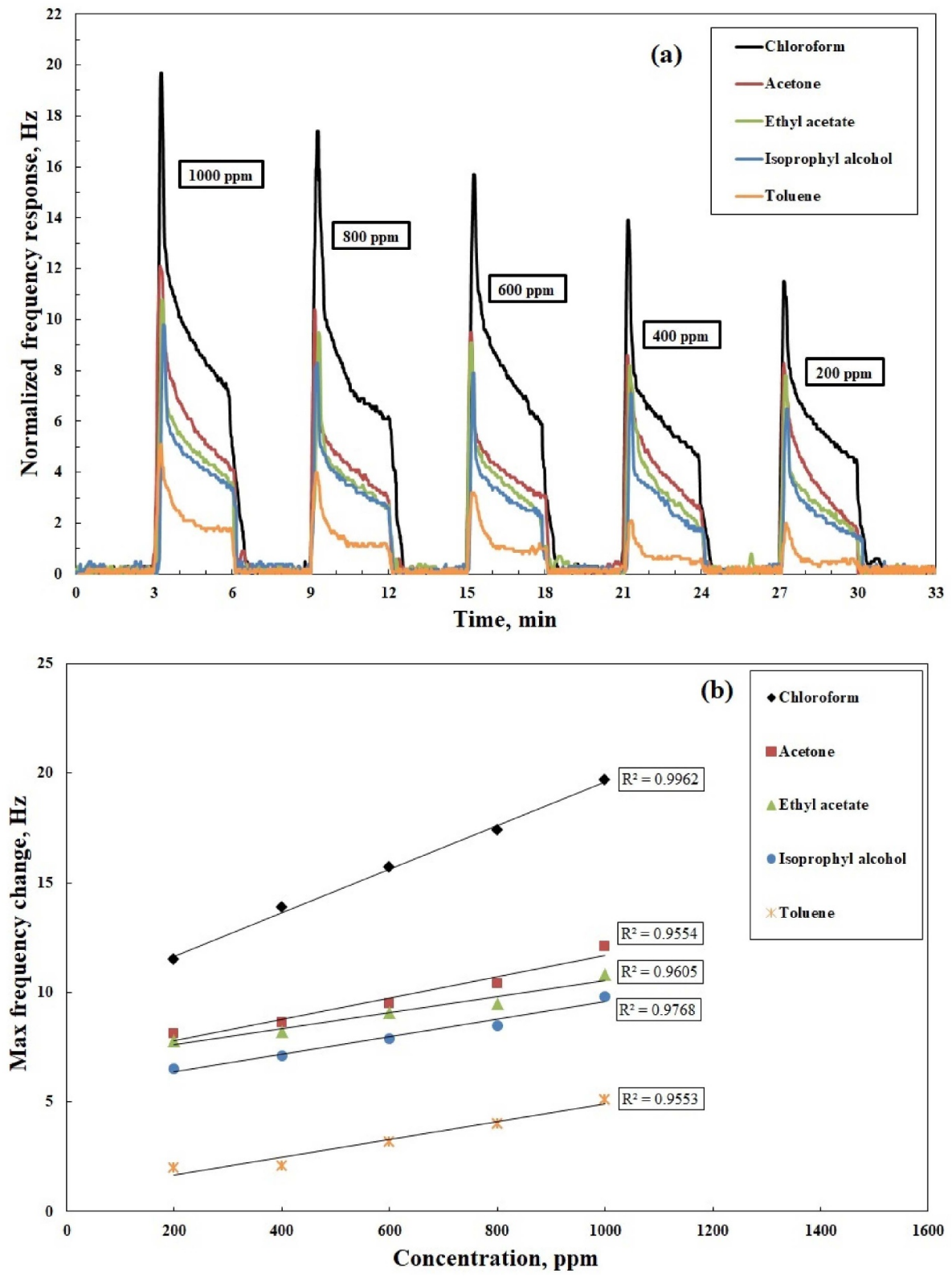
Figure 4. (a) Reproducibility of PPO thin film for five different vapor concentrations, (b) maximum frequency change versus vapor concentration.

film matrix more than others. Slower recovery process of chloroform vapor also indicates that the interaction between chloroform and PPO thin film is distinctive.

Figure 4(a) shows the dynamic measurements of VOCs for PPO thin films at different concentrations from 200 ppm to 1000 ppm. As seen on figure, although the variety of

Table 3. Sensitivity and LOD parameters of VOCs.

VOCs	Concentrations (ppm)	200	400	600	800	1000
Chloroform	Sensitivity (ppm^{-1})	0.0575	0.0347	0.0261	0.0217	0.0197
	LOD (ppm)	5.21	8.63	11.46	13.79	15.22
Acetone	Sensitivity (ppm^{-1})	0.0405	0.0215	0.0158	0.0130	0.0121
	LOD (ppm)	7.40	13.95	18.94	23.07	24.79
Ethyl acetate	Sensitivity (ppm^{-1})	0.0390	0.0205	0.0151	0.0118	0.0108
	LOD (ppm)	7.96	14.63	19.78	25.26	27.77
Isopropyl alcohol	Sensitivity (ppm^{-1})	0.0325	0.0177	0.0131	0.0106	0.0098
	LOD (ppm)	9.23	16.9	22.78	28.23	30.61
Toluene	Sensitivity (ppm^{-1})	0.0100	0.0052	0.0053	0.0050	0.0051
	LOD (ppm)	30.00	57.14	56.25	60.00	58.82

concentration the response characteristics of vapors are almost same between each other. Besides, max frequency change increases with increasing vapor concentration and the maximum values are given in figure 4(b). The data of concentration dependence of a gas sensor states two crucial parameters which are the sensitivity (S) and the limit of detection (LOD). For a gas sensor, sensitivity is defined as the change of response per ppm (ppm^{-1}) [12] and the LOD is defined as the lowest quantity (ppm) of the analyte can be detected by the sensor [11]. LOD can be described by the following equation (4) and parameters are summarized in table 3,

$$\text{LOD} = \frac{3\sigma}{S} \qquad (4)$$

where σ is the standard deviation of the signal and S is the sensitivity to specified analyte.

Table 3 represents the sensitivity and the LOD parameters of PPO thin film against various concentrations of VOCs. As seen in table, PPO thin film is more sensitive to chloroform vapor than others which is an expected result. It is obvious that the estimated LOD values of chloroform are much lower than the other VOCs for a specific concentration. Another conspicuous point of the table is decreasing sensitivity and increasing LOD values with increasing vapor concentration. This can be also experienced in figure 4(b) with cutting the y-axis at non-zero point. That corresponds to the decreasing the sensor response per ppm of analyte vapor while the total response is increasing. This relationship can be explained as saturation of PPO thin film with higher amounts of vapor concentrations. Thin film structure in three dimensional matrix has relatively limited volume and accessible binding sites of PPO sites decrease with increasing vapor concentration. Afterward all the binding sites of PPO thin film are occupied by vapor molecules, the thin film will be completely saturated. Thus the sensor will be reached maximum response owing to the vapor interaction of all possible sites of thin film.

It has been previously reported that the interaction of vapor molecules starts with the interaction of the active adsorption sites on the surface of thin film and then continues with diffusion into film structure [11]. Therefore the interaction mechanism between the vapor and the thin film can be considered in three stages which are surface adsorption, diffusion and desorption process [12]. The first sharp increase on the response curve is ascribed to surface adsorption of vapor molecules when they introduced into gas cell. This stage causes a rapid response increase because; easily accessible thin film molecules on the surface have been suddenly exposed to the analyte. Afterwards other two stages, diffusion and desorption start to dominate the interaction characteristics because of the vapor molecules start to diffuse into thin film matrix. During that diffusion process, adsorption and desorption of vapor molecules occur simultaneously which is a dynamic process consists of diffusion in and out of the sample. In the process of adsorption and desorption, sensor response starts to settle down and this can be seen as a decrease in frequency change until equilibrium. When the vapor molecule adsorption and desorption stops, thin films' response reach saturation and this can be observed as a fixed frequency change. The end of these three stages, dry air is injected into gas cell and fully desorption process starts until removal of all adsorbed vapor molecules for recovery of sample.

The characteristics of diffusion have been investigated using the Fick's second law of diffusion. That law defines that the rate of change in concentration with time is proportional to the rate at which the concentration gradient changes with the distance in a given direction [29]. The Fick's second law of diffusion can be simplified as the following equation [12],

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{D}{\pi a_0^2}} t_s^{1/2} \qquad (5)$$

where a_0 is the thickness of the thin film, D is the diffusion coefficient, M_t and M_∞ represent the amount of diffusant entering the plane sheet at time t and infinity (saturation), respectively and t_s represents the swelling time.

The resonance frequency of quartz crystal is very sensitive to very small mass changes and the amount of the mass diffusing into film structure produces a frequency change. Therefore the equation (5) can be expressed as follows [47],
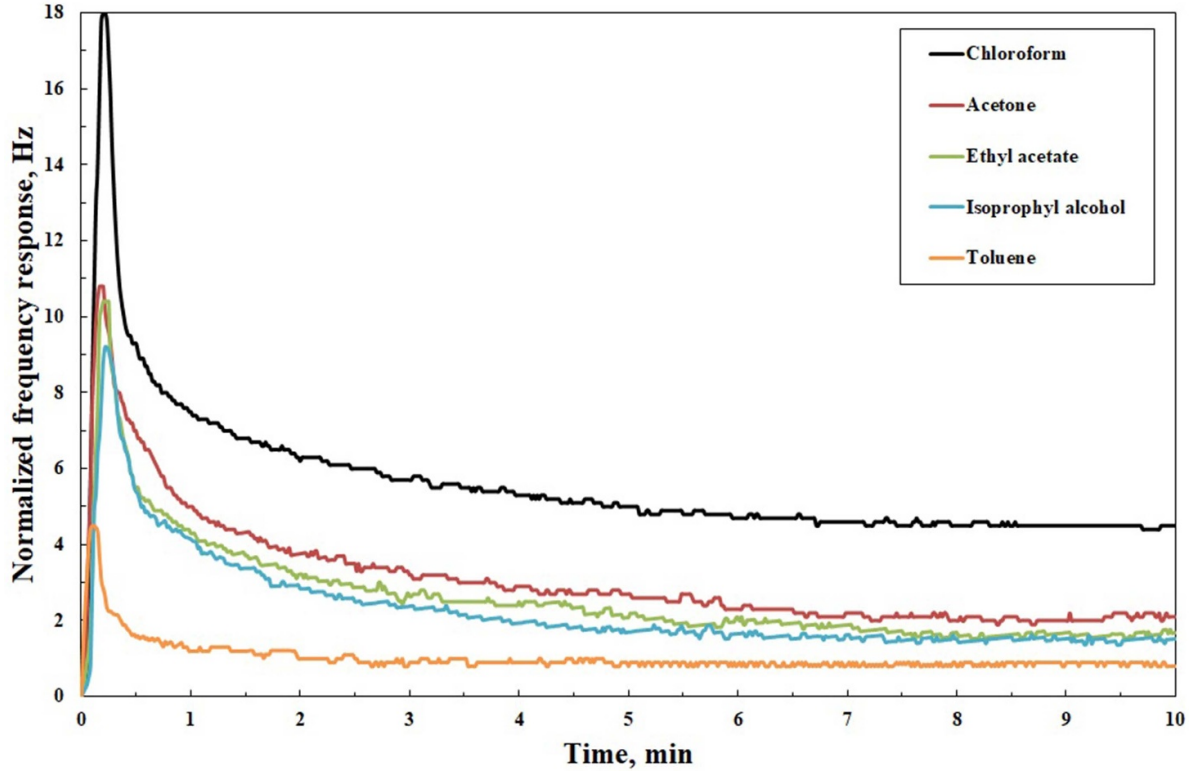
Figure 5. Extended kinetic measurements of enzyme film on exposure to 800 ppm of VOCs.

$$\frac{M_t}{M_\infty} \sim \frac{\Delta f_t}{\Delta f_\infty} = 4\sqrt{\frac{D}{\pi a_0^2}} t_s^{1/2}. \qquad (6)$$

In order to control the diffusion characteristics using equation (6), the exposure time was extended to 10 min and the sensor was allowed to reach saturation. Figure 5 shows the kinetic measurements of enzyme film on exposure to 800 ppm of VOCs, where the frequency change is plotted as a function of time. All conditions in this experiment were kept the same as the experiments in figure 3 except for the exposure time. It can be clearly seen that the response rates and characteristics of all analytes are almost identical to the results in figure 3 for the first 3 min. This is a confirmation of the reusability of enzyme thin film sensor device. But the intention of repeating the measurement was to observe the saturation point which was recorded in figure 5 for all vapors. After 10 min exposure period, the enzyme thin film was saturated by all VOCs.
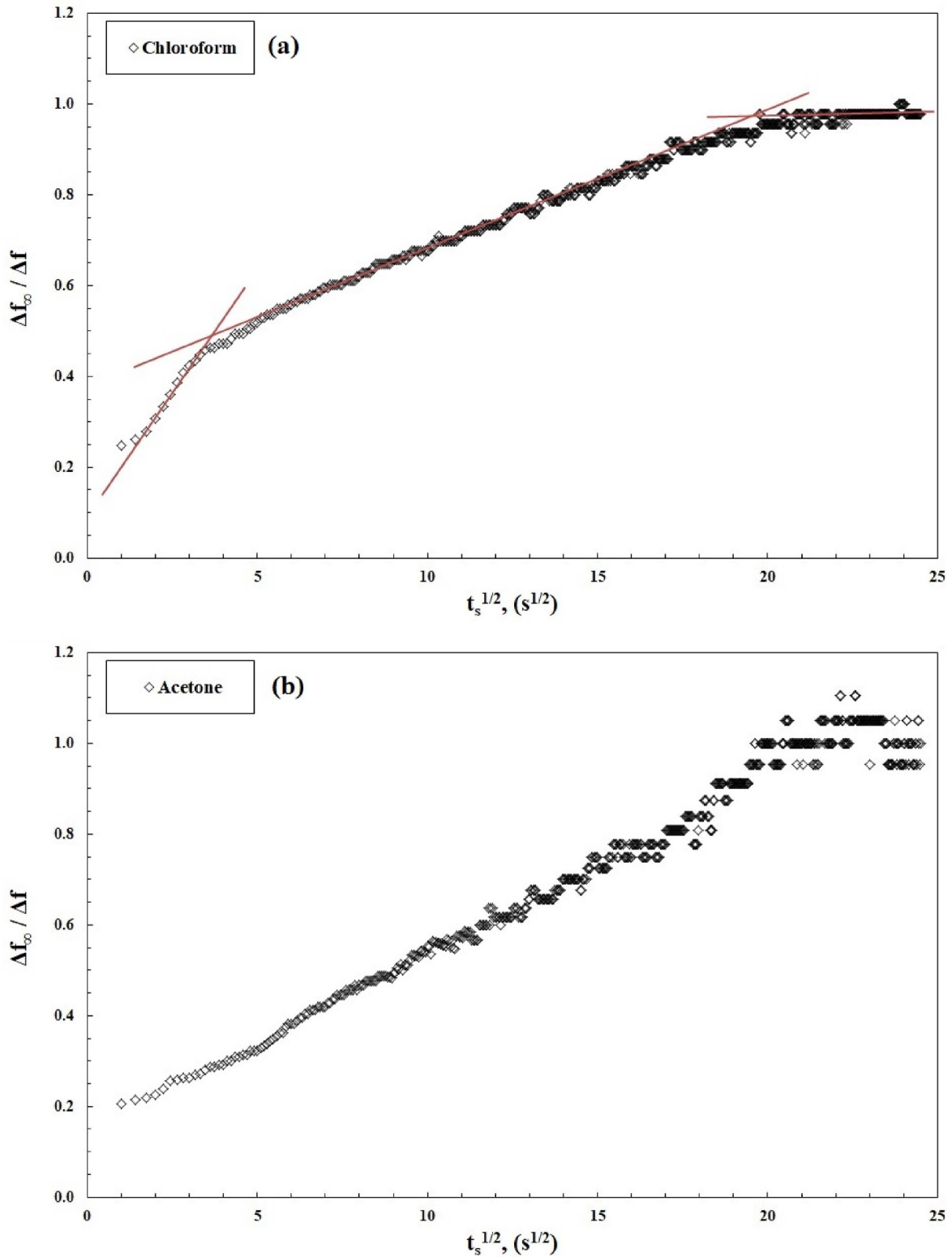
Figure 6 shows the normalized frequency changes versus square root of the time according to equation (6). The values of diffusion coefficients (D) determined from $\left(\frac{\Delta f_t}{\Delta f_\infty}\right)$ plotted as a function time $t_s^{1/2}$ are summarized in table 4. The larger values of diffusion coefficient correspond to fast diffusion of the vapor molecule. An increase in this chart indicates that the diffusion continues until it reaches the steady state. The steady state corresponds to the saturation of the sensor system. The slop of the increasing line gives the magnitude of diffusion.

The plots of each vapor are shown in separate charts in figure 6 in order to interpret respectively. Acetone,

ethyl acetate and isoprophyl alcohol exhibit similar characteristics that can be clearly seen in figures 6(b)–(d) and table 4 as close diffusion coefficient values. According to the table 4, toluene has slightly higher diffusion coefficient value than these three VOCs which is estimated as 0.38. As seen in figure 6(e), diffusion of toluene vapor takes a shorter period than other vapors although the higher diffusion coefficient. This can be expressed as the only surface interaction between sensor film and toluene vapor. Toluene has lowest sensor response and all of the interaction which generates the response is surface interaction with negligible diffusion. Therefore it takes a very short period for the toluene molecules to reach the surface and upper layers of sensor film and start the interaction.

According to the figure 6(a), the curve of chloroform can be investigated to have two different linear regions with two different slopes resulting two different diffusion coefficients (D_1, D_2). It is clear that the diffusion coefficient of chloroform is changing during the exposure period until saturation point. Two consecutive slopes are shown in figure 6(a) and estimated diffusion coefficients are given in table 4 as D_1 and D_2.

Elovichian model can be employed to provide an explanation of this result. This model states that the vapor adsorption rate should decay exponentially as the amount of adsorbed vapor increases [12]. This can be interpreted in terms of decreasing accessible thin film regions as a function of number of vapor molecules that have already been adsorbed by thin film. This model is a convenient way to differentiate between

Figure 6. Plot of normalized frequency change against square root of swelling time for (a) chloroform, (b) acetone, (c) ethyl acetate, (d) isopropyl alcohol, (e) toluene.

surface interaction and diffusion. It is well known that the interaction rapidly starts with the surface of the thin film and the change in slope indicates that the surface interaction is completed. Following this, the dominant interaction becomes the diffusion interaction. Therefore, two slopes and two different diffusion coefficients can be seen in cases where the diffusion interaction takes part after surface interaction. The response results of chloroform vapor indicate that remarkable
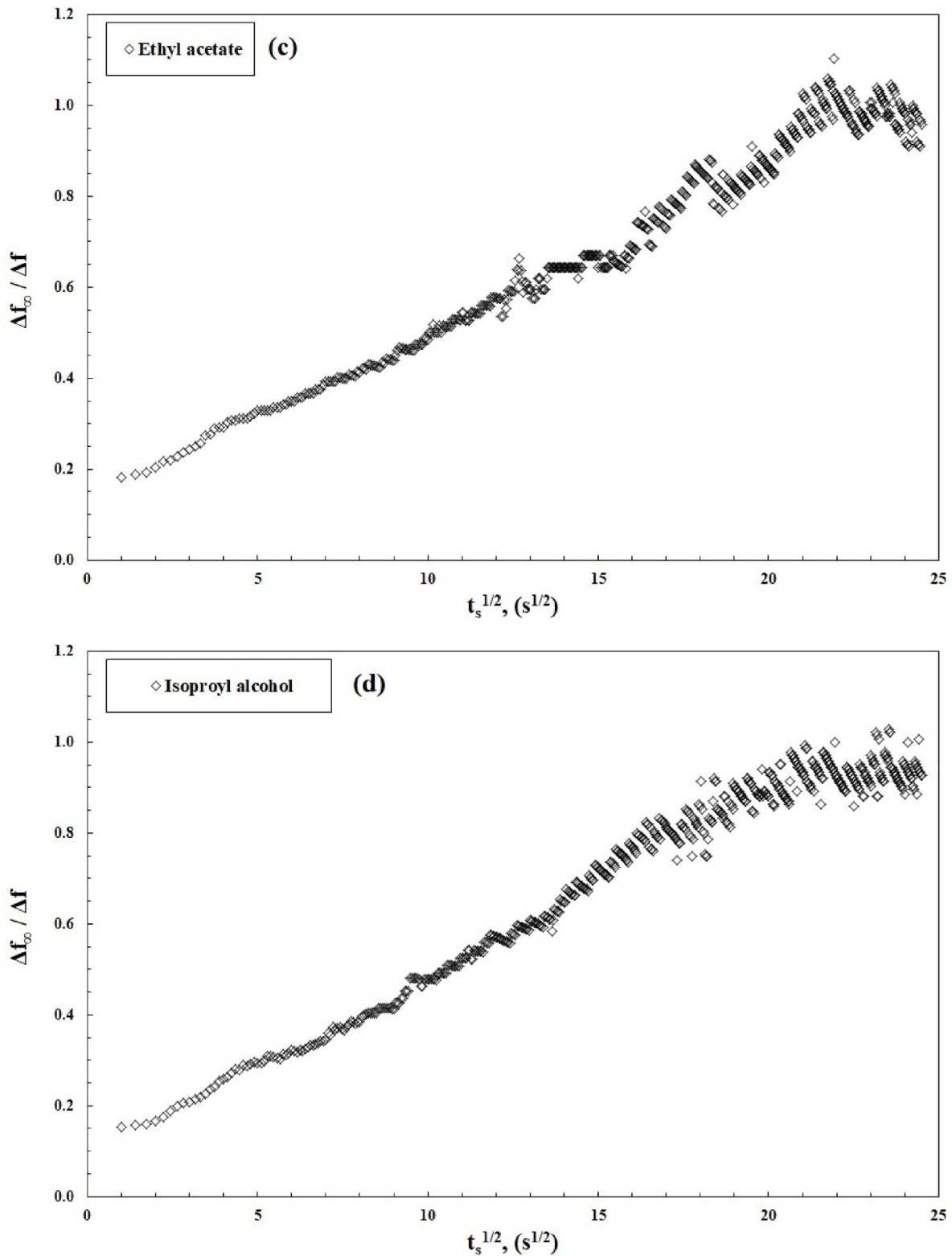
Figure 6. (Continued.)

amount of diffusion is carried out during chloroform vapor exposure. Thus the chloroform vapor has two different diffusion coefficients.

Interaction between PPO film and chloroform starts with a remarkably faster diffusion (D_1) compared to the other investigated VOCs. As it was mentioned before chloroform and sensor interaction starts with that fast surface interaction because PPO film is more sensitive to chloroform and results with the fast diffusion coefficient. Following that chloroform molecules starts to diffuse into PPO structure with a
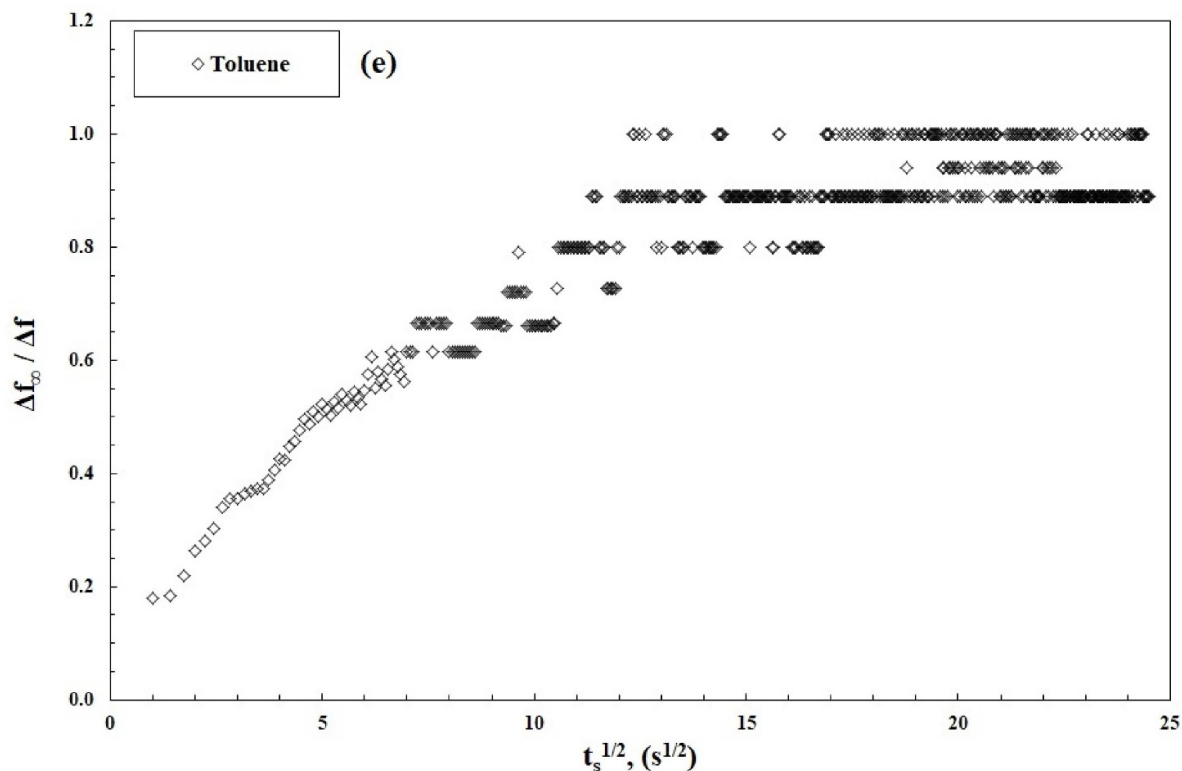
Figure 6. (Continued.)

Table 4. Diffusion coefficients (D) of VOCs.

	Chloroform		Acetone	Ethyl acetate	Isopropyl alcohol	Toluene
	D_1	D_2				
Diffusion coefficients (cm^2 s^{-1}) $\times 10^{-15}$	1.48	0.14	0.24	0.22	0.26	0.38

lower diffusion coefficient (D_2). Since the diffusion of vapor molecules into thin film matrix is relatively difficult than surface interaction, kinetic behavior of molecules start to decelerate. In addition, desorption of vapor molecules start during that period and as a result of these diffusion proceeds until saturation point. The diffusion effect of vapor molecules depends on several parameters because there is an obstructed path for the vapor molecules compared to the surface, which decreases the sensor response speed. The diffusion rate slows down as the accessible PPO molecules are occupied by the vapor molecules. Therefore, the interaction mechanism needs to be considered in three steps: which are (1) surface interaction, (2) diffusion effect and (3) saturation. In all vapor results, depending on the response size, the surface interaction and the saturation regions have been are clearly seen.

In cases where the diffusion occurs with a considerable lower number of molecules, the second slope and also the second diffusion constant may not be formed. This indicates that the majority of the produced sensor response is due to the interaction of vapor molecules with the surface of the thin film. According to the results in figure 6 and table 4, vapors other than chloroform cannot diffuse into the thin film

in sufficient quantity, the diffusion step cannot become dominant and hence no secondary gradient occurs. It has been observed in our previous studies that the alkyl chain size and functional group of the analyte vapor affect the diffusion mechanism [11, 28]. As in our current study, some vapors have two different diffusion constants, while others can only interact with the surface.

4. Conclusion

In this study, PPO enzyme has been selected as a new thin film material and successfully deposited onto surface of glass and quartz crystal using spin coating method. In order to obtain a sufficient thin solid enzyme layer on the solid substrate, bio-composite (chitosan-gelatine) first layer was deposited as lower layer. Solid-state thin film processes were successfully achieved via spin coating technique and thin films' thicknesses were evaluated 3 nm for bio-composite and 9 nm for PPO layer.

Enzyme sensor film was exposed to five different VOCs (chloroform, acetone, ethyl acetate, toluene and isopropyl

alcohol) at different concentrations. The response of the sensor film was monitored by QCM system and data were recorded as time-dependent frequency change. Concentration and time dependence sensor responses were used for to investigate the sensing characteristics and diffusion process.

PPO thin film exhibited excellent sensor responses against all examined analytes which were found to be fast and reversible. Besides, the responses of all VOCs were dependent upon the concentration of exposed vapor. PPO sensor film was shown to be more sensitive to chloroform vapor as can be seen from the equal concentrations of responses. In order to analyze the interaction between the PPO film and vapor molecule, diffusion characteristics were examined in the terms of diffusion coefficients of vapors during the exposure.

Our results showed that the sensor response and sensitivity largely dependent on the physical properties of the analyte molecules. Molecular size of that vapors can affect the interaction rate due to the pores in the film structure can control the diffusion of the vapor molecules. In addition to the diffusion advantage of chloroform, it can specifically bind to discrete sites of proteins. The sensitivity of PPO thin film against chloroform is attributed to the specific interaction characteristic and more diffusing behavior.

With the help of these promising results, enzymes can be integrated into vapor sensor systems in order to improve selectivity and sensitivity.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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