DETERMINATION OF PHOSPHATE IN ALGAL CULTURE MEDIA BY PRECIPITATION TITRATION USING LEAD ION SELECTIVE ELECTRODE

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This paper presents a method for determining phosphate ions in Chlorella homosphaera 424 algal culture media by precipitation titration using a lead ion selective electrode. The chosen reaction conditions eliminated the complex ionic medium interferences, where the analyte is the minor component, in the 0.19-0.38 g/L concentrations range, and HCO_3^- is the major species, as high as 12.2 g/L. Several performance parameters were evaluated: standard deviation under repeatability conditions (s_r) ≤0.005 g/L, accuracys (δ) 0.003 g/L, limit of detection 0.009 g/L, limit of quantification 0.03 g/L, and expanded uncertainty of 0.01 g/L.

Keywords: phosphate ion, precipitation titration, lead ion selective electrode, algal culture media

1. Introduction

Phosphorus is an essential element for all life forms, influencing plant growth and photosynthesis in algae [1]. Many phosphorus species, including ortho-, pyro-, poly-, meta-, organic, colloidal, and suspended phosphorus are present in natural waters. Each of these forms can be determined either partially or fully (depending on the reaction conditions) as orthophosphate via hydrolysis.

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Phosphate ion stimulates the growth of plankton and aquatic plants, providing food for fish. This may cause an increase in the fish population and improve the overall water quality. However, if an excess of phosphate enters a waterway, algae and aquatic plants grow excessively, choking up the waterway and using large amounts of oxygen. This condition is known as eutrophication or over-fertilization of the receiving waters. This process, in turn, causes the death of aquatic life, as it lowers the dissolved oxygen levels. Extensive monitoring is needed for effective water quality management. Accurate, specific, and reliable methods for analyzing phosphates represent a key approach in water quality management [2]. In the recent years, many rapid and simple methods were used for the determination of phosphorus as phosphate in water samples [2-6]. R. de Marco et al. [2,3] developed a method to determine phosphate in hydroponic nutrient solution, waste water, and fertilizers by direct flow injection potentiometric (FIP) analysis, using a cobalt-wire ion selective electrode. Hirokazu Hara et all [7] developed a continuous-flow system for the determination of phosphate in river water using a lead ISE. Another approach was proposed by A. T. Lawal et al. [8], who developed a biosensor based on purine nucleoside phosphorylase, xanthine oxidase (XOD), and potassium ferrocyanide fixed into a polypyrrole film via galvanostatic polymerization.

Chlorella homosphaera 424 culture media used as feedstock for biofuel production consists of electrolyte solutions of varying concentrations, quite different from those of natural water environment, due to the high concentration of HCO_3^-. A typical composition culture media is presented in Table 1.

Table 1

Algal culture media composition [9]

Component substances	Concentration g/L
$NaHCO_3$	16.80
$NaNO_3$	2.500
NaCl	1.000
K_2SO_4	1.000
K_2HPO_4	0.500
$MgSO_4 \times 7H_2O$	0.200
$CaCl_2 \times 2H_2O$	0.040
Chelated iron	0.005
Microelements	< 0.003

Phosphate ions are vital nutrients for algae, their concentration in the culture medium, 0.270 g/L, being about 100 times smaller than the HCO_3^- concentration and comparable to the Mg^{2+} concentration. Both species interfere with Pb^{2+} quantification by precipitation. Our research focused on eliminating the interfering species in the algae growth environment, along with ensuring

performance parameters such as repeatability, accuracy, detection limit, quantification limit, uncertainty.

2. Experimental part

Reagents and materials: lead nitrate, 99%, calcium chloride dihydrate dibasic phosphate, 98-100.5%, magnesium sulphate heptahydrate ACS Reagent, puriss. p.a., ≥ 99%, sodium chloride, 99.5%, hydrochloric acid, 37%, nitric acid, 70%, potassium hydroxide fixanal, 1.0 M, manganese sulphate, 99-101%, copper sulphate pentahydrate purris. p.a., ≥ 98%, cobalt nitrate hexahydrate, 98%, iron sulphate heptahydrate, ≥ 99%, Na_2EDTA dehydrate, 99-101%, and sodium tetraborate decahydrate purris. p.a., ≥ 99.5% were obtained from Sigma-Aldrich (Germany). Sodium acidic carbonate, 99.5-100.5%, sodium nitrate ACS, ISO, Ph. Eur. reagent, Na_2EDTA fixanal, 0.1 mol/L were obtained from Merck (Germany). Potassium sulphate puriss. p.a., ACS reagent, was obtained from Riedel-de–Haën (Germany), glacial acetic acid, ACS reagent, 99.8%, boric acid, ACS reagent, zinc sulphate heptahydrate, extrapure ACS reagent, molybdenum oxide, 85%, and calcium chloride dihydrate, ACS reagent, 99-105%, were obtained from Scharlau (Spain). Ammonia, 25% solution, was obtained from Chimreactiv (Romania).
All solutions were prepared with boiled ultrapure water (18.2 MΩ), using A grade glassware.

Microelements stock solution: 2.86g H_3BO_3, 2.03g $MnSO_4 \times 4H_2O$, 0.222g $ZnSO_4 \times 7H_2O$, 0.018g MoO_3, 0.0790 g $CuSO_4 \times 5H_2O$, 0.494g $Co(NO_3)_2 \times 6H_2O$ were dissolved in about 800 mL ultrapure water, free of carbon dioxide and diluted to 1000 mL.

Chelated iron stock solution: 0.69g $FeSO_4 \times 7H_2O$ and 0.93g Na_2EDTA were dissolved in 80 mL ultrapure water and heated to boiling for a short time. After cooling to room temperature, solution was made up to 100 mL [9].

Sample solutions: The measurements were performed in algal growth solutions with a phosphate content varying in the 0.0696 - 0.696 g range. They were prepared as follows:

– 16.80 g $NaHCO_3$, 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4 \times 7H_2O$ and 0.04 g $CaCl_2 \times 2H_2O$ were dissolved in about 800 mL ultrapure water, free of carbon dioxide;

– 1 mL microelements solution and 5 mL chelated iron solution were added to the mixture, and then made up to 1000 mL with ultrapure water.

Titration reagents: lead nitrate was dried for 2 hours at 110^0C. 4.1405 g were weighed out, dissolved in ultrapure water (18.2 MΩ), free of carbon dioxide, in a 250 mL volumetric flask and made up to the mark. The solution was standardised by titration with Na_2EDTA, 0.1 mol/L solution.

Borax neutralization solution: 40 g $Na_2B_4O_7 \times 10H_2O$ were dissolved in ultrapure water, free of carbon dioxide, and diluted to 500 mL.

NH_3 neutralization solution: 50 mL NH_3, 25% solution, was transferred into a 100 mL volumetric flask and made up to the mark with ultrapure water, free of carbon dioxide.

Equipments: an automatic titrator Graphix DL50, Mettler Toledo equipped with a lead ion selective electrode (Metrohm) and Ag/AgCl reference electrode with double junction (Mettler Toledo) was used for titrations.

Ion chromatographic determination of phosphate content was carried out with an ICS 3000 Dionex system consisting of an isocratic eluent delivery pump, sample injection port, separation column (AS4A4×250mm), suppressor (ASRS 300×4 mm), and conductivity detector. The eluent was prepared by diluting with deionised water 1:10 an eluent concentrate (Dionex) containing 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$. The eluent flow rate was 0.6 mL/min, and the suppressor worked at 8mA. The calibration curve was obtained in the 0.0001–0.0300 g/L concentration range, using a stock standard solution for ion chromatography (Merck), traceable to SRM NIST. A typical calibration curve for phosphate is given in Fig. 1. Typically 1 mL aliquot from algae growth media samples was added into 100 mL ultrapure water, filtered through a 0.45 µm membrane, and further analyzed by ICS.

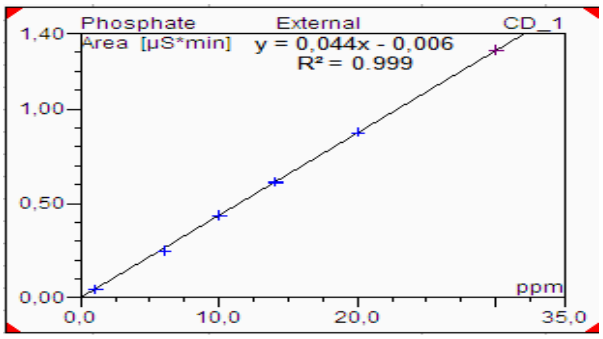

Fig.. 1 Calibration curve for phosphate in the 0.0001 –0.030 g/L concentration range, using 1.8 mM Na_2CO_3 and 1.7 mM NaHCO eluent, at a 0.6 mL/min flow rate, at 30°C.

2. Results and discussion

The determination of phosphate is based on the precipitation reaction of Pb^{2+} with PO_4^{3-}, the equivalence point being indicated potentiometrically, with the aid of a lead ion selective electrode. In a weak alkaline solution, all phosphate species precipitate, forming neutral lead phosphate, according to the reactions [10]:

$$2PO_4^{3-} + 3Pb^{2+} = Pb_3(PO_4)_2(s)$$

respectively:

$$2HPO_4^{2-} + 3Pb^{2+} = Pb_3(PO_4)_2(s) + 2H^+$$

or

$$2H_2PO_4^{-} + 3Pb^{2+} = Pb_3(PO_4)_2(s) + 4H^+$$

The nutrient medium used for algae growth has a complex ionic composition, creating serious interferences during the precipitation of determination of phosphate, as the titration curve in Fig. 2 demonstrates.

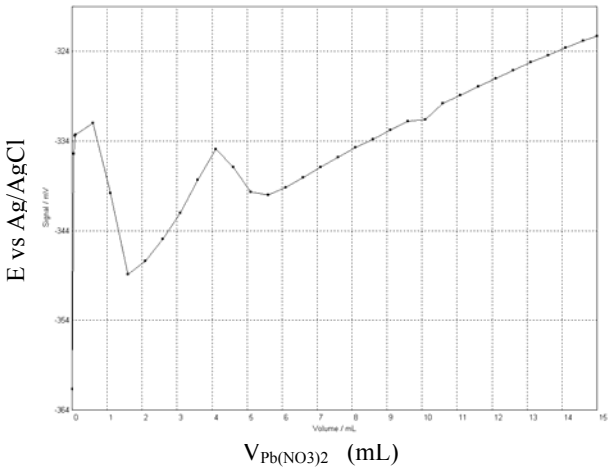

Fig. 2. Potentiometric titration of PO_4^{3-} against $Pb(NO_3)_2$, 5.0×10^{-2} mol/L, without elimination of interferences

Given the selected solubility data in Table 2 and the ionic concentration levels characteristic to the algae culture medium, the possible interferent species are:

– anions: HCO_3^-, HO^-, and SO_4^{2-}, prone to precipitation with Pb^{2+},
– cations: Ca^{2+}, Mg^{2+}, and Fe^{3+}, likely to form precipitates with PO_4^{3-}.

Table 2

Solubility products, K_{SP}, of sparingly soluble compounds [10, 12]

Ionic species analyte/interferent	Precipitate	Solubility product	Solubility
PO_4^{3-}	$Pb_3(PO_4)_2$	1.1×10^{-33}	1.00×10^{-7}
HCO_3^-	$PbCO_3$	1.5×10^{-13}	3.90×10^{-7}
OH^-	$Pb(OH)_2$	2.8×10^{-16}	4.20×10^{-6}
SO_4^{2-}	$PbSO_4$	2.2×10^{-8}	1.50×10^{-4}
Fe^{3+}	$FePO_4$	1.3×10^{-22}	1.14×10^{-11}
Ca^{2+}	$Ca_3(PO_4)_2$	2.0×10^{-29}	7.10×10^{-7}
Mg^{2+}, NH_4^+	$MgNH_4PO_4$	2.5×10^{-13}	5.00×10^{-7}
Mg^{2+}	$Mg_3(PO_4)_2$	1.0×10^{-13}	9.80×10^{-4}

Since the lead carbonate formation changes the titration curve shape and renders equivalence point detection, and HCO_3^- concentration is about 100 times larger than that of the analyte, HCO_3^- was removed by acidic decomposition. HNO_3, HCl, and CH_3COOH were tested. The influence of different acids on the method accuracy was studied at the same time with the buffer influence (Figs. 3, 4, and 5). Quantitative elimination of CO_2 formed by acidic decomposition was achieved by boiling for 5 minutes.

As $PbSO_4$ solubility is larger than that of $Pb_3(PO_4)_2$ and concentrations are of similar order of magnitude, interference from SO_4^{2-} is not significant. For the same reasons, the interferences of Ca and Mg cations were neglected.

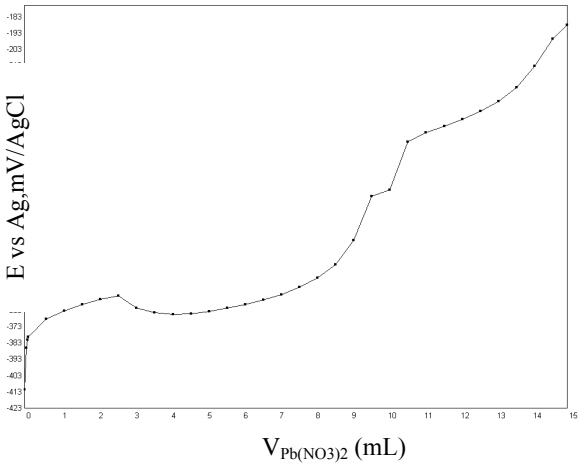

Fig. 3. Potentiometric titration of PO_4^{3-} against $Pb(NO_3)_2$, 5.0×10^{-2} mol/L, after treatment HNO_3 to eliminate the acidic carbonate.

Despite the fact that Fe^{3+} ions form insoluble phosphates, they do not interfere in the titration with Pb^{2+}, as in the algal culture media they form stable complexes both with EDTA and $H_2PO_4^-$, and HPO_4^{2-} ions are present in the 8.0-9.0 pH range.

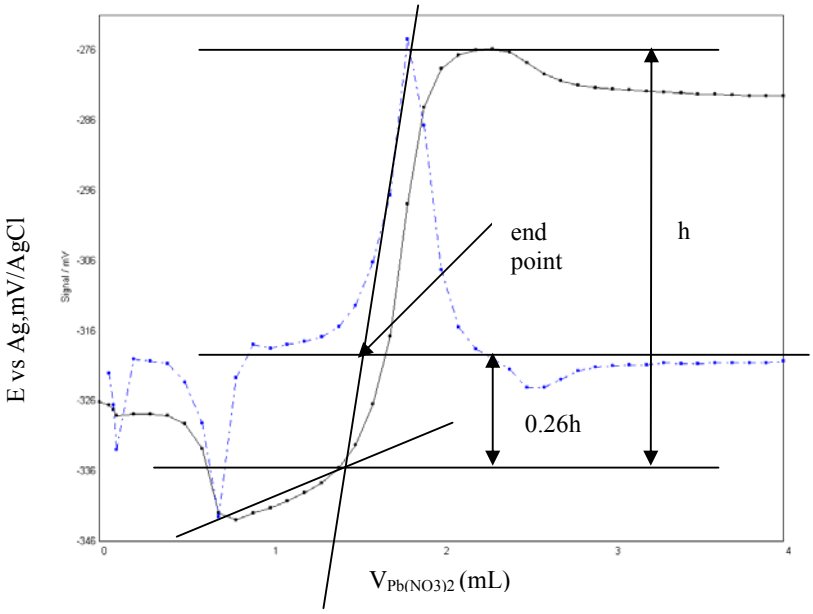

Fig. 4. Potentiometric titration of PO_4^{3-} against $Pb(NO_3)_2$, 5.0×10^{-2} mol/L, using NH_3 as neutralizing agent at pH 8.0-8.5. The blue line represents the titration curve first derivative.

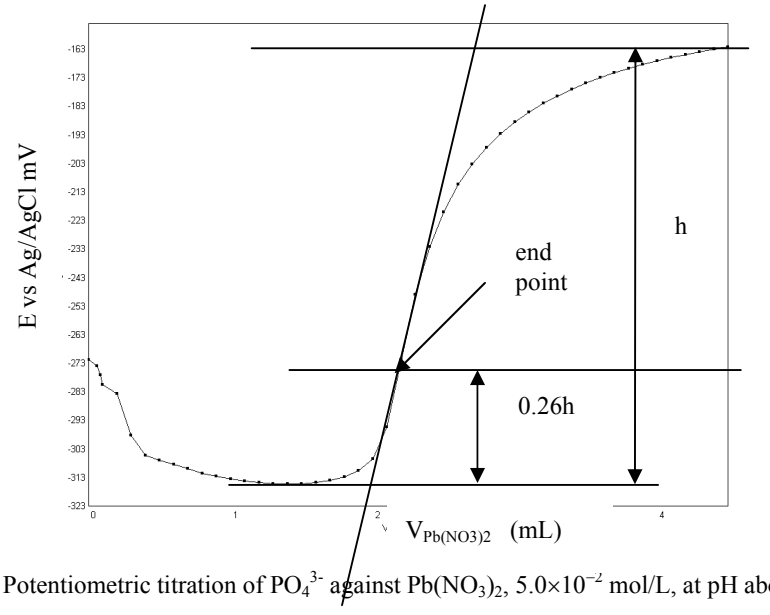

Fig. 5. Potentiometric titration of PO_4^{3-} against $Pb(NO_3)_2$, 5.0×10^{-2} mol/L, at pH above 8.5.

Mg^{2+} interference becomes significant when ammonia is used due to the formation of the sparingly soluble compound $MgNH_4PO_4$. Fig. 4 presents the titration curve of PO_4^{3-} against $Pb(NO_3)_2$, 5.0×10^{-2} mol/L, using NH_3 as neutralizing agent, for a sample composition as given in Table 1. The interferent presence led to a negative error of 30%.

NH_3, KOH, and $Na_2B_4O_7$ at pH values ranging from 6.5 to 10 were tested as neutralizing agents. The selection was performed based on two criteria: method accuracy and titration duration. Although the lead ion selective electrode works best in the 2-6 pH range, titrations were performed in a weak basic medium in order to achieve quantitative precipitation of lead phosphate at the chosen reaction rate. This way a complete analysis could be carried out within 15 minutes. At pH smaller than 7 the phosphate precipitates slowly, quantitative titration requiring more than 30 minutes. At pH higher than 8.5, the sparingly soluble $Pb(OH)_2$ is formed. With a solubility product of 2.8×10^{-16}, hydroxide formation causes additional reagent consumption, as shown in Fig. 5. The pH range in which phosphate is precipitated quantitatively at high speed, without interference from the species present in the culture medium, ranges from 8.0 to 8.5. When KOH was used as neutralizing agent, pH adjustment could not be achieved with sufficient precision and the analysis lacked reproducibility. Usage of NH_3 as neutralizing agent caused quantitative magnesium precipitation, with a negative error as high as 30%. For this reason the NH_3 was abandoned as neutralizing agent. The neutralizing agent, ensuring quantitative phosphate precipitation at satisfactory speed was borax.

Based on the preliminary tests a method for the determination of phosphate species was developed and evaluated. The sample was filtered for algae separation and 25 mL were sampled from the filtrate, to the nearest 0.1 mL. Acid carbonate was decomposed with 2 mL glacial acetic acid, 1:1, and the sample was boiled 5 minutes for complete removal of CO_2, followed by cooling and neutralization with borax to pH 8.0 – 8.5. It was further titrated with 5.0×10^{-2} mol/L $Pb(NO_3)_2$ standard solution, using a Graphix DL50 Mettler Toledo titrator, at a reagent delivering speed of 0.1 mL/30 s, equipped with a lead ion selective indicating electrode and a silver/silver chloride reference electrode.

The titration curves, $E(mV) = f(V_t)$, (fig. 6), based on precipitation reactions are asymmetric, the equivalence point being determined at 0.26 of the potential jump height (**h**) [10]. The phosphate concentration was calculated as:

$$C_{PO_4^{3-}} = \frac{2}{3} \times \frac{M \times V_t \times F \times 95}{V_s},\ \text{g/L}$$

where: M = molarity of the titration reagent solution, mol/L

V_t = used volume of titration reagent, mL

F = volumetric correction factor of the titration reagent

V_s = sample volume, mL

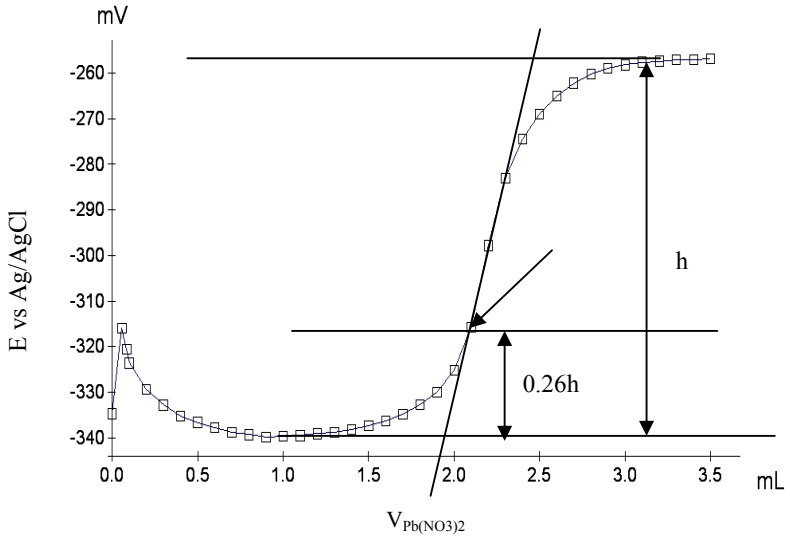

Fig. 6. Potentiometric titration of PO_4^{3-} against $Pb(NO_3)_2$, 5.0×10^{-2} mol/L, at pH 8.0-8.5, using $Na_2B_4O_7$ as neutralizing agent.

3. Method performance parameters study

The acceptance criteria for the relative standard deviation in the determination of total phosphate ions in *Chlorella homosphaera 424* algal growth medium was establish based on Horwitz equation, $RSD < 2^{(1 - 0.5\lg C)}$, where C, the sample analyte concentration is expressed as mass fraction. The *limit of detection* (LOD) was calculated as $3\times RSD\times C/100$, the *limit of quantification* (LOQ) as $10\times RSD\times C/100$, and the expanded uncertainty as $2\times RSD\times C/100$ [14]. Studies in the literature [15] recommended an accuracy, δ, not larger than 0.004 g/L. Monitoring of complete algal development required a 0.038 – 0.38 g/L working range, and a titration time not larger than 15 minutes.

Repeatability

Table 3

Repeatability data

$10^{-3}\times C$ (mass fraction)	No. of replicates	$10^{-3}\times s_r$ (mass fraction)	RSD (%) (experimental)	RSD (%) (Horwitz)
0.038	8	0.003	7.7	9.3
0.080	7	0.005	3.4	8.3
0.190	7	0.005	2.5	7.3

0.270	7	0.005	1.8	6.9
0.380	7	0.005	1.3	6.5

The repeatability test checks the consistency of calculated results for the analyte over a short time period. Experimental data for method repeatability were obtained by performing titrations on an algal growth medium at 5 different phosphate concentration levels (0.038 – 0.380 g/L), in repeatability conditions (same method, same day, same analyst, same equipment). The results and calculated parameters, s_r and RSD, are presented in Table 3. The repeatability test was passed ($RSD < 2^{(1 - 0.5\lg c)}$) over the entire concentration range.

Accuracy

Accuracy of an analytical method is usually determined by studying relevant reference materials or by spiking studies [13]. In this paper accuracy was determined as the difference **(δ)** between the average concentration for the 7 titrations (Table 4) and the theoretical value of the phosphate reference material (0.270 ± 0.003 g/L).

Table 4

Accuracy data

N	*C* (g/L)	Average (g/L)	δ (g/L)
1	0.270	0.267	0.003
2	0.270		
3	0.270		
4	0.260		
5	0.260		
6	0.270		
7	0.270		

The result, δ = 0.003 g/L, meets the acceptance criteria for the proposed determination method, δ ≤ 0.004 g/L.

Limit of detection (LOD) and *limit of quantification (LOQ)*

Table 5

Detection and quantification limit data

N	*c*, (g/L)
1	0.041
2	0.033
3	0.043
4	0.039
5	0.038
6	0.038

7	0.038
8	0.039
s_r, g/L	0.003
LOD, g/L	0.009
LOQ, g/L	0.030

The method detection limit was determined by multiplying by 3 the repeatability standard deviation, s_r, calculated for the solution containing the smallest amount of phosphate (0.038 g/L). The method quantification limit was determined by multiplying by 10 the repeatability standard deviation calculated for a synthetic solution containing the smallest phosphate amount (0.038 g/L), s_r. The results (Table 5) meet the imposed acceptance criteria, LOD ≤ 0.014 g/L and LOQ ≤ 0.047 g/L.

Uncertainty

The significant uncertainty sources affecting the measured concentration identified are presented in Table 6.

Table 6

Uncertainty budget

Components u(x)	Sources	Value	U.M.	Standard uncertainty	Relativ standard deviation
*u(*Rep*)*	Repeatability	0.267	g/L	0.005	0.018
u(Vp)	Sample volume	25	mL	0.017	0.00069
u(Vt)	Reagent volume	2.09	mL	0.028	0.006
u(F)	Factor	1	-	0.0001	0.0001
u(δ)	Accuracy	0.003	g/L	0.0018	0.0063
u(P)	Phosphate standard purity	99	%	0.0058	0.0058

Uncertainties associated to each component were calculated according to the uncertainty propagation rules [11]:

$$u_c = r \times \sqrt{\left(\frac{u(\mathrm{Rep})}{\mathrm{Rep}}\right)^2 + \left(\frac{u(Vp)}{Vp}\right)^2 + \left(\frac{u(Vt)}{Vt}\right)^2 + \left(\frac{u(F)}{F}\right)^2 + \left(\frac{u(\delta)}{\delta}\right)^2 + \left(\frac{u(P)}{P}\right)^2}$$
$$= 0.267 \times 0.02 = 0.0053\ \mathrm{g/L}$$

where ***r*** is the average phosphate concentration, 0.267 g/L, and all other symbols are defined in Table 6. The expanded uncertainty was obtained by applying a coverage factor, k = 2, corresponding to a confidence level of 95%.

$$U = 0.0053 \times 2 = 0.011\ \mathrm{g/L}$$

The expanded uncertainty value obtained meets the acceptance criteria for the determination method, $U \leq 0.038$ g/L.

The results obtained by precipitation titration using lead ion selective electrode results were crossed–checked by ion chromatography. The results obtained are presented in Table 7.

Table 7

Results obtained by titration and IC

Potentiometric titration				Ion Chromathography (IC)				Standard concentration (g/L)
*C** (g/L)	s_r (g/L)	RSD (%)	δ (g/L)	*C** (g/L)	s_r (g/L)	RSD (%)	δ (gl/L)	
0.267	0.005	1.8	0.003	0.245	0.004	1.4	0.025	0.270

*average of 7 determination

The F test was applied to check if there are significant differences between the two analysis approaches [16]. The experimental F value, 1.56, is less than the tabulated value, 4.28, corresponding to a 95 % confidence level and 6 degrees of freedom. Therefore there is no significant difference between the two analysis methods.

$$F_{\exp} = \frac{s_{r1}^2}{s_{r2}^2} = \frac{(0.005)^2}{(0.004)^2} = 1.56$$

Also the precipitation titration method ensured a smaller deviation than ion chromatography.

4. Conclusion

A titration potentiometric method for the determination of phosphate ionic species in algal culture media is proposed. Systematic studies on interferences addressed the practical issues caused by the ionic medium complexity. Two stages of sample processing eliminated the major interference by filtration for algal mass and decomposition of acid carbonate ion.

The buffer system selection and pH working range eliminated the HO^-, Mg^{2+}, NH_4^+ ions interferences and ensured proper working conditions for the chosen indicating electrode and reaction speed, with impact on the analysis duration.

The results demonstrated that the method can be used for determination of all phosphate species in the algal culture medium in the 0.038 – 0.380 g/L concentration range with the following performances: standard deviation under repeatability conditions (s_r) ≤ 0.005 g/L, 0.003 g/L accuracy (δ), 0.009 g/L detection limit, 0.03 g/L quantification limit and 0.01 g/L expanded uncertainty for a 0.270 g/L concentration level. The results obtained by precipitation titration

using lead ion selective electrode results were crossed–checked by ion chromatography.

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