Factorial design analysis on the solubility of total mercury in reduction process

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ABSTRACT. The dental wastewater can contribute to the total daily mercury load on the environment. Factorial design of experiments is useful to analyze factors that influence this solubility. The aim of the present study was to design experiments to examine the effects of operational variables, humic acid, temperature, pH and contact time that may affect the solubility of total mercury as dental amalgam residue in reduction process. Based on the factorial design of experiments, the humic acid concentration was the most significant factor in this process, followed by other factors. The parameters affecting the solubility of total mercury showed that when the [HA], T and CT increases and pH decreases there is an important increase of total mercury concentration in process. For the tested conditions, the high total mercury concentration was obtained using the humic acid concentration = 1.0 g L^{-1}, temperature = 35ºC, pH = 4.0 and contact time = 10 days.

Key words: dental amalgam, total mercury, humic acid, temperature, pH, factorial design of experiments.

RESUMO. Análise do processo experimental na solubilidade do mercúrio total em processo redutivo. O esgoto odontológico pode contribuir na carga total de mercúrio no ambiente. O estudo do planejamento experimental é útil para analisar os fatores que influenciam nesta solubilidade. O objetivo deste trabalho foi realizar um planejamento experimental para analisar os efeitos das variáveis operacionais, ácido húmico, temperatura, pH e tempo de contato, que podem afetar a solubilidade do mercúrio total como amálgama odontológico em um processo de redução. Baseado no planejamento experimental, a concentração de ácido húmico foi o fator mais significativo no processo, seguido dos demais fatores. Os parâmetros que afetam a solubilidade do mercúrio total mostram que quando a [AH], T e TC aumentam e o pH diminui há um aumento significativo na concentração de mercúrio total no processo. A maior concentração de mercúrio total foi obtido nas condições de concentração de ácido húmico = 1,0 g L^{-1}, temperatura = 35ºC, pH = 4,0 e tempo de contato = 10 dias.

Palavras-chave: amálgama odontológica, mercúrio total, ácido húmico, temperatura, pH, planejamento experimental.

Introduction

The use of dental amalgam in most developed countries for over 150 years triggered a scientific and political discussion about the rationale for using mercury in amalgam (Saquy, 1996; Mutter *et al*., 2004).

Dental amalgam consists of about 50% metallic mercury (Elizaur Benitez *et al*., 1995; Saquy, 1996; Drummond *et al*., 2003; Mutter *et al*., 2004; Dalla Costa *et al*., 2005).

Dentists have had a more relaxed attitude towards their own exposure and, to some extent, the possibility of causing environmental problems. In recent years, the dental personnel have been aware of their environmental responsibility (Hörsted-Bindslev, 2004).

Its release into traditional waste streams, such as the municipal solid waste stream or the sewer system, and its potential point source discharge into the environment is becoming a major concern.

Studies have found that the dental wastewater stream can contribute from 10 to 70% of the total daily mercury load on wastewater treatment facilities (Berglund, 1999 *apud* Drummond *et al*., 2003).

Installations of effective separators have shown to eliminate up to 91-99% of the mercury in the wastewater from the dental clinic. Thus, this installation does not guarantee low concentration of mercury in the wastewater (Hörsted-Bindslev, 2004).

Mercury (Hg) is moreover distributed in the environment by human activities such as metal smelting and coal production and as uncontrolled waste disposal (dental waste) (Drummond *et al.*, 2003; Hörsted-Bindslev, 2004).

Mercury is one the most hazardous environmental pollutants (Drummond *et al.*, 2003; Leermakers *et al.*, 2005; Coelho-Souza *et al.*, 2005). It exists in a large number of physical and chemical forms with a large variety of properties that determine its complex distribution, biological enrichment and toxicity (Leermakers *et al.*, 2005).

The most important chemical forms are elemental mercury (Hg^0), inorganic mercury (Hg^{2+}), monomethylmercury (MMHg, CH_3Hg^+) and dimethylmercury (DMHg, CH_3HgCH_3) (Leermakers *et al.*, 2005).

In the biogeochemical cycle of mercury, these species may interchange in atmospheric, aquatic and terrestrial environments (Leermakers *et al.*, 2005).

Several main different processes and environmental variables (temperature, pH and chemical composition) affecting the solubility, mobility and chemical form influence metal ions in the aquatic and the geological environmental (Bäckström *et al.*, 2003; Boening, 2000).

The methylation of inorganic mercury occurs by abiotic and biotic processes (Hansen and Danscher, 1997; Coelho-Souza *et al.*, 2005). Abiotic methylation of mercury by methylcobalamin, methyltin compounds, and/or natural organic matter is very likely. Among these three compounds (or classes of compounds) natural organic matter is the most provable methylating agent (Weber, 1993; Melamed and Villas Bôas, 2002).

Natural organic matter (NOM) is the most promising potential methyl donor to mercury. It is a mixture of natural, metal-complexing organic compound present in the aquatic environment or extracted from it.

Humic matter carbon represents 20% of dissolved organic carbon in seawater, 60% in river water and 70% in wetlands (Weber, 1993). This NOM, such as humic and fulvic acids, contains a larger number of functional moieties such as carboxylic, phenolic and alcoholic groups that interact with surface groups as well as ion in solution. Most active groups, in this respect, are titrable and will at the natural pH range associate with dissolved metal ions through inner- as well as outer-sphere complexation (Bäckström *et al.*, 2003).

The presence of dissolved humic acid increases the solubility of total mercury through a mechanism of dissolution-complexation and favors the dispersion of mercury from the source to the environment (Melamed and Villas Bôas, 2002). Similarly, Bäckström *et al.* (2003) reported an increase of mercury concentration when fulvic acid is present and at low pH.

Jahanbakht *et al.* (2002) reported an increase of mercury concentration in water when pH decreased and when temperature increased (0.155 mg m^{-3} to 24ºC and 20.5 mg m^{-3} to 30ºC). Messaitfa (1997) *apud* Jahanbakht *et al.* (2002) noted Hg decrease in water when pH ranged between 7.4 and 8.4.

Melamed and Villas Bôas (2002) reported a significant increase of total mercury concentration in samples when contact time increased.

Approximately 50 to 90% of total mercury in estuaries and coastal water is bound to NOM. Natural organic matter is associated with movement of mercury in water, complexes and methylates mercury (Weber, 1993).

When mercury was methylated, it was accumulated in food chains, particularly in the aquatic milieu where a high degree of biomagnification occurs. The food chain seems to be the predominant route of human exposure to organic mercury compounds (Melamed and Villas Bôas, 2002; Azevedo, 2003; Hörsted-Bindslev, 2004).

The ecological and toxicological effects of mercury are strongly dependent on the chemical species present (Drummond *et al.*, 2003; Leermakers *et al.*, 2005).

MMHg compounds are considerably more toxic than elemental mercury and its inorganic salts. MMHg is efficiently adsorbed from the gastro-intestinal tract, and it passes the blood-brain and placenta barriers. MMHg primarily affects the central nervous system (Cardoso *et al.*, 1999; Azevedo, 2003; Hörsted-Bindslev, 2004; Leermakers *et al.*, 2005).

Due to the toxicity of mercury and the resulting environmental and occupational problems, several countries have adopted regulations to reduce or ban the sale and use of mercury products (Hörsted-Bindslev, 2004).

There are many variables affecting the solubility of mercury in the environment and a factorial design experiment can be used to identify the most important variables (Clark and Stephenson, 1999).

The aim of the present study was to design experiments to examine the effects of operational variables, which may affect the solubility of total mercury as dental amalgam residue in reduction process (abiotic environment without oxygen, which occurs the solubilization and methylation of mercury). Particularly, the significance of the effect of four main experimental variables were examined: humic acid concentration, temperature, pH and contact time, which has been selected as a representative parameter of solubility of total mercury.

Material and Methods

Dental amalgam residue

Dental amalgam residues used in this study were obtained in dental clinic at the State University of Maringá. This residue is an interaction of mercury (50-52%), silver (20-34%), tin (8-15%) and cupper (1-15%) (Dalla Costa *et al*., 2005).

Experimental design

The response variable in this study was the concentration of total mercury (T-Hg).

In order to determine the effect of the operating variables on the concentration of mercury compound of the reduction process, a set of designed experiments was performed.

Table 1 shows the independent factors - x_i, (X_1, X_2, X_3 and X_4), levels and experimental design in terms of coded and uncoded variables.

Table 1. Experimental design for solubility of total mercury in reduction process.

	Natural Variable				Coded Variable			
Expt no.	[HA]	T	pH	CT	X_1	X_2	X_3	X_4
1	0.1	10	4.0	5	-1	-1	-1	-1
2	0.1	10	4.0	10	-1	-1	-1	+1
3	0.1	10	10.0	5	-1	-1	+1	-1
4	0.1	10	10.0	10	-1	-1	+1	+1
5	0.1	35	4.0	5	-1	+1	-1	-1
6	0.1	35	4.0	10	-1	+1	-1	+1
7	0.1	35	10.0	5	-1	+1	+1	-1
8	0.1	35	10.0	10	-1	+1	+1	+1
9	1.0	10	4.0	5	+1	-1	-1	-1
10	1.0	10	4.0	10	+1	-1	-1	+1
11	1.0	10	10.0	5	+1	-1	+1	-1
12	1.0	10	10.0	10	+1	-1	+1	+1
13	1.0	35	4.0	5	+1	+1	-1	-1
14	1.0	35	4.0	10	+1	+1	-1	+1
15	1.0	35	10.0	5	+1	+1	+1	-1
16	1.0	35	10.0	10	+1	+1	+1	+1

A two-level-four-factor factorial design was employed in this study, requiring 16 tests, performed in duplicate.

The variables and their levels selected for the study of mercury solubility were: humic acid concentration [HA] (0.1-1.0 g L^{-1}); temperature [T] (10-35°C); pH (4.0-10.0); and contact time [CT] (5-10 days).

Experimental procedure

Experiments were carried out in 250 mL erlenmeyers with a solution volume of 150 mL that consisted of humic acid solution and 1 g of dental amalgam residue.

The pH was adjusted to the desired value (4.0 and 10.0) using hydrochloric acid (HCl) and sodium hydroxide (NaOH).

Erlenmeyers were incubated in desired temperature (10 and 35°C), below constant agitation (150 rpm) into shaker (Marioni MA 830).

Samples were removed at predetermined intervals (5 and 10 days) in factorial design analysis.

Subsequently to digestion with oxidant mixture – water, hydrochloric acid and nitric acid (3 H_2O: 2 HCl: 1 HNO_3) in "cold finger" reactor, samples were separated by vacuum filtration through a cellulose porous material, with a 0.45 μm pore diameter.

Analytical methods

Mercury was analyzed by cold vapor atomic absorption spectrometry.

Total mercury (T-Hg) was determined by oxidizing all forms of Hg with bromine chloride solution (1 mL of HCl 50% + 1 mL of KB_2O_3 1.5%), before reduction with $SnCl_2$ (Melamed and Villas Bôas, 2002; Wilken and Hintelmann, 1991).

Results and discussion

Table 2 shows the experimental results of reduction process as an average of the duplicate experiments, whose results at each operation condition are shown in Table 1.

A statistical analysis software package (SAS Institute, Inc., Cary, N.C. – version 6.12) was used to analyze the results.

Table 2. Results of total mercury solubility in reduction process.

Expt n°.	[HA] (mg L^{-1})	T (°C)	pH	CT (day)	T-Hg (μm L^{-1})
1	0.1	10	4.0	5	21.7
2	0.1	10	4.0	10	27.7
3	0.1	10	10.0	5	11.7
4	0.1	10	10.0	10	15.0
5	0.1	35	4.0	5	36.0
6	0.1	35	4.0	10	51.3
7	0.1	35	10.0	5	21.3
8	0.1	35	10.0	10	30.3
9	1.0	10	4.0	5	88.0
10	1.0	10	4.0	10	98.3
11	1.0	10	10.0	5	90.0
12	1.0	10	10.0	10	81.0
13	1.0	35	4.0	5	163.7
14	1.0	35	4.0	10	168.7
15	1.0	35	10.0	5	129.7
16	1.0	35	10.0	10	141.3

The analysis of variance for main effects and interactions indicates that the main effects of the variables X_1 (concentration of humic acid), X_2 (Temperature), X_3 (pH) and X_4 (Contact Time), as well as their interaction ($X_1 \cdot X_2$, $X_2 \cdot X_3$, $X_2 \cdot X_4$, $X_1 \cdot X_2 \cdot X_3$, $X_2 \cdot X_3 \cdot X_4$ and $X_1 \cdot X_2 \cdot X_3 \cdot X_4$) affect significantly (P-value$<\alpha=0.05$) the solubility of total mercury in reduction process, with a confidence level of 95%.

The interaction $X_3 \cdot X_4$ was also considered, because P-value is very next to 0.05. Other interactions were not significant to the process.

The analysis of variance to concentration of total mercury (Table 3) indicates that the statistical model can be satisfactorily used ($R^2 = 0.9932$, P-value$<\alpha$).

Table 3. Analysis of variance.

Source of variation	Degree of freedom	Sum of square	Mean square	*F*-value	*P* > *F*
X_1 = [HA]	1	104253.52	104253.52	4663.72	<0.0001
X_2 = T	1	17902.68	17902.68	800.87	<0.0001
X_3 = Ph	1	3417.18	3417.18	152.87	<0.0001
X_4 = CT	1	500.52	500.52	22.39	<0.0001
$X_1.X_2$	1	6279.18	6279.18	280.90	<0.0001
$X_2.X_3$	1	652.68	652.68	29.20	<0.0001
$X_2.X_4$	1	172.52	172.52	7.72	0.0091
$X_3.X_4$	1	88.02	88.02	3.94	0.0559
$X_1.X_2.X_3$	1	204.18	204.18	9.13	0.0049
$X_2.X_3.X_4$	1	93.52	93.52	4.18	0.0491
$X_1.X_2.X_3.X_4$	1	165.02	165.02	7.38	0.0105
Error	32	715.33	22.35		
Corrected total	47	134555.97			

$R^2 = 0.9932$

In the factorial design of experiments, it is useful to consider the factor response relationship in terms of a mathematical model such as a response function. Considering the effecting factors and interactions, it is possible to fit an adequate regression model to the data by using a linear parameter model (Equation 1) as described by Myers and Montgomery (1995) and obtain a first order polynomial equation, so that the response at intermediate factor levels can be predicted.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_1.X_2 + \beta_6 X_1.X_3 + \beta_7 X_1.X_4 + \beta_8 X_2.X_3 + \beta_9 X_2.X_4 + \beta_{10} X_3.X_4 + \beta_{11} X_1.X_2 X_3 + \beta_{12} X_1.X_3 X_4 + \beta_{13} X_1.X_2 X_4 + \beta_{14} X_2.X_3 X_4 + \beta_{15} X_1.X_2 X_3 X_4 + \varepsilon \quad (1)$$

where y is the total mercury concentration, βs are the main interaction coefficients, X_1, X_2, X_3 and X_4 are the coded variables according to Table 1.

The estimated main effects of the four factors and their interaction with the total mercury concentration are summarized in Table 4.

The values of these coefficients were incorporated in Equation 2, which takes the following form:

$$\hat{y} = 73{,}4791 + 46{,}6041\, x_{HA} + 19{,}3125\, x_T - 8{,}4375\, x_{pH} + 3{,}2291\, x_{TC} + 11{,}4375\, x_{HA}.x_T - 3{,}6875\, x_T.x_{pH} + 1{,}8958\, x_T.x_{TC} - 1{,}3541\, x_{pH}.x_{TC} - 2{,}0625\, x_{HA}.x_T.x_{pH} + 1{,}3958\, x_T.x_{pH}.x_{TC} + 1{,}8541\, x_{HA}.x_T.x_{pH}.x_{TC} \quad (2)$$

The effect values presented in Table 4 show that the humic acid concentration [HA] is the most important factor in this process, followed by temperature, pH and contact time.

Table 4. Effects for 2^4 factorial design.

Effects	Estimated effect ± standard error
Mean	73.4791 ± 0.6917
Main effects	
X_1 = [HA]	46.6041 ± 0.6917
X_2 = T	19.3125 ± 0.6917
X_3 = pH	-8.4375 ± 0.6917
X_4 = CT	3.2291 ± 0.6917
Two-factor interaction	
$X_1.X_2$	11.4375 ± 0.6917
$X_2.X_3$	-3.6875 ± 0.6917
$X_2.X_4$	1.8958 ± 0.6917
$X_3.X_4$	-1.3541 ± 0.6917
Three-factor interaction	
$X_1.X_2.X_3$	-2.0625 ± 0.6917
$X_2.X_3.X_4$	1.3958 ± 0.6917
Four-factor interaction	
$X_1.X_2.X_3.X_4$	1.8541 ± 0.6917

In addition, it can be seen that [HA], T and CT have a positive effect, while the pH has a negative effect on the total mercury concentration in reduction process in the range of variation of each variable selected in this study. The interaction between T and pH, between pH and CT, and [HA], T and pH have a negative effect whereas the interaction effect between [HA] and T, between T and CT, between T, pH and CT, and interaction among the four selected parameters have a positive effect on T-Hg concentration. Then, it can be stated by way of statistical analysis that the studied factors were influenced significantly in the total mercury concentration in reduction process.

Applying the Tukey's test, with a level of significance of 5%, the difference between the averages of the two levels of the factors (Myers and Montgomery, 1995) can be determined. Means total mercury concentrations for the two levels of the factors are presented in Table 5.

Table 5 shows the significant difference of the total mercury concentration between the two levels of the factors HA, T and pH (around 93, 38 and 16 μg L^{-1}, respectively). An increase in the humic acid concentration, temperature and contact time, and a decrease in the pH resulted in an increase in solubility of total mercury.

Table 5. Means of total mercury concentration in 2^4 factorial design.

Levels	Variation			
	HA	T	pH	CT
-1	26.875	54.167	81.917	70.25
1	120.083	92.792	65.042	76.708

Tukey's test (α = 0.05)

Figure 1 presents plots of the four main effects in order to assist the practical interpretation of the experiments. The main effect plots are figures of the marginal response averages at the levels of the four factors. Notice that three variables ([HA], T and CT) have positive main effects, that is, increasing the variable, the average deviation from the fill target moves upward, while one variable (pH) has negative main effect, that is, increasing the variable, the average deviation from the fill target moves downward. As can be seen in Figure 1, a change in the levels of each factor produces a different variation in the solubility. An increase in the humic acid concentration from 0.1 to 1.0 mg L^{-1} resulted in an increase in solubility of total mercury of 93.2% (Figure 1a). The same can be observed with the temperature and contact time, in which an increase of temperature from 10 to 35 resulted in an increase in solubility of 38.6% (Figure 1b), and an increase in contact time from 5 to 10 resulted in an increase of 6.5% (Figure 1d). On the other hand, an increase in the pH from 4.0 to 10.0 caused a decrease in the process efficiency of 16.9% (Figure 1c).

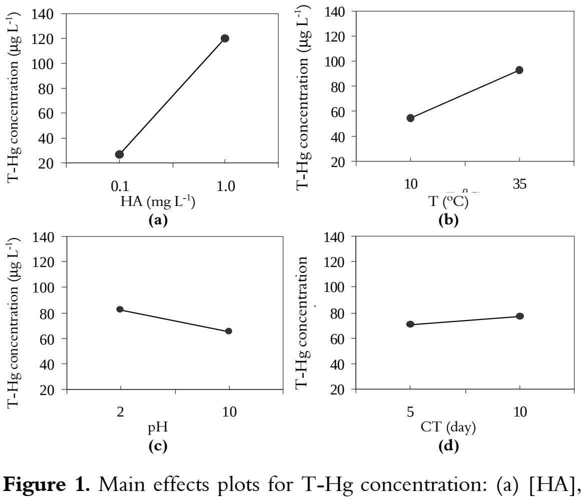

Figure 1. Main effects plots for T-Hg concentration: (a) [HA], (b) T, (c) pH, (d) CT.

As the two-factor interactions among X_1 ([HA]), X_2 (T), X_3 (pH) and X_4 (CT) were also significant, it is necessary to examine the interaction effects, shown in Figure 2. The interaction plots are graphs of the response averages for the T at the fixed levels of the [HA] (Figure 2a), for CT at the fixed levels of the T (Figure 2b) and at the fixed levels of the pH (Figure 2d), and for pH at the fixed levels of the T (Figure 2c). The [HA]-T interaction indicates that the temperature effect is very small when the humic acid concentration is at the low level and large when the [HA] is at the high level, with the high T-Hg concentration obtained with high [HA] and T. Similarly, the contact time interaction indicates that the temperature effect is small when the CT is at the small level and large when the CT is at the high level, with the high T-Hg concentration obtained with high [HA] and T. The T-pH interaction indicates that the T effect is very small when the pH is at the high level and large when the pH is at the low level, with the high T-Hg concentration high temperature and small pH. The pH-CT interaction indicates that the CT effect is very small when the pH is at the high level and large when the pH is at the low level, with high T-Hg concentration obtained with high contact time and small pH.

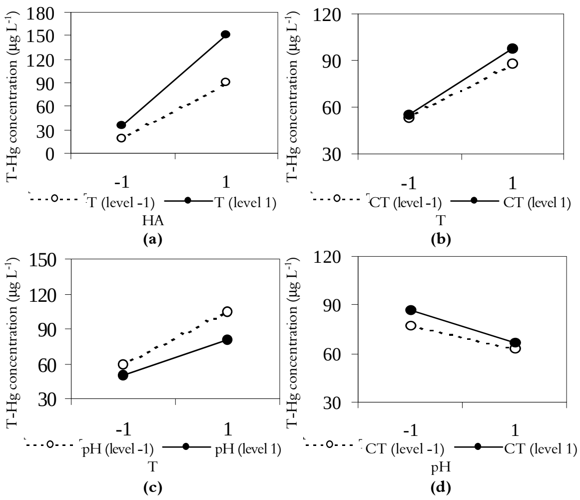

Figure 2. Two-factor interaction plots for T-Hg concentration: (a) [HA]-T, (b) T-CT, (c) T-pH, (d) pH-CT.

As the interaction between AH-T-pH is also significant, by fixing AH at its levels, the averages for pH in the fixed levels of T were calculated (Figure 3a and b); by fixing T at its levels, the averages for pH at the fixed levels of HA were calculated (Figure 3c and d); and by fixing pH in its levels, the averages for the T in the fixed levels of HA were calculated (Figure 3e and f). The AH-T-pH interaction effects indicate that high T-Hg concentration was at the low level of pH and at the high level of AH and T.

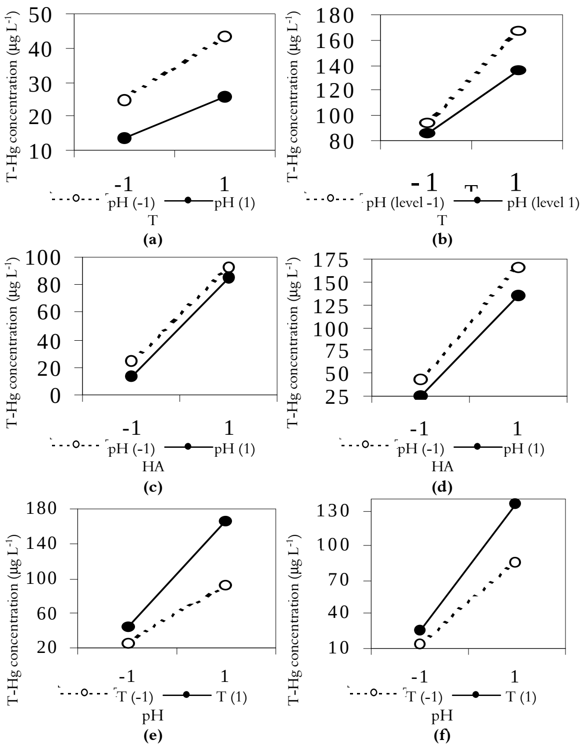

Figure 3. Three-factor interaction plots for T-Hg concentration: (a) [HA]-T-pH to [HA] level -1, (b) [HA]-T-pH to [HA] level +1, (c) [HA]-T-pH to T level -1, (d) [HA]-T-pH to T level +1, (d) [HA]-T-pH to pH level -1, (e) [HA]-T-pH to pH level +1.

Figure 4 shows the T-pH-CT interaction. By fixing T in its levels, the averages for CT in the fixed levels of pH were calculated (Figure 4a and b); by fixing pH in its levels, the averages for CT in the fixed levels of T were calculated (Figure 4.c and d); and by fixing CT in its levels, the averages for the pH in the fixed levels of T were calculated (Figure 4e and f). The T-pH-CT interaction effect indicates that high T-Hg concentration was at the low level of pH and at the high level of T and CT.

Therefore, the high T-Hg concentration was obtained using the [HA] = 1.0 g L^{-1}, T = 35ºC, pH = 4.0 and CT = 10 days.

The results showed that the solubility of total mercury increased with higher humic acid concentration, temperature and contact time and low pH. This was also observed by Jahanbakht *et al.* (2002), Melamed and Villas Bôas (2002), Weber (1993) and Wilken, and Hintelmann (1991).

When the dental amalgam residue is discarded at a low pH and high temperature environment, the existing mercury in this residue solubilizes easily, reacting and forming inorganic mercury. In contact with the humic acid, this compound is methylated and becomes the most toxic compound (methyl-mercury).

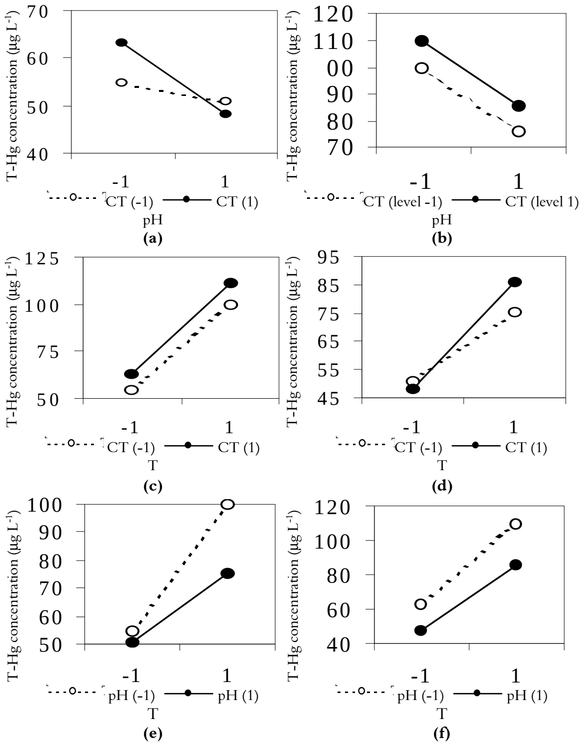

Figure 4. Three-factor interaction plots for T-Hg concentration: (a) T-pH-CT to T level -1, (b) T-pH-CT to T level +1, (c) T-pH-CT to pH level -1, (d) T-pH-CT to pH level +1, (d) T-pH-CT to CT level -1, (e) T-pH-CT to CT level +1.

Conclusion

Based on the factorial design of experiments for total mercury solubility as dental amalgam residue in reduction process, the effects of four factors were identified in the solubility of total mercury: the humic acid concentration, the temperature, the pH and contact time. According to the significance effect obtained in variance analysis, the humic acid concentration was the most significant factor in this process, followed by the temperature, the pH and contact time. The parameters that affect the solubility of total mercury showed that when the [HA], T and CT increase and pH decreases, there is an important increase of total mercury concentration in process. Thus, new experiments should be conducted to analyze the mercury solubility in other systems.

Acknowledgements

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