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VOLTAMMETRIC INVESTIGATION OF THE DISTRIBUTION OF HYDROXO-, CHLORO-, EDTA AND CARBOHYDRATE COMPLEXES OF LEAD, CHROMIUM, ZINC, CADMIUM AND COPPER: POTENTIAL APPLICATION TO METAL SPECIATION STUDIES IN BREWERY WASTEWATER

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ABSTRACT. This paper reports results on complex formation reactions between OH^-, Cl^-, EDTA and carbohydrate ligands with Pb^{2+} ions at various $[L_T]:[M_T]$ ratios and at different pH values (1.5-13.0). Differential pulse anodic stripping voltammetry (DPASV) employing an *ex situ* plated thin mercury film electrode (TMFE) was used to measure the shifts in peak potentials. Formation of simple, polyligand as well as mixed ligand complexes are reported. The reactions between the Pb(II) and the carbohydrate ligands showed pronounced pH dependency on metal forms compared to reactions with simple inorganic ions such as chloride. Modeling of the experimental data obtained with the DPASV method was done using computer software (3D-VISE). The calculated complex formation curves (CCFC) based on mass balance equations were fitted to the experimental complex formation curves (ECFC) and the goodness of the fit evaluated (RSD < 5%). These studies were applied to Pb, Cr, Zn, Cd and Cu speciation in brewery wastewater in which differences between total metal determined by flame atomic absorption spectrometry (FAAS) after acid digestion and labile fraction determined by DPASV were used to estimate the percentage of non-labile fraction (mainly metal-organic complexes). Up to 90% of the metal was found to exist as the "inert" fraction, implying that the effluent system from the brewery industry poses minimal health risks to the environment with regard to toxic forms of the metals as the organically bound metal forms are generally known to have low toxicity compared to the aquo or labile metal forms.

KEY WORDS: Metal speciation, Carbohydrates, Brewery wastewater, Differential pulse voltammetry

INTRODUCTION

During the past three decades [1-4], increased attention has been paid to metal-organic association in natural aquatic systems. Metal ions form both soluble and insoluble complexes with organic materials (natural as well as anthropogenic). This greatly affects their transportation and accumulation in the environment [5]. The effects of metal speciation on living organisms may be twofold; (i) decrease in the toxicity of certain metals and (ii) increase in the bioavailability of other metals. Studies using organic chelates such as nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) have demonstrated that bioavailability of trace metals to phytoplankton (with respect to both toxicity and biolimitation) are generally correlated to the free metal ion concentration rather than the total metal concentration [5-9]. Typically, in wastewater especially from food industries, the ratio of concentration of organic ligands relative to that of the metals is high [10]. Therefore metals present in the wastewater from brewery are assumed to exist largely as organic complexes.

The objective of this study was to establish the nature of complex formation reactions between heavy metals and organic substances such as carbohydrates for application in metal speciation studies in brewery effluents. Thus different classes of carbohydrates including monosaccharides (e.g. glucose and galactose), disaccharides (e.g. maltose and sucrose) and

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*Corresponding author. Tel +267 3552488; Fax +267 355 2836; E-mail: ngilajc@mopipi.ub.bw polysaccharides (e.g. starch and cellulose) have been studied. Other related organic substances likely to be found in brewery effluent such as ethanol and glycine, were also studied. Established [11] reactions between Pb^{2+} versus chloride and EDTA were repeated in the preliminary studies of this work as a starting point for the voltammetric experiments on metal-inorganic and metal-organic complex formation reactions. Other metal ions studied were Cu^{2+}, Cd^{2+}, Zn^{2+} and $Cr^{3+/6+}$.

Background on brewery effluent

Brewery wastewater samples were obtained from Kgalagadi Breweries plant located in Gaborone city, Botswana. Samples were collected from specific points of wastewater effluent system of the breweries. Our earlier survey [12] conducted on brewery effluent composition indicated the effluent to consist of the following compounds; carbohydrates (glucose, sucrose, etc.), ethanol, proteins, lactic acid, malic acid, phenols, propyleneglycol, vitamins, lipids, yeast, caramel and malt as well as inorganic ions such as chloride, sulphate, phosphate and carbonate from water treatment reagents. The above list is also typical of by-products from the ingredients used in liquor and soft drink production [13, 14]. The presence of metals in the brewery effluent is predicted to leach out from the metal boilers during the cleaning of apparatus and also from raw water from supplies to the plant [12].

Modeling of DPV data

Speciation studies using differential pulse polarography (DPP) and differential pulse voltammetry (DPV) for determining the *labile*, *semi-labile* and *fully inert* forms of the metal-ligand systems have been reported [15-20]. Of particular interest to this work is the study by Cukrowski [15] who used Eqn. 1 for manipulating data obtained by DPP/DPV to work out the concentration of the free metal $[M_{free}]$ and complexed metal $[M_{comp}]$ forms. Equation 2 gives the relationship between peak potential shift and the peak potentials of the free metal ion and that of the metal complex.

$$\Delta E_p(i) - \left(\frac{RT}{nF}\right) \ln\left\{\frac{i_p([M_{comp}])_{(i)}}{i_p([M_{free}])_{(i)}}\right\} = \left(\frac{RT}{nF}\right) \ln\left\{\frac{[M_T]_{(i)}}{[M_{free}]_{(i)}}\right\} \qquad (1)$$

where

$$\Delta E_P = E_P(M_{free}) - E_P(M_{comp}) \qquad (2)$$

$\Delta E_p(i)$ is the shift in the potential value as a result of complex formation reaction in the solution at the i^{th} pH value. $[M_T]$ is the total concentration of metal, present in the sample. i_{Mcomp} is the peak current of the labile complex and i_{Mfree} is the peak current of the free metal ion. R, T, F and n have the usual meaning as applied in the Nernst equation. The 'free' metal ion concentration cannot be obtained from DPP/DPV experimental data but is calculated from the *mass-balance* equations written for the assumed metal-ligand model [19]. In equation 2 $E_p(M_{free})$ is the DPP peak potential of an "uncomplexed" metal ion while $E_p(M_{comp})(i)$ stands for the peak potential obtained at each i^{th} pH value for a given metal-ligand system. The curves prepared using experimental values obtained from the left hand side of Eqn. 1, known as the *experimental complex formation curve* (ECFC), are fitted to the calculated values using the expression on the right hand side of Eqn. 1, known as the *calculated complex formation curve* (CCFC) [16].

Using the CCFC, the stability constant value initially predicted for a given complex is repeatedly refined to obtain a good fit (RSD < 5%) to the experimental curves. In this study, stability constants for Pb complexes with some organic ligands (Table 1) obtained from the literature [21-23] was used as the starting values entered in the *mass balance* equation for processing the CCFC.

Table 1. Lead complexes with organic ligands [21-23].

(A) Overall stability constants (log β).

Ligand (L)	Log β					
	ML	ML_2	ML_3	MHL	$MH_{-1}L_2$	$ML_2(OH)$
[a]IDA	7.36	9.78			-2.21	
[b]EDTA	16.9			19.8		
Picolinic acid	4.58	7.92				11.46
OH ($\mu = 0.5$ M)	6.0	10.3	13.3			

(B) Stepwise formation constants (log k_1).

Ligand	Log k_1 of ML
Ethylenediamine	5.04
Glycine	4.87
Oxine	4.91
[c]TMDTA	13.40
Mercaptoethanol	6.6
Imidazole	1.6

M = Pb^{2+}, H = proton, L = ligand. [a]IDA = iminodiacetic acid, [b]EDTA = ethylenediaminetetraacetic acid, [c]TMDTA = trimethylenediametetraacetic acid. Ionic strength, $\mu = 0.1$ M.

EXPERIMENTAL

Sampling and sample preparation

Wastewater samples were collected from a brewery plant located in Gaborone city, Botswana. Sampling was carried out using one-litre plastic bottles, which had been previously cleaned by soaking in 10% nitric acid, and thoroughly rinsed with distilled-deionized water. Before retaining a sample, the containers were rinsed with the wastewater for at least three times. Wastewater samples S2, S5, S6, S13, S16 and ST were collected from different points of the brewery wastewater system. The sample numbering (2, 5, 6, 13, 16 and T) indicated the sequential sampling locations chosen according to the flow of wastewater from the brewing system down to the final disposal tank, T.

Reagents

All the chemicals used were of Analar grade. For analysis with flame atomic absorption spectrometer (FAAS), commercial (SAARCHEM, South Africa) standard stock solutions of metal ions (1000 mg L^{-1}) were used. Nitrate salts (SAARCHEM, South Africa) were used to prepare metal ion stock solutions (0.1 M) for differential pulse anodic stripping voltammetric (DPASV) experiments. Working solutions of Cu^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+} and Zn^{2+} were then prepared by appropriate dilution of the stock solutions. The supporting electrolyte used in the quantitative analysis with DPASV was 1.5 M KCl/0.5 M sodium acetate (pH 4.6) [24] while the supporting electrolyte used in exploratory experiments with DPV was 0.1 M HNO_3 or 0.1 M $NaNO_3$. The ligands used in the DPV experiments were Cl^- (as NaCl), EDTA (disodium salt), glucose, galactose, fructose, maltose, sucrose, starch, cellulose and glycine (purchased from Sigma-Aldrich, USA). The stock solutions (0.1 M) for the ligands were prepared in distilled-deionized water. Alumina powder (Metrohm, Switzerland) was used for cleaning the glassy-carbon working electrode. The effect of pH was studied using suitable concentrations (0.1 to 10.0 M) of either HNO_3 or NaOH (Sigma-Aldrich, USA) to adjust pH. Ultrapure deionized water (obtained by passing distilled water through a reverse osmosis system, Milli-Q water system, millipore) was used in all standards and sample preparations.

Instrumentation

A Varian SpectrAA 220 FS atomic absorption spectrometer (Varian, Australia) equipped with a deuterium background correction was used to determine total dissolved metal content. An electrochemical analyzer comprising of Autolab recorder (PGSTAT10, Ecochemie, Netherlands) and voltammetric stand (663 VA, Metrohm, Switzerland) was used for voltammetric analysis. A software (757 VA Computrace, Metrohm, Switzerland) running in a Dell Dimension V400 computer was used for data acquisition and manipulation. A three-electrode system voltammetric cell, 150-mL capacity (Metrohm, Switzerland) was used. The working electrode was a rotating disk glassy carbon (GC) electrode for DPV measurements and a mercury-plated GC electrode for DPASV. A silver-silver chloride reference electrode (Metrohm, Switzerland) with an internal filling solution of 3 M KCl saturated with AgCl was used. A carbon rod served as the auxiliary electrode. The pH measurements were done with a combined glass electrode (HI 1332 model) and pH/mV meter, both from Hanna Instruments (Portugal).

Voltammetric analysis

The glassy carbon electrode was cleaned and polished with alumina powder and then put into a cell containing 4.0 mg L^{-1} $Hg(NO_3)_2$ in 0.1 M $NaNO_3$ [24]. After the solution was purged with nitrogen for 5 min, a blanket of nitrogen was maintained over the solution. An *ex situ* plating of the mercury film was achieved by holding the potential at −1000 mV for 300 s. A mixture of metal and ligand ($[L_T]$:$[M_T]$ at different ratios, were obtained. The $[L_T]$:$[M_T]$ ratio of 1:1 was obtained by mixing 10 μL of 0.1 M metal solution with 10 μL of 0.1 M of ligand added into 20 mL of blank solution (0.1 M $NaNO_3$ or 0.1 M HNO_3), to keep ionic strength at 0.1 M. In order to investigate the effect of pH (1.5 to 13.0) on the metal-ligand mixture, NaOH was used to increase pH by 0.05 to 0.1 unit steps. A set of 40 to 50 voltammograms was recorded for each ligand-to-metal $[L_T]$:$[M_T]$ ratio ranging 1:1 to 50:1 and also for high metal to ligand ratio of 1:1 to 1:10. Electrode equilibration time of 3 to 5 min (depending on the solution pH required) was allowed after each addition of NaOH. DPASV analysis was accomplished under the following instrumental conditions; pulse amplitude 50 mV, step height 4 mV, pulse width 0.2 s and sweep rate 60 mV/s.

Atomic absorption analysis

Total dissolved metal was determined after acid digestion of filtered samples. Wastewater samples were filtered through a 0.45 μm cellulose acetate membrane filter. Each time, the collection flask was rinsed with the first portion of the filtrate and discarded before retaining the sample filtrate. An aliquot of the sample (50 mL) was put into a 250-mL conical flask and digested with 5 mL mixture (3:1) of concentrated nitric acid (65%) and perchloric acid (70%). When digestion was complete, 10 mL of 0.5 M HCl was added to re-dissolve the residue, boiled for 5 min and the resultant solution filtered into a 50-mL volumetric flask. Ultrapure water was used to bring the volume to the mark. Total dissolved metal was then determined by FAAS at their respective analytical wavelengths and slit width (those recommended by the manufacturer).

RESULTS AND DISCUSSION

Effect of pH and ionic strength on Pb^{2+} peaks

The effect of pH on peak characteristics of Pb^{2+} was investigated by titrating Pb^{2+} solution (prepared in acidic solution, pH 1.5 using 5.0 M HNO_3) against NaOH. The peak current of Pb^{2+} was observed to decrease while the peak potential (E_{PC}) showed a shift from low to high cathodic values (E_{PC}) with increase in pH. Voltammograms in Figure 1 show a peak potential shift to a more negative value while the peak broadness increases with reduced intensity for peaks 1 to 5. However, peak 6 appeared to increase in height suggesting presence of a more labile species.

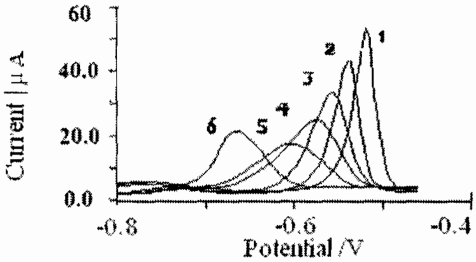

Figure 1. Voltammograms showing the effect of pH on the peak characteristics of Pb starting with pH 1.5 adjusted with conc. HNO_3 (15.0 M). The pH was increased by adding varying volumes of NaOH (10.0 M). Peaks 1 to 6 represent pH values 1.5, 3.0, 5.0, 7.0, 9.0 and 11.0, respectively. Supporting electrolyte was 20 mL of 0.1 M $NaNO_3$.

These observations suggest that $[Pb^{2+}]_{free}$ decrease with increase in $[OH^-]$ as a result of formation of metal-OH complexes, $Pb(OH)_y^{(2-y)+}$ which are less labile species compared to 'free' Pb^{2+} (Eqn. 3)

$$Pb^{2+} + y\,OH^- \rightleftharpoons Pb(OH)_y^{(2-y)+} \qquad (3)$$

The decrease in peak height (I_p) suggests either the formation of inactive, semi or fully inert complex(es) or $Pb(OH)_{2(s)}$ precipitate with respect to the DPV technique.

The effect of ionic strength on the Pb^{2+} peak characteristics, was also investigated by varying the concentration of the supporting electrolyte using different volumes of 1.5 M KCl/0.5 M NaAc. Similar to effect of pH, the E_{pc} was observed to shift to a more negative value as ionic strength was increased from 0.10 to 0.40 M. The explanation may be that, increasing the concentration of the supporting electrolyte increases conductivity of the medium. This raises the E_{pc} value for Pb^{2+} as a result of reduced diffusion rates of metal ion due to concentration polarization effect (overpotential resulting in high E_{pc}) [11].

Pb-chloride complexes

In order to compare metal-inorganic (most of which are well established reactions [21, 22]) with metal-organic complex formation reactions, Pb-chloride system was studied by varying the pH of Pb-Cl system at a constant ligand-to-metal concentration ratio as well as the amount of Cl^- (0.1 M) added in to Pb^{2+} solution (0.1 M). Figure 2A shows voltammograms of Cl^- added in Pb^{2+} solution using DPASV. Each step (1 to 6) in Figure 2B is assumed to correspond to a particular $PbCl_x^{(2-x)+}$ species as shown by the pattern of stepwise formation constants (log k_x) in Table 2.

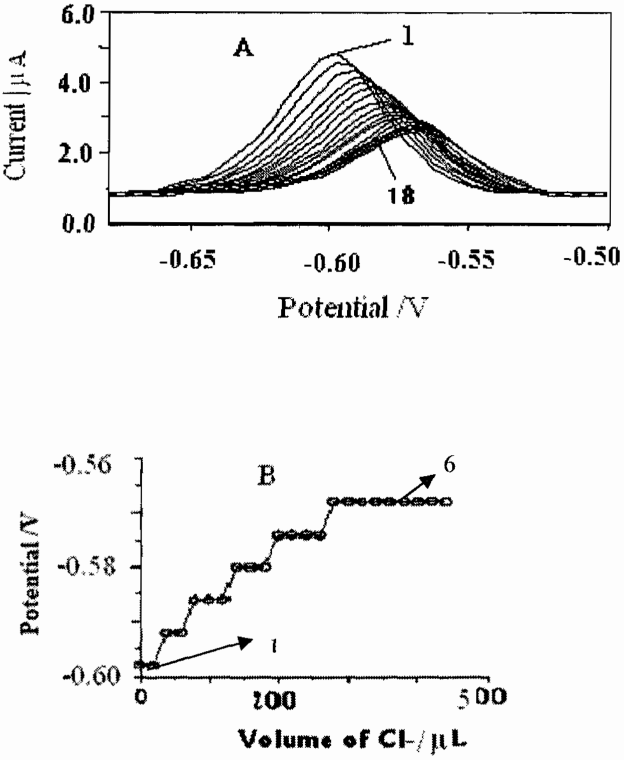

Figure 2. Pb-Cl system. (A) DPASV scans showing reactions between Pb^{2+} and Cl^-. (1) 1st scan; and (18) is 18th scan, using DPASV. (B) Plot of peak potential: E_p (mV) versus added volume (10 μL) of 0.1 M Cl^- in 5 x 10^{-5} M [Pb^{2+}]. (1) Lowest and (6) highest [Cl^-] added.

Table 2. Lead-chloride, $PbCl_x^{(2-x)+}$ complexes: experimentally calculated (using Eqn.3) stepwise formation constants (log k_x).

x	Complex formation reaction	log k_x
1	$Pb^{2+} + Cl^- \rightleftharpoons PbCl^+$	5.59
2	$PbCl^+ + Cl^- \rightleftharpoons PbCl_2$	6.13
3	$PbCl_2 + Cl^- \rightleftharpoons PbCl_3^-$	6.53
4	$PbCl_3^- + Cl^- \rightleftharpoons PbCl_4^{2-}$	6.78
5	$PbCl_4^{2-} + Cl^- \rightleftharpoons PbCl_5^{3-}$	7.07
6	$PbCl_5^{3-} + Cl^- \rightleftharpoons PbCl_6^{4-}$	7.40

The log k_x values were calculated using Eqn. 4 [11], where k_1 corresponds to $PbCl^+$ and k_6 to $PbCl_6^{4-}$.

$$\log k_X = -\left(\frac{n\Delta E_p}{0.05916} + p\log[L^{-b}]\right) \tag{4}$$

The coefficient p of the log term represents the stoichiometric number of the ligands in the reaction. Complex formation reactions between heavy metals and simple inorganic and organic ligands have been widely studied. Their stability constants are well documented in the literature [11, 25, 26]. Therefore no attempt was made to determine stability constants in this work. Greater emphasis was on species distribution in view of its relevance to metal speciation in water systems. The Pb-Cl system was shown to exhibit labile complex characteristics at varying pH (1.5 to 10.5) for a given $[L_T]:[M_T]$ ratios.

Speciation distribution diagrams (Figure 3), generated by matching the CCFC with ECFC in an attempt to obtain a good fit (RSD < 5%) using 3D-VISE software [16], confirm formation of different Pb species with changes in pH.

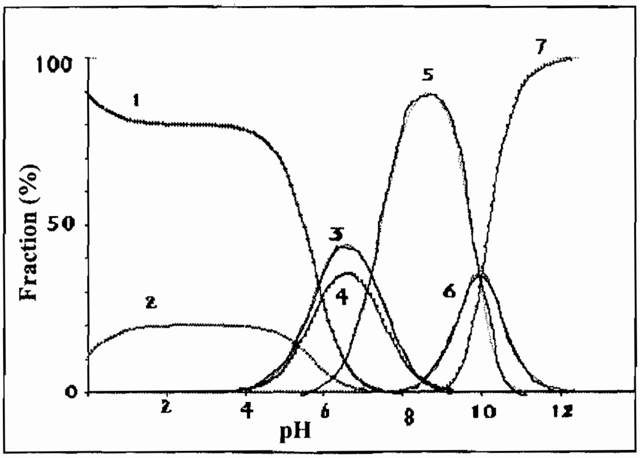

Figure 3. Speciation distribution diagram of lead-chloride, showing fraction (%) of species present in solution as a function of pH: (1) Pb_{free}, (2) MLH, (3) ML(OH), (4) M(OH), (5) $M(OH)_2$, (6) $M(OH)_3$, (7) $M(OH)_4$. Pb:Cl = $[L_T]:[M_T]$ = 10; M = Pb and L = Cl. Supporting electrolyte was 20 mL of 0.1 M $NaNO_3$.

Pb-EDTA complexes

Addition of EDTA in to Pb^{2+} solution resulting in high ligand to metal concentration $[L_T:]:[M_T]$ ratios 5:1, 10:1 and 100:1, show a decrease in peak current intensity of 'free' Pb^{2+} as EDTA at a fairly constant peak potential of –0.420 V. Voltammograms 1 to 5 in Figure 4A, indicate subsequent decrease in $[Pb^{2+}]$ while voltammograms 6 and 7 show background cathodic current to increase with increase of pH above 9.0. Peak 7 at ~ –0.6 V is not well resolved. Peak broadness was attributed to coexistence of more than one complex, e.g. Pb-EDTA and $Pb(OH)_x^{(2-x)+}$, a situation that is statistically favoured [27]. The baseline current initially shifted from –2.0 µA at pH 1.5 to a lower value of –0.2 µA at pH 3.0, and then to a higher value of –1.5 µA at pH 5.0, –2.7 µA at pH 8.5, and –4.0 µA at pH 12.9. This may be as a result of increased conductivity due to deprotonated EDTA species as well as Na^+ and OH^- released into the solution as $NaOH_{aq}$ is added. Pb-EDTA systems with higher metal to ligand concentrations, $[L_T]:[M_T]$ ratios of 1:1, 1:2, 1:5, 1:10 and 1:50, were obtained by addition of Pb^{2+} into the EDTA solution. Peaks 1 to 4 in Figure 4B show increased I_p with increase in Pb concentration. Peak 5 shows relatively larger current compared to the rest of the peaks (1 to 4). This may be due to formation of a polynuclear Pb-complexes or merely due to increased 'free' Pb^{2+} concentration. In both Figure 4A and B, group **a** peaks (appearing between –0.4 and –0.5 V) represent reduction reaction of 'free' Pb^{2+} while group **b** peaks (appearing between –0.5 and –0.6 V) represents reduction reaction of 'labile' Pb-complexes.

Pb-glucose and Pb-sucrose complexes

Pb-glucose system was studied under similar conditions as Pb-EDTA. The concentrations of ligand to metal, $[L_T:]:[M_T]$, was kept at ratio 5:1. The effect of pH (1.5 to 12.9) on Pb-glucose was demonstrated by observing peak characteristics namely, potential and current values. The cathodic peak potentials (E_{PC}) are shown (Figure 5) to be fairly constant (–0.45 V) as pH increases. Peaks 1 to 5 indicate subsequent decrease in $[Pb^{2+}]$ while voltammograms 6 and 7, show background cathodic current to increase with increase of pH above 9.0. Peak 7 at ~ –0.55 V is too broad. Peak broadness was attributed to coexistence of more than one complex, e.g. Pb-glucose and $Pb(OH)_x^{(2-x)+}$. The shift in background current (non-Faradaic) may be due to presence of deprotonated glucose (ionic) at high pH and also due to increased $[OH^-]$ and $[Na^+]$ from NaOH resulting in high conductivity of the solution.

Comparing the effect of increasing $[Pb^{2+}]$ at a constant [ligand] for glucose and sucrose systems, it was found that the amount of glucose consumed in the reaction with Pb^{2+}, was twice that of sucrose i.e. glucose to sucrose ratio of 2:1. This ratio confirms the known stoichiometry that sucrose is a dimer of glucose [28].

The speciation distribution diagrams for Pb-sucrose at $L_T:M_T = 5:1$ in Figure 6 suggest the following species: M_{free}, ML, ML(OH) and $M(OH)_x$, x = 1 to 4 where M is Pb^{2+} and L is sucrose (charges omitted for simplicity). It is noted that M_1L_1 and $M_1(OH)_1$ were found to occur in rather low percentage (3 to 4%) at low pH (2.0 to 5.0). The 'free' or aquo metal species is observed only at low pH conditions since the extent of reaction between Pb and ligand is small [26]. It is shown (Figure 6) that $M_1L_1(OH)_1$ is predominant (65-68%) in the pH range 3.9-7.9. The absence of $M_1L_2(OH)_1$ species in the 5:1 system otherwise expected for a high $[L_T]:[M_T]$ ratio, could be attributed to the large size of sucrose molecule which may result to steric hindrance if two molecules are involved [26]. It is interesting to note that similar studies involving Zn-glycine and Zn-fructosyl glycine systems [29] reported only ML_1, ML_2, ML_3 systems but did not report the existence of mixed complexes such as $ML_1(OH)$, that are reported in this work. This highlights the weak metal binding to the hydroxyl groups in sugars. The weak binding ability of sugars has been clearly demonstrated by complexation reactions between metal ions with fructosyl glycine in which the molecule binds exclusively through the amino acid moiety [30].

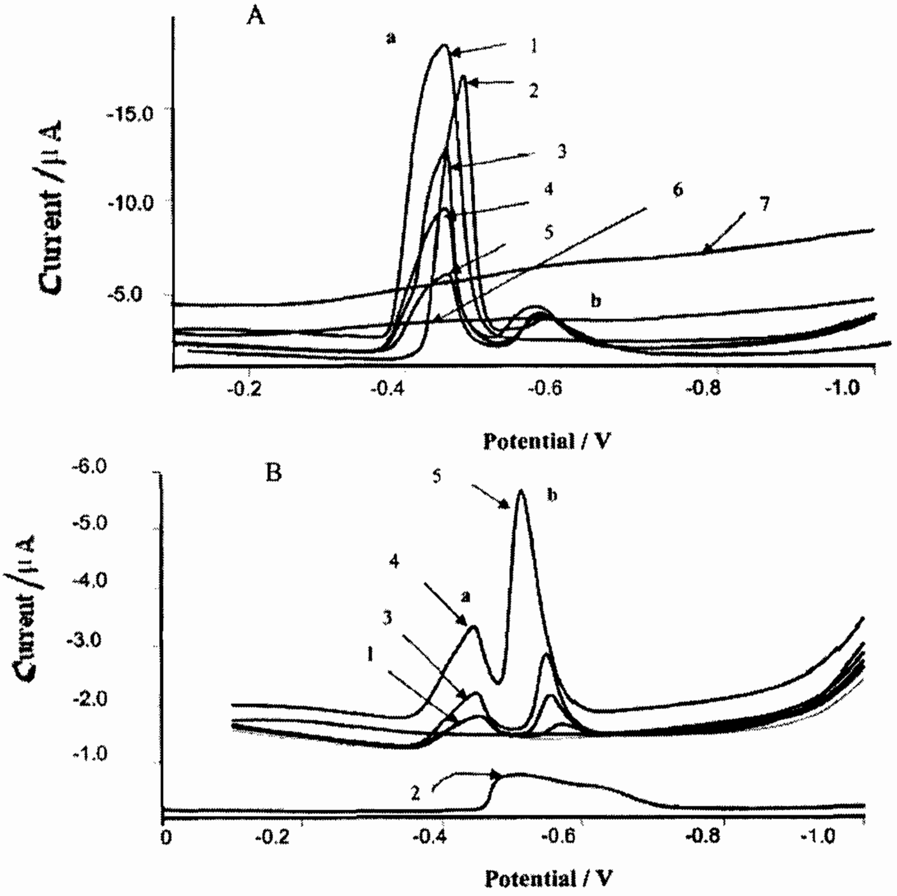

Figure 4. Pb-EDTA voltammograms: (A) Effect of pH on peak potential and current, at L_T: M_T = 10. Peaks (1) to (5) indicate decrease in [Pb^{2+}]; (6) and (7) increased background current (cathodic) above pH 9.0. (B) Peaks 1 to 5 show increasing [Pb^{2+}] added in EDTA solution. Group **a** peaks represent reduction of 'free' Pb^{2+} and group **b** are 'labile' Pb-complexes. Supporting electrolyte is 20 mL of 0.1 M $NaNO_3$.

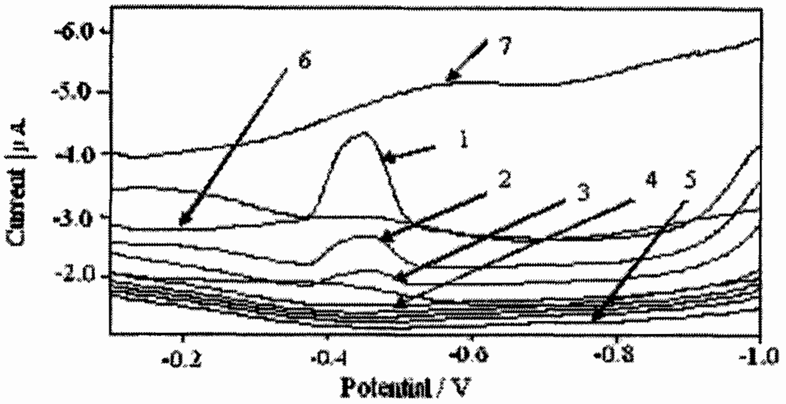

Figure 5. Pb-glucose voltammograms showing effect of pH at $[L_T]:[M_T] = 5:1$. Peaks (1) to (5) indicate decrease in $[Pb^{2+}]$; (6) and (7) increased background cathodic current above pH 9.0. Supporting electrolyte was 20 mL of 0.1 M $NaNO_3$.

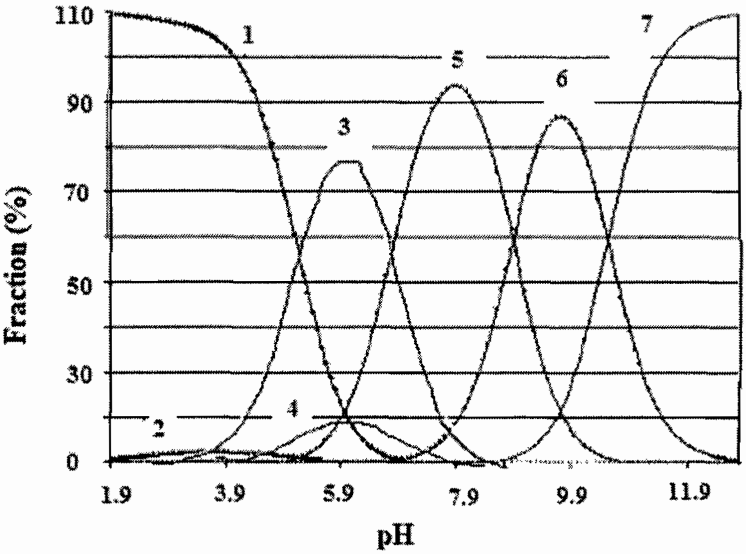

Figure 6. Pb-Sucrose speciation distribution diagram; M = Pb and L = sucrose. (1) $M_{free} = Pb^{2+}$, (2) M_1L_1, (3) $M_1L_1OH_1$, (4) M_1OH_1, (5) $M_1(OH)_2$, (6) $M_1(OH)_3$, (7) $M_1(OH)_4$. Supporting electrolyte was 20 mL of 0.1 M $NaNO_3$.

Comparison of the speciation diagrams for the L_T:M_T = 1:1 and 5:1 systems show that in the former (1:1), the distribution of 'free' Pb^{2+} species occur in a relatively wider pH range (pH 1.5 to 8.0) whereas in the latter (5:1) system, it occurred up to pH 7.0 only. This is because at high $[L_T]$:$[M_T]$ ratio, formation of a wide range of complexes is possible. This observation is supported by the absence of species such as M_lL_l in the 1:1 system, yet present in the 5:1 system. From these observations, one could also conclude that increasing the ligand concentration increases the degree of complex formation with the metal ion.

Pb(II)-complexation with other carbohydrate ligands

Results of complex formation reactions between Pb versus galactose and maltose showed similar behaviour to that of Pb-glucose and Pb-sucrose systems, respectively. However, interpretation of reactions between Pb versus starch and cellulose was limited by their poor solubility in aqueous media, particularly at low pH but with improved solubility at high pH (> 8.0). Addition of cellulose or starch solutions to the Pb^{2+} solution at low pH, showed reduced intensity of the 'free' Pb^{2+} peak. We attributed this to the presence of the insoluble ligand particles that may occlude Pb as well as interfere with the electrode surface. At pH >8.0, broad and small peaks were observed as in Pb-sucrose system under the same pH conditions.

Complexation reactions of other heavy metals with carbohydrate ligands

Other metals, Cd^{2+}, Cu^{2+}, Zn^{2+} and Cr^{3+}, were each reacted with each of the following ligands: EDTA, glucose, sucrose, starch, cellulose, glycine and ethanol. Ligand to metal, $[L_T]$:$[M_T]$ systems studied were 1:1, 2:1, 5:1 and 10:1 as well as systems with relatively higher metal to ligand ratios, $[M_T]$:$[L_T]$ of 2:1, 5:1 and 10:1. The peak characteristics observed show a similar trend to that of Pb-carbohydrate systems. These observations imply that reactions between heavy metals and carbohydrates do occur giving products with varying solubility depending on the pH. Mixing different metals together in a solution containing glucose or sucrose revealed that the presence of secondary metal ions masks the peak of the analyte (primary) metal ion. This phenomenon may be due to formation of various metal species with overlapping peaks. If this is the case, the appearance of a certain peak will depend on the relative percentage composition of that species in a mixture. This prediction was extended to real sample matrix by spiking septic tank wastewater sample (ST in the Experimental section) with a standard metal solution in order to observe the behaviour of peaks of the other metal ions. While the non-spiked sample showed five peaks (Figure not displayed), upon spiking a given metal standard solution, peaks of some of the other metal ions became invisible. For example, peak 3 in Figure 7 assigned to Cr^{3+} which was initially visible, is hardly visible as Pb^{2+} standard (peak 2) was spiked. The phenomenon of peak masking was confirmed by varying the composition of metals in the standard mixture containing a given ligand and spiking a particular metal standard. This was intended to rule out the problem of plot scale for peaks with varying heights. The peaks appearing in Figure 7 (ST sample spiked with Pb^{2+}) were assigned to: (1) Cu^{2+}, (2) Pb^{2+}, (3) Cr^{3+}/Cr^{6+}, (4) Cd^{2+}, and (5) Zn^{2+}. Addition of standard solutions of sugars (glucose, fructose, maltose, galactose and sucrose) to the wastewater had no effect on the observed peak potentials. This could be due to the fact that the wastewater is already rich in these sugars such that there is no change in the overall concentration after spiking.

Metal speciation in brewery wastewater

Wastewater samples obtained from the brewery plant effluent were analyzed using both FAAS and DPASV techniques. The FAAS technique was used to determine the levels of total dissolved metals (Cu, Zn, Cr, Pb and Cd) in wastewater samples (S2, S5, S6, S13, S16 and ST), collected from the brewery plant effluent. The results obtained are given in Table 3. The data show the FAAS data to have generally higher metal concentration values (RSD = 0.5 to 4.0%) compared to that by the DPASV technique (RSD = 0.1 to 2.0%). This is expected since the DPASV technique detects only the labile metal forms that are 'voltammetrically active' [17] unless the samples are first digested by UV-irradiation [31] to decompose organic matter and release the strongly complexed metal fraction for DPASV determination. UV-irradiation (not done in this work due to lack of facilities) is a good choice in sample preparation for the determination of total metal in speciation analysis because of its efficiency and the fact that additional chemicals are not necessary, thus minimizing errors [32]. However, in the absence of a UV-digester, FAAS or GFAAS is equally useful in the determination of total metal content.

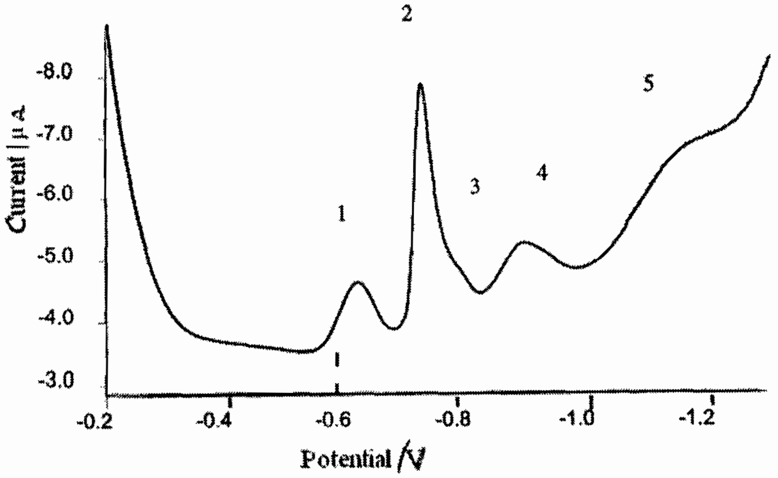

Figure 7. Differential pulse anodic stripping voltammogram of brewery wastewater sample (septic tank, ST) spiked with Pb^{2+} standard: (1) Cu^{2+}, (2) Pb^{2+}, (3) $Cr^{3/6+}$, (4) Cd^{2+}, (5) Zn^{2+}.

Table 3. (A) Data of metal concentrations (mg L^{-1}) found in wastewater samples collected at different brewery effluent locations (S2 to ST), analyzed with FAAS and DPASV. The expression, % Δc, refers to the percent difference between AAS and DPASV values.

Sample[a]	Pb			Zn			Cr[b]	Cd[c]	Cu[c]
	AAS	DP	% Δc	AAS	DP	% Δc	AAS	DP	DP
S2	ND	0.190	-	ND	0.143	-	1.020	0.009	0.005
S5	0.459	0.220	52.1	0.250	0.059	76.4	2.490	0.030	0.020
S6	0.537	0.210	60.9	0.286	0.090	68.5	2.860	0.050	0.020
S13	0.660	0.080	87.9	0.286	0.256	10.5	3.470	0.070	0.035
S16	0.790	0.240	69.6	0.440	0.104	76.6	3.660	0.080	0.070
ST	1.067	0.100	90.6	0.967	0.090	90.7	4.060	0.100	0.090

[a] Sample code = S2, S5, S6, S15, S16, ST representing sampling site, S; 2, 5, 6, 13, 16 and septic tank (ST). ND- not detectable; (-) - not done; [b] AAS values only; [c] DPASV values only.

The difference between the FAAS and DPASV values (Δc = FAAS – DPASV) gives an estimation of the fraction of the metals that is DPASV- nonlabile [32, 33]. The % Δc (= 100 x Δc/FAAS), should be proportional to the concentration of organically bound metal complexes. An attempt was made to establish the nature of complexes responsible for loss of lability. Standard solutions of the metals were spiked with sugar solutions and no loss in lability was observed, suggesting that the sugar complexes of these metals are labile. Similar experiments with amino acids solutions also showed no loss in lability. Loss in lability was however observed when a protein standard (albumin) was used to demonstrate the nature of complexation reactions between metal ions and protein-based materials. Thus the DPASV-inert metal fraction is expected to be mainly metal-protein complexes and the fraction of the metal that is adsorbed and/or complexed with colloidal materials. On the basis of % Δc, Pb showed a relatively higher degree of complex formation than Zn. Comparison of Cr, Cd and Cu in % Δc terms was not possible as data were not complete. Thus, Cr was determined with only FAAS while for Cd and Cu only DPASV could be used as their levels were below detection limit of FAAS technique. However, going by DPASV values for Pb, Zn, Cd and Cu (Table 3), it was noted that generally, Pb had relatively the highest levels of labile forms compared to the rest except for sample S13 where Zn level is higher.

The trends of FAAS data showed metal concentrations to increase from sample S2 to S16. This could be due to the fact that S2 to S16 represent different points of the wastewater transportation (in terms of distance from the point of discharge) towards the storage tank, with S2 representing upstream and S16 the last point downstream of the wastewater system. ST is the sample collected from the septic tank where the wastewater is stored awaiting the final disposal. Contrary to FAAS, the DPASV data showed a relatively lower level of metals in sample ST compared to S13 and S16. This may be attributed to dynamism of metal-ligand complex formation reactions where each location of the wastewater transportation from S2 to ST, represents a different speciation pattern.

CONCLUSIONS

Our study, using synthetic solutions, has shown the speciation distribution, particularly for metal-carbohydrate complexes, to be highly dependent on the pH where the degree of complex formation between the metal and the ligand was found to be high at pH > 9.0. The significance of effect of pH and ionic strength highlight the potential changes that the complexes could undergo on entering the receiving waters of the environment under different conditions. Depending on pH, the mobility and bioavailability of metal ions may be altered with significant environmental consequences. The brief investigations on wastewater samples from the brewery effluent, showed evidence of predominance of non-labile metal-organic forms which imply that the effluent poses minimal health risks since organic-metal forms are generally less toxic to the environment. Results of this work are expected to provide a reference for future studies on speciation of heavy metals with organic materials in wastewater from food industries such as breweries and leather tanning processes among others. However, we note that in real applications, speciation determination of a given metal in the presence of other metals and various ligands in the same sample remains a big challenge.

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REFERENCES

1. Handa, N. *J. Oceonogr. Soc. Jpn.* **1966**, 22, 79.
2. Ramamoorthy, S.; Kushuner, D.J. *Nature* **1975**, 256, 399.
3. Yuchi, A.; Wada, H.; Nakagawa, G. *Anal. Sci.* **1985**, 1, 19.
4. Midorikawa, T.; Tanoye, E.; Sugimura, Y. *Anal. Chem.* **1990**, 62, 1737.
5. Buffle, J. *Complexation Reaction in Aquatic Systems: An Analytical Approach*, Ellis Horwood, Chichester; **1988**; p 87.
6. Sunda, W.G. *J. Mar. Res.* **1976**, 34, 511.
7. Anderson, M.A.; Morel, F.M.; Guillard, R.R.L. *Nature* **1978**, 276, 70.
8. Brand, L.E.; Sunda, W.G.; Guillard, R.R.L. *J. Exp. Mar. Biol. Ecol.* **1986**, 96, 225.
9. Sunda W.G.; Tester, P.A.; Huntsman, S.A. *Estuar. Costal Shelf Sci.* **1990**, 30, 207.
10. Metcalf, E.; Eddy, D. *Wastewater Engineering, Treatment, Disposal and Re-use*, EEC Technical Report, **1991**.
11. Harris, D.C. *Quantitative Chemical Analysis*, 5th ed., W.H. Freeman and Company: New York; **1999**; pp 312-327.
12. Torto, N.; Ngila J.C.; Peloewetse E. *A Survey of Brewery Operation and Identification of Effluent Parameters* prepared for Kgalagadi Breweries Limited, *Botswana*; 12 April **2002**.
13. Webb, R. *The Beginner's guide to advanced and all-grain brewing*, http://home1.gte.net/richwebb/guide_do.htm
14. Van Loon, J.C.; Barefoot, R.R. *Analyst* **1992**, 117, 563.
15. Cukrowski, I. *Anal. Chim. Acta* **1996**, 336, 23.
16. Cukrowski, I.; Hancock, R.D.; Luckay, R.C. *Anal. Chim. Acta* **1996**, 319, 39.
17. Cukrowski, I. *Electroanalysis* **1999**, 11, 606.
18. Cukrowski, I.; Cukrowska, E.; Hancock, R.D.; Anderegg, G. *Anal. Chim. Acta* **1995**, 312, 307.
19. Cukrowski, I. *Electroanalysis* **1997**, 9, 699.
20. Cukrowski, I. *J. Electroanal. Chem.* **1999**, 460, 197.
21. Martell, A.E.; Motekaitis, R.J. *The Determination and Use of Stability constants*, VCH Publishers: New York; **1988**; p 47.
22. Martell, A.E; Smith, R.M. *Critical Stability Constants*, Vol. 1-6, Plenum Press: New York; **1974-1989**.
23. Cukrowski, I.; Loader, S.A. *Electroanalysis* **1998**, 10, 883.
24. Metrohm Application Bulletin, No. 254/1e, *Determination of zinc, cadmium, lead and copper by anodic stripping voltammetry using carbon electrodes*, Metrohm; Switzerland; **1997**; p 1.
25. Powell, K.J.; Pettit, L.D. *IUPAC Stability Constants Database*, Academic Software, UK, **1999**.
26. Jenkin, D.J.; Potter, B.V.L. *Carbohydr. Res.* **1996**, 287, 167.
27. Stolzberg, R.J. *Anal. Chim. Acta* **1977**, 92, 193.
28. Ferrier, R.J. *Carbohydrate Chemistry*, Vol. 26, The Royal Society of Chemistry: London; **1992**; pp 25-31.
29. O'Brien, J., Morrissey, P.A. *Food Chem.* **1997**, 58, 17.
30. Clydesdale, F.M., *Mineral Interactions in Foods* in *Nutrient Interactions*, Bodwell, C.E. Erdman, J.W. (Eds.), Marcel Dekker: New York; **1988**; pp 73-113.
31. Golmowski, J.; Valenta, P.; Nurnberg, H.W. *Fresenius J. Anal. Chem.* **1985**, 322, 315.
32. Li, Y.; Xue, H. *Anal. Chim. Acta* **2001**, 448, 121.
33. Turner, D.R.; Whitfield, M.; Dickson, A.G. *Geochim.Cosmochim. Acta* **1981**, 45, 855.