engineering proceedings

MDPI

Proceeding Paper

Development of a Chemical Sensor Based on Deep Eutectic Solvents and Its Application for Milk Analysis †

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† Presented at the 2nd International Electronic Conference on Chemical Sensors and Analytical Chemistry, 16–30 September 2023; Available online: https://csac2023.sciforum.net/.

Abstract: Deep eutectic solvents (DESs) have unique physical and chemical properties, such as low vapor pressure, ease of synthesis, stability, and non-toxicity. Although they have found application in areas of research such as organic synthesis, electrochemistry, biocatalysis, and the development of biosensors, their use as sensitive coatings for chemical sensors has not been previously considered. This study examines the fundamental principles of generating sensitive coatings for piezoelectric quartz sensors utilizing hydrophilic deep eutectic solvents (choline + polyalcohols). Thin films from DESs with a melting point above 50 °C, including those in the composite coatings with amorphous silicon oxide, have been studied. The sorption characteristics of the coatings were thoroughly examined via piezoelectric quartz microbalance. It has been demonstrated that the limits of detection and determination of volatile organic compounds in aqueous solutions by films based on DESs exhibit lower limits than other polymer coatings. A novel approach is proposed for processing the kinetic curve of the sorption of volatile substances by films based on DES to improve the reliability and detection of volatile compounds in the gas phase above aqueous solutions. The use of DES-based piezoelectric quarts sensors has been demonstrated for assessing microbiological indicators of milk.

Keywords: chemical sensors; deep eutectic solvents; gas analysis; kinetic parameter; microbiological indicator; milk

Citation: Shuba, A.; Anokhina, E.; Umarkhanov, R.; Bogdanova, E. Development of a Chemical Sensor Based on Deep Eutectic Solvents and Its Application for Milk Analysis. *Eng. Proc.* **2023**, *48*, 34. https://doi.org/10.3390/CSAC2023-14889

Academic Editor: Jose Vicente Ros-Lis

Published: 20 September 2023

1. Introduction

Creating and developing new sensors and devices for analyzing various objects is a constantly developing area of modern sensor science. Gas sensors have a sensitive surface layer, which determines their analytical characteristics. The continuous pursuit and synthesis of novel materials to produce sensitive sensor coatings is ongoing [1,2]. Polymer sorbents [3,4], metal–organic structures [5], metal oxides and salts [6,7], carbon nanomaterials [8,9], composite materials [10], and various combinations of the studied sorbents [11].

Deep eutectic solvents (DESs) are widely used in analytical practice for the analysis of real objects with complex composition due to their unique properties, such as low vapor pressure, ease of synthesis, stability, non-toxicity, and the ability to control the degree of hydrophilicity by choosing initial components. Therefore, their use as sorbents for creating coatings for gas sensors is promising for extracting volatile components of different polarity from the gas phase.

The goal is to investigate the sorption properties of thin films based on hydrophilic deep eutectic solvents (choline + polyalcohols) and their application to the analysis of the gas phase of milk.

2. Materials and Methods

2.1. Preparation of Deep Eutectic Solvents

Hydrophilic eutectic solvents based on choline bitartrate (IROOX, Kachkanar, Russia) and polyalcohols (erythritol, xylitol, and sorbitol) (Sigma–Aldrich, Burlington, MA, USA) were chosen to create sorption coatings. The thermal method was used to obtain DESs. Choline bitartrate and one of the polyalcohols were placed in a glass vessel and stirred for two hours while heating in a water bath to 80 °C. A viscous flowing transparent liquid was formed, then cooled to room temperature. The obtained deep eutectic solvents had a melting point above ambient temperature, causing the DESs to solidify to form a white plastic mass under normal conditions.

2.2. Formation and Analysis of Sensitive Layers of Sensors Based on a Deep Eutectic Solvent

To fabricate sensor coatings, a mixture of 2 mL of ethanol (95% vol.) and 10 mg of a DES was incorporated until a transparent solution was obtained. This solution was then applied to the electrode of a piezoelectric quartz resonator (16 MHz, Meteor Plant JSC, Volzhsky, Russia) via dispersion spraying [12]. Subsequently, the coating was placed in an oven at 50 °C for 20 min to remove any unbound ethanol. Moreover, to fabricate a more stable coating with a higher specific surface, 10 mg of amorphous silicon oxide (ASO) (a polydisperse powder with a particle size of up to 0.09 mm and 99% purity) was added to the resulting solution and sonicated (90 W, 60 s) to obtain a stable sol. After this, the obtained sol was also deposited on the electrode surface via dispersion spraying. The surface of the obtained coatings was examined using the Solver-Pro NT-MDT scanning tunneling microscopy.

2.3. Estimation of Volatile Compounds Sorption Features via Coating Based on a Deep Eutectic Solvent

The study of the volatile compound sorption was carried out on the device "MAG-8" (OOO "Sensors—New Technologies", Voronezh, Russia) with piezoelectric quartz sensors and an injector input of the gas phase [13]. The sensors were trained on volatile organic compounds of various classes, including alcohols, ketones, ethyl acetate, acetaldehyde, carboxylic acids (analytical grade, Reakhim LLC, Moscow, Russia), and bidistilled water, to evaluate their sorption characteristics. The measurement time of sorption of the equilibrium gas phase over pure compounds or samples of raw milk (20 mL) was 80 s. A software of MAG-8 recorded the values of the shift of frequency of the piezoelectric sensor during the sorption of the volatile compounds with a frequency of 1 s, according to which the maximum sensor signal ($\Delta F_{max,i}$, Hz) was obtained. The effectiveness of sorption via the obtained coatings was evaluated using specific mass sensitivity [11] and a selectivity coefficient [12].

Based on the sensor signals after the measurement, the parameter β has been proposed, which was calculated using the equation:

$$\beta_i = (\Delta F_{max,i} - \Delta F_{80s,i})/(80 - \tau_{max,i}), \quad (1)$$

where $F_{max,i}$ and $\Delta F_{80s,i}$ are the maximum signal and the signal at 80 s of measurement for the *i*-th sensor, respectively, and $\tau_{max,i}$—time of achievement of the maximum signal by the *i*-th sensor. The proposed parameter reflects the sorption kinetics of volatile compounds on piezosensor coatings and can be used to identify the volatile compounds in the gas phase over samples. In order to determine the detection limit of volatile compounds in aqueous solutions, we studied the gas phase sorption of certain substances (acetic and butyric acids, butanone-2, and isopentanol) in the concentration range of 0.001–10% by volume.

2.4. Milk Analysis

Samples of raw cow's milk (n = 14) were obtained from various farms at different seasons (March–July 2023), cooled immediately after milking to T = (4 ± 2) °C, and delivered to the laboratory for no more than 3 h of storage.

2.4.1. Determination of Physical and Chemical Properties of the Milk

Mass fraction of dry solids in the samples was determined by drying [14] in a Binder ED 53 oven (BINDER Inc., Tuttlingen, Germany) to a constant mass at T = (105 ± 2) °C; the mass fraction of fat—using the Gerber acid method [15], mass fraction of total protein—using formol titration [16], density—using the areometric method [17], titratable acidity—using the titrimetric method with phenolphthalein indicator [18], and purity group—using the gravimetric method [19]. The experimental studies of each sample were carried out three to five times. The number of repetitions of each experiment to determine one value was three times.

2.4.2. Determination of Microbiological Indicators

Microbiological indicators (the quantity of mesophilic aerobic and facultative anaerobic microorganisms QMAFAnM and the quantity of yeasts and molds) were determined using microbiological inoculation on universal nutrient media (plate count agar, Sabouraud agar, Obolensk, Russia) according to standard methods described in GOST [20,21]. QMAFAnM was estimated as the average value from three milk dilutions (from 10^6 to 10^4). The raw milk sample was diluted 10 times to estimate the quantity of yeast and mold.

2.5. Data Processing

Calculations were carried out using methods of mathematical statistics via the XLSTAT application (Lumivero, Denver, CO, USA) for Microsoft Office 365 Family (Microsoft Corporation, Redmond, WA, USA). The significance of the findings was determined by utilizing the *p*-value, which was less than or equal to 0.05. The detection limit of volatile compounds is estimated by the difference (three criteria) between the sensor signal for sensor vapor and the sensor signal for water vapor. To assess the correlation of the output data of the sensors with physical, chemical, and microbiological indicators, the Pearson correlation coefficient was calculated with an assessment of its statistical significance via the Student's t-criteria [22].

3. Results

When analyzing the gas phase over aqueous solutions, the resulting coatings based on deep eutectic solvents show different levels of baseline drift during three months of intensive operation (Table 1).

Table 1. Performance characteristics of sensor coatings and specific mass sensitivity of sensor coatings to vapors of some test substances.

Covering	Acetic Acid	Butyric Acid	Ethanol	Butanol	Butanol-2	Acetaldehyde	Film Masses	Baseline Drift *, kHz
choline + erythritol	11.96	17.59	0.54	0.57	0.13	2.20	8.81	4.22
choline + xylitol	12.21	9.56	0.45	0.39	0.11	2.13	8.58	1.415
choline + sorbitol	11.25	10.13	0.46	0.34	0.08	1.93	15.47	0.644
choline + erythritol + ASO	6.54	23.84	0.50	1.17	0.14	2.06	5.29	0.213
choline + xylitol + ASO	4.63	32.20	0.52	2.75	0.19	1.65	4.56	0.563
choline + sorbitol + ASO	4.94	24.93	0.43	1.49	0.15	1.69	5.71	0.544

*—determined by the shift in the oscillation frequency of the sensors after three months of operation.

According to the type of chronofrequencyograms, different nature of the sorption of volatile compounds on DES films was established, depending on the ability of the substance to form hydrogen bonds, for example, the sorption of ethanol and acetone vapors (Figure 1).

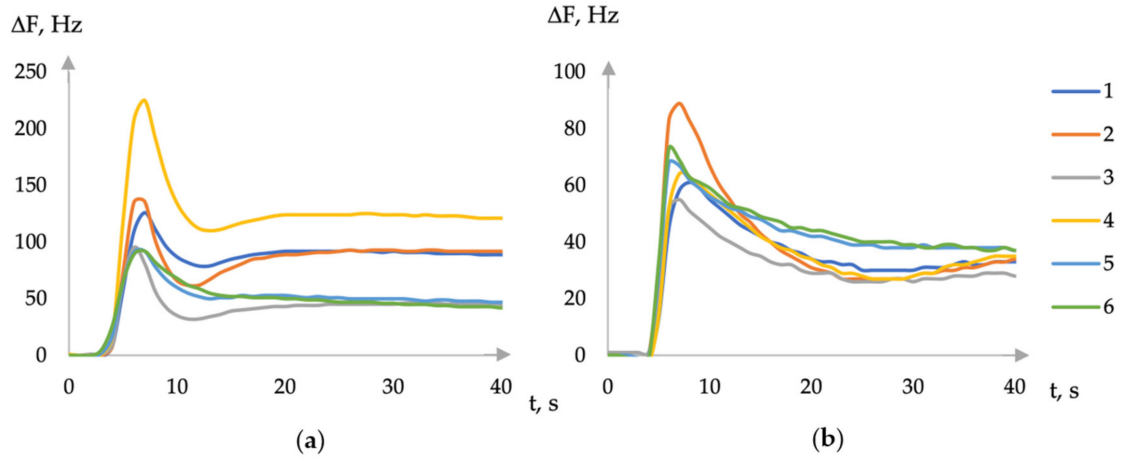

Figure 1. The first part of chronofrequencygram of ethanol (**a**) and acetone (**b**) vapor sorption on an array of sensors with coatings (1—choline + xylitol, 2—choline + erythritol, 3—ASO + choline + erythritol, 4—choline + sorbitol, 5—ASO + choline + sorbitol, and 6—ASO + choline + xylitol).

The parameters β for volatile substances were calculated from the signals of sensors with films based on DESs (Figure 2).

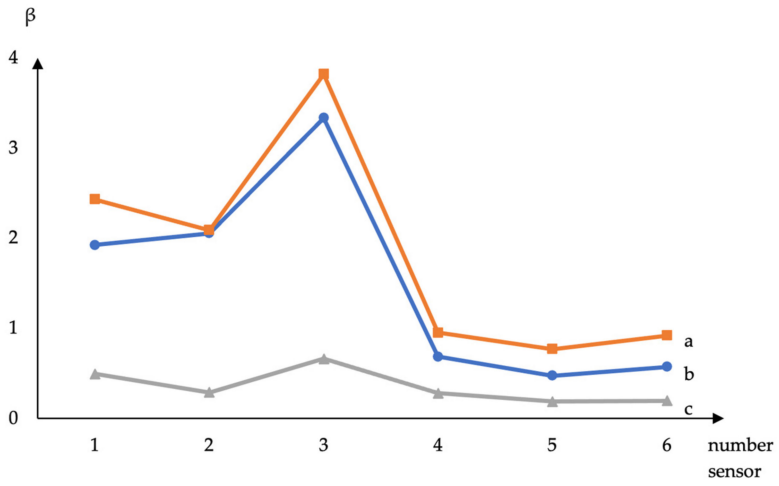

Figure 2. Calculated from sensor signals (1—choline + xylitol, 2—choline + erythritol, 3—ASO + choline + erythritol, 4—choline + sorbitol, 5—ASO + choline + sorbitol, and 6—ASO + choline + xylitol) parameter β for volatile compounds: a—ethyl acetate, b—acetic acid, and c—methyl ethyl ketone.

The statistically significant correlations (r) between signals and sensor parameters and the outcomes of determining the microbiological parameters of milk samples were established (Table 2).

Table 2. Statistically significant correlation coefficients (r) of sensor signals and microbiological parameters of milk samples.

Sensors Parameter	Titratable Acidity, °T	Sensor Parameter	Quantity of Mold CFU/mL	Sensor Parameter	Quantity of Yeast CFU/mL
$\Delta F_{max,\ choline+sorbitol}$	0.609	$\Delta F_{80s,\ choline+sorbitol}$	0.643	$\Delta F_{max,\ choline+erythritol+ASO}$	0.830
$\beta_{choline+sorbitol}$	0.679			$\beta_{choline+erythritol+ASO}$	0.868

The results of determining the physical, chemical, and microbiological parameters of milk are presented in Table A1 (Appendix A).

4. Discussion

It has been established that adding amorphous silicon oxide increases the stability of the film on the electrodes of the piezoresonator, therefore increasing the service life of sensors based on them. Additionally, the sorption efficiency of volatile compounds is increased (Table 1). It has been demonstrated that the reduction of the specific mass sensitivity of the micro-weighing of vapors of volatile compounds is associated with the increase in the carbohydrate radical of polyalcohol in the composition of the DES (Table 1). Furthermore, the addition of silicon oxide abnormally increases the mass-specific sensitivity of micro-weighing with a choline + xylitol + ASO film. This phenomenon is believed to be attributable to the more effective distribution of DESs on silicon oxide particles during the coating formation process and the steric availability of hydroxyl groups of xylitol for sorption. Despite the lower mass, the sorption efficiency on the choline + xylitol + ASO film is higher for polar volatile compounds with a hydrocarbon radical length exceeding C_3 (Table 1).

The addition of amorphous silicon oxide in the formation of a film with DES partially eliminates the differences in the chronofrequencygram of proton and aprotic substances (Figure 1). The length of the hydrocarbon radical of polyalcohol, which is part of the DES, does not affect the type of chronofrequency gram. Therefore, the kinetic features of sorption are also retained in this case. The study of the absorption of volatile vapors in the gas phase over aqueous solutions revealed that, for all films based on DES, the sensitivity of micro-weighing of vapors of substances abruptly changes when the concentration of the substance in the solution reaches 0.1 or 1% by volume. Based on the findings obtained from a comparative analysis of the sorption of volatile compounds in aqueous solutions for films based on DES and macromolecular sorbents (crown ethers, Triton X-100), it was determined that the detection limit of volatile compounds is significantly lower (0.001–0.01% vol.) than that of previously employed sorbents (0.01–0.1% vol.)

It has been established that the β parameter differs for substances depending on the composition of the DES. However, when ASO is added, the β parameter practically does not differ for coatings of different compositions and is close to the β parameter for an amorphous silicon oxide film. It was found that the sensor data with choline + sorbitol film correlated with the indicators of titratable acidity and the content of fungi and mold in raw milk (Table 2). The parameters of the sensor with choline + erythritol + ASO film significantly correlate with the yeast content in milk (Table 2). The addition of signals and sensor parameters into the regression model to predict the total microbial count of milk samples can increase the accuracy and reduce prediction error to 6%.

Author Contributions: Conceptualization, A.S.; methodology, A.S., E.A. and E.B.; validation, R.U., E.A. and E.B.; formal analysis, A.S. and E.B.; investigation, all authors.; writing—original draft preparation, A.S., R.U. and E.B.; writing—review and editing, A.S. and E.A.; visualization, A.S. and R.U.; project administration, R.U.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Russian Science Foundation grant no. 22-76-10048.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Korneeva O.S. for providing a microbiology laboratory for investigation and Kuchmenko T.A. for providing the equipment for gas phase analysis.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Physical, chemical, and microbiological indicators of raw milk samples.

No	Mass Fraction of Dry Solids, %	Mass Fraction of Fat, %,	Mass Fraction of Total Protein, %	Titratable Acidity, °T	QMAFAnM *, CFU/mL	Quantity of Yeast CFU/mL	Quantity of Mold CFU/mL
1	16.02 ± 0.12	7.5 ± 0.3	3.46 ± 0.15	19 ± 0.5	10,000,000	100,000	0
2	12.22 ± 0.13	3.8 ± 0.1	3.74 ± 0.10	20 ± 0.5	4,000,000	10,000	0
3	13.36 ± 0.08	4.8 ± 0.1	3.45 ± 0.10	19 ± 0.5	4,500,000	1000	10
4	15.15 ± 0.14	7.5 ± 0.5	3.26 ± 0.10	15 ± 0.5	340,000	0	0
5	11.63 ± 0.13	3.5 ± 0.1	3.01 ± 0.10	19 ± 0.5	2,400,000	1500	160
6	11.77 ± 0.11	3.1 ± 0.1	3.30 ± 0.15	19 ± 0.5	590,000	650	900
7	10.83 ± 0.09	3.9 ± 0.1	2.40 ± 0.10	15 ± 0.5	4,640,000	5680	0
8	12.31 ± 0.12	3.7 ± 0.1	3.10 ± 0.15	18 ± 0.5	98,000,000	8004	60
9	11.41 ± 0.06	3.2 ± 0.1	2.00 ± 0.05	15 ± 0.5	480,000	0	10
10	12.14 ± 0.10	4.1 ± 0.1	2.88 ± 0.10	16 ± 0.5	5,700,000	34,200	300
11	11.72 ± 0.07	3.4 ± 0.1	1.16 ± 0.10	15 ± 0.5	42,000,000	1800	0
12	10.92 ± 0.09	3.3 ± 0.1	1.35 ± 0.10	11 ± 0.5	2,000,000	2300	10
13	11.44 ± 0.11	3.6 ± 0.1	2.59 ± 0.15	17 ± 0.5	3,400,000	17,400	10
14	15.07 ± 0.15	6.5 ± 0.3	3.07 ± 0.10	16 ± 0.5	39,000,000	100,000	0

*—the number of CFU is calculated as the arithmetic mean value when counting on Petri dishes with different dilutions if it was possible or from appropriate dilution.

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