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Chemometric approach for the spectrophotometric determination of chloramphenicol in various food matrices: Using natural deep eutectic solvents

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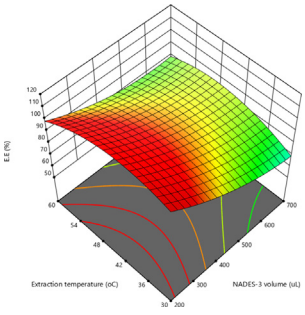
HIGHLIGHTS

- Cheap, green and safe analytical method for the determination of chloramphenicol.
- The main extraction factors were optimized by chemometric modelling.
- An eco-friendly natural deep eutectic solvent was prepared.
- Temperature controlled-natural deep eutectic solvent emulsification liquid–liquid microextraction was developed.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel, simple and green temperature controlled-natural deep eutectic solvent emulsification liquid–liquid microextraction (TC-NADES-LLME) coupled with UV–vis spectrophotometry was optimized for preconcentration and measurement of chloramphenicol (CAP) in eggs, milks honeys and chicken meat. Four different NADES were prepared and investigated for the efficient extraction of CAP. The important parameters (pH, NADES-3 vol, Fe(III) amount and extraction temperature) affecting the extraction efficiency of the TC-NADES-LLME procedure were investigated and optimized using a chemometric approach. In this study, Fe(III), NADES-3 and extraction temperature were used as complexing agent, extraction solvent and emulator accelerator, respectively. Using optimized values, the linear range of the TC-NADES-LLME procedure was in the range of 0.1–300 μg L^{-1} with a coefficient of determination of 0.9991. The detection limit and enhancement factor were 0.03 μg L^{-1} and 285, respectively. The precision of the method has been confirmed in repeatability and reproducibility studies. Relative standard deviation of these studies was<4.2 %. The matrix effect was investigated by adding three different CAP concentrations to the selected samples, and the results indicated the low matrix effect of the method. The TC-NADES-LLME procedure was successfully applied to determine and extract CAP in the selected samples.

1. Introduction

Chloramphenicol (CAP) was first isolated as a metabolic product of *streptomyces venezuelae bacteria* [1]. Today, it is an antibiotic synthesized by artificial means. It is highly effective against many pathogenic bacteria, rickettsia and mycoplasma; It has been pro-

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ven that its effect is shown by disrupting the protein synthesis in the microorganism [2]. Significant migration of CAP into serous spaces and body fluids has been reported. For example, the CAP passes to the brain approximately 9-fold the plasma level and nearly half to the cerebrospinal fluid [3]. The three best-known toxic features of CAP are aplastic anaemia, gray syndrome, and bone marrow suppression, respectively [4]. Because of this high toxicity, the use of CAP in food-producing animals has been banned by the European Community since 1994 [5]. In addition, CAP is included in Group A of Council Directive 96/23/EC [6], including substances with a "zero tolerance residue limit" in edible tissues. For these reasons, it is important to develop new analytical methods for the selective, accurate and rapid determination of the CAP in real samples.

Sample preparation methods are directly effective in the accuracy, precision and determination limits of the analysis. It is also the step that usually determines the speed of many analytical methods [7]. Aqueous samples are subjected to a sample preparation process to remove the organic species to be determined from the interference environment and to provide a sample suitable for instrumental analysis [8]. So far, sample preparation methods such as ultrasound assisted dispersive liquid–liquid microextraction [9], ionic liquid-anionic surfactant based aqueous two-phase extraction [10], switchable hydrophilicity solvent-based homogeneous liquid–liquid microextraction [11] and dispersive liquid–liquid microextraction [12] have been used for the extraction and separation of CAP in different matrix. The selection of the extraction solvent is very important in sample preparation studies. It is preferred that the extraction solvent is environmentally friendly, easy to prepare, accessible and inexpensive. In this context, the preparation and development of new generation extraction solvents has been an important issue in sample preparation methods [13].

In recent years, natural deep eutectic solvents (NADES) have been prepared and used as extraction solvents in sample preparation methods [14]. NADES are formed through hydrogen bond interactions [15]. There are two conditions for an H-bond to occur. These are high-polarity molecules that donate protons to the H-bond, and small molecules that donate electrons to the H-bond (proton acceptors) [16]. The melting point of the formed NADES is lower than its components. NADESs are mostly in liquid form between room temperature and 70 °C [17]. The use of NADES as extraction solvents for sample preparation studies is due to the fact that they have many advantages over conventional solvents [18]. First of all, since their polarity is quite high, the NADEs have the ability to dissolve many organic or inorganic substances, such as cellulose, which are insoluble in conventional solvents (organic solvent, surfactant, supramolecular) [19]. In addition, the NADES are composed of organic substances and are inexpensive, biodegradable, non-flammable, non-volatile, environmentally friendly, easy to prepare, odorless and colorless solvents [20–23].

Chemometric approach is a form of process analysis in which experimental parameters are changed in a controlled manner to detect their effects on a response of interest [24]. Statistical experiment design methods (full factorial design, central composite, Box-Behnken, Doehlert matrix..etc) experimentally describe the regression model between one or more measurable input variables [25]. These methods provide great advantages in terms of optimizing the ambient conditions, increasing the efficiency, reducing the number of experiments and reducing the cost [26].

In this study, novel, simple and green temperature controlled-natural deep eutectic solvent emulsification liquid–liquid microextraction (TC-NADES-LLME) coupled with UV–vis spectrophotometry was optimized for preconcentration and measurement of CAP in eggs, milks honeys and chicken meat. Four different NADES were prepared and investigated for the efficient extraction of CAP. Then the important parameters affecting the extraction efficiency of the TC-NADES-LLME procedure were investigated and optimized with chemometric approach. The precision of the method has been confirmed in repeatability and reproducibility studies. The proposed method provides several advantages such as high enhancement factor, low limit of detection, and good repeatability.

2 . Experimental

2.1 . Chemicals and reagents

All chemicals used in optimization and determination studies were of at least analytical purity and purchased from Sigma Aldrich (St. Louis, USA) and Merck (Darmstadt, Germany). Ultra-pure water was obtained from Millipore Milli-Q purification system (Millipore, Bedford, MA, USA). A 100 mg L^{-1} stock solution of chloramphenicol (CAP) was prepared by dissolving the appropriate amount of its solid (Sigma) in ethanol. Working solutions (0.01, 0.03, 0.09, 0.5, 1, 10, 25, 75, 150, 300, 400 and 500 μg L^{-1}) of the CAP were obtained daily by proper stepwise dilution of the stock solution with the water. A 25 mmol L^{-1} of Fe(III) solution (as complexing agent) was prepared from $Fe(NO_3)_3$ salt (Merck) in the water. Choline chloride (Merck), urea (Merck), citric acid (Sigma), ethylene glycol (Sigma) and lactic acid (Merck) were used for the preparation of NADES solutions. 0.1 mol L^{-1} pH = 6.6 potassium phosphate buffer at 25 °C was prepared by mixing 38.1 mL of K_2HPO_4 and 61.9 mL of KH_2PO_4.

2.2 . Devices

The following devices were used in the optimization, sample preparation and determination steps. Temperature control ultrasonic bath was purchased from Kudos company (SK5210LHC Shanghai, China). pH controls were provided with a digital pH meter containing a glass-calomel electrode (Metrohm® pH 827, Herisau, Switzerland)). Hettich Universal-320 model (London, England) centrifuge was used to achieve phase separation. The CAP analysis was performed with a computer-controlled Shimadzu UV-1800 UV–vis spectrophotometer (Tokyo, Japan) equipped with a quartz cuvette with a path length of 10 mm.

2.3 . Sample preparation

Pasteurized cow milk, raw bovine milk, skimmed cow milk, chestnut honey, cow milk, flower honey, chicken meat, and egg were chosen for analysis. Milk, chicken meat, and egg samples were collected from the markets in Sivas/Turkey. Honey samples were collected from local producers in Erzurum and Erzincan/Turkey. Milk samples were prepared according to the following procedure [27]. 10 mL of milk samples were added to a centrifuge tube containing 250 μL of perchloric acid (0.25 mol L^{-1}). Tubes were shaken by vortex for 15 min, then centrifuged at 3000 rpm for 10 min. The resulting mixture was filtered through filter paper, and then the pH of its solutions was adjusted to 7.0. The sample preparation steps applied for the honey sample are listed below [27]. First, 1.0 g honey samples were carefully weighed and added to the centrifuge tube containing 5 mL phosphate buffer. Then, 4 mL of ethyl acetate was added to the mixture and vortexed until complete mixing was achieved. The resulting mixture was centrifuged at 3000 rpm for 5 min, then filtered through a membrane filter and the pH was adjusted to 7.0. Chicken meat and egg samples were prepared according to the following procedure [28]. Chicken meat and egg samples were first homogenized with a laboratory clarifier. Then, 5 g of homogenized samples were transferred to centrifuge tubes containing 5 mL of acetonitrile and 5 mL of 4%(w/v) NaCl. The mixture was vortexed for 3 min, then it was centrifuged at 5000 rpm

for 5 min. The supernatant phase was transferred to a clean tube and 10 mL of n-hexane was added. Afterwards, the mixture was vortexed again for 1 min and centrifuged at 5000 rpm for 5 min, and the aqueous phase was used for the application of the TC-NADES-ELLME procedure.

2.4. Chemometric approach

For the optimization of the important experimental steps of the TC-NADES-ELLME procedure, Box–Behnken design (BBD) based on the chemometric approach was used. As a result of the preliminary experiments, important variables including pH, NADES-3 vol, Fe (III) amount and extraction temperature were selected for optimization by the BBD. In this context, a four-variable 3-level full BBD experimental plan was created. A total of 29 experiments, five of which were central experiments, were conducted. The variables, levels, units and symbols of the established BBD model are presented in Supplementary Material Table S1. Experimental studies are expressed by the following quadratic equation-1.

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_1^2 + \sum_{1 \le i \le j}^{k} b_{ij} x_i x_j + \varepsilon \quad (1)$$

Where y is response, x_i was factors, k was factor number, b_0 was constant, b_i, b_{ij} and b_{ii} were regression parameters for the effects of linear, interaction and quadratic coefficients, respectively, and ε was residue. In addition, the terms X_iX_j and X_i^2 represent the interaction and quadratic terms, respectively. The E.E% values obtained as a result of the application of the established experimental model and the E.E% values predicted by the model were given in Supplementary Material Table S2.

2.5. Preparation of NADESs

NADES solutions were prepared using the method reported in the literature [21]. In this study, Choline chloride was used as the HBA, while urea, citric acid, ethylene glycol and lactic acid was used as the HBD. The experimental steps for the four NADES prepared using these components were presented below. Choline chloride was first added to four separate beakers, then different HBDs were added on top of each beaker. Then the beakers are placed on the heating plate and heated until a homogeneous liquid is obtained. Homogeneous solutions were obtained in 10 min at approximately 80 °C. Finally, the NADES preparation step was completed by adding 20% (v/v) water to the homogeneous solutions. In this study, the mole ratios and compositions of the added HBA and HBD were presented in Table 1.

2.6. TC-NADES-ELLME procedure

The experimental steps for the TC-NADES-ELLME procedure were carried out as follows. A 5.0 mL of the sample solution containing CAP (100 μg L^{-1}) was added to 15-mL centrifuge tube. The pH of the sample solution was adjusted to pH = 6.6 with potassium phosphate buffer. Then, 15 mmol L^{-1} of Fe(III) was added in the obtained mixture to ensure the complexation of the CAP in the sample. After the complexation, 250 μL NADES-3 (as an extraction solvent) and 100 μL THF (as an emulsifier) were added to the mixture in order to separate the CAP-Fe complex from the sample solution. Subsequently, tubes were placed in an ultrasonic bath and sonicated at 43 °C for 2 min. The main purpose of the sonication step is to ensure that the emulating agent and extraction solvent were dispersed effectively and quickly in the sample. In this way, a cloudy solution was obtained, proving the formation of insoluble self-aggregation at nanoscales. The tubes were centrifuged at 4000 rpm for 5 min and the NADES-3 phase containing analyte was separated from the aqueous phase. After the aqueous phase was decanted, the remaining NADES-3 phase was made up to 200 μL with ethanol. Finally, the obtained solution was transferred to quartz cuvettes and spectrophotometric measurements were taken at 450 nm. The same experimental steps were used for the blank solutions.

2.7 . Enhancement factor and extraction efficiency calculation methods

Enhancement factor (EF) and extraction efficiency (E.E%) were two important reference indicators of the efficiency of the optimization step.

The EF of the overall TC-NADES-ELLME procedure was calculated by the following equation-2:.

$$EF = C_{final}/C_{initial} \quad (2)$$

Where C_{final} was the amount of CAP obtained in the NADES phased that was injected in UV–vis spectrophotometer, and so it can be calculated with the calibration graphs after the TC-NADES-ELLME procedure. $C_{initial}$ was the added amount of CAP in sample solution.

The E.E% of the overall TC-NADES-ELLME procedure was calculated by the following equation 3:.

$$E.E\ (\%) = 100 \times C_{final} \times V_{final}/C_o \times V_o \quad (3)$$

Where V_{final}, V_o and C_o refer to the volume of the final phase, the volume of the sample solution and the initial amount of CAP in the sample solution, respectively.

3 . Results And discussion

3.1. Preliminary experiments

Prior to BBD design, preliminary studies were carried out to select the appropriate NADES and its molar ratio. In this study, four NADES, whose composition was given in Table 1, were prepared. The prepared NADES were investigated using the mole ratios given in Table 1 for the separation and preconcentration of the CAP in the sample solution. As a result of the study, the E.E% of CAP for the NADES used was NADES-4 (95.1%) >NADES-2 (85.3%) >NADES-3 (73.9%) >NADES-1 (62.7%), respectively. In the light of the results obtained, the NADES-4, which was prepared from the mixture of choline chloride and lactic acid, was chosen as the appropriate NADES. Once the appropriate NADES-4 has been selected, its molar ratio is an important parameter influencing the E.E% of CAP. For this reason, solutions of different molar ratios of NADE-4 were pre-

Table 1
The composition, physical properties and mole ratios of the four NADES prepared.

Abbreviations	HBA	HBD	Molar Ratio	Water addition (%)	Physical Aspect Color	E.E % of CAT
NADES-1	Choline chloride	Urea	1:2	20	Colorless transparent oil	62.7
NADES-2	Choline chloride	Citric acid	1:1	20	Pale yellow oil	85.3
NADES-3	Choline chloride	Ethylene glycol	1:1	20	Colorless transparent oil	73.9
NADES-4	Choline chloride	Lactic acid	1:1	20	Colorless transparent oil	95.1

pared and then tested for the E.E% of CAP. The molar ratios tested for NADES-4 were 2:1, 1:1, 2:1 and 3:1, respectively. As a result of the study, the highest E.E% of the CAP was obtained at a molar ratio of 1:2. Here, there was a drastic decrease in the E.E% when the lactic acid in the mixture of choline chloride and lactic acid forming NADES-4 was increased more. This is probably because excess lactic acid builds up on the formed NADES. For all these reasons, NADES-4 at a molar ratio of 1:2 was chosen for the BBD design.

3.2. Statistical assessment of main numeric factors

The ANOVA data obtained as a result of the application of the four-variable three-level BBD design were presented in Table 2. The following conclusions were drawn from the interpretation of the ANOVA table. First, it was evaluated whether the established BBD model was significant for the extraction efficiency of CAP. In this evaluation, since the p-value was<0.05, the BBD model was considered statistically significant at the 95% confidence level. The F-value is taken into account when evaluating the parameters that contribute to the BBD model. Here, the numerical magnitude of the F-value represents the magnitude of the contribution to the BBD model. In the light of this explanation, when Table 2 was evaluated, the parameters that contributed the most and least to the BBD model were pH and extraction temperature, respectively. Additionally, parameters such as Fe(III) amount (p-value: 0.0844) and extraction temperature (p-value: 0.8683) with p-values greater than 0.05 were insignificant for the BBD model. Also, all binary and quadratic interactions were significant for the BBD model. The p-value of Lack of Fit is taken into account to decide whether there were significant errors in the BBD model. Lack of fit (p-value) of 0.8764 from Table 2 also strengthened the absence of lack of fit and the reliability of the BBD model. Fit statistics numerical values for the BBD used were presented in Supplementary Material Table S3. The coefficient of determination R^2, the adjusted coefficient of determination (R^2_{Adj}), the predicted coefficient of determination (R^2_{pred}) and the coefficient of variation (CV %) were 0.995, 0.990, 0.982 and 1.3, respectively. These results indicated that the accuracy and general usability of the quadratic polynomial model is sufficient. The Adeq. Precision of 60.25 indicates that the BBD model can be used to navigate the design space. The reliability of the BBD model can also be seen from the high agreement between the prediction and the actual values (see Fig. 1a). As a result of these data, the quadratic equation for the BBD model was expressed by the following equation 4.

$$E.E(\%) = +79.04 + 6.11A + 1.74B + 0.550C - 0.050D - 8.57AB - 7.53AC + 4.28AD + 8.40BC + 5.65BD + 4.67CD - 2.05A^2 + 6.93B^2 + 3.49C^2 - 10.86D^2 \quad (4)$$

3.3. Response surface curves and optimum conditions

The effects of binary interactions on the E.E% of CAP were investigated by drawing 3D surface response graphs. Fig. 2a shows the effect of pH and Fe(III) amount on the E.E % of CAP. Quantitative extraction efficiency was observed in the pH range of 5–7 and the amount of Fe(III) <20 mmol L^{-1}. The reason for the decrease in E.E% especially at low pH may be due to the reduction of Fe (III) ions in the acidic region. In addition, the decrease in E.E% at high Fe(III) concentrations may be due to precipitation of Fe(III) ions. The effect of the interaction between Fe(III) amount and NADES-3 on E.E of CAP was presented in Fig. 2b. It can be seen from the related figure that the highest E.E% is obtained in the presence of low NADES volumes (350 μL >) and low Fe(III) amount (25 mmol L^{-1}>). Also, the E.E% decreased as the amount of both variables increased. The effect of the interaction between extraction temperature and NADES-3 vol on E.E% of CAP was presented in Fig. 2c. Here, it was seen that the change of the extraction temperature did not cause a significant change on the E.E % of CAP Especially at low NADES-3 volumes, higher E.E % of CAP was obtained when the extraction temperature was in the range of 30–45 °C. The effect of the interaction between NADES-3 vol and pH on the E.E% of CAP was presented in Fig. 2d. Here, quantitative E.E% of CAP was provided when the pH was in the range of 5–7 and the volume of NADES-3 was<300 μL. Non-quantitative results at high NADES-3 volumes can be attributed to the excessive presence of NADES-3 in sample solution.

The microextraction parameters (A = 6.6, B = 250 μL, C = 15 mmol L^{-1} and D = 43 °C) were optimized using the BBD model equation by solving a regression equation with the predicted E.E % of 97.8 for CAP. These selected optimum data were evaluated to test the predictions from the BBD model. As a result of three repetitive applications, 97.2% of CAP was extracted and this result confirmed that the model was suitable and reliable for optimization.

Table 2
ANOVA analysis results.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3031.35	14	216.52	205.78	< 0.0001	significant
A	447.74	1	447.74	425.52	< 0.0001	
B	36.40	1	36.40	34.59	< 0.0001	
C	3.63	1	3.63	3.45	0.0844	
D	0.0300	1	0.0300	0.0285	0.8683	
AB	294.12	1	294.12	279.52	< 0.0001	
AC	226.50	1	226.50	215.26	< 0.0001	
AD	73.10	1	73.10	69.47	< 0.0001	
BC	282.24	1	282.24	268.23	< 0.0001	
BD	127.69	1	127.69	121.35	< 0.0001	
CD	87.42	1	87.42	83.08	< 0.0001	
A^2	27.24	1	27.24	25.89	0.0002	
B^2	311.14	1	311.14	295.70	< 0.0001	
C^2	78.93	1	78.93	75.01	< 0.0001	
D^2	765.25	1	765.25	727.27	< 0.0001	
Residual	14.73	14	1.05			
Lack of Fit	7.58	10	0.7579	0.4239	0.8764	not significant
Pure Error	7.15	4	1.79			
Cor Total	3046.08	28				

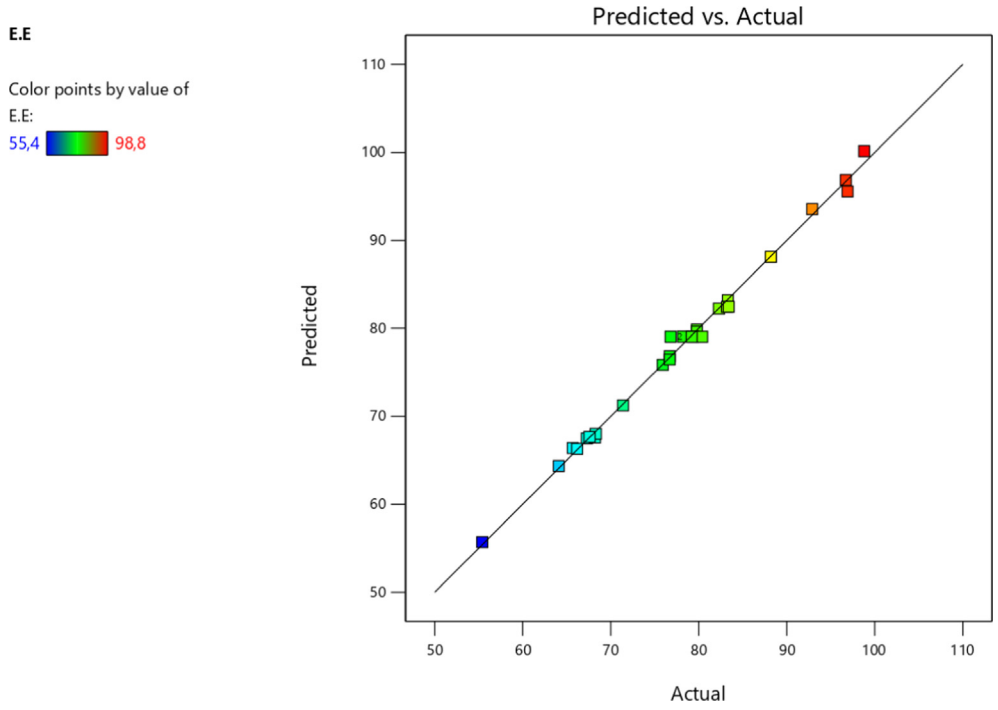


Fig. 1. Agreement between the experimental results obtained and the actual value.

3.4. Analytical performance

Analytical performance of the TC-NADES-ELLME procedure using a new NADES named as Choline chloride:lactic acid for the extraction and preconcentration of CAP was assayed. The analytical data obtained were presented in Table 3a. Linear range was in the range of 0.1–300 μg L^{-1} with a coefficient of determination of 0.9991. Limits of detection (LOD) and limit of quantification (LOQ) were calculated as three and ten times the standard deviation of the sample blank signal, respectively. The LOD and LOQ were calculated as 0.03 μg L^{-1} and 0.1 μg L^{-1}, respectively. As a result of the analysis of the samples, the average recovery and average relative standard deviation (RSD) were calculated as 96.5% and 2.4%, respectively. Also, the EF was 285.

3.5. Interference

Since optimization studies are performed on model solutions, different chemical components may affect the extraction step. Considering this fact, a study has been made for possible interference components. In this study, the components in Table 3b were added at different rates on the model solutions and then the TC-NADES-ELLME procedure was applied to them. Then, RSD, recovery and tolerable limits were found for the studied components. In the results in Table 3b, the RSD and recovery for the studied components were calculated in the range of 2.0–3.7% and 94.2–99.4%, respectively. These results were analytically reliable. The tolerable limit is calculated from the ratio of the amount of studied components to the amount of CAP (50 μL L^{-1}), which causes a ± 5% change in the analytical signal obtained in the absence of the components ion. It can be seen that a high tolerable limit is achieved in the results.

3.6. Repeatability and reproducibility

The precision of the TC-NADES-ELLME procedure was evaluated with the repeatability and reproducibility approaches. The experimental steps for these approaches were performed as follows. Five different concentrations (1, 5, 50, 100 and 250 μg L^{-1}) of the CAP were added to the selected samples in both approaches. Then, for the repeatability approach, the TC-NADES-ELLME procedure was applied to all added samples three times in one day. For the reproducibility approach, the TC-NADES-ELLME procedure was applied to the same samples in triplicate in three consecutive days. Recovery and RSD values were calculated for the added concentrations in both approaches. As a result of the study, recovery and RSD for the repeatability approach ranged between 96.4 and 99.0% and 1.8–3.4%, respectively, while recovery and RSD for the reproducibility approach ranged between 94.3 and 98.7% and 2.5–4.2%, respectively. These analytical results (see Table 3c) indicate that the method exhibits high precision.

3.7. Recovery

The matrix effect of the TC-NADES-ELLME procedure was investigated by the study on selected samples. Three different concentrations of CAP were added to the selected samples, and then the recovery values were calculated by applying the TC-NADES-ELLME procedure. The standard CAP concentration added was chosen to include the lower (5 μg L^{-1}), middle (100 μg L^{-1}) and high (200 μg L^{-1}) values of the linear range. As a result of the study, the recovery for the added low, medium and high CAP concentrations was in the range of 91 ± 4–97 ± 3%, 93 ± 5–98 ± 2% and 95 ± 3–99 ± 4%, respectively. As can be seen from the results (see Table 3d), quantitative recoveries were obtained for all three concentrations, which indicates that the TC-NADES-ELLME procedure has good accuracy.

3.8. Sample analysis

After the necessary validation studies were carried out, the applicability of the TC-NADES-ELLME procedure was tested for analysis of CAP in selected samples including such as pasteurized milk, raw bovine milk, skimmed milk, chestnut honey, cow milk,

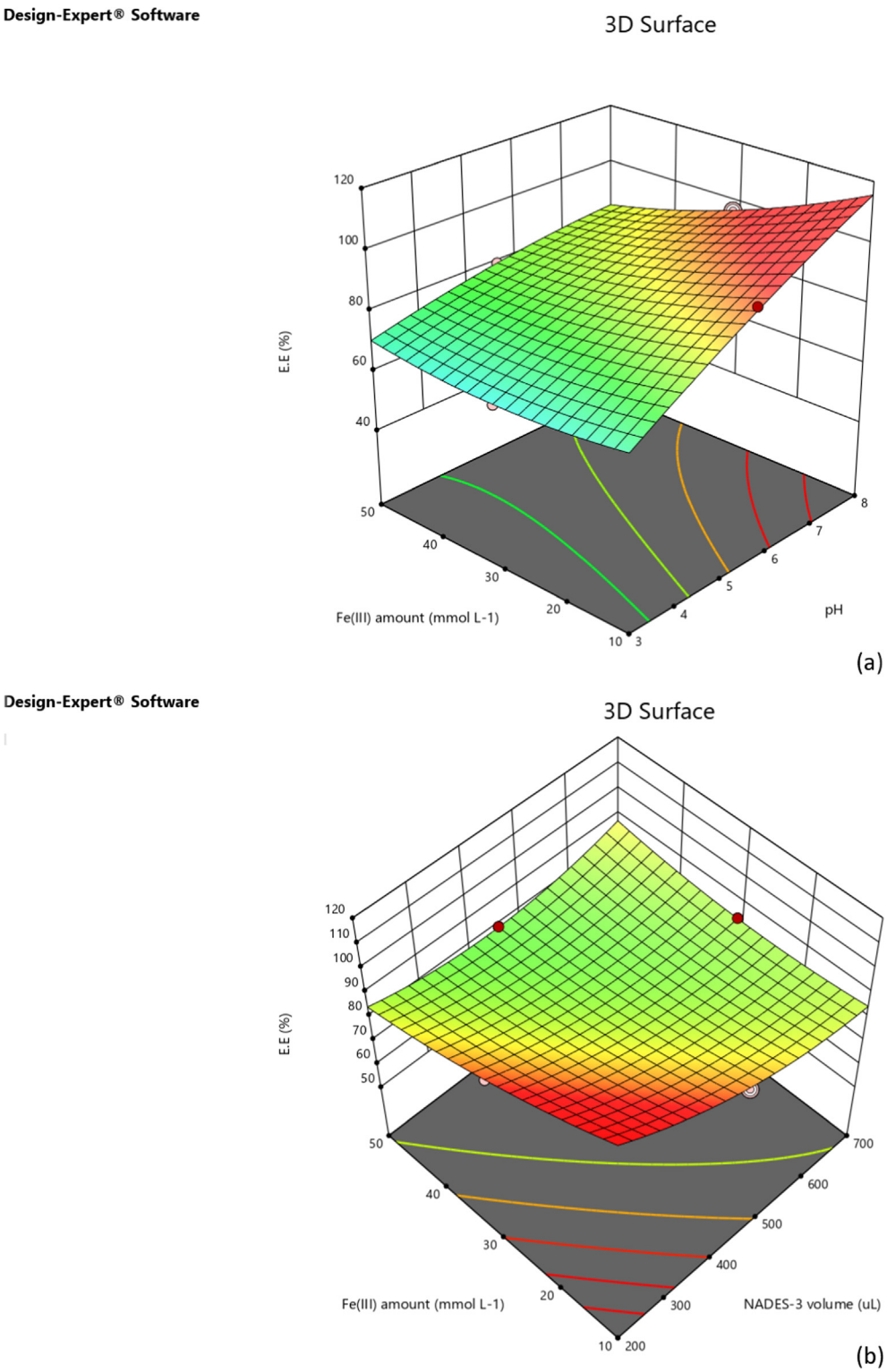
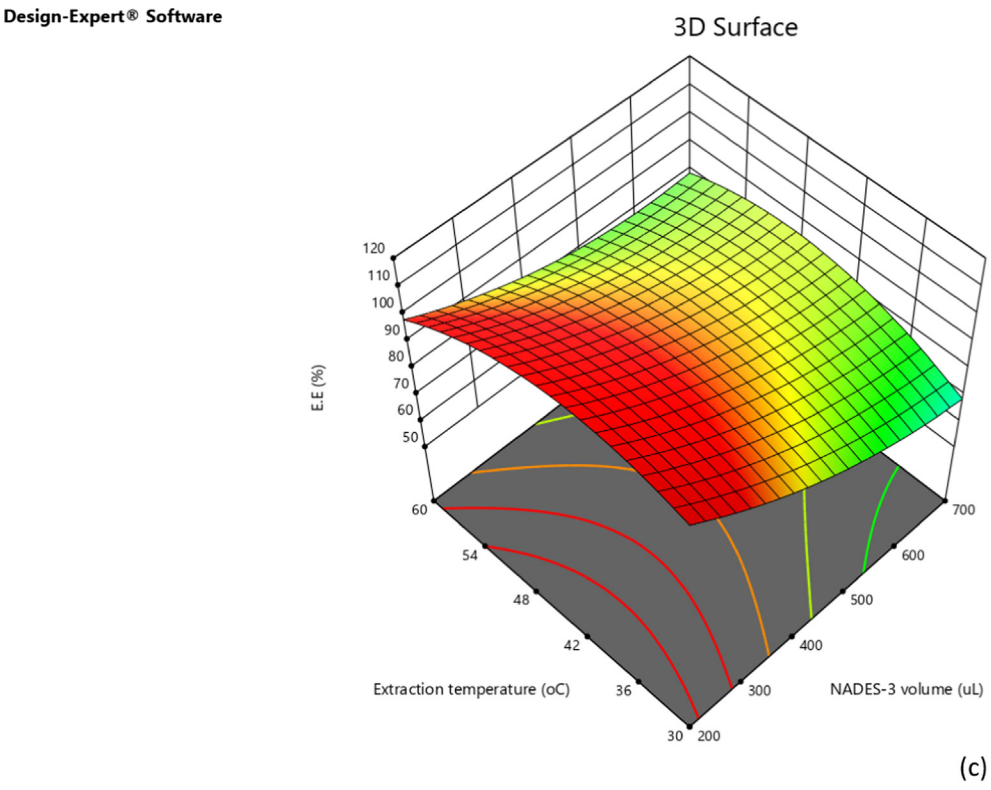


Fig. 2. (a-d). 3D response surfaces for: (a) Fe(III) amount- pH, (b) Fe(III) amount- NADES-3, (c) extraction time-NADES-3, (d) NADES-3-pH.

flower honey, chicken meat, and egg. The CAP could not be detected in some samples including raw bovine milk, flower honey-1, egg-2and egg-3. High CAP content (11.27 ± 1.03 μg kg^{-1}) was detected in chicken meat-2. All the analytical data obtained were presented in Table 4. These results demonstrate the high quality of the TC-NADES-ELLME procedure for the extraction and determination of CAP in complex matrices. In addition to these, it can be said that the TC-NADES-ELLME procedure can be safely applied to a wide range of solid and liquid samples.

An analytical comparison among the TC-NADES-ELLME-UV–Vis method and other applicable analytical methods for the extraction and quantification of the CAP was given in Table 5. The TC-NADES-ELLME procedure has a comparable linear range, a relatively low RSD, and a good EF compared to other complex and expensive ana-



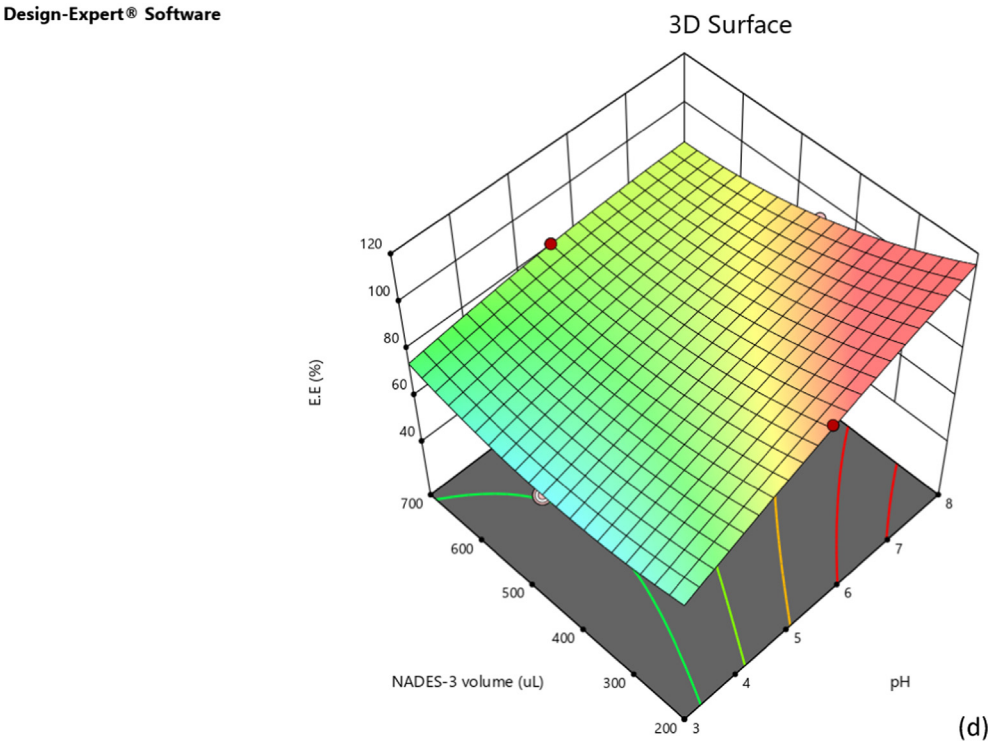


Fig. 2 (*continued*)

lytical techniques including LC/MS-MS and HPLC-PDA. Compared to other extraction procedure, the presented procedure requires very low extraction time for extraction of CAP. These efficient analytical results can be easily achieved through a new, effective, simple and fast emulsification microextraction using a highly green and biodegradable extracting agent (NADES-3) followed by a simple, fast and inexpensive UV–vis spectrophotometer detection technique.

Table 3a
Analytical parameters results of the TC-NADES-ELLME method.

Analytical parameters	Optimal value
Regression equation A=(a ± SD_a) c + (b ± SD_b)	A=(0.2895 ± 0.0004)c +(0.1729 ± 0.06944)
Liner range (μg L^{-1})	0.1–300
Coefficient of determination (r^2)	0.9991
LOD (μg L^{-1})	0.03
LOQ (μg L^{-1})	0.1
EF	285
Average RSD (%)	2.4
Average Recoveries (%) in added samples	96.5
Measurement wavelength (nm)	544

A: Absorbance; c:Chloramphenicol concentration (μg L^{-1}); a:slope; b:intercept; SDa and SDb, standard deviations of slope and intercept, respectively.
EF: Enhancement factor; LOD: Limits of detection; LOQ: limits of quantification; RSD: relative standard deviation.

Table 3b
Selectivity of the TC-NADES-ELLME method in the presence of different components.

Components	RSD (%)	Recovery (%)	Tolerable limit
Na^+	2.1	99.4	2000
K^+	2.0	99.2	2000
SO_4^{2-}	2.8	98.1	2000
CO_3^{2-}	2.3	98.7	1500
Mn^{2+}	2.7	99.0	1500
Zn^{2+}	2.6	98.5	1000
F^-	2.1	98.7	1000
I^-	2.8	98.0	750
Co^{2+}	2.5	97.1	500
Cu^{2+}	2.7	97.4	500
Se^{4+}	2.9	97.2	500
Hydrazine	3.0	96.2	250
Tartrazine	3.6	95.4	250
Oxalate	3.1	96.7	250
Florfenicol	3.4	95.7	100
Trimethoprim	3.2	94.2	100
Thiamphenicol	3.7	95.8	100

Table 3c
Repeatability and reproducibility studies.

Spiked (μg L^{-1})	Repeatability (N = 3)		Reproducibility (N = 3 × 3)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	96.4	1.8	94.3	2.5
5	97.2	2.3	95.7	2.9
50	98.7	2.7	96.8	3.1
100	98.6	3.0	97.4	3.8
250	99.0	3.4	98.7	4.2

Table 3d
Results of assays to test the sample matrices effect for the CAP.

Samples	Added the CAP amount		
	5 μg L^{-1}	100 μg L^{-1}	200 μg L^{-1}
Pasteurized cow milk	94 ± 4*	95 ± 3	96 ± 4
Raw bovine milk	96 ± 2	97 ± 4	98 ± 5
Skimmed cow milk	92 ± 4	93 ± 4	96 ± 5
Cow milk	97 ± 3	96 ± 3	98 ± 4
Chestnut honey-1	95 ± 3	96 ± 4	98 ± 5
Chestnut honey-2	92 ± 4	93 ± 5	96 ± 3
Flower honey-1	96 ± 2	97 ± 4	99 ± 4
Flower honey-2	97 ± 2	98 ± 2	99 ± 2
Egg-1	95 ± 4	97 ± 2	98 ± 3
Egg-2	92 ± 5	94 ± 4	97 ± 3
Egg-3	93 ± 3	94 ± 3	96 ± 4
Chicken meat-1	96 ± 2	97 ± 2	98 ± 2
Chicken meat-2	91 ± 4	93 ± 3	95 ± 3

* Mean relative recovery ± standard deviation (N = 3).

Table 4
Analytical data of the CAP in selected samples by the TC-NADES-ELLME method.

Samples	Measured value (μg L^{-1} for liquid samples and μg kg^{-1} for solid samples)
Pasteurized milk	0.84 ± 0.06
Raw bovine milk	n.d*
Skimmed milk	0.46 ± 0.04
Cow milk	1.75 ± 0.22
Chestnut honey-1	2.39 ± 0.08
Chestnut honey-2	3.57 ± 0.08
Flower honey-1	n.d
Flower honey-2	2.36 ± 0.54
Egg-1	4.65 ± 0.86
Egg-2	n.d
Egg-3	n.d
Chicken meat-1	7.53 ± 0.96
Chicken meat-2	11.27 ± 1.03

* could not be determined.
**Mean amount ± standard deviation (N = 3).

4. Conclusions

For the first time, a simple TC-NADES-LLME procedure coupled with UV–vis spectrophotometry was optimized for preconcentration and measurement of CAP in eggs, milks honeys and chicken meat. Four different NADES were prepared and investigated for the efficient extraction of CAP. Then the important parameters affecting the extraction efficiency were optimized by BBD approach. Using optimized values, the linear range of the TC-NADES-LLME procedure was in the range of 0.1–300 μg L^{-1} with a coefficient of determination of 0.9991. The detection limit and

Table 5
Comparison of the TC-NADES-ELLME method with other microextraction procedures for the determination of CAP.

Analytical method	Liner range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	EF	RSD (%)	Extraction time (min)	References
TC-NADES-ELLME/UV–VIS	0.1–300	0.03	285	2.4	2	Current method
EME/HPLC-UV	0.04–250	0.012	195	<5.5	15	[29]
HA-DLPME/IMS	0.5–20	0.13	812	4.8	4	[30]
SPE/HPLC-PDA	20–100	0.1	–	9.9	50	[31]
LC/MS-MS	0.075–0.9	0.015	–	14.9	10	[32]
DLLME-MNPsUV– VIS	50–1000	16.5	50	6.29	10	[33]

EME/HPLC-UV: Electromembrane extraction/ high-performance liquid chromatography method with ultraviolet detection; HA-DLPME/IMS: homogenizer assisted dispersive liquid-phase microextraction/ ion mobility spectrometry; SPE/HPLC-PDA: solid phase extraction/high performance liquid chromatography with the photo diode arrays detector; LC/MS-MS: Liquid chromatography-tandem mass spectrometry; DLLME-MNPs/UV– VIS: Magnetic nanoparticles assisted dispersive liquid–liquid microextraction/ spectrophotometer.

enhancement factor were 0.03 $\mu g\ L^{-1}$ and 285, respectively. The precision of the method has been confirmed in repeatability and reproducibility studies. Relative standard deviation of these studies was<4.2 %. The NADES has remarkable advantages over other conventional organic extraction solvents, such as unique physical–chemical properties, simple preparation, low cost and favorable biocompatibility. No obvious matrix effect of the developed extraction process was observed. The TC-NADES-ELLME procedure was economical, easy to use and requires only simple equipment for extraction/analysis. At the same time, good linearity, low RSD values and high relative recovery were obtained. It was clear that the TC-NADES-ELLME procedure could be highly competent for accurate and precise analysis of CAP at trace levels.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2022.121198.

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