

# Comparison of classic and chemometric methods used for phosphate removal from fresh human urine under optimum conditions

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Human urine constitutes 1% of domestic wastewater and can be used to recover nitrogen and phosphorus when collected separately at source. Sustainable nutrient (nitrogen, phosphate, potassium) cycling requires the recovery of these valuable resources from human urine. As nitrogen and phosphorus are valuable nutrients needed for plant growth, these components of urine are an excellent fertilizer. Phosphorus can be applied in natural fertilizers and in addition the pollution load of wastewater treatment plants is reduced.

Different nutrient removal and recovery methods from urine have been studied at lab scale, but none so far has reached technological competence and none has been extended to practical use. The focus of this study was to evaluate electrocoagulation as a process for the removal of phosphate from fresh human urine using iron plate electrodes. The effect of pH and current density on phosphate removal was investigated. While determining the optimum conditions for removal, classical and chemometric methods were compared. Using the central composite design (CCD), optimum conditions were determined with only 13 experiments, and time and labour savings were achieved compared to the classical method. Initial pH values and current density were controlled within the range of pH 5–9 and current density 12–40 mA/cm<sup>2</sup>. From the obtained results, it was found that optimal initial current density is 40 mA/cm<sup>2</sup> for both methods, and optimal pH is 7 for the classical method and 6.24 for CCD, which is the natural pH of human urine. Realization of phosphate removal using optimum conditions discovered with CCD, provides savings on experimental effort, time, chemicals and energy consumption, and will contribute to resource recovery, reduction of wastewater load and sustainable fertilizer production.

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## INTRODUCTION

Increasing human population, industrialization and bad agricultural practices, such as excessive use of pesticides and water, cause rapid pollution of clean water resources (Sugeçti et al., 2016; Tunçsoy et al., 2021). One of the important recent problems of water resources is eutrophication caused by nutrient run-off. Eutrophication affects water quality by depletion of oxygen, which results in loss of aquatic life. Recovery of nitrogen and phosphorus at source in domestic wastewater is a very important step for wastewater management. In most cases, phosphorus is the limiting factor in the eutrophication process (Lacasa et al., 2011). In addition, the price of phosphate rocks has increased two- to threefold in the past decade and this phosphorus reserve is a non-renewable resource that will be exhausted in 50 to 100 years (Wei et al., 2018; Tao et al., 2019; Kemacheevakul et al., 2011). Therefore, most of the recent nutrient removal studies have focused on the removal and recovery of phosphorus from wastewaters.

Human urine, which is known as yellow water, constitutes 1% of domestic wastewater and contains 80% nitrogen and 50% phosphorus when collected separately at source (Liu et al., 2016). An adult person produces about 500 L of urine and 50 L of faeces in 1 year (Ganrot, 2005). A healthy person's urine is sterile when it leaves the body. Human urine can be used as a fertilizer following an appropriate recovery method after being collected separately at the source (Palmquist and Jönsson, 2003).

In recent years, human urine has begun to be seen as a source of nutrients and a number of technologies have investigated nutrient removal and recovery from human urine. Electrochemical technologies for the recovery of nutrients are preferential to alternative technologies such as precipitation, biological processes, adsorption, membrane systems and freeze/thaw concentration (Liu et al., 2016). For example, due to its physical-chemical properties, aluminium filling is considered to be a suitable material for use in continuous flow reactors (as filter media) or batch sorption units. In some studies, it has been reported that the thermally treated (at 900°C) aluminium filler (TT/AF900), which is used as an adsorbent to remove P from aqueous solutions, has fast adsorption capacity over a wide pH range (4–10) in aqueous solution (Qasemi et al., 2018). In another study, a mild and non-acidic method was proposed using thermally activated dolomite under standard conditions (25°C, 101.3 kPa) to prepare granular phosphate sorbents. NaH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub> and Na<sub>3</sub>PO<sub>4</sub> aqueous solutions were used as phosphating reagents. The effects of the properties of the reagents, contact time, the phase, and chemical composition of the obtained sorbents on adsorption properties were investigated. Up to 90–95% of Pb<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Ni<sup>2+</sup>, Sr<sup>2+</sup> and Co<sup>2+</sup> were removed in dynamic mode from multicomponent aqueous solutions (at initial concentrations of 5 mg/L, linear velocity 10 m/h) (Ivanets et al., 2016).

Compared to conventional wastewater treatment methods, electrocoagulation processes have many advantages such as ease of automation, a short operation time, not requiring the addition of chemicals, a negligible amount of chemical sludge, low operating and maintenance costs, compact arrangement, high removal efficiency, the requirement for a small area and in-situ generation of coagulants (Lacasa et al., 2011; Koparal and Ögütveren, 2002; Bakshi et al., 2019; Chen, 2004). Electrocoagulation processes, which are not widely used due to the need for electricity, are very promising in terms of high removal efficiency and being environmental friendly. The electrical current supplied to the system dissolves the metal electrodes and causes the metal cations to be introduced into the solution. Thus, no external coagulant is added to the system. While metal ions are released in the anode, hydrogen gas is produced in the cathode. The hydrogen gas produced also causes the flocculated pollutants to float (Chen, 2004; Reilly et al., 2019; Attour et al., 2014).

In analytical studies, method optimization is routinely done for high sensitivity or selectivity. In classical optimization techniques, optimization of each factor usually requires a lot of experimentation, labour and time. But new optimization methods can significantly reduce the number of these experiments and reduce chemical and energy use, analysis time and manpower required. Commonly used experimental design techniques are full factorial, fractional factorial and central composite design. Fractional factorial design requires less experimentation than full factorial. However, full factorial and partial factorial designs are complex and seldom used (Johnson et al., 2002).

Central composite design (CCD) is a better alternative to full factorial design as it provides more information and requires fewer experiments (Komarova et al., 2001). With the central composite design method, the effect of factors and the interactions between factors can be examined. Error calculations can be made with repeated experiments. Parabolic effects can be studied. Optimum conditions of the factors can be found. Many articles published in recent years also confirm that the classical method and chemometric methods are widely used together (Peris-Diaz and Krezel, 2021). For this reason, this study aimed to determine the relationship between phosphate concentration in fresh human urine, pH and current density parameters, and to determine the best possible phosphate removal conditions using the CCD method. Firstly, optimum conditions were determined with the classical method, and then it was revealed that optimum conditions could be determined with fewer experiments by applying the chemometric method at the specified intervals. The results obtained with the chemometric method and the classical methods were compared and it was concluded that the optimum values determined in both methods were close.

## EXPERIMENTAL

### Chemicals and reagents

All chemicals used in the experiments were of analytical or HPLC purity. The chemical characterization of fresh human urine is presented in Table 1. The pH was adjusted with NaOH and H<sub>2</sub>SO<sub>4</sub>. All reagents were of analytical grade. After each run, the effective surface area of the electrodes was observed to be reduced due to the formation of metal oxides on the surface. This phenomenon is known as passivation and leads to incremental energy usage. Therefore, after each run, the oxide layers were removed using the combination of acid reagent and distilled water. The EC system was cleaned immediately after each experiment using the following procedure: tap water rinse, 10% H<sub>2</sub>SO<sub>4</sub> for 15 min, deionisation water rinses.

**Table 1.** Characterization of fresh human urine

Parameters	Fresh human urine
pH	6.5–7.5
Conductivity (mS/cm <sup>2</sup> )	15–20
PO <sub>4</sub> -P (mg/L)	600–800
NO <sub>3</sub> -N (mg/L)	350–450
NO <sub>2</sub> -N (mg/L)	0.2–0.4
NH <sub>3</sub> -N (mg/L)	1 500–2 000
TOC (mg/L)	3 000–3 500
Colour	Light yellow

### Instrumental

The pH and conductivity were measured continuously (WTW pH/cond 720). Each run was timed starting with the DC power supply switching on. An electrochemical reactor has two anodes and two cathodes. The electrodes were all the same size (dimensions: 5 × 12.5 cm; 2 mm thickness) and made of iron material. Phosphate concentration was analysed with a Shimadzu UV-1800 instrument according to Standard Methods (APHA, 1998).

### Sample preparation

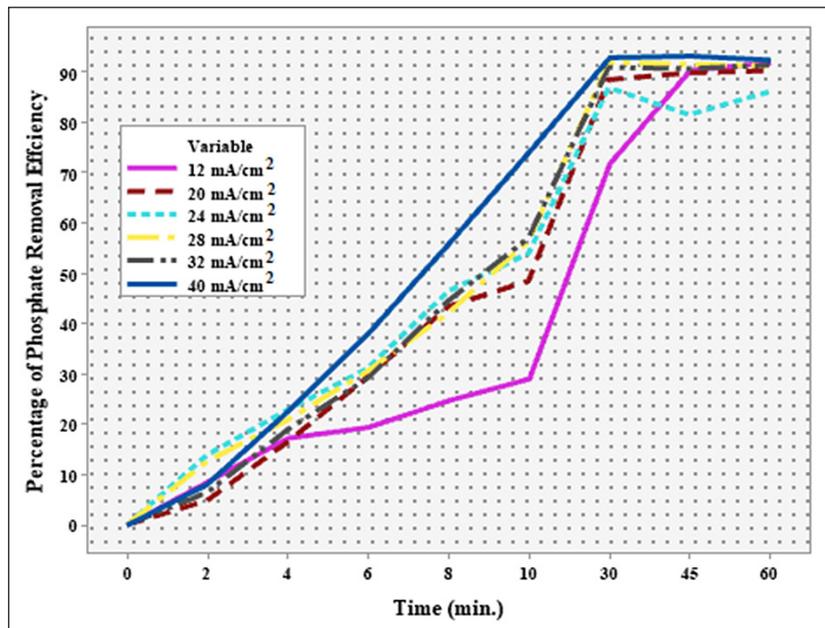
Source-separated urine was collected from male donors who were not taking any medication. Human urine was collected from a waterless urinal placed at the Zonguldak Bulent Ecevit University, Department of Environmental Engineering, and stored at 4°C until the experimental work. Undiluted and fresh urine was used immediately in experiments. The samples were collected in polypropylene containers. The fresh human urine was filtered through 0.45 µm cellulose acetate filters before measurements, then stirred for 60 min at 200 r/min (Wisestir) in an electrochemical unit and the pH and conductivity measured and characterized. The experiments were carried out in a batch electrochemical reactor with 900 mL of human urine at different current densities of 12, 20, 24, 28, 32 and 40 mA/cm<sup>2</sup>, and at room temperature (20–25°C). The second series of electrochemical treatment experiments were performed at the different pH values (5, 6, 7, 8, 9) using optimal current density. The experiments were operated for 1 h. Samples were taken at 0, 2, 4, 6, 8, 10, 15, 25, 30, 45 and 60 min from the EC unit and centrifuged at 1 000 r/min for 10 min. Conductivity and pH were measured immediately. The experiments were repeated twice.

## RESULTS AND DISCUSSION

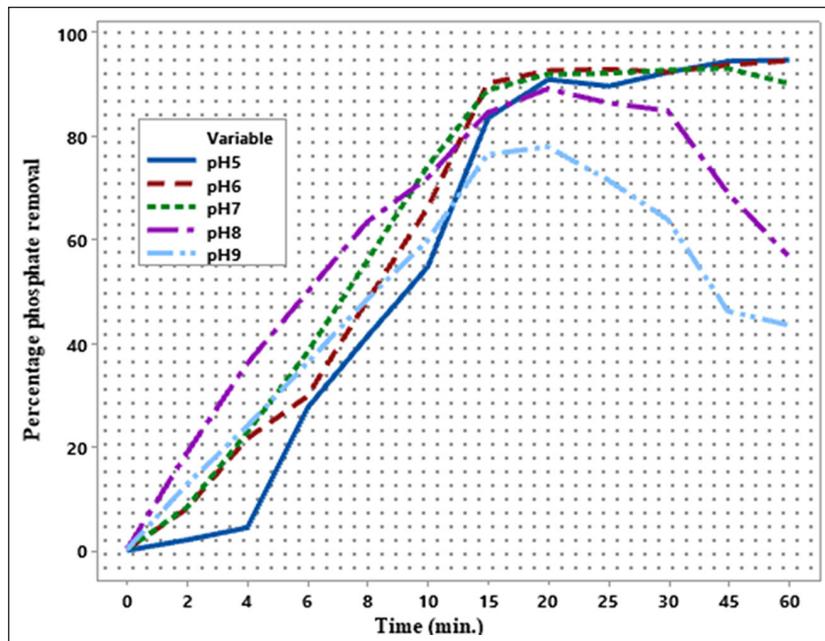
To determine the optimum conditions for phosphate removal from human urine, many experimental procedures are required with the classical method. The highest percent phosphate removal was determined by using different current densities and different pH values. Optimized parameters were evaluated by the function of PO<sub>4</sub>-P removal efficiency.

### Optimization with the classical method

In this study, a series of experiments were carried out using a current density of 12 to 40 mA/cm<sup>2</sup> to examine the effect of current density on phosphate removal. Other experimental conditions were selected as the different and natural pH value of fresh human urine, 2 cm iron electrode distance, magnetic stirring speed of 200 r/min, non-chemical addition and electrolysis time of 60 min. Variation of phosphate removal efficiency versus time for different current densities (12, 20, 24, 28, 32 and 40 mA/cm<sup>2</sup>) with iron plate electrodes is shown in Fig. 1.



**Figure 1.** Percentage phosphate ( $\text{PO}_4\text{-P}$ ) removal versus time for different current densities



**Figure 2.** Percentage phosphate ( $\text{PO}_4\text{-P}$ ) removal versus time at different initial pH values

Current density is a critical parameter in electrocoagulation processes that can be controlled directly. It affects the amount of metal (Fe or Al) ions dissolved from the anode, the rate at which bubbles are produced at the cathode during electrolysis.  $\text{H}_2$  gas formation rate and floc sizes vary according to the current density. These parameters also affect the treatment efficiency of the electrocoagulation process (Elazzouzi et al., 2017). The need for low energy consumption should also be considered when selecting the optimum current density. At this stage, it is very important that the operating time at which high removal efficiency is obtained should be short. For these reasons,  $40 \text{ mA/cm}^2$  was chosen as the current density value at which high removal efficiency was obtained in a shorter time.

It is well-known that the initial pH value of the solution plays a major role in system behavior (Attour et al., 2014; Elazzouzi et al., 2017). It has been established that pH has a considerable influence

on the performance of electrocoagulation processes (Bouamra et al., 2012; İrdemez et al., 2006). İrdemez et al. (2006) conducted an optimization study for removal of phosphate from wastewater and worked in the pH 4–7 range. In a different study, the electrolysis method was used to simultaneously remove nitrogen and organic substances from the urine and, in addition to determining the optimum current density, an optimization study was carried out to find the most suitable electrode material. The importance of the effect of different pH applications on the anodic oxidation and cathodic reduction processes of nitrogen species in wastewater is also discussed (Zöllig et al., 2017).

Experiments were carried out at various values of pH 5, 6, 7, 8 and 9 using  $1 \text{ M NaOH}$  or  $1 \text{ M H}_2\text{SO}_4$  under a constant current density which was kept at  $40 \text{ mA/cm}^2$ . Variation of phosphate removal efficiency versus time at different initial pH values is shown in Fig. 2.

The increase in pH from 6 to 9 results in a reduction of PO<sub>4</sub>-P from 94.62% to 43.50%, respectively. A pH of 7 is the natural human urine pH. At pH 7, the phosphate removal efficiency was around 74%, whereas it was 66, 54, 71 and 59% when the urine pH was 5, 6, 8 and 9, respectively, for the first 10 minutes of the experiment. Overall, it was found that the electrocoagulation treatment was most effective in neutral pH conditions. Since sufficient OH<sup>-</sup> is not produced under acidic conditions, metal hydroxide species do not form and the removal efficiency is therefore estimated to be low. On the other hand, it is considered that the metal hydroxides formed dissolve faster at pH 8 and above, and return to metal cations, and it is estimated that this is the reason for low removal efficiency at high pH values. Using a processing time of 25 min, the best PO<sub>4</sub>-P removal efficiency is achieved by controlling the initial pH value to be neutral (PO<sub>4</sub>-P removal efficiency is 92%). After 10 min the remaining concentration was 164.3 mg PO<sub>4</sub>-P/L at pH 7 and after 25 min only 49.6 mg PO<sub>4</sub>-P/L (Fig. 3).

In a study by Franco et al. (2017), in which phosphorus removal from surface waters was carried out by electrocoagulation process, the pH value resulting in a high removal efficiency (99%) in the shortest time was determined as 7.5, for high P concentration samples. At lower and higher pH values, a longer time was required to achieve the desired removal efficiency. A study by Irdemez et al. (2006), which examined phosphate removal from wastewater by electrocoagulation, emphasized the effect of pH on the system: at a pH above 10, phosphate removal slowed down considerably and the energy requirement increased. It has been shown that the predominant species of dissolved iron in solution above pH 10 is Fe(OH)<sub>4</sub><sup>-</sup>, which adversely affects phosphate removal. According to Kim et al. (2020), the OH<sup>-</sup> concentration in acidic conditions is not sufficient for metal hydroxide formation. Therefore, the removal efficiency is also not very high at acidic pH values. However, under alkaline conditions, metal hydroxides form more easily and contribute to the removal of contaminants. In addition, when the pH value rises above 9, metal hydroxides can return to their metal cations.

At high pH values, the formation of metal hydroxide increases and this accumulates on the surface of the anode. This leads to a reduction in phosphate removal efficiency and an increase in electricity consumption. Therefore, lower pH values are

preferred (Attour et al., 2014). This finding supports the optimal pH values obtained in this study. Due to the high removal efficiency at the natural pH obtained in this study, there is no need for pH adjustment and use of additional chemical in phosphorus removal. In addition, it was concluded that many of the important iron complexes (Fe(OH)<sup>+</sup>, Fe(OH)<sub>2</sub>, Fe(OH)<sub>4</sub><sup>2-</sup>) that coagulate were formed at this optimal pH (Bouamra et al., 2012; Chen et al., 2014).

### Optimization with the chemometric method

In the study, pH and current density parameters, which are known to have an effect on phosphate removal from the urine sample, were determined as factors, and a 2-factor central composite design was used. In the central composite design, each factor has 5 levels: low factorial (-1), high factorial (+1), center (0), low axial (-α) and high axial (+α). In the central composite design, the α value depends on the factor number (k), and was found to be 1.414 with the α = 2k/4 equation (Salehi et al., 2012). The levels of selected factors are given in Table 2. The experimental plan and phosphate concentrations (actual and predicted responses) are given in Table 3; pH, and current density were coded as x<sub>1</sub> and x<sub>2</sub>.

The results of the experiments determined in Table 2 were evaluated in the Minitab 16 program and the results obtained from an analysis of variance (ANOVA) are given in Table 3; 5% (α = 0.05) significance level was used. In Table 4, the F test and p values were examined in order to determine the factor effects. In order for the effect of a factor to be statistically significant, the p value obtained by analysis of variance must be less than the determined significance level (5%). When the main effects were examined (Table 3), the pH factor was not found to be significant (p > 0.05), but current density was found to be significant (p < 0.05). When factor interactions are examined; pH \* pH and pH \* current density interactions were found to be statistically significant (p < 0.05). The p values shown in bold in Table 4 were statistically significant (p < 0.01); other interactions were not found to be significant.

The factor effects graph is given in Fig. 4, from which it can clearly be seen that the current density values have an effect on average phosphate concentrations but alone pH alone is not associated with large differences in average phosphate concentrations.

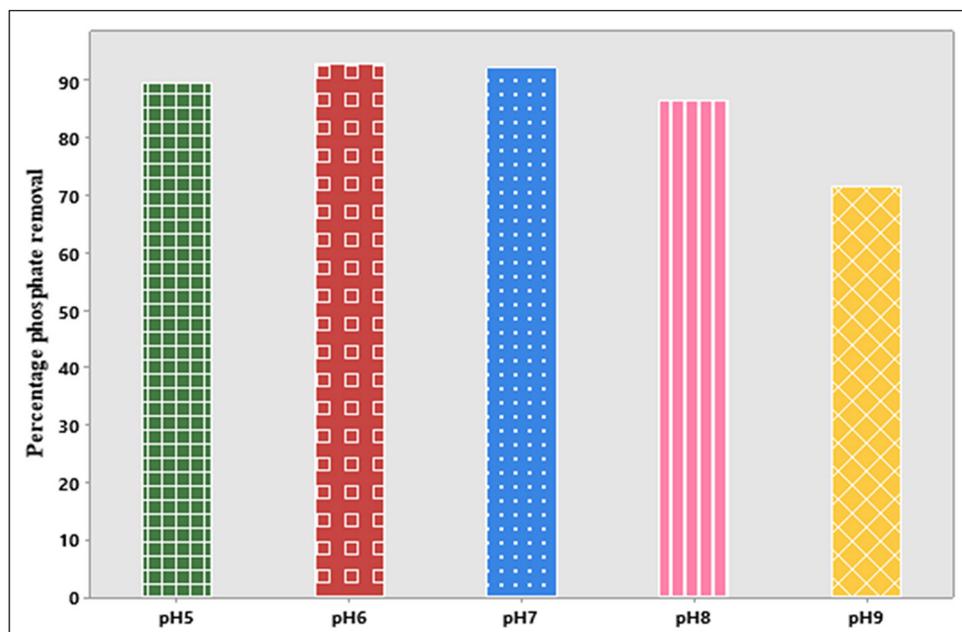


Figure 3. Percentage phosphate (PO<sub>4</sub>-P) removal after first 25 minutes of electrocoagulation

**Table 2.** The levels of selected factors used in the 2-factor central composite design

	Low axial $-a = -1.414$	Low factorial (-1)	Center (0)	High factorial (+1)	High axial $+a = +1.414$
pH	5.585	6	7	8	8.414
Current density (mA/cm <sup>2</sup> )	20.686	24	32	40	43.313

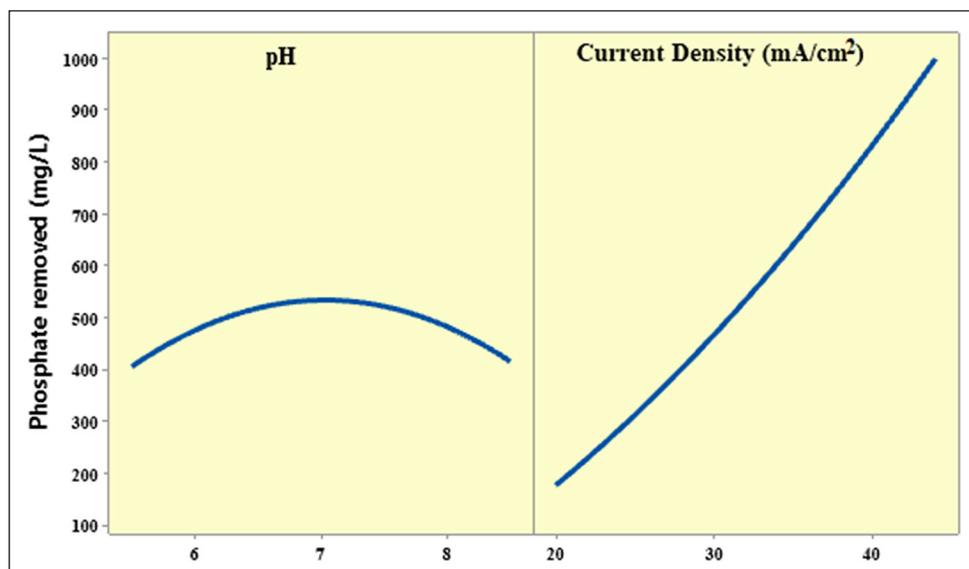
**Table 3.** Coded parameter levels and actual and predicted phosphate concentrations obtained at these levels

Run No	$x_1$	$x_2$	Actual phosphate concentration (mg/L)	Predicted phosphate concentration (mg/L)
1	-1	-1	132.80	164.35
2	1	-1	304.64	293.79
3	-1	1	828.45	832.20
4	1	1	755.00	719.34
5	$-a$	0	447.53	419.59
6	$+a$	0	397.17	429.20
7	0	$-a$	209.80	194.31
8	0	$+a$	950.00	969.59
9	0	0	570.50	533.80
10	0	0	501.12	533.80
11	0	0	534.60	533.80
12	0	0	542.20	533.80
13	0	0	520.60	533.80

**Table 4.** ANOVA results for the factors and factor interactions. Values in bold indicate statistical significance,  $p < 0.01$ .

Source	DF	Sum of squares	Mean square	F-value	p-value
Model	5	643 895	128 779	119.87	<b>0.000</b>
Linear	2	601 145	300 572	279.78	<b>0.000</b>
$x_1$	1	92	92	0.09	0.778
$x_2$	1	601 052	601 052	559.48	<b>0.000</b>
Square	2	27 709	13 854	12.90	<b>0.004</b>
$x_1 * x_1$	1	20 817	20 817	19.38	<b>0.003</b>
$x_2 * x_2$	1	4 031	4 031	3.75	0.094
2-way interaction	1	15 042	15 042	14	<b>0.007</b>
$x_1 * x_2$	1	15 042	15 042	14	<b>0.007</b>
Lack-of-fit	3	4 860	1 620	2.44	0.205
Pure error	4	2 660	665		
Total	12	651 415			

DF: Degrees of freedom



**Figure 4.** Plot of main factor effects

The binary interactions of the factors are given in Fig. 5. There is an increase in the average phosphate concentrations with current density alone in Figure 4 and there is also an increase in the average phosphate concentrations with the interaction of current density values and pH (Fig. 5).

For the regression analysis, the function that reveals the effect of the parameters on the phosphate concentration was used. The formula (Eq. 1) via the Minitab 16 package program is given below:

$$\text{Phosphate concentration} = -4\,599 + 1\,015 x_1 + 63.8 x_2 - 54.7 x_1 x_2 + 0.376 x_2^2 x_1 - 7.67 x_1^2 x_2 \quad (1)$$

where  $x_1$  is pH and  $x_2$  is current density

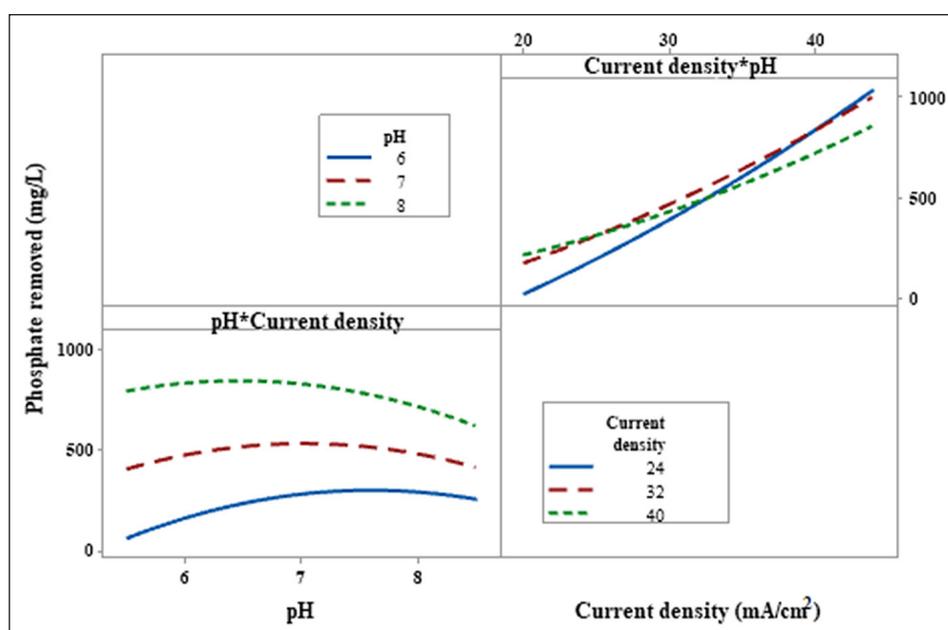
As can be seen from the developed model, phosphate removal increases as the current density increases. When the values of the model statistics given in Table 5 are examined, it is seen that the model works very well, as  $R^2$  and adjusted  $R^2$  have high values. The fact that the  $R^2$  and adjusted  $R^2$  values are close to each other

**Table 5.** Result of regression analysis

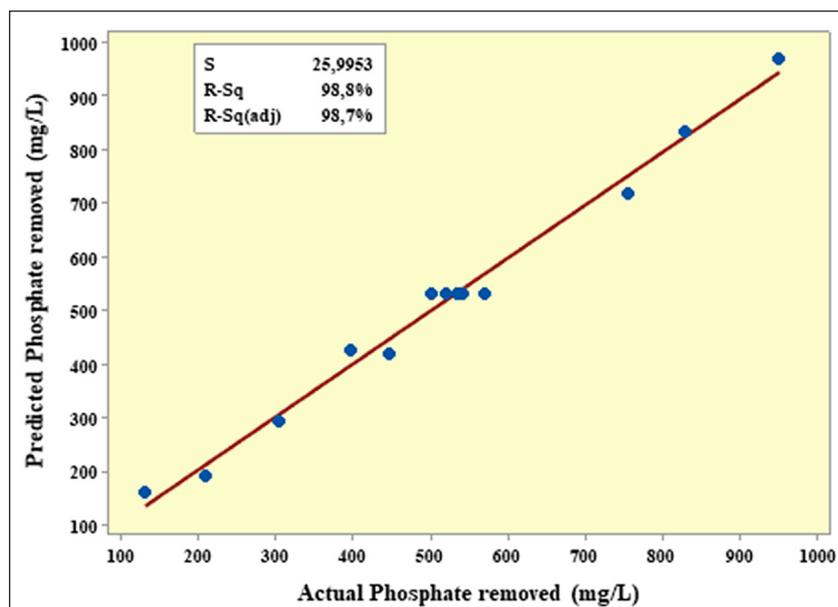
$R^2$	$R^2$ (adjusted)	$R^2$ (predicted)
98.85%	98.02%	94.06%

shows that the model has good predictive ability. The graph of the phosphate concentrations measured experimentally (actual) against the predicted phosphate concentrations (predicted), and the obtained regression equation, is given in Fig. 6.

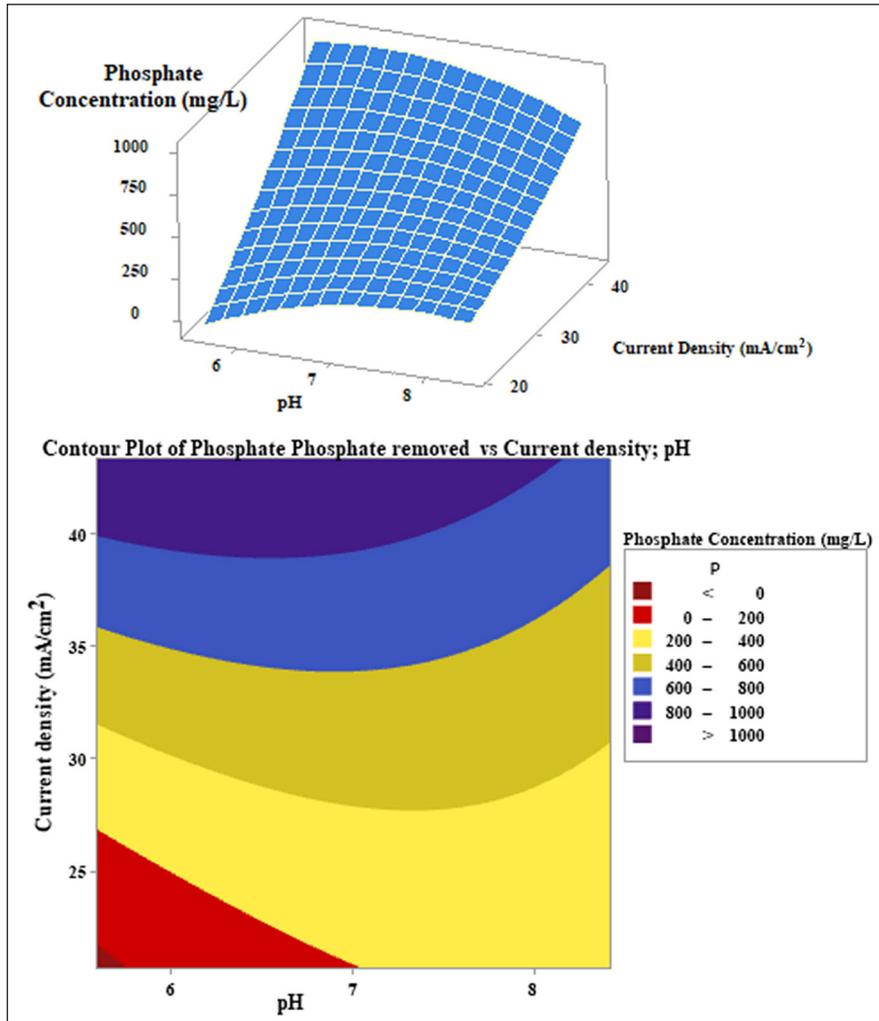
The 3D response surface and contour plot showing the variation of urine phosphate concentration with pH and current density is given in Fig. 7. This 3D response surface plot was taken into account when determining the optimum conditions for phosphate removal in urine. The graph shows that as the current density increases, the phosphate removal also increases. Optimum conditions were determined as 6.24 for pH and 43.31 mA/cm<sup>2</sup> for current density.



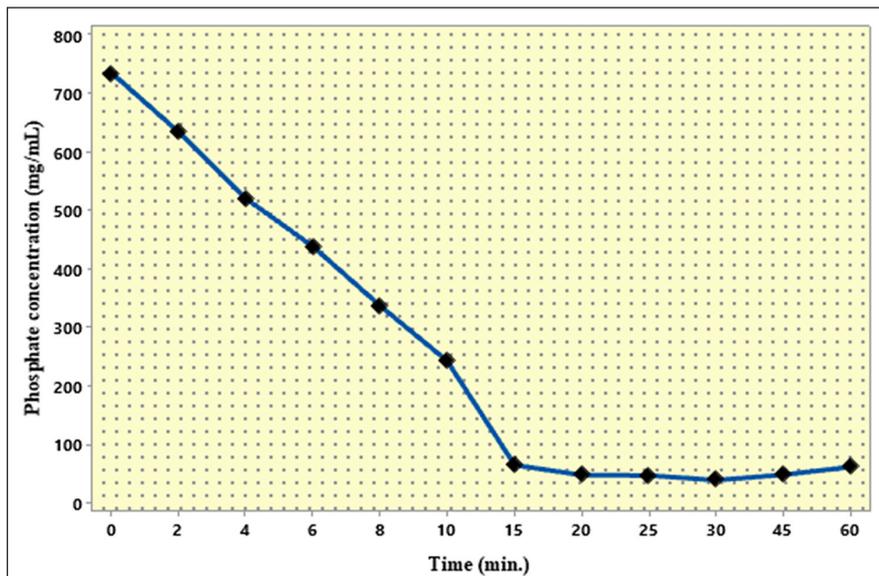
**Figure 5.** The effect of parameter interactions on phosphate concentrations



**Figure 6.** Predicted values based on the model in Eq. 1 versus actual values for phosphate concentration



**Figure 7.** 3D Surface and contour plot of phosphate concentration vs current density and pH



**Figure 8.** Phosphate concentration versus time under chemometrically determined optimum conditions

**Phosphate removal from human urine at optimum conditions**

Urine phosphate concentration in the urine sample versus time for the analysis performed under chemometrically determined optimum conditions is given in Fig. 8.

It was observed in the Fig. 8 that the phosphate concentration decreased linearly and the concentration remained constant after 15 min.

## CONCLUSION

Technological developments in analytical chemistry bring with them the need to produce more chemical data. In addition, the development of new methods and tools is important for the elimination of many fundamental problems. However, various difficulties are encountered in the process of carrying out such studies with high efficiency and performing adequate data analysis. Issues such as excessive use of chemicals and equipment, labour requirements, and lack of knowledge in the development of the method to obtain optimum results at the experimental stage prolong the experimental period, require more effort and adversely affect the study. For this reason, chemometry has been the focus of attention in recent years and has come to the fore in understanding data and obtaining accurate results in experimental studies. In this study, classical and chemometric methods were applied; the obtained optimum conditions were compared and the accuracy of the methods was discussed. The optimum conditions obtained in both methods are similar and it was demonstrated that the electrochemical method can achieve high removal efficiencies (>90%) in phosphate removal from human urine.

In this study, effects of initial pH and current density on phosphate removal by electrocoagulation using iron plate electrodes were investigated and effects of these parameters on removal efficiency were analysed. Studies were carried out in a specific order at laboratory scale. By evaluating the results obtained, the current density and pH at which the highest removal efficiency was obtained were determined. In experimental studies using iron electrodes and fresh human urine, 12, 20, 24, 28, 32 and 40 mA/cm<sup>2</sup> current densities and 5, 6, 7, 8 and 9 pH values were used. For both classical and chemometric methods, in the experiments using 40 mA/cm<sup>2</sup> and pH 7 (neutral pH), PO<sub>4</sub>-P% removal efficiency was about 90% at the end of the 60 min operation time. According to results obtained from the experiments and chemometric methods, removal rates and removal efficiencies were increased by increasing current density using iron plate electrodes. But system energy consumption increased by increasing current density. However, when high current density is used, achieving a high removal efficiency in a shorter time will also reduce the energy requirement. In the experimental study, it was determined that the phosphorus removal efficiency was above 70% at the end of the first 10 min when a current density of 40 mA/cm<sup>2</sup> was used. When other current densities were used, this value could not exceed 60% at the end of the 10 min process. In the experimental study, the highest removal efficiency was obtained at the natural pH of the sample, revealing that use of this system does not require addition of further chemicals.

Due to the high amount of phosphate it contains, human urine is a fraction of wastewater that can be reused after being collected separately at source. In this study, an electrocoagulation process was used for phosphate removal and recovery with iron electrodes. The results showed that nutrient phosphate recovery from human urine can be performed successfully by electrocoagulation.

This is the first study to be performed using the central composite design method in an optimization study for phosphate removal in urine. The data obtained from the CCD was evaluated with 3D surface response diagrams and an optimization decision was made. Study data showed that the method is suitable for routine applications.

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