

Research Article

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A novel lactose biosensor based on electrochemically synthesized 3,4-ethylenedioxythiophene/thiophene (EDOT/Th) copolymer<https://doi.org/10.1515/chem-2020-0100>

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Abstract: In this study, a new lactose biosensor has been developed in which the 3,4-ethylenedioxythiophene/thiophene (EDOT/Th) copolymer is used as a transducer. The EDOT/Th copolymer was deposited on the glassy carbon electrode to be used as the working electrode. In addition to the working electrode, the three-electrode system was used in both the electrochemical synthesis and in the biosensor measurements. Lactase (β -galactosidase) that catalyzes the breakdown of lactose into monosaccharides (glucose and galactose) and galactose oxidase that catalyzes the oxidation of the resulting galactose were attached to the copolymer by a cross-linker on the modified working electrode. The response of the enzyme electrode to lactose was determined by cyclic voltammetry (CV) at +0.12 V. Enzyme electrode optimization parameters (pH, temperature, enzyme concentration, etc.) were performed. Fourier transform infrared spectroscopy, scanning electron microscopy and CV methods were used to support copolymer formation. In addition, the characteristics of the enzyme electrode prepared in this study (K_m , 0.02 mM; activation energy E_a , 38 kJ/mol; linear working range, up to 1.72 mM; limit of detection, 1.9×10^{-5} M and

effects of interferents [uric acid and ascorbic acid]) were determined.

Keywords: copolymerization, 3,4-ethylenedioxythiophene, lactose, polythiophene, sensor

1 Introduction

In recent years, electrochemical sensors have an increasing attention due to their excellent properties, especially in electroanalytical determination of important analytes. Electrochemical sensors are relatively new technologies due to their ability to be highly sensitive to small changes in analyte concentrations [1]. Various electrode materials have been used in the construction of electrochemical sensors. Among them conductive polymers, which were first discovered in 1977 by Shirakawa and co-workers, have found special attention due to their excellent characteristics such as low cost, environmental stability, ease of synthesis, high surface area, long-term stability and high electron affinity [2–4]. Polythiophenes are an attractive class of conducting polymers, which are thermally stable and environmentally friendly. The discovery of electrical conductivity in polythiophenes was reported in 1980 by Yamamoto et al. [5] and Lin and Dudek [6]. There are few studies on the synthesis of copolymers of thiophene and their derivatives with physical properties that are different from those of homopolymers prepared by electrochemical methods [7,8]. Further functionalization of polythiophenes could enable its sensing selectivity and improve electrocatalytic properties. Polythiophenes could be fabricated as the recognition units of biosensors by the direct polymerization of monomers or the substitution of the prepared polythiophene backbones [9]. Polythiophenes have a lot of application areas such as transistors [10], electrochromic devices [11], light-emitting devices [12], batteries [13], adsorption materials [14], sensors and biosensors [15,16]. Electrochemical sensor platforms based on such materials are widely used in the detection of biomolecules due to their long-term stability, low detection limits and wide linear ranges and reproducibility. However, the conductivity of

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polythiophene decreases during long-time usage. Thus, the decreased conductivity limits its industrial applications. The electrochemical stability of polythiophene should be increased by introducing an alkoxy group (such as ether group). This group decreases the polymerization potential of thiophene due to its electron-donating character. The lower oxidizing potential provides a more stable polymer structure. In the case of ether-substituted thiophene, 3-alkoxy, 3,4-alkoxy and pseudocrown ether thiophenes are the important compounds to be considered. Besides, studying polythiophenes with long-side-chain substituents show electrical properties similar to that of polythiophenes because of the distortion in the polymer chain. Hence, many scientists are interested in poly-3,4-(crown ether) thiophene (PEDOT) with a short chain. The electrical stability of polythiophene is also increased by co-polymerization, and this leads to an increase in the biosensitive performance of polythiophene-based sensors [4,17–21].

Lactose (β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucose) is a disaccharide found in significant amounts in mammalian milk (~4.7% in cow's milk and ~7% in breast milk) [22]. Lactose concentration in milk is important for the evaluation of raw/processed milk quality. Low lactose level in raw milk can be an indicator of mastitis infection. The effective analysis of lactose is also significant during the processing of milk and/or whey to different dairy products [23].

There are only a few studies on thiophene-based lactose sensors. In a study by Sharma et al. [24], amperometric biosensor has been developed by immobilization of lactase and galactose oxidase enzymes on poly(3-hexylthiophene)/stearic acid Langmuir–Blodgett (LB) films, and it has been proposed that it can be used to determine the lactose/galactose ratio in the food and biological fluids. Recently, we reported the polypyrrole/PEDOT copolymer using electrochemical polymerization in biosensor application for lactose determination. The resulting electrochemical method was totally characterized in terms of its limit of detection (1.4×10^{-5} M) and response time of biosensor (8–10 s) [23]. Thiophene is of low cost than polypyrrole, and the redox potential of polythiophene is higher than polypyrrole. However, polythiophene has lower stability during charge and discharge cycles [25]. Copolymerization of thiophene and 3,4-ethylenedioxythiophene (EDOT) potentially will improve the stability of polythiophene. Thus, the usage of thiophene and EDOT copolymer during sensor fabrication will be a promising alternative to develop low-cost lactose biosensor. Although there are some studies on biosensors using polythiophene and its copolymers in the literature [26,27], no biosensors have been developed for lactose determination to the best of our knowledge.

2 Materials and methods

2.1 Materials

Thiophene (Aldrich) was purified by distillation at reduced pressure before use. 3,4-EDOT (Aldrich) was used without further purification. Galactose oxidase (GaOx, EC 1.1.3.4, 1,79,000 units/g, type VII-S from *Aspergillus niger*; Sigma) and β -galactosidase (EC 3.2.1.23, $\geq 8,000$ units/g solid, lactase from *Aspergillus oryzae*; Sigma) were used as biocomponents to modify polymer modified glassy carbon (GC) disc electrode. $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (Riedel De Haen) and $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (Riedel De Haen) were used to prepare the buffer solution. Alumina polishing suspension agglomerate (0.05 μm ; Baikowski) was used as an electrode polisher. Double-distilled water was used to prepare the buffer solution. All other compounds were of analytical reagent grade.

2.2 Instrumental

Fourier transform infrared spectroscopy (FTIR) spectrum was obtained using FTIR spectrophotometer between 400 and 4,000 cm^{-1} with a 4 cm^{-1} resolution on a PerkinElmer model (Beaconsfield, Buckinghamshire, HP91QA, England). Scanning electron microscopy (SEM) was performed using Phillips XL-30S FEG scanning electron microscope. Polymerization studies and biosensor measurements were conducted in a three-electrode cell equipped with potentiostat/galvanostat (CompactStat, Ivium Technologies, Netherlands). Polymer-modified GC disc electrode was used as a working electrode, and platinum wire and Ag/AgCl electrodes were used as counter and reference electrodes, respectively. Indium thin oxide electrode was used for the deposition of polymer in the analysis of FTIR and SEM.

2.3 Fabrication of electrode

The GC working electrode was polished with alumina polishing suspension and washed with ethanol and phosphate buffer solutions. Electrochemical copolymerization of thiophene and EDOT was performed using acetonitrile containing LiClO_4 . Th and EDOT monomers (total concentration was 0.5 M) were polymerized by the cyclic voltammetric method in the range of -0.10 to 1.50 V with three cycles at room temperature (Figure 1). After electropolymerization, the electrode was washed several times with acetonitrile to remove any remaining monomer. A total of 2.94 mg of

galactose oxidase and 2 mg of β -galactosidase were dissolved in 500 μ L of phosphate buffer solution (pH 6.7). The appropriate amount of glutaraldehyde with enzyme solution was attached to the polymer-modified electrode by the dropping and drying method. It was then stored at 4°C in the phosphate buffer (pH 6.7). To find the best lactose sensing, various molar ratios of the Th and EDOT monomers (in the range 20–80%) were tried. The highest response was provided at 80% EDOT in copolymer, and this Th/EDOT ratio was selected for the novel lactose biosensor fabrication. The similar procedure was applied to the working electrode that consists of PEDOT to estimate lactose. Since polythiophene cannot be polymerized under these conditions, biosensor experiments have not been conducted.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 FTIR results

Figure 2 shows the FTIR spectrum of PEDOT and EDOT/Th (80–20%) copolymer to indicate the formation of the copolymer structure. The bands between 1198 and 1089 cm^{-1} correspond to C–O–C asymmetric and symmetric stretching of the ethylenedioxy group in PEDOT spectra [28].

The bands at 1198 and 1180 cm^{-1} together form a shoulder at 1186 cm^{-1} in the spectra of copolymer. The bands at 684 and 690 cm^{-1} are attributed to typical C–S stretching [29]. The bands at 1514 and 1515 cm^{-1} show the C=C stretching for PEDOT and copolymer, respectively [30]. The band corresponding to the C–C stretching of the quinoidal structure of the thiophene ring was observed at 1325 cm^{-1} for the copolymer, whereas at 1352 cm^{-1} for the PEDOT [31]. The presence of this band indicates the better polymerization of thiophene in the copolymer structure [32]. There is an effective red shift for this band in the spectra of copolymer. It shows that the formation of copolymer decreases the structural energy of polymer [33]. Similar effect has been encountered at 684 cm^{-1} in the spectra of copolymer.

3.2 SEM results

Figure 3a and b shows SEM images of PEDOT and EDOT/Th copolymer. Figure 3a shows that PEDOT has the vesicular structure with lap-straked globules about 0.2 μm that create batches [34]. That structure has a lot of gaps and pores, which is a desired property to immobilize the enzyme. However, shown clearly in Figure 3b, the EDOT/Th copolymer structure is also porous, but it became more layered, regular and homogeneous. The smoothness of the surface morphology ensures that the electron skipping phenomenon is easy on both the chain and between chains and layers.

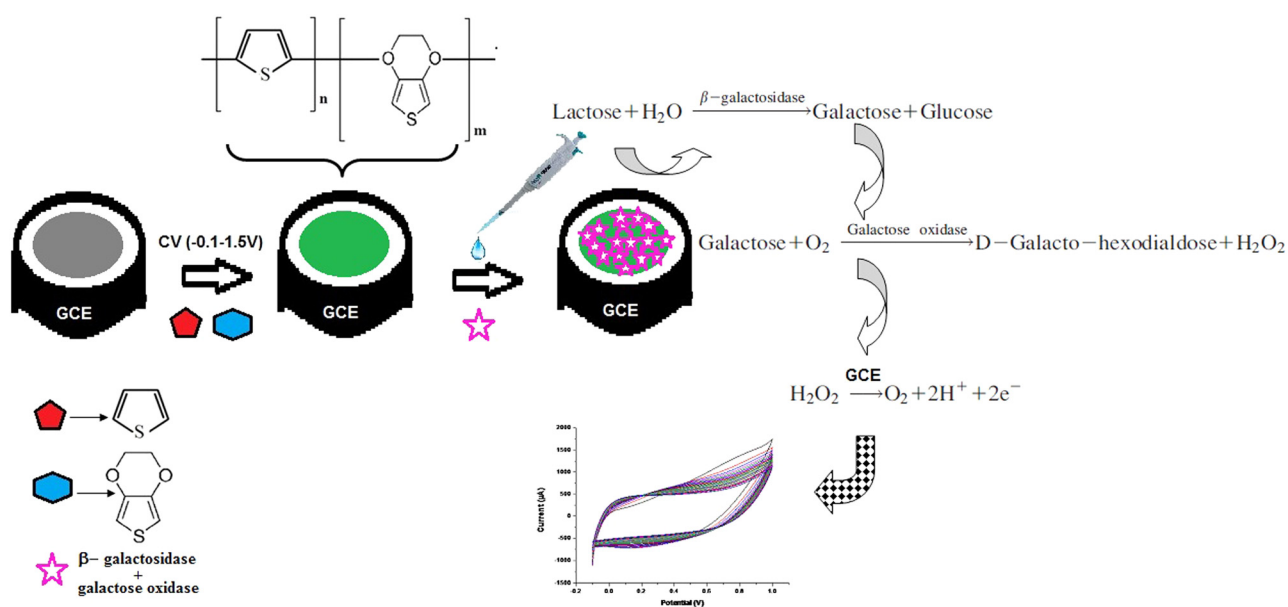


Figure 1: Copolymerization of EDOT/Th and working mechanism of the enzyme electrode.

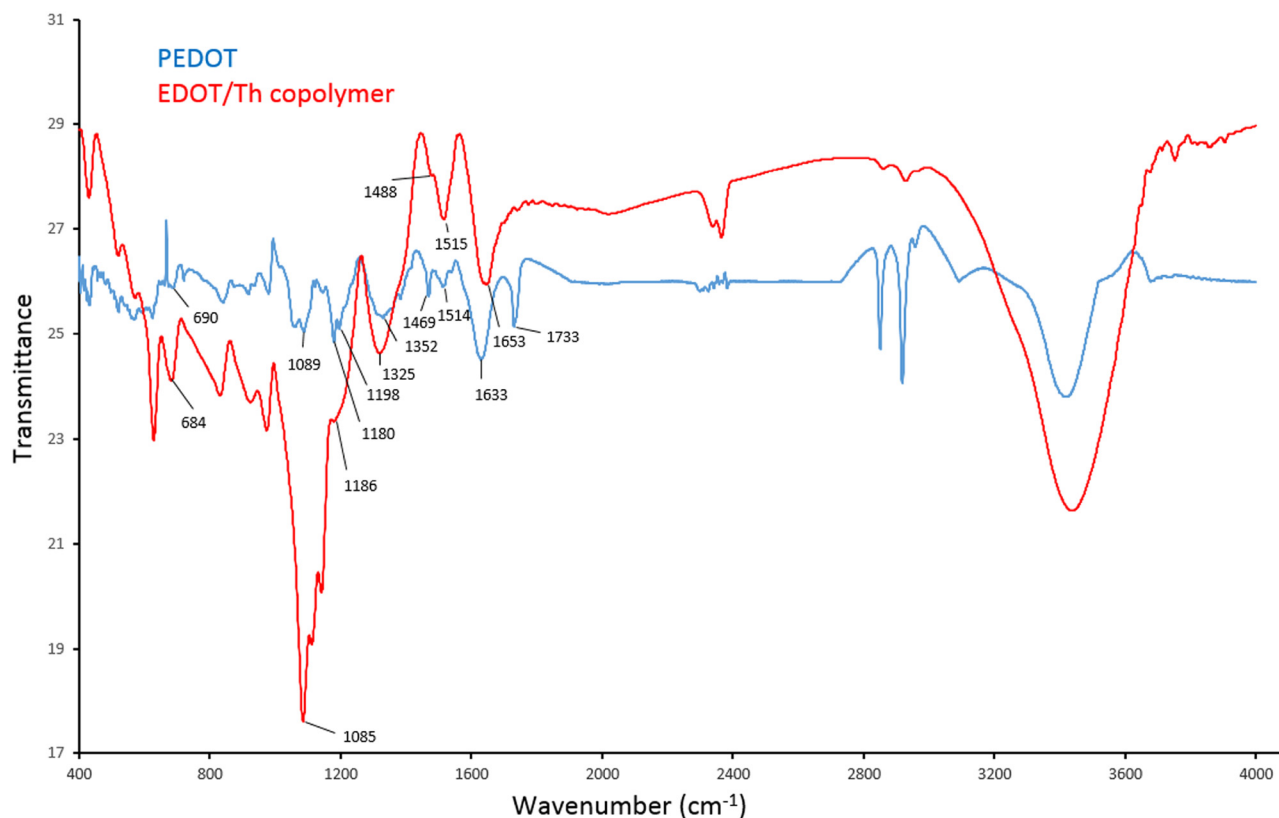
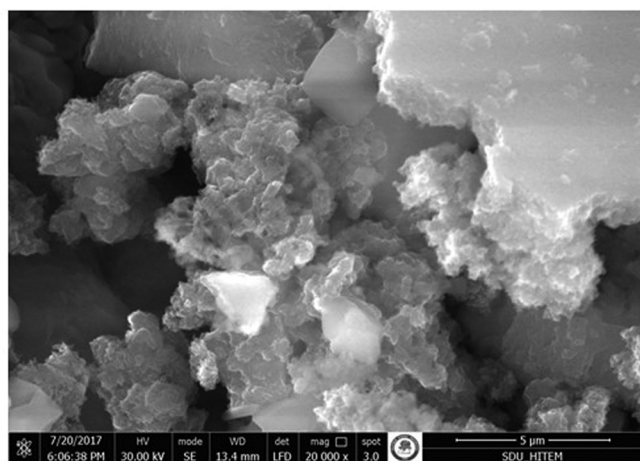


Figure 2: FTIR spectra of PEDOT and EDOT/Th copolymer.

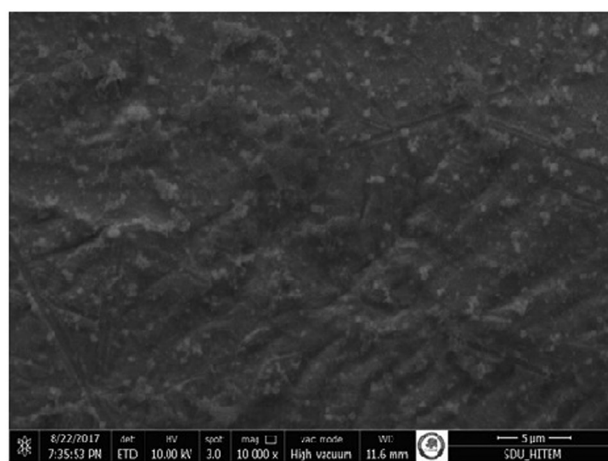
The ability of PEDOT to arrest the enzyme and facilitate the electron transport of the copolymer structure suggests that it would be appropriate to use the copolymer as a lactose biosensor. This is also evident from the fact that the copolymer responds better to lactose versus PEDOT.

3.3 Cyclic voltammetry (CV) studies

Figure 4a shows the cyclic voltammograms of formation of PEDOT, PTh and EDOT/Th copolymer. All experiments were carried out in unstirred solution at the fixed scan rate of 50 mV/s. The onset point of the cyclic



(a)



(b)

Figure 3: SEM images of (a) PEDOT and (b) EDOT/Th copolymer.

voltammograms of PEDOT and copolymer begins at 0.8 V, while it starts just about 1.2 V at cyclic voltammogram of PTh. The knot observed for all polymers is typical of the electrosynthesis of conducting polymers. It is clearer for PEDOT and copolymer because the CV of the copolymer shows similar redox behavior and oxidation potentials with PEDOT. Besides, the maximum current value of copolymer is at the average value of both monomers according to their CV curves. These electrochemical behaviors are attributed to the formation of copolymer structure onto the GC electrode.

The electrochemical responses of both copolymer-modified and enzyme immobilized copolymer-modified GC electrodes were examined using the CV method. The scanning rate of 50 mV/s is applied to take CVs of the electrodes as shown in Figure 4b. At the beginning, the copolymer-modified GC electrode was immersed into buffer solution and -0.1 to 1.0 V was applied to this system. The same procedure was carried out for enzyme immobilized copolymer-modified GC electrode. There was a difference between the cyclic voltammograms of electrodes. As shown in Figure 4b, the oxidation and reduction potentials of copolymer were shift to the left with the presence of enzyme. This result supported the immobilization of enzyme to the copolymer surface.

CV is a useful tool to estimate quantitative information about a redox system in solution. The value of the potential scanning rate between working and reference electrodes is changed at each cycle. The expression of the peak current in a reversible system at 298 K is given by the Randles–Sevcik equation [35,36].

$$i_p = (2.69 \times 10^5) \cdot n^{3/2} \cdot A \cdot D^{1/2} \cdot C_o \cdot v^{1/2},$$

where i_p is the peak current (A), n is the number of electrons exchanged during the redox process ($n = 2$ as shown in Figure 1), D is the diffusion coefficient (m^2/s), C_o is the concentration of the analyte (mol/m^3), A is the area of the electrode (m^2), and v is the experimental scan rate (V/s). In CV, this equation describes the effect of the scanning speed on peak current. The peak current depends not only on the diffusion properties of the concentration and electroactive species but also on the scanning speed [37]. Figure 5(a–h) is used to determine whether the cyclic voltammograms of the enzyme immobilized copolymer-modified and copolymer-modified working electrodes continue their stability depending on the scanning rate (25–250 mV/s). Anodic peak currents were increased with the scanning speed. This shows that the copolymer is very well attached to the electrode and still maintains similar stability after the enzyme has been attached. Besides, the linearity of plot indicates that the electrochemical process is diffusion assisted and quite reversible and stable even at high scanning rate [38]. In addition, it was observed that the anodic peak potentials are linear with the logarithm of scan rate at higher scan rates from 100 to 250 mV/s (Figure 5). These results suggest the quasi-reversible electron transfer system for both enzyme immobilized copolymer-modified and copolymer-modified working electrodes [39].

3.4 Lactose detection

Electrochemical cell and electrodes used in lactose determination are the same as those used in copolymer synthesis. The response of the enzyme electrode was determined in the range of -0.1 to 1.0 V by the CV

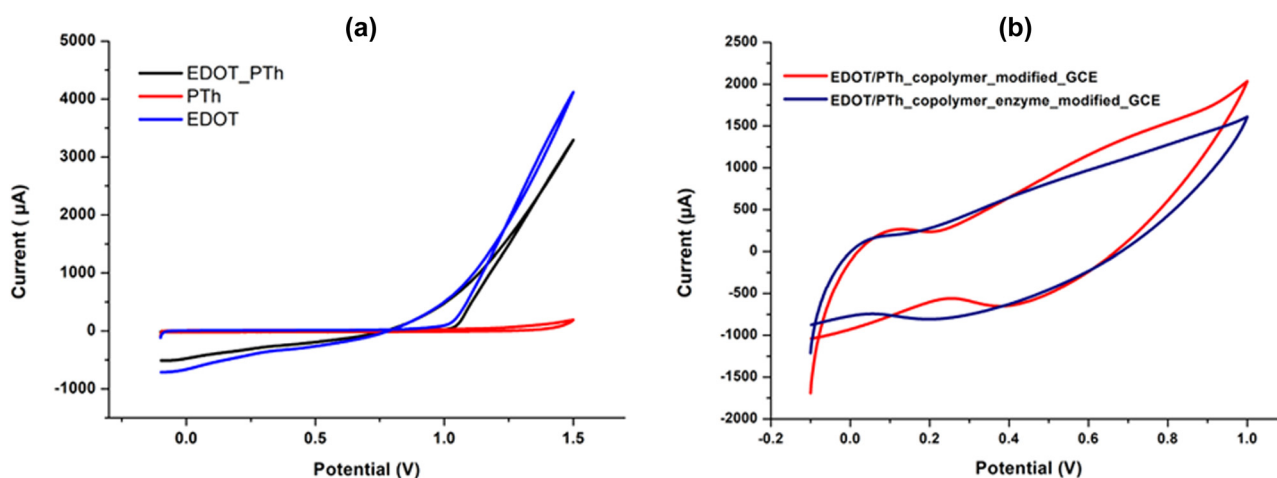


Figure 4: (a) Cyclic formation voltammograms of PEDOT, PTh and EDOT/Th copolymer. (b) Cyclic voltammograms of copolymer-modified and enzyme immobilized copolymer-modified GC electrodes with 50 mV/s scan rate in the buffer solution.

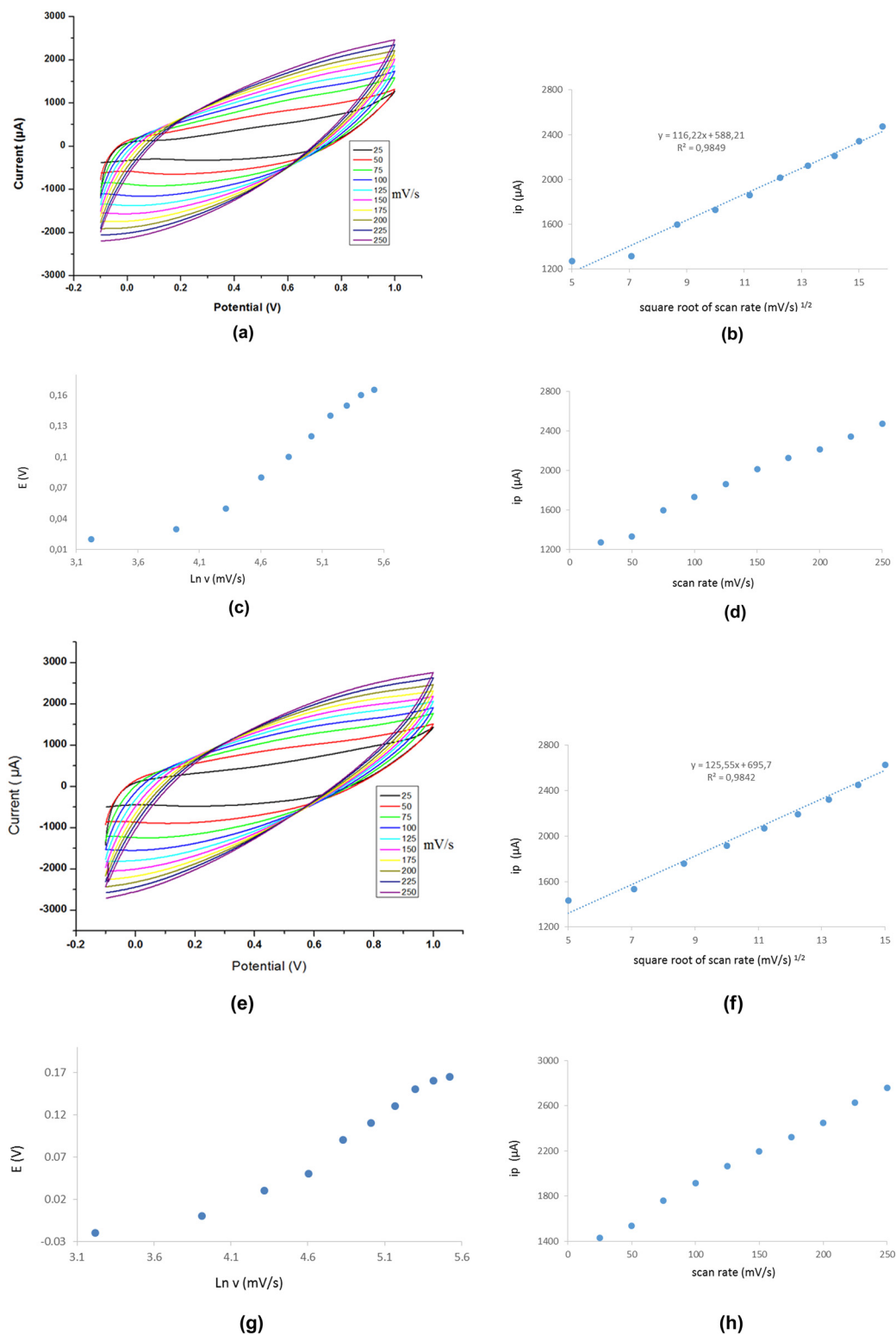


Figure 5: Cyclic voltammograms (I - V), current versus square root of scan speed (I - $v^{1/2}$), potential versus \ln of scan rate (E - $\ln v$) and current versus scan rate (I - v) of copolymer-modified (a-d) and enzyme immobilized copolymer-modified (e-h) GC electrodes with various scanning rates.

method according to the current values to be obtained at +0.12 V, in which lactose is oxidized against the known

amounts of lactose addition as shown in Figure 6 [40]. To determine the response of the enzyme-modified

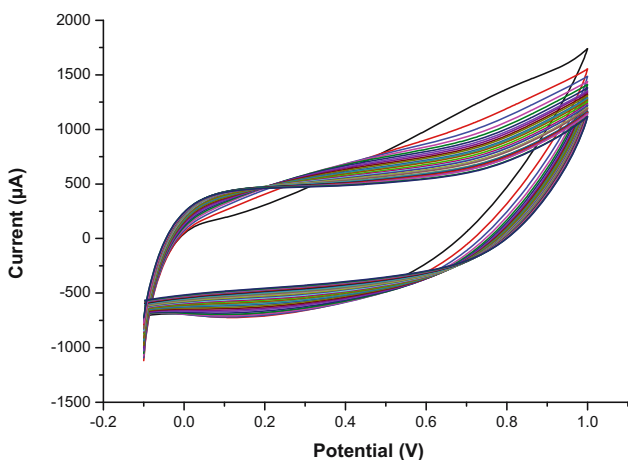
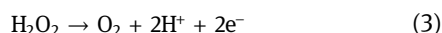
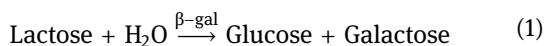


Figure 6: Typical cyclic voltammetric responses of β -gal/GaOx immobilized film of EDOT/Th copolymer to lactose in the buffer solution (pH 6.7).

electrode, first, a voltammogram was taken in the lactose-free buffer solution. Then, the known amount of lactose solution was added to the buffer solution, and cyclic voltammogram was recorded. This process was continued until there was no current increase at +0.12 V. The lactose added to the working medium is oxidized in the O_2 -saturated buffer solution coupled with β -galactosidase and galactose oxidase enzymes immobilization onto the polymer according to equations (1)–(3). The lactose concentration spent during the enzymatic reactions is proportional to the current change. Studies were repeated at various working parameters and optimum operating conditions of the electrode were determined.



3.5 Effect of copolymer ratio and pH

To determine the optimum copolymer ratio for lactose determination, the amount of EDOT in copolymer was varied between 20% and 80% and the response of the enzyme electrode was measured. The effect of homopolymers (PTh and PEDOT) was also examined to make comparison. Since polythiophene did not accumulate on the electrode under these conditions, the effect of polythiophene could not be determined. Among PEDOT and copolymer-modified working electrodes, 80% EDOT:20% Th copolymer-modified working electrode has

shown the maximum current value against lactose concentration. Figure 7a shows the effect of the copolymer composition on the response of the enzyme electrode.

pH is one of the most important parameters to be examined for enzyme biosensors as it affects the working performance of the enzyme. Therefore, the pH of the buffer solution was changed from 6 to 8. Figure 7b shows the pH dependence of the Th/EDOT copolymer-modified enzyme electrode to lactose in 0.1 M NaH_2PO_4 buffer solution. The current increased from pH 6.0 until 6.7, and after that value, it decreased gently until pH 8.0. The developed lactose biosensor exhibited maximum sensitivity at pH 6.7. That pH value is also nearly the pH value of the milk. In subsequent studies, the buffer pH of 6.7 was used.

3.6 Effect of coating cycle number

The coating thickness of the copolymer on the working electrode has been optimized by using the cycle number of the CV method, which is used at polymerization of

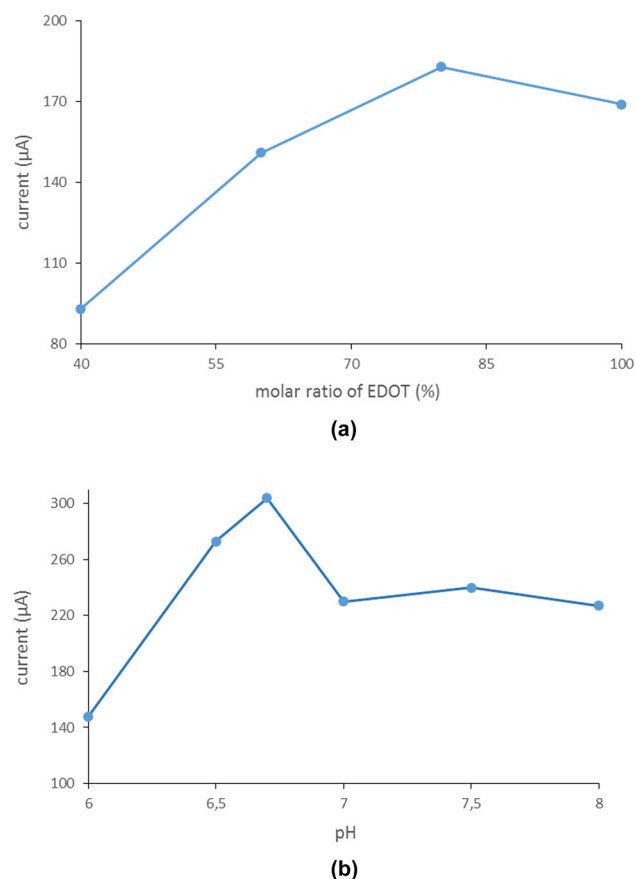


Figure 7: (a) Effect of the EDOT molar ratio on the response of the enzyme electrode. (b) Effect of pH on the response of the enzyme electrode.

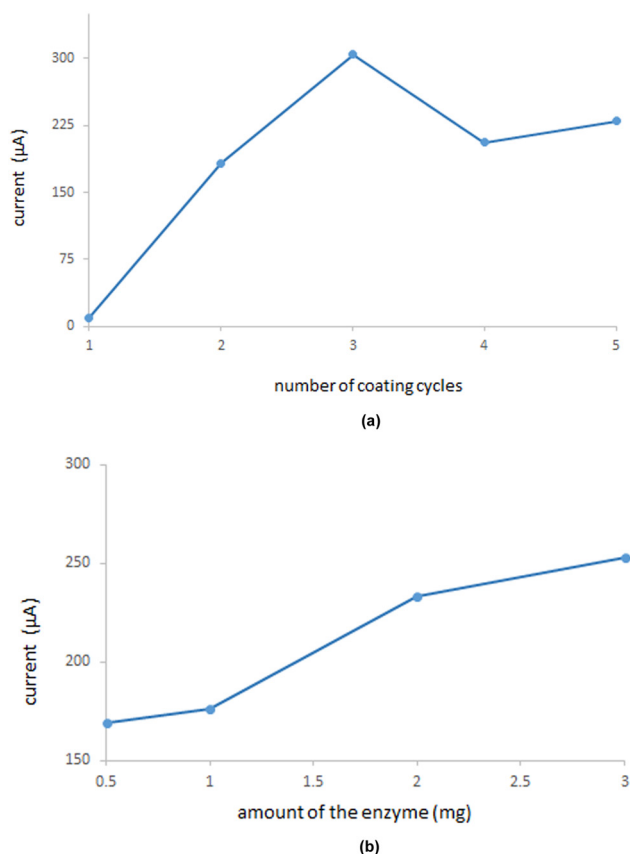


Figure 8: Effect of (a) coating cycles and (b) enzyme amount on the response of the enzyme electrode.

copolymer onto the working electrode. When the polymer layer is too thin, its interaction with the enzyme solution decreases and only less enzyme is retained on the surface. Therefore, the sensor response is reduced. When the layer is too thick, the transmission of electrons is restricted. As a result, the response of the enzyme electrode against lactose decreases. Therefore, the determination of the optimum coating thickness is important for the performance of the enzyme electrode. As shown in Figure 8a, the highest current response to lactose was obtained by coating the copolymer on the working electrode surface in 3 cycles.

3.7 Effect of enzyme concentration

The effect of the enzyme pair (galactose oxidase and β -galactosidase) on the electrode response was investigated by changing the amounts provided that their ratios remained constant among themselves (Figure 8b). The

highest current response was obtained in a solution containing 4.41 mg of galactose oxidase and 3 mg of β -galactosidase. However, this current value is very close to the current value obtained for a solution containing 2.94 mg of galactose oxidase and 2 mg of β -galactosidase. Therefore, considering that the sensor should be economical, 2.94 mg of galactose oxidase and 2 mg of β -galactosidase enzyme solution were used in the following studies.

3.8 Effect of temperature

The temperature is an efficient factor on the response of the enzyme electrode. Therefore, it is important to determine the effect of the temperature on the working performance of the copolymer-modified enzyme electrode. Figure 9 shows the current values obtained for the biosensor at different temperatures. This figure also shows that the current value increased linearly up to 50°C and reached the maximum value at this point. This result is better than the result previously found for the biosensor developed using this enzyme system for the determination of lactose [24]. After this value, the current values decreased considerably because of the various conformations attributed to temperature for the immobilized enzyme. However, enzymes are known to be able to denature at high temperatures. Although the maximum current response was obtained at 50°C by the working electrode, we used ambient temperature for measurements due to the long-term thermal stability of the enzymes [41].

Using the experimental data in the range in which the reaction rate increased with the temperature, the activation energy of the reaction carried out with the immobilized

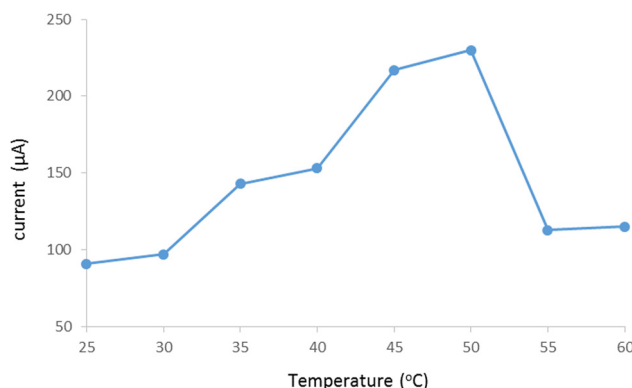


Figure 9: Effect of temperature on the response of the enzyme electrode at +0.12 V in the range of -0.1 to 1.0 V applied potential, pH 6.7.

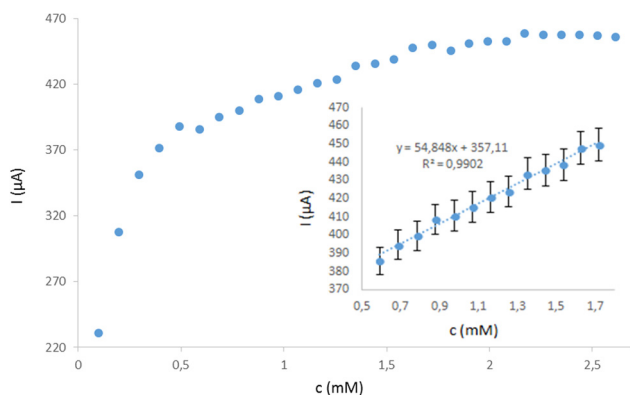


Figure 10: Effect of lactose concentration on response and linear working range (insider graph) for the modified electrode at +0.12 V in range of -0.1 to 1.0 V applied potential, pH 6.7.

β -galactosidase and the galactose oxidase was determined using the Arrhenius equation. For this purpose, $\ln I$ values against $1/T$ were plotted (not shown here). The activation energy of β -galactosidase and galactose oxidase enzyme systems was found to be 38.00 kJ/mol. This value is comparable to the value found with the free enzyme [42]. According to the experimental results, we can say that the EDOT/Th copolymer supplies a suitable working medium for β -galactosidase and galactose oxidase enzyme systems, which make the sensor stable even at high temperatures.

3.9 Effect of substrate concentration

The prerequisite for a healthy measurement with enzyme electrodes is to obtain current values proportional to the

substrate concentration in the measuring medium. The substrate concentration range in which this condition is met is defined as the linear operating range of the enzyme electrode, and in practice, this range is desirable to be broad. After optimizing the working conditions, the response of the prepared biosensor to the standard lactose solution was examined. Figure 10 shows the response of the modified working electrode as a function of each $100 \mu\text{L}$ of 0.02 M lactose solution in the range of -0.1 to 1.0 V by the CV method according to the current values to be obtained at $+0.12$ V (pH 6.7, three coating cycles, 25°C , with the molar ratio of 80% EDOT:20% Th).

As shown in Figure 10, the developed enzyme electrode has given proportional current values to lactose concentration up to 1.72 mM. Therefore, the linear working limit of the prepared enzyme electrode was determined as 1.72 mM lactose concentration. On the other hand, the electrode response to the lactose concentration reaches a constant value of about 2.17 mM, and there is no increase in the current value at higher concentrations. This shows that the enzyme in the polymer film reaches saturation against the substrate at a concentration of 2.17 mM lactose for the electrode prepared under the conditions of the measuring medium. Therefore, diffusion restriction in the electrode activity will not be important at lactose concentration of 2.17 mM and higher under these conditions. The detection limit of the modified enzyme electrode was found as 1.9×10^{-5} M lactose concentration. Besides, the sensitivity of the enzyme electrode was also calculated from the plot of current density (current/area) against concentration, which was $0.06 \mu\text{A}/\text{mM cm}^2$. This value is comparable with the study by Marrakci *et al.* [43]. The maximum reaction rate (V_{\max}) of the copolymer modified was detected at $500 \mu\text{A}$.

Table 1: Performance values of some lactose sensors

Method	Electrode modifier	Enzyme ^a	Detection limit and/or linear working range	Sensitivity ($\mu\text{A}/\text{mM cm}^2$)	Ref.
Photoelectrochemical	Gold nanoparticles, MnO_2 and $\text{g-C}_3\text{N}_4$ decorated TiO_2	GOx/ β -GAL	$0.23 \mu\text{M}$, 0.008 – 2.5 mM	1.66	[47]
Amperometric	Polymer multilayer (cellobiose dehydrogenase [CDH])	GOx/CAT	10 – $100 \mu\text{M}$	—	[48]
Conductometric	Thin-film conductometric sensor	GOx/ β -GAL	1×10^{-4} M	0.01	[43]
Cyclic voltammetry	Pt/graphene/P(1,5-DAN)	GOx/ β -GAL	Up to 14 mM	1.33	[49]
	Gold and silver nanoparticles (NPs)	CDG	$1.3 \mu\text{g}/\text{mL}$ ($3.8 \mu\text{M}$)	5.4	[50]
	PEDOT/PTh copolymer	β -GAL/GAOx	250 nM/ 0.5 – 200 mM	0.06	This study
			Up to 1.72 mM		

^a GOx, glucose oxidase; CAT, catalase; β -GAL, beta-galactosidase; GAOx, galactose oxidase; CDG, cellobiose dehydrogenase.

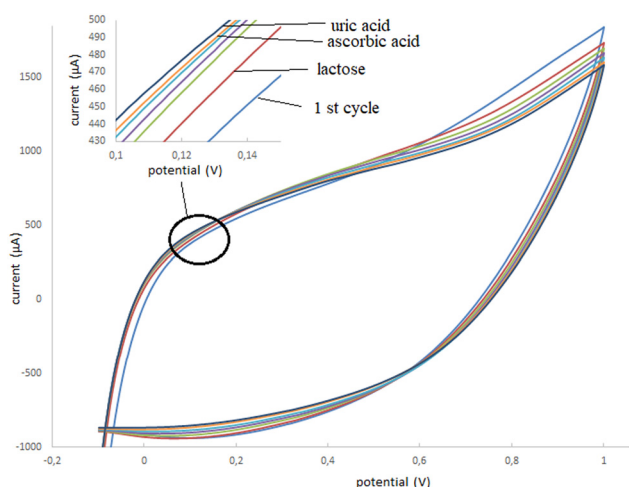


Figure 11: Effect of interferents on the response of lactose biosensor at +0.12 V in the range of -0.1 to 1.0 V applied potential, pH 6.7.

The relevance between the inverse of the current value (i^{-1}) and the inverse of lactose concentration (C^{-1}) was plotted according to the Lineweaver–Burk form of the Michaelis–Menten equation, which is not shown here [44]. The Michaelis–Menten constant is an important parameter for characterizing the enzyme electrode. This constant shows that the rate of conversion of the substrate to the product depends on the rate of degradation of the enzyme substrate complex. As the K_m value decreases, the interaction between the enzyme and the substrate increased [45]. In this study, the apparent Michaelis – Menten constant was calculated for copolymer-modified enzyme-entrapped working electrode using the Lineweaver–Burk equation. The value of 0.20 mM was about ten times lower than that found in the literature for the free enzyme system (2.30 mM) [46]. This result shows that the polymer structure does not lead to diffusion restriction. It may also indicate that the electronic activity of the EDOT/Th copolymer has the synergistic effect on the response of the enzyme system. Table 1 presents the comparison of some lactose biosensor performances with biosensor developed in this study for lactose determination. As a result, the lactose biosensor based on the EDOT/Th copolymer has various advantages, such as broad linear range with a low detection limit, thermal stability, fast response, low K_M (means robust relation between enzyme and substrate), simple and economic fabricating procedure.

3.10 Interferences and reproducibility

The effect of some commonly encountered species that could intervene electrochemically on the current

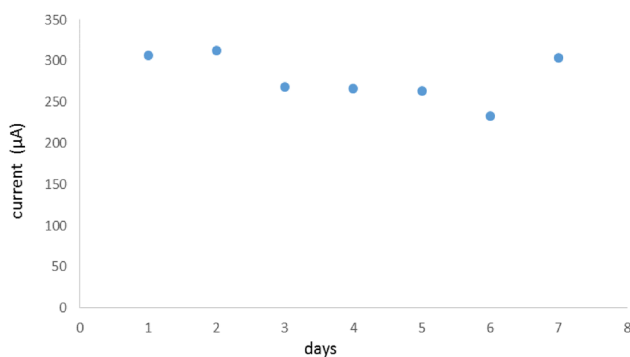


Figure 12: Reproducibility of the lactose biosensor at +0.12 V in the range of -0.1 to 1.0 V applied potential, pH 6.7.

response of lactose biosensor to lactose was investigated. Ascorbic acid and uric acid were chosen to investigate the effect of these species on the response of the enzyme electrode. It was determined that the effect of ascorbic and uric acids on the electrode response was insignificant (Figure 11). Similar results were reported by Gürsoy et al. [51].

The reproducibility of the biosensor was performed by the seven different biosensors prepared under the same conditions (Figure 12). The results showed that the biosensor had a good reproducibility with the variation coefficient of 9.8%.

4 Conclusion

This study indicates the usability of EDOT/Th copolymer-modified galactose oxidase/ β -galactosidase entrapped enzyme electrodes for the determination of lactose. This biosensor is an alternative to determine lactose in cheaper, faster and simple way. Copolymerization of monomers was achieved on the GC electrode by CV in the range of -0.10 to 1.50 V at room temperature. The growth of the copolymer film was checked by CV, FTIR and SEM analysis. The appropriate amount of glutaraldehyde and enzyme solution was attached to the polymer-modified electrode. After optimization of working conditions of the electrode, linear working range and detection limit were calculated to be up to 1.72 mM and 1.9×10^{-5} M, respectively. The developed lactose biosensor has a very fast response time (4 – 5 s) due to the fast electron transport system of copolymer. K_M values of immobilized (0.02 mM) and free enzymes (2.30 mM) indicate that there are no diffusional limitations and denaturing effect in the immobilization procedure of enzymes. Besides, the influences of

ascorbic acid and uric acid, which are the major interferents in the milk, on the response biosensor have no significant effect. Further studies are needed to show the applicability of the biosensor to determine lactose concentration in milk samples.

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