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Potentiometric determination of bile phosphates using a lead selective electrode

LUIGI CAMPANELLA*, MAURO TOMASSETTI, GIUSEPPE D'ASCENZO, GIORGIO DE ANGELIS, ROBERTO MORABITO and LORENZO SORRENTINO

Institute of Analytical Chemistry, University of Rome, Rome, Italy

Abstract: A potentiometric method based on phosphate precipitation with Pb^{2+} and on a lead ion selective electrode is applied to the determination of bile phosphates. The method is rapid and simple and does not require extensive sample pretreatment.

Keywords: *Bile phosphates; lead ion selective electrode; potentiometric titration; phosphorus determination; lecithin.*

Introduction

The lithogenic index (IL), a numerical expression for the relative lithogenicity of bile, is determined by the analysis of the bile acids, cholesterol and phospholipids present in the gallbladder bile [1, 2]. The phospholipid concentration can be obtained by total phosphorus analysis at levels above 0.005 mol/l [3]. This analysis is easier than that of individual phospholipids and within experimental error it yields the same values for IL [4]. The two principal methods for the determination of total bile phosphorus are the molybdate–vanadate method [4, 5] and Bartlett's method [6] using the Fiske–Subbarow reagent [7]. These methods require prior sample treatment using perchloric acid or a perchloric acid–hydrogen peroxide mixture at 170–180°C. In the present paper a potentiometric method based on a phosphate precipitation titration with lead(II), with the employment of a lead ion selective electrode is proposed and compared with Bartlett's method [6]. Preliminary studies of the optimum experimental conditions (sample volume, concentration range, pH, and stoichiometry of the precipitate) are described. The method does not require sample pretreatment and is rapid and simple to carry out.

Experimental

Merck reagent grade products including egg lecithin were employed. The Fiske-Subbarow reagent was supplied by the Sigma Chemical Company. The lead electrode membrane was of the heterogeneous solid type: it contained 80% of a mixture of Ag_2S and PbS (1:1 w/w) and 20% polyethylene. This type of electrode is produced by Amel

* To whom correspondence should be addressed.

(Milan, Italy). Its internal solution is $Pb(NO_3)_2$ (10^{-3} mol/l) and KCl (10^{-2} mol/l); the built-in reference electrode is $Ag/AgCl/Cl^-$. An Orion microprocessor-controlled potentiometer, Model 901, was employed. Spectrophotometric measurements were performed using a Perkin–Elmer Model 320 spectrophotometer with 10 mm quartz cells. Thermogravimetric analysis of precipitates utilized a Du Pont Model 951 thermobalance.

Potentiometric titrations were tested using aqueous KH_2PO_4 solutions (5×10^{-4} to 10^{-2} mol/l) and aqueous emulsions of egg lecithin. They were applied to bile samples obtained by surgery or by duodenal intubation from subjects with gallstones. Titrations were performed with ease between pH 9.5 and 11.5; this range is suitable for the analysis of bile samples since both conjugated and unconjugated bile acids are soluble in alkaline media. Aqueous phosphate solutions and lecithin emulsions (50 ml), or bile samples diluted 10–40 times, were adjusted with sodium hydroxide to pH 9.5–11.5 and titrated while stirring with lead nitrate solutions (10^{-4} to 5×10^{-2} mol/l). Changes in potential were determined using the lead selective membrane electrode. The potentiometric data were compared with spectrophotometric analysis [6]; prior to the latter procedure bile samples (100 μl) were heated at 170°C on an oil bath, with 0.5 ml of 70% $HClO_4$ [8]. All precipitates were studied by titrimetric, spectrophotometric and thermogravimetric methods.

Results and Discussion

The precipitation of phosphate by lead(II) probably occurs according to the reaction:

$$n\ Pb^{2+} + m\ PO_4^{3-} + p\ OH^- \rightarrow Pb_n(PO_4)_m(OH)_p$$

with $2\ n = (3\ m + p)$.

The pH has two effects on the Pb^{2+} equilibrium, affecting hydroxocomplex formation, and the protonation of phosphate. The most useful pH range will thus be in mildly alkaline solutions: at lower pH values PO_4^{3-} is protonated and at higher pH Pb^{2+} is hydrolysed to form soluble hydroxocomplexes. Examples of titration curves, involving pure phosphate solutions, lecithin emulsions and bile samples, are shown in Fig. 1 (a–c): all the samples yielded similar titration curves. In the bile samples, interference from cholic acids did not occur, as lead cholate was not precipitated. As no buffer was employed the pH varied during the titration (Fig. 1d) but this apparently did not affect the stoichiometry of the precipitation reaction. The latter was determined by the initial pH which is little changed for 80–90% of the titration. Stoichiometries at different initial pH values were determined from the titration data and from the analysis of precipitates for Pb^{2+}, PO_4^{3-} and OH^- by EDTA titration, spectrophotometry and thermogravimetry respectively. The results are compared in Table 1. The precipitate PO_4^{3-}:OH^- ratios at different pH values (Fig. 2) exhibit a linear behaviour whether determined titrimetrically or by thermogravimetric and spectrophotometric methods. Such a linear dependence provides evidence for the proposed precipitate formula. The pH also affects the reproducibility of the quantitative phosphate determinations: some typical titration curves (Fig. 3) show different gradients with optimum shape at pH 11.0. This result is confirmed by the data in Table 2.

During studies of bile samples it was apparent that freezing the samples, even for short periods, results in the formation of precipitates which do not readily redissolve at room temperature. Results can thus be affected by systematic errors unless fresh samples are employed. However, the method involves no pretreatment step, so it is rapid, does not

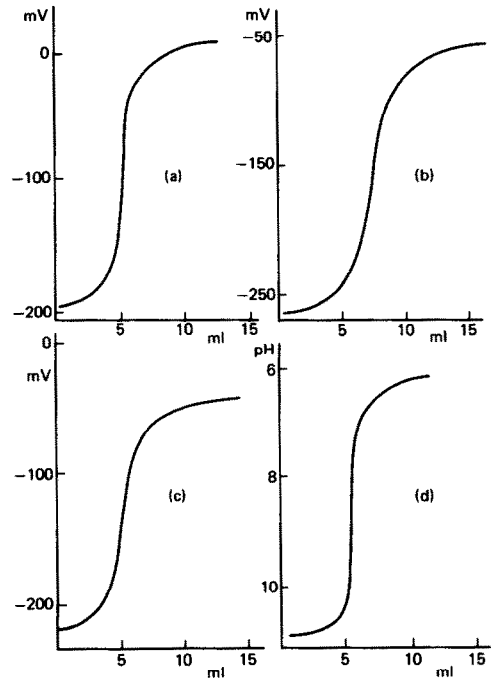

Figure 1
Titration curves of standard phosphate (a), lecithin (b), and bile liquid (c) solutions at pH 11.0. Curve (d) shows the changes of pH occurring during the phosphate titration.

Table 1
Analysis of precipitates obtained at different pH values

% Composition	pH			
	11.5	11.0	10.5	9.5
PO_4^{3-} (potentiometric titrimetry)	4.3	14.7	18.0	20.3
PO_4^{3-} (Bartlett's method)	5.0	12.5	20.0	22.0
OH^- (potentiometric titrimetry)	11.5	5.2	3.2	1.8
OH^- (thermogravimetry)	10.8	5.8	3.0	1.7
Pb^{2+} (potentiometric titrimetry)	84.2	80.1	78.8	77.9
Pb^{2+} (complexometric titrimetry)	85.0	80.4	78.2	77.6
Calculated formula of precipate	$Pb_9PO_4(OH)_{15}$	$Pb_5(PO_4)_2(OH)_4$	Pb_2PO_4OH	$Pb_7(PO_4)_4(OH)_2$

Table 2
Potentiometric determination of 5×10^{-4} mol/l phosphate: Absolute errors and standard deviations (S.D.) as a function of the initial pH.

pH	Error (%)	S.D. (mol/l) (n = 5)
9.5	+6	$0.3\ 10^{-4}$
10.5	+7	$0.3\ 10^{-4}$
11.0	+5	$0.1\ 10^{-4}$
11.5	+15	$0.3\ 10^{-4}$

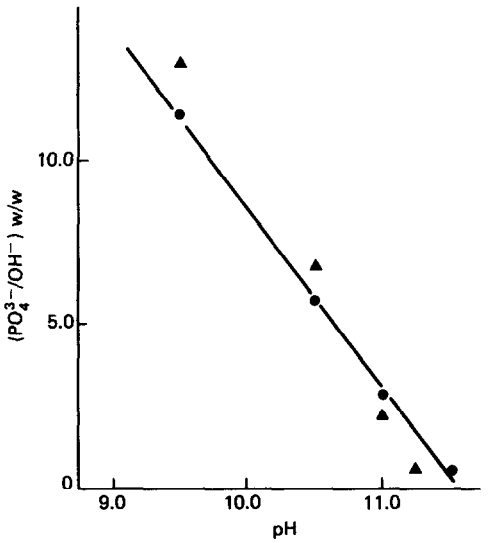

Figure 2
The precipitated weight ratio PO_4^{3-}:OH^- as a function of pH; titrimetric (●), and spectrophotometric and thermogravimetric (▲) data.

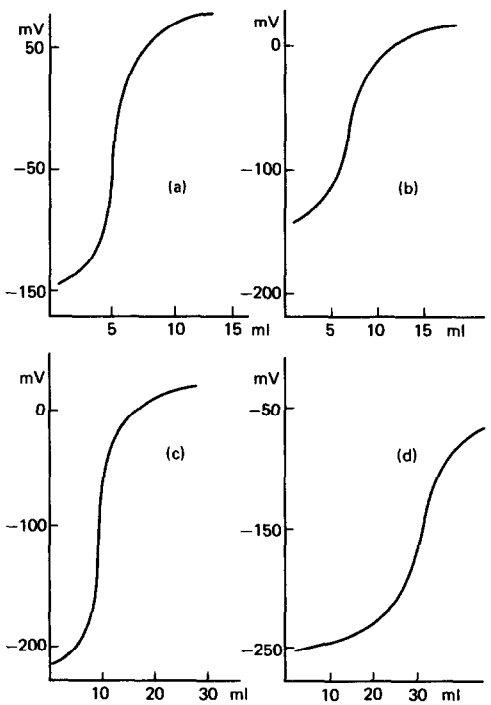

Figure 3
Titration curves of standard KH_2PO_4 solutions (5.0×10^{-4} mol/l) at different initial pH values; a: pH 9.5, b: pH 10.5, c: pH 11.0, d: pH 11.5.

require corrosive reagents and avoids the systematic errors possibly arising during pretreatment. Ten samples of human bile with phosphorus concentrations of 5×10^{-3} to 8×10^{-2} mol/l were analysed and the results compared with those obtained by Bartlett's method [6]. Table 3 shows the precision and accuracy of the potentiometric

Table 3
Comparison of phosphorus determination by the potentiometric method and by Barlett's spectrophotometric method

	Potentiometric method		Spectrophotometric method	
Total phosphorus (mmol/l)	Precision (RSD %)	Accuracy (% recovery)	Precision (RSD %)	Accuracy (% recovery)
egg lecithin (1–25)	5	−8	4	−10
bile samples (5–80)	5	(+6–+10)	2.5	(−2.5–+2.5)

method, describes the results obtained with the bile samples and with lecithin solutions, and summarizes the comparison with Bartlett's method. The agreement between the potentiometric and spectrophotometric methods is good at phosphorus concentrations above 3.5×10^{-2} mol/l (error 5–7%), but for lower phosphorus concentrations the agreement is poor.

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