

Modeling contaminant transport in polyethylene and metal speciation in saliva

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ABSTRACT

Properties of both chemical contaminants and polymers can impact contaminant diffusivity and solubility in new and aged polyethylene materials for pipes and geomembranes. Diffusivity, solubility, polymer and chemical properties were measured for thirteen contaminants and six polyethylene materials that were new and/or aged in chlorinated water. Tree regression was used to select variables, and linear regression was used to develop predictive equations for contaminant diffusivity and solubility in polyethylene. Organic contaminant properties had greater predictive capability than polyethylene properties. Model coefficients significantly changed between new materials to chlorine-aged materials, indicating changes of polyethylene properties impact the interaction between contaminants and polymers.

The metallic flavor of copper in drinking water influences the taste of water and can cause the taste problems for water utilities. The mechanism of metallic flavor caused by these metals is related to free or soluble ions. Free copper concentrations were measured at different pH in diluted artificial saliva using a cupric ion selective electrode. Three major proteins in human saliva: α -amylase, mucin and lactoferrin, were added in the artificial saliva and the impacts on the chemical speciation of copper were analyzed. Inorganic saliva components, typically phosphate, carbonate and hydroxide combined with copper and greatly influenced the levels of free copper in the oral cavity. Proteins such as α -amylase, mucin and lactoferrin also impacted the chemical speciation of copper, with different affinity to copper. Mucin had the greatest affinity with copper than α -amylase.

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Author's Preface & Attribution

This thesis is composed of two manuscripts. Each chapter is a separate manuscript to which several colleagues contributed. A brief description of their background and their contributions are included here.

Chapter 1 presents a predictive model and manuscript developed through the integrated work of Mr. Jia Tang (MS student), Dr. Andrea M. Dietrich, (Professor, Dept. Civil & Environmental Engineering, Virginia Tech and Committee Chair), and Dr. Daniel L. Gallagher, P.E. (Associate Professor Dept. Civil & Environmental Engineering, Virginia Tech and Committee Member). The input data for the model development were obtained from the dissertation of Dr. Andrew Whelton, who was advised by Drs. Dietrich and Gallagher, and their published articles.

Chapter 2 was primarily the combined work of Mr. Jia Tang (MS student) and Dr. Andrea M. Dietrich, (Professor, Dept. Civil & Environmental Engineering, Virginia Tech, and Committee Chair). They were aided by helpful suggestions and insights from Dr. Marc Edwards, (Professor, Dept. Civil & Environmental Engineering, Virginia Tech, Committee Member), Dr. Daniel L. Gallagher, P.E., (Associate Professor Dept. Civil & Environmental Engineering, Virginia Tech and Committee Member), and Dr. Susan E. Duncan (Professor, Dept. Food Sciences and Technology, Virginia Tech).

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Chapter 1

Modeling organic contaminant diffusivity and solubility in polyethylene based on polymer and organic contaminant properties

Abstract

Properties of both chemical contaminants and polymers can impact contaminant diffusivity and solubility in new and aged polyethylene (PE) materials for water pipes and geomembranes. Diffusivity, solubility, polymer and chemical properties were measured for thirteen contaminants and six PE materials that were new and/or aged in chlorinated water. Tree regression was used to select variables, and linear regression was used to develop predictive equations for contaminant diffusivity and solubility in PE. Organic contaminant properties, especially dipole moment and octanol-water partition coefficient, had greater predictive capability than pipe properties. Values of $R^2 > 0.8$ were obtained for new and aged pipe. Model coefficients significantly changed between new PE materials to chlorine-aged PE, indicating changes of PE properties impacted the interaction between contaminants and PE. Bulk density of PE material played a more important role for diffusion but had little influence on solubility for both new and aged PE pipes. Diffusion of polar contaminants was greater in chlorinated water aged polyethylene than new polymers, although the solubility was similar in both new and aged polymers. These results provide guidance for material selection and contamination potential for both water pipes and geomembranes.

Keywords: Modeling, PE pipe, geomembrane, diffusivity, solubility, polymer

1. Introduction

Polyethylene (PE) is a polymer that is widely used throughout the world in drinking water distribution systems and landfill liner applications. PE water pipes are increasingly being applied in buried and building plumbing globally (AWWA 2003; AWWA 1996). High-density polyethylene (HDPE) water pipes have been used for buried water service since the 1940s but their use expanded greatly in the 1990s (AWWSC 2002) and further expanded in the 21st century with the approval of crosslinked polyethylene (PEX) pipes for buried water service (AWWA 2007). HDPE is also widely used as a geomembrane due to its high short-term chemical resistance and impermeability liquid wastes (Tisinger et al. 1991; Koerner 1998; Rowe et al. 2004) and excellent mechanical strength (Aminabhavi and Naik, 1998). PE geomembranes are used as liners for the containment of hazardous and municipal wastes in conjunction with geotextiles or mesh underliners that allow gases to escape and leachates to be collected. These PE materials are flexible, inexpensive, resist corrosion, and are expected to provide decades of service in drinking water systems (Davis et al. 2006) and lifetimes of over 100 years at 35 °C for HDPE landfill liners (Rowe et al. 2009).

A potential problem for the wide use of PE materials is that organic chemicals can diffuse in, out, and through PE and adversely affect water quality of drinking water and groundwater. Aminabhavi and Naik (1999) investigated the transport of 14 non-polar and polar organic solvents that are frequently found as leachates in landfill and impoundment sites and found that all of the chemicals permeated PE materials and could result in soil and groundwater contamination. Non-polar aromatic components of gasoline (benzene and alkylbenzenes) were demonstrated to permeate HDPE drinking water pipes exposed to gasoline contaminated soil in the field (Thompson and Jenkins 1987) and under laboratory applications (Mao et al. 2010). Drinking water distribution systems are vulnerable to intentional or unintentional contamination with neat solvents or organic contaminants; when this occurs, knowledge of permeation of contaminant into the polymer pipe is needed to assess the future use of the distribution system (Clark and Deininger 2002; USEPA 2002). PEs that sorb organic chemicals can also release them to the water or ambient air resulting in widespread environmental contaminant (Saquing et al. 2010).

Environmental conditions, contaminant properties, and polymer properties affect diffusivity and solubility of contaminants (Crank and Park 1968; Comyn 1985; Whelton et al. 2010a; Rowe et al. 2009). Increased temperature results in faster diffusion through a polymer and can also enable polymer chain mobility which increases diffusivity and solubility (Aminabhavi and Naik 1999). Contaminant diffusion and contaminant dissolution in polymers is restricted to polymer free volume or amorphous regions, and contaminants do not diffuse through or reside in highly crystalline/dense regions. Crosslinks generally inhibit contaminant transport and swelling (Guillot et al. 2004; Desai et al. 1998; Sheu et al. 1989; Haxo et al. 1988) and contaminants can interact with polymer additives (e.g., carbon black) (Comyn 2004). An increase in PE bulk density results in a reduction of nonpolar contaminant diffusivity and solubility in PE water pipes (Dietrich et al. 2010; Whelton et al. 2010a). Contaminant size, shape, symmetry, and polarity can also influence polymer interactions and polar contaminants are sparingly soluble in hydrophobic polymers like MDPE and HDPE (Comyn 1985). For instance, polar contaminants (e.g., water, methanol) are sparingly soluble in hydrophobic polymers (Comyn 1985) whereas nonpolar compounds (e.g., toluene and trichloromethane) have moderate to great solubility in PEs.

Oxidation of PE from exposure to disinfectant-containing water, oxygen, or leachate can change polymer surface and bulk properties leading to mechanical failure and changes in contaminant diffusivity and solubility in PE pipes and liners (Rowe et al. 1999; Dietrich et al. 2010). Two concerns for the drinking water and landfill liner industries are: 1) that the current state of knowledge concerning PE performance is much less than that of traditional water distribution materials such as iron and concrete; 2) the effects of PE aging by disinfectants and oxidation on contaminant permeation in PE are not well understood (Imran et al. 2009).

Differences between new and aged PE surface and bulk characteristics must be investigated to determine how PE aging impacts contaminant fate and transport. PE water pipes are known to be attacked by free radicals, including those produced by the disinfectant chlorine dioxide (Colin et al. 2009 a and b), chlorine (Dietrich et al. 2010; Whelton et al. 2010b; Mitroka et al. 2010), and oxygenated hot water (Karlsson et al. 1992) resulting in increased polar carbonyl groups [$>C=O$] on the surface, loss of antioxidants and oxidative resistance (referred to as Oxidation Induction Time or OIT) at the surface and in the bulk polymer, and polymer chain scission that can eventually lead to physical break-down of the polymer (Colin et al. 2009 a and

b). HDPE landfill geomembranes exposed to air, water and leachate also exhibit loss of OIT due to consumption or migration of antioxidants and formation of oxygenated functional groups on the polymer surface (Rimal and Rowe 2009; Rowe et al. 2009).

Contaminant fate in polymers is dependent on the interactions between the contaminant and polymer and is commonly described in terms of solubility (S ; g/cm^3) and diffusivity (D , cm^2/sec) (Crank and Park, 1968). Contaminant solubility can be measured (Equation 1) and diffusion can be calculated by fitting data to a regression using Equation 2 according to Crank (1975) as long as the sample thickness is known. Diffusion and solubility have been previously reported by others to describe neat contaminant interactions with buried HDPE and PEX pipes and HDPE landfill liners (Dietrich et al. 2010; Whelton et al. 2010a; Chao et al. 2007; Chao et al. 2006; Joo et al. 2005; Joo et al. 2004; Aminabhavi and Naik 1999; Park and Nibras 1993).

Equation 1

$$S = \frac{M_{\infty} - M_0}{M_p} \times \rho_{\text{Polymer}}$$

Where

M_{∞} = the mass of contaminant in the saturated polymer (M)

M_p = the initial mass of the polymer (M)

M_0 = the initial mass of contaminant in the polymer which is equal to zero (M)

ρ_{polymer} = the polymer's bulk density (M/L^3).

Equation 2

$$\frac{M_t}{M_{\infty}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left(\frac{-D(2n+1)^2 \pi^2 t}{4\ell^2}\right)$$

Where

M_t = Mass of contaminant in polymer at time t (M)

M_{∞} = Mass of contaminant in the saturated polymer at equilibrium (M)

t = Elapsed time (T)

D = Diffusion coefficient (L^2/T)

ℓ = Half sample thickness (L)

There is very little work focused on comprehensive influence of contaminant and PE properties for predicting contaminant diffusion and solubility in PE materials even though these materials are vulnerable to chemical contamination that may lead to adverse impacts on water quality. The goal of this study was to (1) evaluate published data to determine the polymer and contaminant properties that have essential influence on contaminant diffusion and solubility; (2) develop a model based on these polymer and contaminant properties to predict contaminant diffusion and solubility in different PEs; (3) validate the predictive model through application to other datasets.

2. Materials and Methods

2.1 Experimental laboratory procedures

Previous published research from our laboratory provides detailed experimental procedures for measuring the interaction of contaminants and PEs (Whelton and Dietrich 2009; Whelton et al 2010a; Dietrich et al. 2010). A brief summary is provided below.

An immersion protocol was used to obtain contaminant diffusivity and solubility values for new and aged PE materials. Dog-bone shaped PE pieces were cut using a Dewes Gumbs Die Company, Inc. (Long Island City, NY) microtensile die and triplicate pieces were immersed in neat contaminant. Immersion testing was conducted by placing PE samples inside screw-tight amber vials with polytetrafluoroethylene septa containing 15-20 mL of neat contaminant at 22 ± 1 °C. Periodically, samples were removed for < 30 seconds, quickly blotted with KimWIPES™ to remove any surface contaminant. Weight measurements were made to 0.0001 g using a Mettler-Toledo (Columbus, OH) balance until a constant mass was obtained. The thickness of the PEs was measured using a Mitutoyo electronic outside micrometers (McMaster-Carr). Laboratory chlorinated-water aged PE samples were exposed on all sides to chlorinated water containing 45 mg/L free available chlorine (Cl_2) and 50 mg/L alkalinity as CaCO_3 which was maintained at pH 6.5, 37 °C and darkness. During aging, water sorption did not occur and the surface area did not change. The oxidation induction time decreased and carbonyls functional groups formed on the surface except for PEX-A.

Weight gain over times data was used to calculate solubility and diffusivity coefficients using Equations 1 and 2. Asymptotic 95% confidence interval was also calculated from the standard error using R version 2.7.1 (R Development Core Team 2008). Type I error of 0.05 was applied in all statistical tests.

2.2 Data for model development and validation

Two sets of data were used for this research. The first set was based on measurements taken in our laboratory and was used to develop the predictive model. The second set was a

combination of additional laboratory data as well as literature data. This second set was used to validate the predictive model.

The predictive model was developed based on previously reported for contaminant solubility and diffusivity data from our research group (Whelton et al. 2009; Dietrich et al 2010; Whelton et al 2010a). The thirteen non-polar and polar contaminants and six of their physical/chemical properties are presented in Table 1-1.

Table 1-1 Properties of Contaminants Used for Model Development and Validation.

Contaminant	Contaminant Property ¹					
	M μ , Debye	Mv, cm ³	Mm, g/mol	Sw, %	ρ , g/cm ³	Log Kow
Polar						
Acetonitrile	3.92	53.3	41.05	100	0.786	-0.34
2-Propanone ^{2,3}	2.88	73.1	58.08	100	0.789	-0.24
Benzaldehyde ²	2.80	120.5	106.12	0.3	1.041	1.48
2-Butanone ^{2,3}	2.76	91.4	72.11	22.3	0.805	0.29
Benzyl Alcohol ²	1.71	125.1	108.14	4	1.041	1.10
1-Butanol ²	1.66	96.2	74.12	7.4	0.809	-0.30
2-Propanol ²	1.56	77.8	60.10	100	0.789	0.05
Nonpolar						
Dichloromethane ³	1.60	60.6	84.93	1.303	1.326	1.25
MTBE ^{2,3}	1.36	119.1	88.15	5.1	0.740	1.24
Trichloromethane ^{2,3}	1.01	74.4	119.37	0.729	1.492	1.97
Toluene ^{2,3}	0.36	117.7	92.14	0.0526	0.867	2.73
<i>m</i> -Xylene ²	0.30	135.9	106.16	0.0161	0.864	3.20
<i>p</i> -Xylene	0.00	135.8	106.16	0.0162	0.867	3.15

1. M μ is dipole moment; Mv is molecular volume; Mm is molecular weight; Sw is water solubility; Log Kow is octanol-water partition coefficient.
2. These ten contaminants were tested in aged PEs; all thirteen contaminants were tested in new PE materials;
3. These four contaminants were used for model validation. Contaminants listed for both development and validation used different PE samples for each.

Six polyethylene materials were evaluated. HDPE resin sheets were obtained from McMaster-Carr, Inc. (Atlanta, GA) and new HDPE, PEX-A, and PEX-B pipes were obtained from a commercial supplier. Six parameters were determined for new and chlorine-aged PE pipes: tensile strength at break, tensile strength at yield, elongation, OIT, bulk density, crystallinity; an additional four parameters were measured for new PE materials: crosslink density, temperature of degradation, thickness, and polymer percentage (Dietrich et al. 2010; Whelton et al. 2010a and b). The bulk density and OIT values are presented in Table 1-2 as only these two values are important to the results and discussion.

Table 1-2 Select Polymer Properties of PE Materials Used for Model Development.

PE Material	Pipe Age	Bulk Density (g/cm ³)	Oxidation Induction Time (OIT) (min)
Resin	New	0.9578	22.4
	Aged	0.9581	13.5
Monomodal HDPE	New	0.9494	92.5
	Aged	0.9513	29.7
Bimodal HDPE	New	0.9547	119.6
PEX-A	New	0.9385	33.5
	Aged	0.9389	27.2
PEX-B (1)	New	0.9524	119.6
	Aged	0.9527	5.4
PEX-B (2)	New	0.9510	> 295

Literature data for diffusivity and solubility of organic contaminants in PEs was used to verify the model. Due to the lack of diffusivity and solubility data for polar compounds in literature, new diffusivity and solubility data for one new PE and 3 aged PEs taken from a potable water distribution system, which are listed in Table 1-3, were measured in this research to verify the model. The organic chemicals used were 2-propanone, 2-butanone, MTBE, and dichloromethane, the properties of which are listed in Table 1-1.

Table 1-3 Properties of potable water pipes used for model validation.

Pipe Material	Density (g/cm³)	Pipe Characteristics
PE 1	0.955 ¹	New HDPE pipe
PE 2 ²	0.9504	7 years in service in water distribution system that only applied combined chlorine
PE 3 ²	0.9513	20 years in service in a water distribution system :18 years free available chlorinated water and 2 years exposure to chloramines
PE 4 ²	0.9504	25 years in service in a water distribution system that only applied free available chlorine

1. Data provided by manufacturer.

2. Data from Dietrich et al. 2010.

2.3 Tree Regression

Tree regression (Lewis 2000; Venables and Ripley 2002; Crawley 2007; Zuur et al 2007; Cheng, Zhang et al. 2009) were generated in R to determine which contaminant and PE property had the greatest effect on contaminant transport. Constructing tree regressions may be seen as a type of variable selection because regression trees indicate the relative importance and interactions of the different factors. Regression trees are a type of predictive model that progressively select the critical regression variables in the order of their impact, or importance, to the model. In the first step, the regression splits the data into two groups or branch based on the most important variable. Tree regressions used for this analysis use binary recursive partitioning to sequentially split the data into two branches so that the difference in the response variable (solubility or diffusivity) between the two branches is greatest. The tree construction process takes the maximum reduction in deviance over all allowed splits of all leaves to choose the next split. The approach is computationally intensive because each split calculates the possible deviance reduction for all possible values of all explanatory variables.

In this study, tree regressions, in which the independent variables comprised both the organic contaminant properties (dipole moment, molecular volume, molecular weight, water solubility, density, octanol-water partition coefficient etc.) and PE material properties (density, oxidation induction time, crystallinity etc.), were performed separately for diffusion and solubility for both new pipe and aged pipe. The results of tree regressions visually demonstrated the significant variables for diffusion and solubility and provided for variable selection for the models.

2.4 Linear regression

In this study, a multiple regression with maximum sixteen independent variables containing six contaminant properties (Table 1-1) and ten polymer properties for new PE pipe and only six polymer properties for aged pipe were used to build the model.

The dependent variables were diffusion coefficients and solubility (6 PEs x 13 contaminants = 78 for new pipes; 4 PEs x 10 contaminants = 40 for aged pipes); the independent variables consisted of contaminant and pipe properties (6 contaminant properties + 10 pipe properties = for new pipes and 6 contaminant properties + 6 pipe properties = 12 for aged pipes). An indicator variable with 0 for new pipe and 1 for aged pipe was created to determine the changes in slopes and intercepts of the models after pipes were exposed to chlorinated water.

Variables were selected based on the correlation between each other, the p value of the slope for each variable, results of tree regressions, and the contribution to the multiple R square for the regression model. Residuals analysis was used to illustrate the goodness of the regression and to check regression assumptions.

A two tailed paired t-test ($\alpha = 0.05$) was used to compare the validation data set of previously published data in literature and our laboratory experiment with the predicted data from our models.

3. Results and discussion

3.1 Evaluating influence of contaminant and PE properties on diffusion and solubility using tree regression

The contaminant solubility and diffusivity data for new PE materials used to develop this model were previously published (Whelton et al. 2010a); data for contaminant interactions with aged PE materials are also published (Dietrich et al, 2010; Whelton 2009; Whelton et al. 2010b). Figure 1-1 shows the tree regression result for the contaminant diffusion in new PE materials. The interpretation of the regression indicates that diffusion was principally and equally controlled by either by water solubility or log Kow of the organic contaminants. At high water solubility ($S_w \geq 2.65\%$) or low octanol-water partition coefficient ($\log K_{ow} < 1.245$), the diffusivity value averaged $7.275 (*10^{-9} \text{ cm}^2/\text{s})$. At low water solubility ($S_w < 2.65\%$) or high octanol-water coefficient ($\log K_{ow} \geq 1.245$), molecular volume also became an important factor. These results could be explained from a chemical perspective: lower water solubility and high octanol-water coefficient are associated with more hydrophobic compounds which are more soluble in PE materials than water; small molecular volumes allowed non-polar molecules to more easily penetrate into the free volume or amorphous regions of PE. This trend was also reported by Joo et al. (2005).

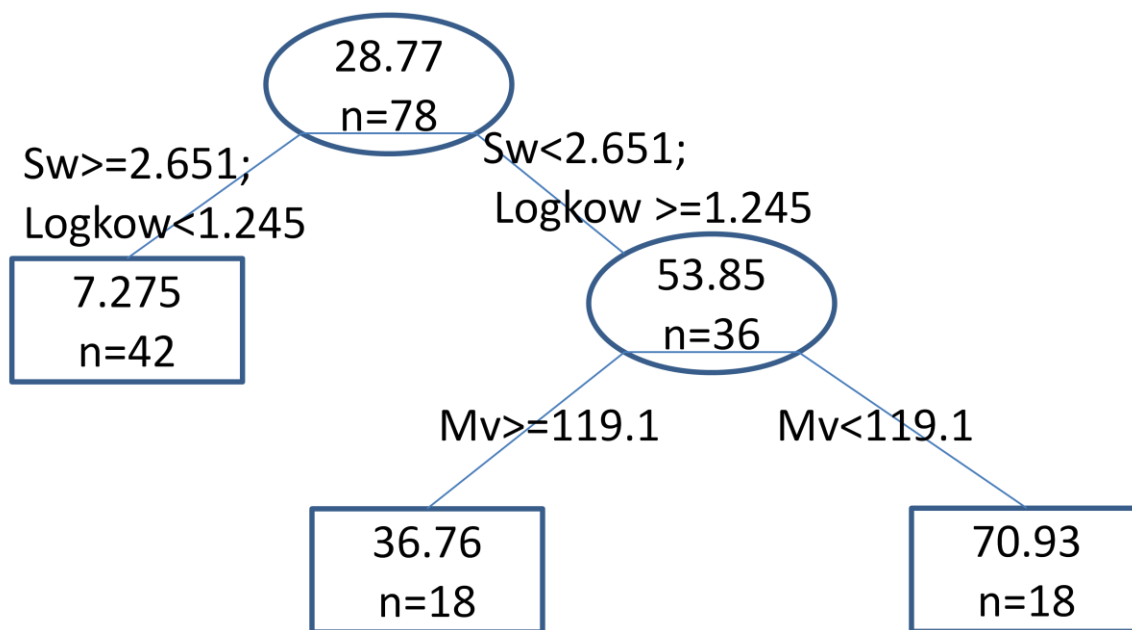


Figure 1-1 Tree regression for contaminant diffusion in new PE materials; Mv is molecular volume; log Kow is log octanol-water coefficient; Sw is water solubility. The number in the box represent mean diffusivity for corresponding group; N= number of data points in group.

The tree regression result for the contaminant solubility in new PE materials is shown in Figure 1-2. The octanol-water partition coefficient, which is a ratio of the solubility of a contaminant in non-polar octanol and polar water and typically reported as LogKow, is the principal property. At higher logKow (≥ 1.17), which indicates the contaminant is more soluble in non-polar materials, chemical polarity, as measured by dipole moment, played an important role. Low polarity chemicals ($\mu \leq 1.185$) had stronger interaction with non-polar polymers, which resulted in higher solubility in PE materials. However, solubility did not increase continuously with the decrease of polarity, but decreased after dipole moment fell below $\mu = 0.33$. To interpret this phenomenon, the properties of the polymers needs to be considered. The polarity of the polymer is not likely zero because its structure is not absolutely symmetrical (Sahebiana et al. 2009) and presence of phenolic antioxidants or X-linking. Thus, contaminants with very low polarity ($\mu < 0.33$) or absolutely no polarity did not interact with polymers as well as those contaminants which have similar polarity with the polymer. In the third node of this figure, several parameters including dipole moment, solubility parameter, molecular volume etc equally divided the node with 24 data points. This may due to the repetition of these parameter in the solubility data with the same organic contaminants or the same PE materials. This cannot distinguish the order of importance of these parameters because the total data points is limited.

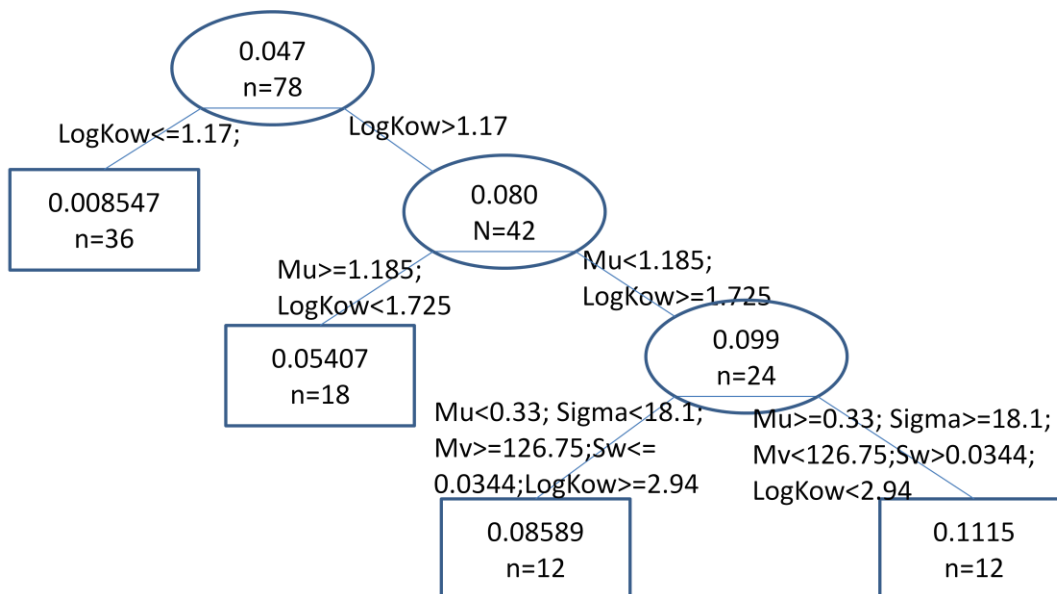


Figure 1-2 Tree regression for contaminant solubility in new PE materials Mu is dipole moment and is a measure of contaminant polarity; logKow is octanol-water partition coefficient; Sigma is solubility parameter . The number in the box represent mean diffusivity for corresponding group; N= number of data points in group.

Figure 1-3 and Figure 1-4 show the tree regressions for diffusion and solubility in aged PE pipe. In both figures, water solubility no longer plays an important role and contaminant polarity, as measured by dipole moment or octanol-water partition coefficient, was the dominant factor controlling the diffusion and solubility. Similar to the effect of contaminant polarity in new PE pipe, there is not a linear relationship between contaminant polarity and diffusion as well as solubility, but a trend of decreasing and then increasing diffusion and solubility with the increasing of contaminant polarity. The surface oxidation of polymers may contribute to this result. As polar groups ($>C=O$, $-OH$ etc.) were produced and polymer chain scission occurred in disinfected water, the PE surface become more polar (Colin et al. 2009; Whelton and Dietrich 2009; Dietrich et al. 2010), which increased the importance of polarity in contaminant diffusion and solubility.

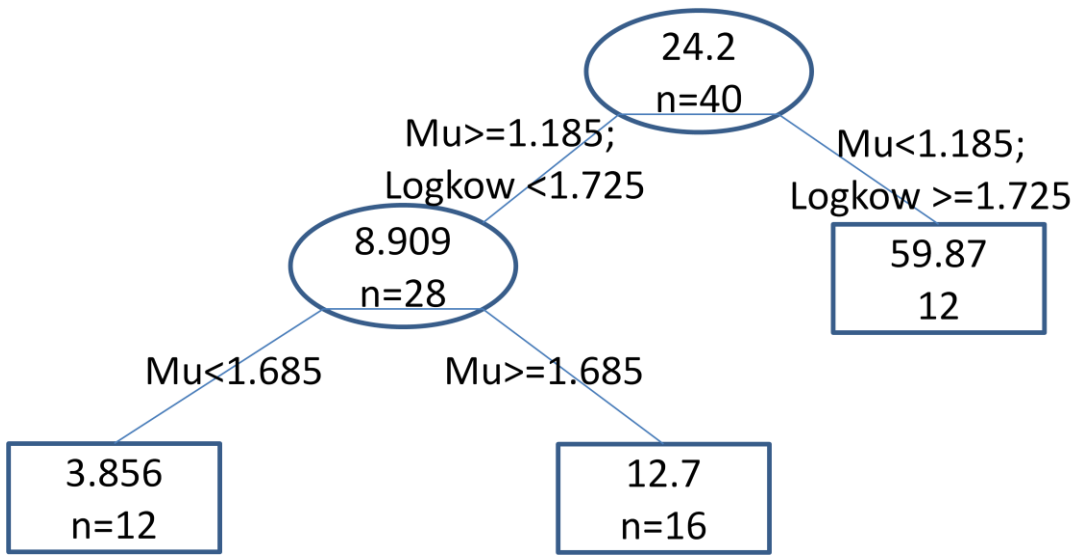


Figure 1-3 Tree regression for contaminant diffusion in aged PE materials; Mu is dipole moment and is a measure of contaminant polarity. The number in the box represent mean diffusivity for corresponding group; N= number of data points in group.

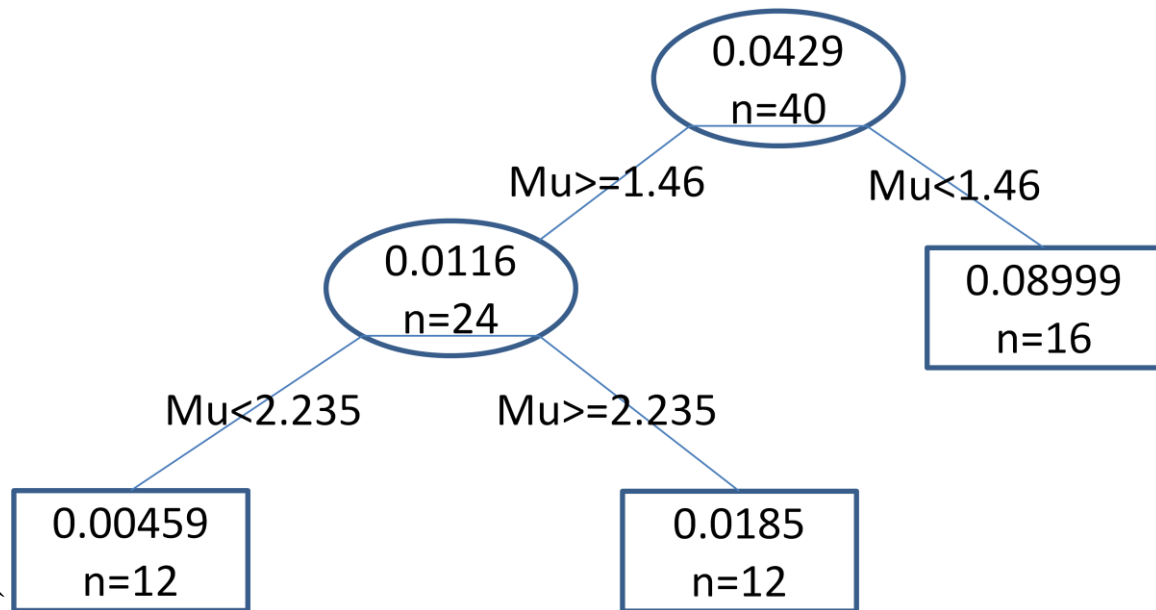


Figure 1-4 Tree regression for solubility in aged PE materials; Mu is dipole moment and is a measure of contaminant polarity. The number in the box represent mean diffusivity for corresponding group; N= number of data points in group.

3.2 Linear regression

Variable selection

Tree regression is the first step to select variables but the approach is not suited for predictive modeling except in the broadest sense. For sixteen variables of new pipe and twelve of aged pipe, a correlation matrix of these explanatory variables was created to check the correlation between each variable pair and those with high correlation coefficient were taken out. After this step, linear regressions were run in R based on the remaining variables. According to the p value for each coefficient (slope and intercept) and the contribution to R^2 , three properties (molecular polarity, molecular volume and octanol-water partition coefficient) of organic chemical and one property (bulk density) of polymer were chosen for the final model (Table 1-4).

Bulk density of the PE material played a more important role for diffusion but had little influence on solubility for both new and aged PE pipes. R^2 for regressions with bulk density as a factor was only 0.05 greater than that without bulk density for diffusion in new pipe while there was only 0.01 increase for solubility, which indicated that bulk density was more important in predicting diffusion than predicting solubility. For aged pipe, the same result was found. This finding was expected since diffusion is a process of the contaminant penetrating into the polymer and it is impacted by the physical pore space of the polymer which is related to bulk density, while solubility is the chemical state of contaminant interaction with the polymer. Interestingly, OIT had no role in predicting contaminant diffusion or solubility even though this property is acknowledged to change greatly in new and aged pipe materials (Table 1-2) and geomembranes (Rowe et al. 2009).

Table 1-4 R² in linear regression.

New PE		Variables in Model			R²
Diffusion	M μ	Mv	Log Kow		0.777
	M μ	Mv	Log Kow	Bulk density	0.828
Solubility	M μ	Mv	Log Kow		0.851
	M μ	Mv	Log Kow	Bulk density	0.860
Aged PE					
Diffusion	M μ	Mv	Log Kow		0.749
	M μ	Mv	Log Kow	Bulk density	0.817
Solubility	M μ	Mv	Log Kow		0.835
	M μ	Mv	Log Kow	Bulk density	0.845

M μ is dipole moment; Mv is molecular volume; log Kow is octanol-water partition coefficient.

Model equations

The relationship between contaminant diffusion and solubility in PE pipe and selected variables was established by linear regression. Data in Table 1-5 presents the coefficients of all the intercepts and slopes for these models expressed by the following equation.

The negative and positive signs of the coefficients indicated that both diffusion and solubility decreased with the increasing of polarity and molecular volume but increased with increasing octanol-water partition coefficient for both new and aged pipes, which agreed with the finding by other researchers (Berens and Hopfenberg 1982; Islam et al. 2008; Müller et al. 1998; Sangam and Rowe 2001, 2005; Joo et al. 2004, 2005). These results can be explained by chemical and physical interactions. Weak-polar or non-polar contaminants have strong interaction with polymers which are also weak-polar or non-polar. Small molecules with small molecular volumes can easily penetrate polymers and also easily stay in the pore cavities of polymers, resulting in the high diffusion and solubility. Higher octanol-water partition coefficient means lower water solubility of contaminants which trends to be compatible with polymers.

Table 1-5 Coefficients for linear regression models.

Models	Model Coefficients					
	Intercept	M μ	M v	Log Kow	Bulk density	R ²
Diffusion in New pipe	1141.0	-7.21	-0.943	25.06	-1092.0	0.828
Diffusion in Aged pipe	979.6	-4.24	-0.718	22.38	-947.4	0.817
Change	-161.8* (-14%)	2.97* (41%)	0.225* (24%)	-2.68* (-11%)	144.5* (13%)	-
Solubility in New pipe	0.7174	-0.0113	-0.0011	0.0358	-0.6163	0.860
Solubility in Aged pipe	0.7363	-0.0059	-0.0013	0.0405	-0.6274	0.845
Change	0.0189* (3%)	0.0054* (48%)	-0.0002* (-18%)	0.0047* (13%)	-0.0111* (-2%)	-

1.* Significant different from 0 ($p>0.05$); units of diffusion, solubility, dipole moment, molecular volume, Log Kow and polymer density are 10^{-9} cm²/s, g/cm³, Debye, cm³/mol, and g/cm³ respectively.

2. R²s of the Regression based on all 19 variables only increased slightly compared with that based on 4 variables.

Comparison between new and aged pipe

By comparing the coefficients of these models between new and aged pipe, the impact of parameters on contaminant-polymer interactions can be analyzed and understood. All the changes of intercepts and slopes were significantly different from 0 ($p>0.05$), indicating that the aging of PE materials impacted the interactions between contaminants and polymers. The solubility and diffusion for new and aged PE materials cannot be modeled by one single equation.

Specifically, the intercept for diffusion decreased from new pipe to aged pipe, coefficients for polarity, molecular volume and bulk density increased, while the coefficient for log Kow decreased. Since slopes for polarity, molecular volume and bulk density were negative, they decreased with respect to absolute value. All decreasing coefficients demonstrated that the diffusion trended to be uniform for aged PE materials, changing slowly with the change of selected factors of influence. However, it is hard to say which was greater between new and aged PEs, since they were depended on the relative values of these properties considered. Islam and Rowe (2008; 2009) found that the diffusive migration of aqueous VOCs through HDPE geomembrane was less for an aged geomembrane than for a new geomembrane, while Dietrich et al. 2010 and Whelton et al. 2010b reported that the neat contaminant diffusion through new HDPE materials was greater than aged HDPE materials. For solubility, the intercept and

coefficients for molecular volume and octanol-water partition coefficient increased, while coefficient for polarity decreased, all with respect to the absolute value. Therefore, aging seemed to weaken the influence of polarity on solubility, which appears to conflict with the results of tree regression. However, the p-value for coefficient of polarity for aged PE materials was 0.228, which was greater than 0.05, indicating a weak linear relationship between contaminant polarity and solubility in PEs. Therefore, polarity had a complex impact on contaminant solubility in PEs rather than linear correlation.

R^2 values for both diffusion and solubility regressions reduced about 0.01 from new to aged PE. This slight decrease demonstrated that the oxidation of polymers raised the complexity of the interactions between contaminants and polymers.

3.3 Model validation

For all the linear regressions, R^2 were greater than 0.8, which displayed acceptable reliability of this model based on experimental data. Joo et al. (2005) reported that mass transport parameters of contaminants through a HDPE geomembrane could be estimated by the properties of organic contaminants such as octanol-water partition coefficient, aqueous solubility, and molecular diameter, which agreed with our finding. Unfortunately, a high R^2 value does not guarantee that the model will be predictive for data of other researchers. Therefore, several sets of contaminant diffusion and solubility data from previous work of other researchers and additional data from our laboratory (Figure 1-5) were investigated and compared with those calculated by this model.

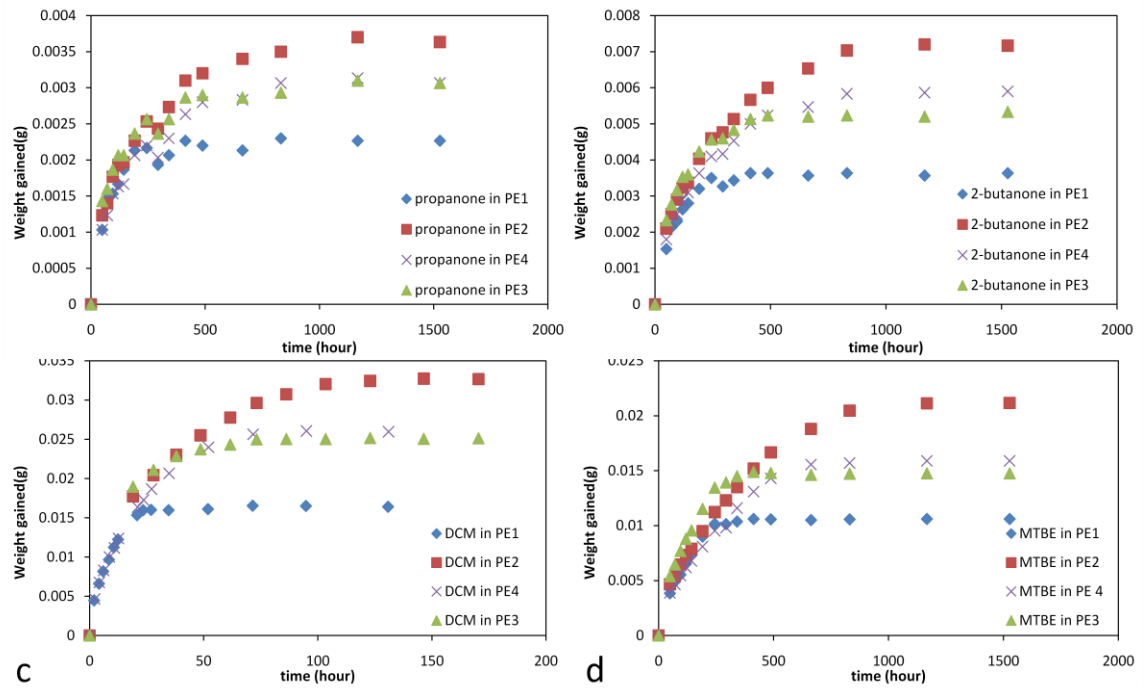


Figure 1-5 Adsorption curves for propanone, 2-butanone, dichloromethane (DCM) and MTBE in 4 PEs. Data is based on the average values of 3 replicates for each chemical in each PE.

Table 1-6 Comparing measured and predicted diffusivity and solubility for PE data from the literature. A two tailed t-test was used for comparison and data were not significantly different ($p>0.05$).

Contaminant	Diffusivity, $10^{-7} \text{ cm}^2/\text{s}$		Solubility, g/cm^3	
	Measured	Predicted	Measured	Predicted
Dichloromethane	0.87 ⁽¹⁾	0.63	0.08 ⁽¹⁾	0.09
	0.82 ⁽²⁾	0.63	0.12 ⁽²⁾	0.09
	0.85 ⁽⁴⁾	0.57	0.0767 ⁽⁴⁾	0.1024
	1.09 ⁽⁴⁾	0.56	0.0748 ⁽⁴⁾	0.1019
	0.71 ⁽⁴⁾	0.57	0.0753 ⁽⁴⁾	0.1024
	0.69 ⁽⁴⁾	0.61	0.0754 ⁽⁴⁾	0.0888
	0.27 ⁽¹⁾	0.52	0.05 ⁽¹⁾	0.07
	0.42 ⁽²⁾	0.52	0.06 ⁽²⁾	0.07
Trichloromethane	0.51 ⁽¹⁾	0.69	0.14 ⁽¹⁾	0.10
	0.79 ⁽³⁾	0.69		
Toluene	0.61 ⁽¹⁾	0.71	0.09 ⁽¹⁾	0.11
	0.68 ⁽²⁾	0.71	0.11 ⁽²⁾	0.11
	0.53 ⁽³⁾	0.71		
2-Butanone	0.10 ⁽⁴⁾	0.097	0.0166 ⁽⁴⁾	0.0190
	0.13 ⁽⁴⁾	0.088	0.0162 ⁽⁴⁾	0.0185
	0.08 ⁽⁴⁾	0.097	0.0163 ⁽⁴⁾	0.0190
	0.08 ⁽⁴⁾	0.010	0.0163 ⁽⁴⁾	0.0095
MTBE	0.055 ⁽⁴⁾	0.16	0.0562 ⁽⁴⁾	0.0274
	0.104 ⁽⁴⁾	0.148	0.0472 ⁽⁴⁾	0.0268
	0.054 ⁽⁴⁾	0.157	0.0479 ⁽⁴⁾	0.0274
	0.057 ⁽⁴⁾	0.071	0.0489 ⁽⁴⁾	0.0268
2-Propanone	0.129 ⁽⁴⁾	0.089	0.0083 ⁽⁴⁾	0.0179
	0.146 ⁽⁴⁾	0.080	0.0087 ⁽⁴⁾	0.0173
	0.090 ⁽⁴⁾	0.089	0.0084 ⁽⁴⁾	0.0178
	0.091 ⁽⁴⁾	0.020	0.0100 ⁽⁴⁾	0.0067
P of t-test	0.3865		0.8617	

- (1). Chao KP; Wang P; Wang YT. 2007. Diffusion coefficients and solubility coefficients of aromatic and chlorinated hydrocarbons in HDPE geomembranes were obtained using the steady state permeation and sorption data from ASTM F739 and immersion methods, respectively;
- (2). Aminabhavi TM; Naik HG. 1999. Aminabhavi et al. measured and calculated diffusivities of 14 organic liquids in HDPE geomembranes;
- (3). Dietrich AM; Whelton AJ; Gallagher DL. 2010. Dietrich et al. measured diffusivity and solubility for aged HDPE pipes removed from disinfected drinking water distribution systems;
- (4). The measured data are from experiments in this research.

Although all the data used to develop the predictive model and its validation used the same immersion method, different researchers determined different diffusivities and solubilities data, which might result from slight difference of experimental conditions. A two tailed paired t-test was used for comparing the literature experimental data with fitted data in the regression models, results of which were displayed in Table 1-6. All the measured experimental data and fitted data were not significant different ($p>0.05$), indicating that the model is a good predictor of the contaminant diffusion and solubility.

Further validation was conducted by regressing the predicted versus measured/literature values for the validation set (Figures 1-6 and 1-7). For both diffusivity and solubility, the slopes were not significantly different than 1 and the intercepts were not significantly different than 0, indicating that the predictive model is suitable for predicting solubilities and diffusivities of contaminant-PE pairs not in the original calibration data set. The residual standard errors, a measure of the average error between predicted and measured values, were $0.17 (10^{-7}\text{cm}^2/\text{s})$ and $0.019 (\text{g}/\text{cm}^3)$ for diffusivity and solubility respectively.

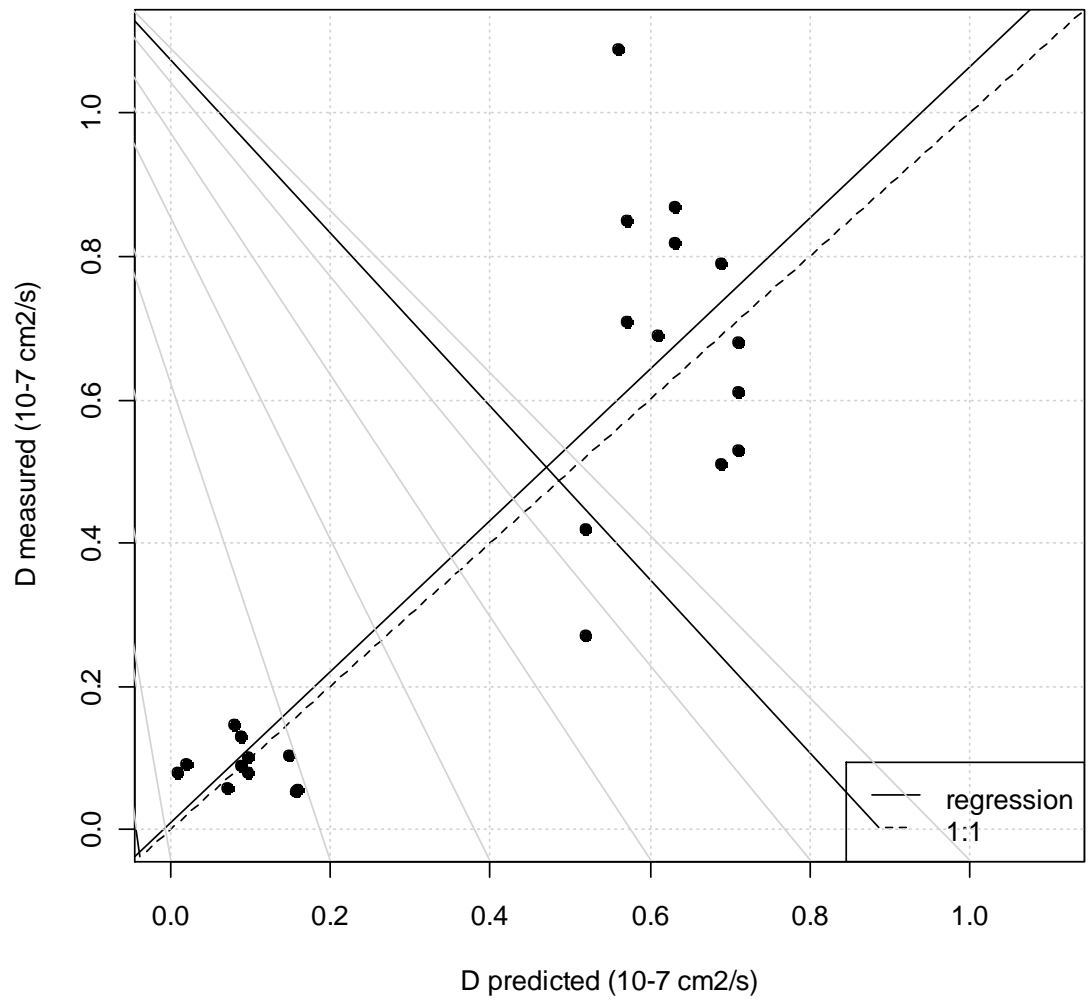


Figure 1-6 Model validation for diffusivity.

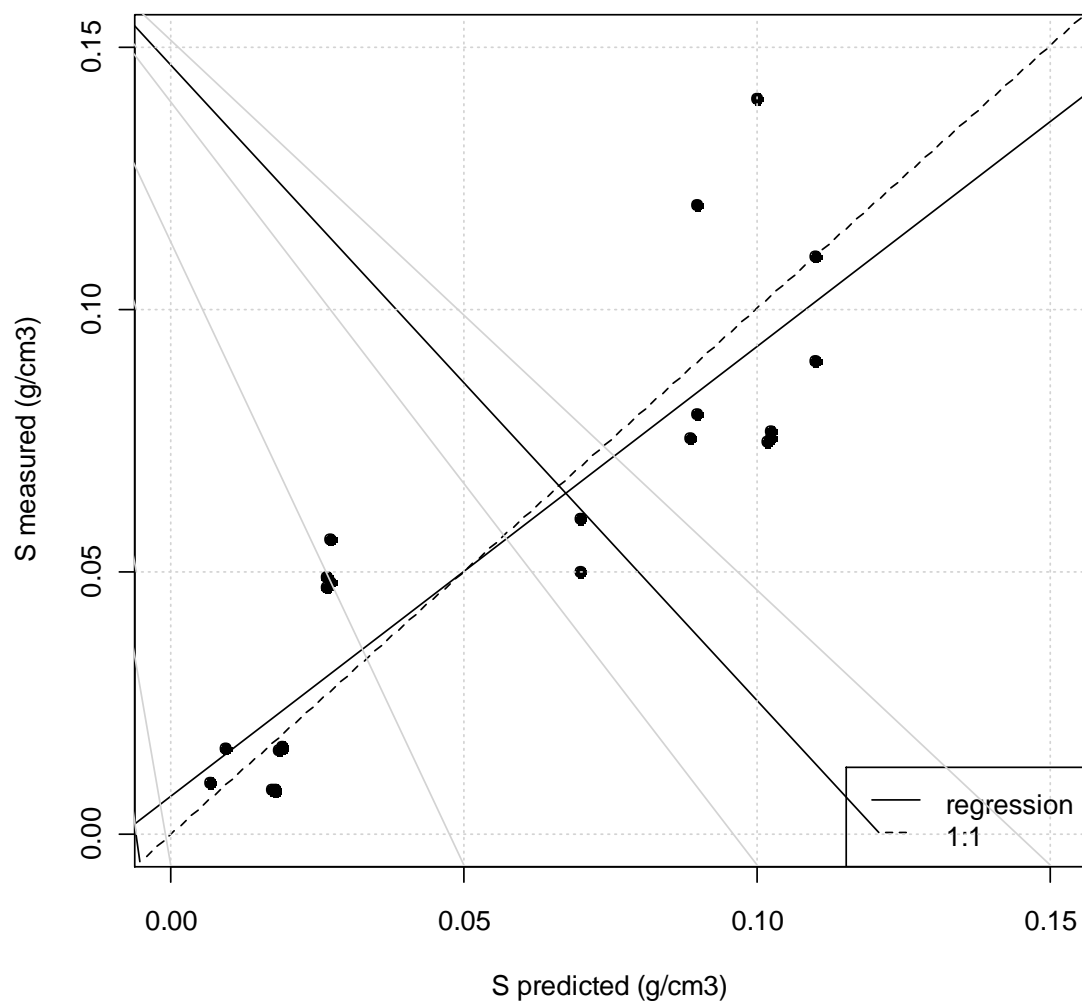


Figure 1- 7 Model validation for solubility.

4. Conclusion

Neat contaminant diffusion and solubility in new and aged polyethylene can be predicted based on the properties of both contaminants and polymers. The contaminant properties of water solubility, octanol-water partition coefficient and molecular volume were major determinants for contaminant solubility and diffusivity; PE bulk density played a minor role with less dense polyethylene materials more susceptible to contamination. The coefficients of the models demonstrated that the aging of PE materials impacted the interactions between contaminants and

polymers. Bulk density of PE material played a more important role for diffusion but had little influence on solubility for both new and aged PE pipes. Polar contaminants diffused faster into chlorinated water aged polyethylene, although the solubility was similar in both new and aged polymers. Two predictive equations are necessary because of the significant differences between new and aged HDPE materials.

These results will aid in selecting materials for both pipes and geomembrane liners and assessing contamination potential in PEs.

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Chapter 2

Chemical speciation of metals in artificial saliva

Abstract

The metallic flavor of copper, iron and zinc in drinking water influences the taste of water and arouses a taste problem for water utilities. The mechanism of metallic flavor caused by these metals is unclear, but previous researchers reported the importance of free or soluble ions on the taste intensity of these three metals, which reflects on the thresholds of metals in different forms of solutions, soluble species or precipitates. Free copper concentrations were measured at different pH values in diluted artificial saliva using a cupric ion selective electrode. Three major proteins in human saliva; α -amylase, mucin and lactoferrin were added to the artificial saliva and their impacts on the chemical speciation of copper were analyzed. Inorganic components, typically phosphate, carbonate and hydroxide combined with copper and greatly influenced the levels of free copper in oral cavity. Proteins such as α -amylase, mucin and lactoferrin also impacted the chemical speciation of copper, with different affinity to copper. Mucin had the greatest affinity with copper, followed by α -amylase, while lactoferrin had very weak binding capacity. Inorganic components dominated in the chemical speciation of copper when no levels of mucin existed in artificial saliva. When the concentration of mucin was raised to > 0.216 mg/L, free copper is controlled by the concentration of mucin.

Keywords: Copper, iron, zinc, artificial saliva, flavor, chemical speciation

1. Introduction

1.1 Copper, Iron and Zinc for Human Health

Copper is an essential element necessary for enzymes and proteins indispensable for life, and adverse health effects may happen due to deficient or excess in copper intake (Dameron 1998). World Health Organization (WHO) recommends a daily intake of 30 µg/kg body weight (WHO 1998). Copper deficiency is a well-documented cause of neurologic disease and hematologic abnormalities (anemia, neutropenia, and leukopenia) in adults known of for many years. In newborns, copper deficiency can cause devastating neurologic disease, most often being seen in Menkes disease (Griffith et al. 2009). Besides the lack of sufficient source of copper intake, copper deficiency can be caused by chronic excessive zinc consumption, such as may occur in those who overuse denture adhesive compounds, because zinc competes with copper for absorption by the gut. From available data on human exposures worldwide, particularly in Europe and the Americas, the risk of health effects from deficiency of copper intake exceeds that from excess copper intake (Dameron 1998).

Copper levels in seawater of 0.15 µg/L and in fresh water of 1-20 µg/L are found in undefiled areas (Dameron 1998). Human activities increase the copper concentrations, and the major sources of release are mining operations, agriculture, solid waste and sludge from treatment works. However, unlike the relatively low range of µg/L concentrations in freshwater, copper concentrations in drinking water vary significantly, from 0.005 to 18 mg/L in variety of flushed and standing drinking water samples from throughout the USA (USEPA 1991; Dietrich et al. 2004). Copper in drinking water makes a substantial additional contribution to the total daily intake of copper, particularly in households where water travel through copper pipes that can be corroded. Intakes of copper from food and drinks varies with different dietary habits as

well as different agricultural and food processing used worldwide (Dameron 1998). Copper concentration in drinking water varies according to the chemical and physical properties of the water, including natural mineral content, pH, hardness, anion concentrations, oxygen concentration, temperature, as well as the characteristics of the plumbing system (Zacarias, et al. 2001).

Although an essential element for human, high copper exposure can cause negative effects to human health. In drinking water studies where consumption and concentration were not controlled, copper at about 1.3 mg/L in tap water was indicated as a source of gastrointestinal related illness (Knobeloch et al. 1994). Between 1998 and 2003, potentially 4.4 million American people in 1400 US communities were exposed to tap water containing copper at levels above 1.3 mg/L (EWR 2005). Pizarro et al. (1999) reported that more than 3 mg/L of copper in drinking water could cause nausea, vomiting, and abdominal pain. In a follow-up study, in which subjects consumed copper-augmented drinking water on a daily basis, a dose-response relationship with higher copper levels producing more GI effects was found. Data from accidental or intentional ingestion of copper salts indicate that acute toxemia and possible death can occur when an adult ingests one gram of copper or more (NRC 2000).

Iron is also an essential element for human. It plays an important role in the synthesis of the oxygen transport proteins, hemoglobin and myoglobin, in the formation of heme enzymes and other iron-containing enzymes that are important in energy production, immune defense and thyroid function (Roeser 1986; Allen 2001). Lack of Fe will lead to lower hemoglobin levels and even anemia. According to the National Heart, Lung and Blood Institute (NHLBI), iron deficiency anemia affects 3.5 million of Americans. In some developing countries, 50% or more of infants, children and women of childbearing age may suffer from anemia (Demaeyer 1985).

Iron-deficiency anemia can decrease mental and physical development in children, decrease work performance and decrease immunity to infection (Scrimshaw 1984; Hercberg 1987; Lozoff 1991). Iron supplements, usually with iron sulfate, ferrous gluconate, or iron amino acid chelate ferrous bisglycinate will usually correct the anemia. Water soluble compounds have the highest bioavailability, but the changes in color and flavor of foods cause sensory problems (Hurrell 1985; Moretti et al. 2005). Iron poisoning would occur by a large excess of iron intake. It has been primarily been associated with young children who consumed large quantities of iron supplement pills, which resemble sweets and are widely used, including by pregnant women. But since 1978 when targeted packaging restrictions in the US for supplement containers with over 250 mg elemental iron have existed, iron poisoning have reduced from several fatalities per year to zero(Litovitz et al. 1989; Tenenbein 2005).

Zinc is another essential trace element, which is essential for normal cellular growth and development, and is an important component of nearly 300 enzymes involved in protein, nucleic acid, carbohydrate and lipid metabolism (Swanson 1987; Valder-Ramos 1992). Dietary Zn deficiency is very prevalent in the developing countries (Sanstead 1995; Cavdar 2000), where nearly two billion people are deficient in zinc (Prasad 2003). In children it causes an increase in infection and diarrhea, contributing to the death of about 800,000 children worldwide per year (Hambidge 2007). The World Health Organization advocates zinc supplementation for severe malnutrition and diarrhea (WHO 2007). Zinc supplements help prevent disease and reduce mortality, especially among children with low birth weight or stunted growth (WHO 2007).

1.2 Standard and Regulation of Copper, Iron and Zinc

Drinking water standards have been established to prevent adverse health effects resulting from excess ingestion of copper. WHO (1998) recommends a limit of 2 mg/l Cu to

prevent adverse health effects from copper exposure. In addition to the adverse health effects of excessive copper ingestion, the water with relatively high concentration of copper can cause bitter, astringent, sour, salty, or metallic flavor (Zacarias et al. 2001; Lawless et al. 2005; Cuppett et al. 2006; Dietrich 2009). WHO guidelines also state that a long-term intake of copper between 1.5 and 3 mg/l has no adverse health effects but levels greater than 5 mg/l in water can impart an undesirable bitter taste (Cuppett et al. 2006). The US Environmental Protection Agency (USEPA) developed a health-based action level of 1.3 mg/l Cu in drinking water (USEPA, 1991) and an aesthetic-based standard of 1 mg/l Cu.

Based on the situation that iron and zinc are not primary contaminants in drinking water, they are not listed in the National Primary Drinking Water Regulations. However, they are in the National Secondary Drinking Water Regulations, which set non-mandatory water quality standards for 15 contaminants. The secondary maximum contaminant level is 0.3 mg/L for iron and 5 mg/L for zinc, both based on the aesthetic effects of these two metals (USEPA 2009).

1.3 Aesthetics

Besides the lack of work on negative effects of copper exposure on human health, little research exists on the aesthetic aspects of metal species in drinking water. Different copper concentrations may produce unpleasant sensations, which are described as metallic, acidic, astringent, salty, or bitter, depending on individual taste acuity (Beguinhruhin et al. 1983; Zacarias et al. 2001; Lawless et al. 2005; Cuppett et al. 2006). The apparent difference between the copper levels that cause aesthetic effects is likely due to varying sensitivities and competing effects on taste from other chemicals present in water (Dietrich et al. 2004; Cuppett et al. 2006). Metallic was reported as the second most important taste problem by water utilities, followed by the organoleptic problem caused by the distribution system (Suffet et al. 1995). Zacarias et al.

(2001) reported the tasting threshold of copper between 2.4 and 3.8 mg/L in tap water, uncarbonated mineral water and distilled deionized water. The study of Cuppett et al. (2006) produced group thresholds for copper in the range of 0.4-0.8 mg/l in both distilled and mineralized water. One early research study in the United States found taste thresholds of 6.6 and 13 mg/l Cu in distilled water and spring water, respectively (Cohen et al. 1960). These previous studies show a wide discrepancy in threshold values for copper with conflicting results in distilled and other water sources, which may due to the differences in water quality, the conformation of copper in aquatic environment as well as the diversity between the panelists.

Iron (II) salts are typically described as metallic, astringent, and sweet at low concentrations and bitter or sour at high concentrations (Lawless et al. 2004; Lim and Lawless 2006). As early as 1934, Shrader and Johnson (1934) reported that as little as 5 ppm iron or copper caused off-flavor in orange juice. Davies (1936) found that iron present in milk in the form of lactate also produced a metallic flavor at a concentration of 15 ppm. Several studies have measured detection thresholds of iron, ranging from 9.6 to 99.2 μM in various types of water (distilled, deionized, spring and mineral spring et.) at different ferrous salts (ferrous sulfate and ferrous chloride).

By investigating the nature of metallic sensations from iron compounds, Frank (1990) suggested that olfaction plays an important role in the metallic flavor of ferrous sulfate, because of a decrease in metallic taste when the nose was occluded, which eliminate retronasal smell cues. The metallic sensation of ferrous sulfate was reduced to baseline when the nose was occluded. At the same time a discrimination test demonstrating that the headspace over solutions of ferrous sulfate was not different from water (Lawless et al. 2004). This result confirmed Frank's results and suggested that metallic taste reports following oral stimulation with FeSO_4 are likely due to development of a retronasal smell, possibly followed by a lipid oxidation reaction in the mouth.

They also found that some people can detect the ferrous under nasal occlusion, passably by tactile or astringent cues. Borocz-Szabo et al. (1980) investigated the thresholds of ferrous and ferric salts in various liquid foods as well as the effect of corrosion products of carbon steel construction materials on sensory properties, finding that except for fruit beverages, all the foods investigated were very sensitive to iron contamination coming from corrosion products in the perception of metallic flavor.

The taste threshold of zinc is rarely reported in the literature and the sensory of zinc is also limited. Interestingly, zinc is not concerned by its taste itself, but its importance in the gustatory sensation of other food. Zinc has high affinity to both thiol and hydroxyl groups and readily complexes with amino acids and proteins. On the one hand, zinc as a taste stimulus interact with taste transduction mechanism in the oral cavity, leading to the signals sent to the brain for perceiving the taste sensations, on the other hand, zinc binds taste receptors, alters the structure of receptor and interferes with the normal function (Keast 2003). Zinc causes the sensation of astringency, by combining with saliva proteins to reduce the salivary lubrication and increase friction between oral membranes (Breslin et al. 1993).

1.4 Chemical Speciation of Copper

Copper in aquatic environments is commonly classified as particulate and dissolved, with the dissolved fraction further classified as free, inorganically or organically complexed (Figure 2-1). For this reason, it is beneficial to know which species of copper form in drinking water in which copper is consumed.

Free, complex, and particulate copper exist under the condition of typical drinking water pH and mineral content. Copper, like many metals, interacts in water to form free metal cations, a variety of soluble complexes, and insoluble particles or precipitates, depending on the chemical

components of the water. Free copper, which is the cupric ion (Cu^{2+}), is soluble and the dominant form at low pH levels (typically below pH 6), in the absence of anionic ligands. In pure water, soluble copper hydroxo complexes ($\text{Cu}(\text{OH})^+$, $\text{Cu}(\text{OH})_2$, $\text{Cu}(\text{OH})_3^-$, $\text{Cu}(\text{OH})_4^{2-}$) form at low and high pH values (Figure 2-2). Figure 2-2 demonstrates how the concentrations of individual hydroxo Cu (II) complexes vary when the total copper concentration is fixed at the USEPA aesthetic standard of 1 mg/l. The metal precipitates most frequently as copper hydroxide [$K_{\text{sp}} \text{Cu}(\text{OH})_2 = 10^{-19.32}$] at intermediate pH levels (typically pH 6.5-12) in pure water but many other complexes precipitates, depending on copper concentration, presence of other anions and cations, temperature, and time to thermodynamic equilibrium, may exist at the presence of anionic ligands (Jensen 2003). Copper will form complexes with common anions, including SO_4^{2-} , OH^- , PO_4^{3-} , HCO_3^- , NO_3^- , and CO_3^{2-} , when the solubility product of possible precipitates is exceeded. A common multianionic precipitate is malachite [$\text{Cu}_2(\text{OH})_2(\text{CO}_3)$].

It is well established that copper speciation affects toxicity and bioavailability in aquatic organisms (from algae to fish) (Dietrich et al. 2004). Free copper (II) ion and monohydroxo Cu(II), Cu^{2+} and CuOH^+ , are considered highly toxic, while other anionic complexes, especially carbonate complexes, are less toxic to aquatic organisms. Particulate copper is not toxic unless it is solubilized in water or the fluids within an organism. Copper-carbonate species are generally not considered toxic (Meador 1991). Increases in carbonate species are usually associated with increases in alkalinity. Therefore, alkaline waters have more of a potential to form copper-carbonate species, thus reducing copper toxicity (Snoeyink and Jenkins 1980). Because inorganic calcium and magnesium ions compete with copper for binding sites on organisms, high water hardness also tends to reduce copper toxicity (Brezonik, King et al. 1991). Although the role of

speciation of copper is known to be important in aquatic toxicity, its role in human sensory response is not well established.

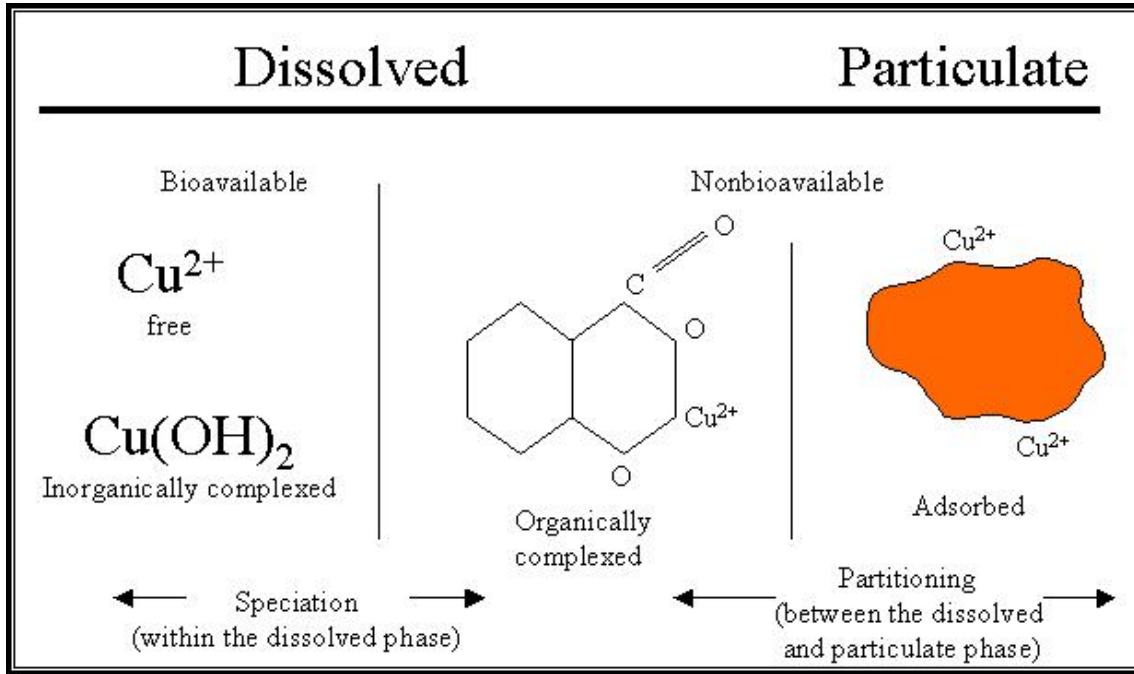


Figure 2-1 Forms of Copper present in Aquatic Environments (Paulson 1993).

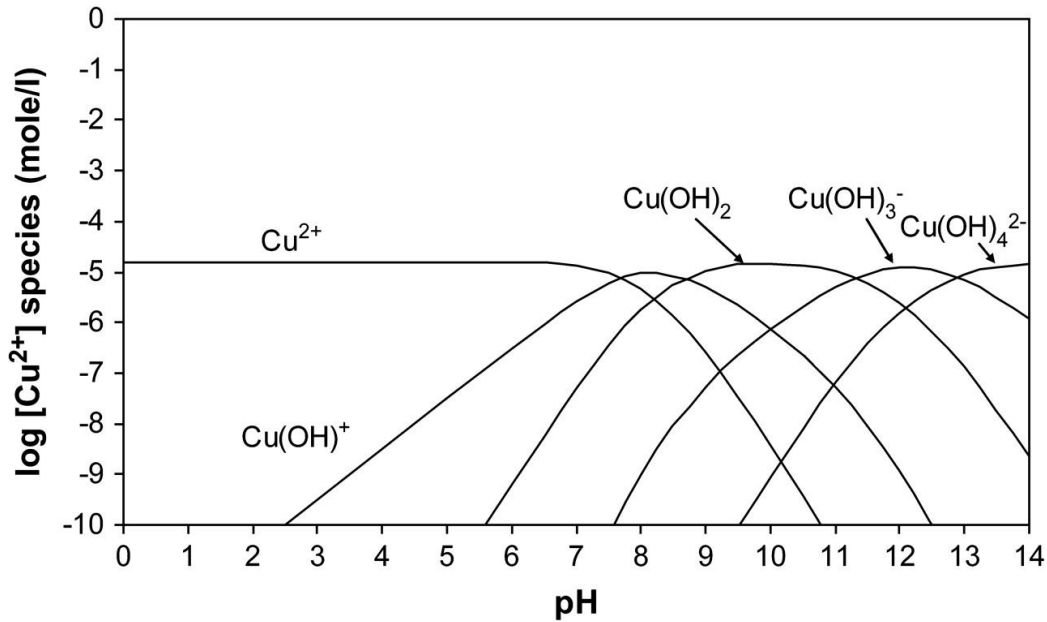


Figure 2-2 Theoretical copper speciation for hydroxo complexes in pure water for a total copper concentration of 1 mg/l, which is the value for the USEPA aesthetic-based standard (Cuppett et al. 2006).

Copper speciation in aquatic environment was found to play an important role in the sensation of copper in previous researches. Hong et al. (2010) reported that copper chemistry in drinking water, which is controlled by pH and other chemical factors, significantly influenced temporal attributes of copper sensation. Decreases in soluble copper species in samples manipulated by increasing pH were significantly associated with decreases in perceived metallic flavor. Cuppett et al. (2006) reported that both free copper ion and soluble copper complexes can be readily tasted, while particulate copper is poorly tasted. The experiments of Zacarias et al. (2001) indicated the copper salt used, sulfate or chloride, had little influence on the sensation of copper. They also found the tasting threshold of copper in uncarbonated mineral water was higher than those in tap water and deionized water, suggesting that chelation between copper and organic anions present in higher amounts in this type of water decreased the taste of copper (Zacarias et al. 2001).

Table 2-1 Solubility product of known metal precipitates and complexes.

	Ksp	Sources
Cu(OH) ₂	2.2×10^{-22}	(Patnaik 2002)
CuCO ₃	3.16×10^{-12}	(Jensen 2003)
Cu ₂ (OH) ₂ CO ₃	1×10^{-32}	(Symes and Kester 1984)
Cu ₃ (PO ₄) ₂	1.40×10^{-37}	http://www.ktf-split.hr/periodni/en/abc/kpt.html
Fe(OH) ₂	3.16×10^{-15}	(Snoeyink and Jenkins 1980)
FeCO ₃	1.58×10^{-11}	(Jensen 2003)
Fe ₃ (PO ₄) ₂	1×10^{-33}	(Snoeyink and Jenkins 1980)

1.5 Composition of Saliva

When metals are ingested into oral cavity, the mixing of water and saliva will change the metal speciation and then influence the perception of metallic flavor. Therefore, the composition of human saliva needs to be known in order to understand the mechanism of the sensation of metals.

Saliva is produced in and secreted from the salivary glands, composed of 98% water, and 2% other compounds such as electrolytes, mucus, antibacterial compounds, and various enzymes. In human saliva, the sub-mandibular gland contributes around 70-75% of secretion, while the parotid gland secretes about 20-25 % and small amounts are secreted from the other salivary glands. It is estimated that over 200 different proteins and peptides are present in the saliva of humans (Beidler, 1995). As part of the initial process of food digestion, the enzymes in the saliva break down some of the starch and fat in the food at the molecular level. Saliva also breaks down food caught in the teeth, protecting them from bacteria that cause decay. Furthermore, saliva lubricates and protects the teeth, the tongue, and the tender tissues inside the mouth. Saliva also plays an important role in tasting food.

Salivary composition and flow rate are affected by the degree of hydration, body position, exposure to light, olfaction, smoking, (previous) stimulation, climatological circumstances and circadian and circannual rhythms (Dawes 1987; Wisniewski et al. 1992). All these factors are responsible for large variations within a subject on a day and day-to-day basis, and also for a wide among-subject variation (Pangborn and Lundgren 1977). The principal inorganic constituents of saliva are sodium, potassium, calcium, chloride and bicarbonate (Young and Schneyer 1981).

α -Amylase (α -1,4- α -D-glucan 4-glucohydrolase) is one of the most important salivary enzymes, which accounts for 40 to 50% of the total salivary gland-produced protein. Most of the enzymes are synthesized in the parotid gland (80% of the total). Thus, human parotid and submandibular saliva contain about 45 mg and 30 mg of α -Amylase (expressed per 100mg of protein) (Tenovuo 1989). Hong et al. (2009) found α -amylase usually showed the thickest blue band in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of saliva, indicating high concentration of α -amylase in whole saliva. Other enzymes are present in human saliva, such as lingual lipase, lactoferrin, salivary lactoperoxidase, invertases, dextranases and other glycosidases. High-molecular-weight mucins, which are a sort of glycoproteins, are the primary contributors to the saliva's viscosity. These and other proline-rich glycoproteins of saliva serve to lubricate hard surfaces.



Figure 2-3 Schematic structure of mucins(Wu et al. 1994)

Because the components of saliva vary among different people and change with time of the day, exact duplication of saliva is impossible in our research. Thus, an average composition of saliva is necessary and reasonable to standardize in a test. Gal et al. (2001) investigated more than 100 recipes of human saliva and found a composition called SAGF medium (Table 2-2), which was similar to real saliva in ionic strength, buffering effects and specific conductivity.

Table 2-2 Composition of the SAGF (Artificial Saliva Gal Fovet) medium

Compound	Concentration in SAGF	1:10 used in this research
	(mg/L)	(mg/L)
NaCl	125.6	12.56
KCl	963.9	96.39
KSCN	189.2	18.92
KH ₂ PO ₄	654.5	65.45
Na ₂ SO ₄ •10H ₂ O	763.2	76.32
NH ₄ Cl	178	17.8
CaCl ₂ •2H ₂ O	227.8	22.78
NaHCO ₃	630.8	63.08
pH = 6.8		

1.6 Role of Saliva in Complexion Metals

In natural environment, metals exist with complexing with different inorganic anions and natural organic ligands. The same circumstance happens when metals is ingested into oral cavity mixed with saliva. Relatively high levels of phosphates and carbonates capture the free metals to form complex or precipitates, while proteins complex with metals forming metal-protein chelation. Hong investigated the results by HPLC and SDS-PAGE of saliva fractionated by HPLC, suggested that salivary proteins such as low molecular weight mucin, α -amylase, and basic PRPs seem to interact with copper (Hong 2009). Dietrich suggested that metals in the mouth may interact with taste receptors, produce odors, and cause changes in saliva and protein composition and result in the sensation of flavor (Dietrich 2009). Ömür-Özbek et al. (2008)

demonstrated that copper (II) caused lipid oxidation, which is measured by the colorimetric thiobarbituric acid reactive substances (TBARs) method, in the mouth at the presence of oxygen (Omur-Ozbek 2008). Besides inducing lipid oxidation in the oral cavity, copper (II) also interacts with salivary proteins and volatile odorants in the mouth to alter sensory perception (Hong et al. 2006, 2008). Epke et al. reported that nasal occlusion lowered the metallic taste and aftertaste ratings of the two iron concentrations (0.001 M and 0.015 M) but the copper metallic taste and aftertaste ratings were slightly influenced by nasal occlusion at the concentration of 0.001 M (64 mg/L) (Epke et al. 2009). Nasal occlusion increased thresholds for copper and iron when retronasal sensations were eliminated (Epke et al. 2007). Those results suggested that nasal perception plays an important role in the sensation of metallic flavor of iron but less important in the sensation of copper, especially at high concentration of copper (II). Hong et al. (2006) suggested that copper (II) interacted with the salivary proteins mucin and α -amylase at pH 7.5 but not pH 7 or 6.5 when added at 2.5 mg/l Cu to an artificial saliva consisting of salts and proteins by detecting the headspace concentrations of hexanal, butyl acetate, 2-heptanone, and ethyl hexanoate.

Hong et al. (2010) found that soluble copper species in a sample played a critical role in perception of copper sensation by controlling metallic flavor, especially at the low copper levels. Olivares et al. (2001) reported that copper imbibed in distilled water caused more gastrointestinal distress than copper consumed in an orange-flavored beverage, which also indicated the importance of free copper in the role of perception of copper.

Based on the lack on the role of free metals in generating metallic flavor, the goal of this research is to:

- Determine the role of free metals in the production of metallic flavor.

- Predict chemical speciation of metals in artificial saliva in MINEQL+ and learn how free metal ion concentration will vary with pH, protein content, other factors in oral cavity.
- Compare predicted values with experimental results.

2. Materials and Methods

2.1 Artificial Saliva Solution Preparation

Distilled-deionized water was generated from a Barnstead Nanopure system that was fed distilled water and subsequently deionized and carbon filtered. This system produced water with a chemical resistivity of 18 MX/cm and pH 5.5.

Artificial saliva was prepared according to the following recipe, the inorganic components of which coming from SAGF, while the organic components of which coming from Hong's reports (Hong et al. 2006). NaCl (0.1256 g), KCl (0.9639 g), KSCN (0.1892 g), KH₂PO₄ (0.6545 g), Na₂SO₄ (0.3366 g), NH₄Cl (0.178 g), CaCl₂ (0.172 g) and NaHCO₃ (0.6308 g) were separately dissolved in 1000 ml nanopure water to make saliva stock solution. Both 0.216 g of mucin (from porcine pancreas, Sigma, St. Louis, MO) and 20, 000 units of α -amylase (from *Aspergillus oryzae*, Aldrich, Milwaukee, WI) were separately dispersed in two 100 mL of nanopure water to make stock solutions of organic components. Nitric acid and sodium hydroxide were used to adjust pH because of their weak affinity to copper. Artificial saliva solution was used for experiments within one hour of preparation.

Table 2-3 Chemicals used in this research (1:10 dilution)

Compound	Concentration(mg/L)	Notes
NaCl	12.56	
KCl	96.39	
KSCN	18.92	
KH ₂ PO ₄	65.45	
Na ₂ SO ₄ •10H ₂ O	76.32	33.66mg/L as Na ₂ SO ₄
NH ₄ Cl	17.8	
CaCl ₂ •2H ₂ O	22.78	17.2mg/L as CaCl ₂
NaHCO ₃	63.08	
α -amylase (from Aspergillus oryzae)	20 000 unit/L	37 units/mg
Mucin (from porcine pancreas)	216	The molecular weight is not provided by the manufacturer.
HNO ₃	-	Used to adjust pH
NaOH	-	Used to adjust pH

2.2 Treatment of copper

A 1000 mg/l copper stock solution was prepared from copper (II) sulfate pentahydrate (catalog number C493-500, Fisher Scientific, Pittsburgh, PA, USA) and diluted to obtain concentrations in the range of 0.1-10 mg/l Cu. All samples were prepared fresh daily to avoid increased precipitation with time and loss of carbonate. All copper solutions were prepared and maintained at room temperature, within 22-24C.

When water is ingested into oral cavity, saliva is diluted by the water consumed. Therefore we must consider the influence of dilution on the chemical speciation of copper in oral cavity. Considering the regular amount of water uptake and the saliva volume, we chose 1:10 (saliva: water) as our dilution rate. In order to investigate the contribution of inorganic components and proteins, total copper concentration and pH on the chemical speciation of copper, we designed our experiments in Table 2-4. We measured the free copper in the five combinations under three kind of copper treatment (2.5mg/L (0.04 mM), 5mg/L (0.08 mM), 10 mg/L (0.16 mM)), under five different pH (5.5, 6, 6.5, 7, 7.5).

Table 2-4 Experimental design table

Components	Copper concentration (mg/L)		
Only Inorganic	2.5	5	10
Inorganic and α -amylase	2.5	5	10
Only α -amylase	2.5	5	10
Inorganic and mucin	2.5	5	10
Only mucin	2.5	5	10

2.3 Ion Selective Electrode measurement

Among the many analytical methods available for determining copper in aquatic environments, only potentiometry with copper ion-selective electrodes (ISEs) can directly measure the free copper ion in situ without perturbing the natural speciation (Belli and Zirino 1993). Free copper ion concentrations were measured using a cupric electrode (Accumet cupric combination electrode, catalog number 13-620-547, Fisher Scientific).

The cupric electrode membrane is composed of sulfides of copper and other metals bonded into a glass body. When the electrode is in contact with free, unbound cupric ions in solution, a potential develops across the membrane, which vary with concentration in a Nernstian fashion:

$$E = E^0 + 2.3 (RT/nF) \log [\text{Cu}^{2+}]$$

where

E is the mV potential from the electrode

E^0 is the sum of all other constant system potentials

R is the gas constant

T is the temperature in degrees Kelvin

n is the number of electrodes required to reduce Cu^{2+}

F is Faraday's constant

The electrode has a range of detection 1×10^{-1} to 1×10^{-8} M Cu^{2+} and works in the pH range of 2-12 and temperature range of 0 to 80°C. Sodium nitrate is a non-complexing component and was used to adjust ionic strength. 5M NaNO_3 is used as Ionic Strength Adjuster (ISA). When measuring the samples, 2 ml ISA was added into 100 ml sample mixed well with a Thermix Stirrer (Fisher Scientific). All free copper ISE measurements were made within 5 minutes of adding copper to saliva.

2.4 Modeling Metal Speciation

In order to compare with our experimental data with predicted results, the concentrations of soluble and precipitated copper in the artificial saliva model systems were estimated by MINEQL+ chemical equilibrium modeling software (version 4, Environmental Research Software, Hallowell, ME). The soluble copper concentration in the artificial saliva model system

with protein could not be calculated by the MINEQL+ software because thermodynamic data of salivary proteins-electrolytes interaction required for calculation are not available.

3. Results and Discussion

3.1 Predicting Speciation of Cu, Fe and Zn with Inorganic Components of Saliva

Soluble free metal ion concentrations in MINEQL+ models for cu, Fe and Zn

Free metals concentrations including copper, ferrous and zinc in diluted SAGF medium were modeled in MINEQL+ at different pH values and displayed in Figure 2-4-a. Dilution rate was 1:10 in all samples assuming that average about 10 ml per drink for normal people and average volume in the mouth of 1.1 ml (Llena-Puy 2006). All the samples were treated with sulfate (CuSO_4 , FeSO_4 and ZnSO_4) with 10 mg/L as metal ion. Closed to atmosphere, which is an option in the software, was applied in MINEQL+ because all the samples were prepared and measured in a short time.

All of the free metal concentrations changed with pH in a similar trend, decreasing as pH increased from 5.5 to 7.5 and tending to zero after 7.5. In the range of 6.5 to 7.5, the typical pH of human saliva (Dietrich 2009), free metal concentrations only are a small part of the total metal (less than 5%), indicating that most of the metals are combined with inorganic components in saliva in the absence of proteins. Comparing Figure 2-4-a and Figure 2-4-b, which shows the free metals in distilled-deionized water at different pH, chemical speciation of metals in water significantly changed after ingested into the oral cavity. The notable decrease in free form of metals would reduce the bioavailability of them in the oral cavity, especially for ferrous and zinc. In other words, the impact of saliva components on ingested aqueous metals must be considered

in order to understand the mechanism of metallic flavor and the fate of metals in the human mouth.

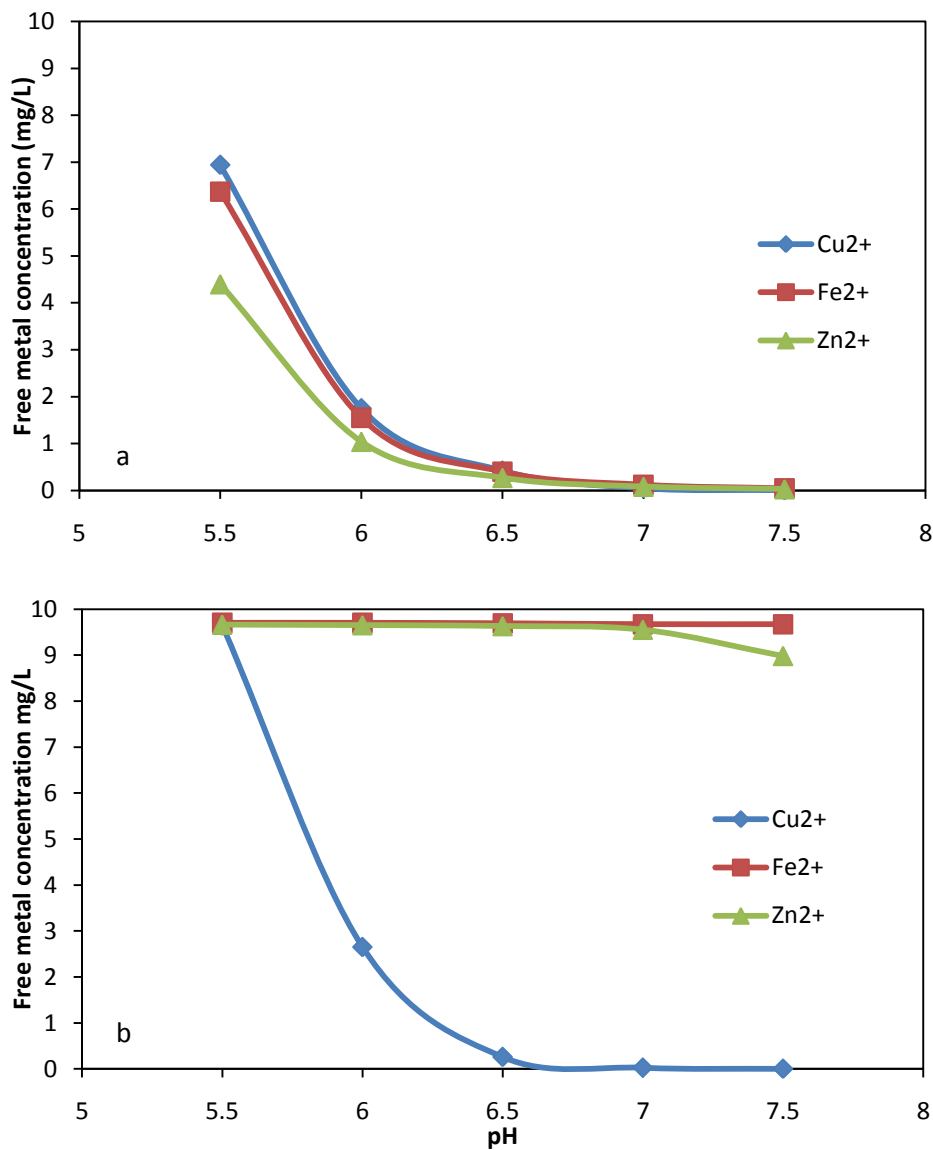


Figure 2-4 Free metal ion concentration as a function of pH modeled by MINEQL+; a: inorganic components of artificial saliva; b: nanopure water; total metal concentration was 10 mg/L as metal.

Soluble and Insoluble Metal Complexes

Metals exist in free, soluble complexes and precipitates in aqueous environment as well as in human saliva. Free, soluble complexes and precipitate forms of metals in artificial saliva

with only inorganic components were modeled and presented in Figure 2-5. Free ion concentration decreased and precipitates increased with the increasing of pH from 5.5 to 7.5, for all the three metals. At pH 5.5, about 50% of the metals are in the free form and at pH of 7 and above, free ion concentrations dropped to nil. There is a small portion of metals in soluble complexes at low pH, but this proportion decreases as pH rises. The trend is similar to the situation of copper in nanopure water in Figure 2-4. Compared with copper and iron, zinc has less solubility in artificial saliva at pH 5.5.

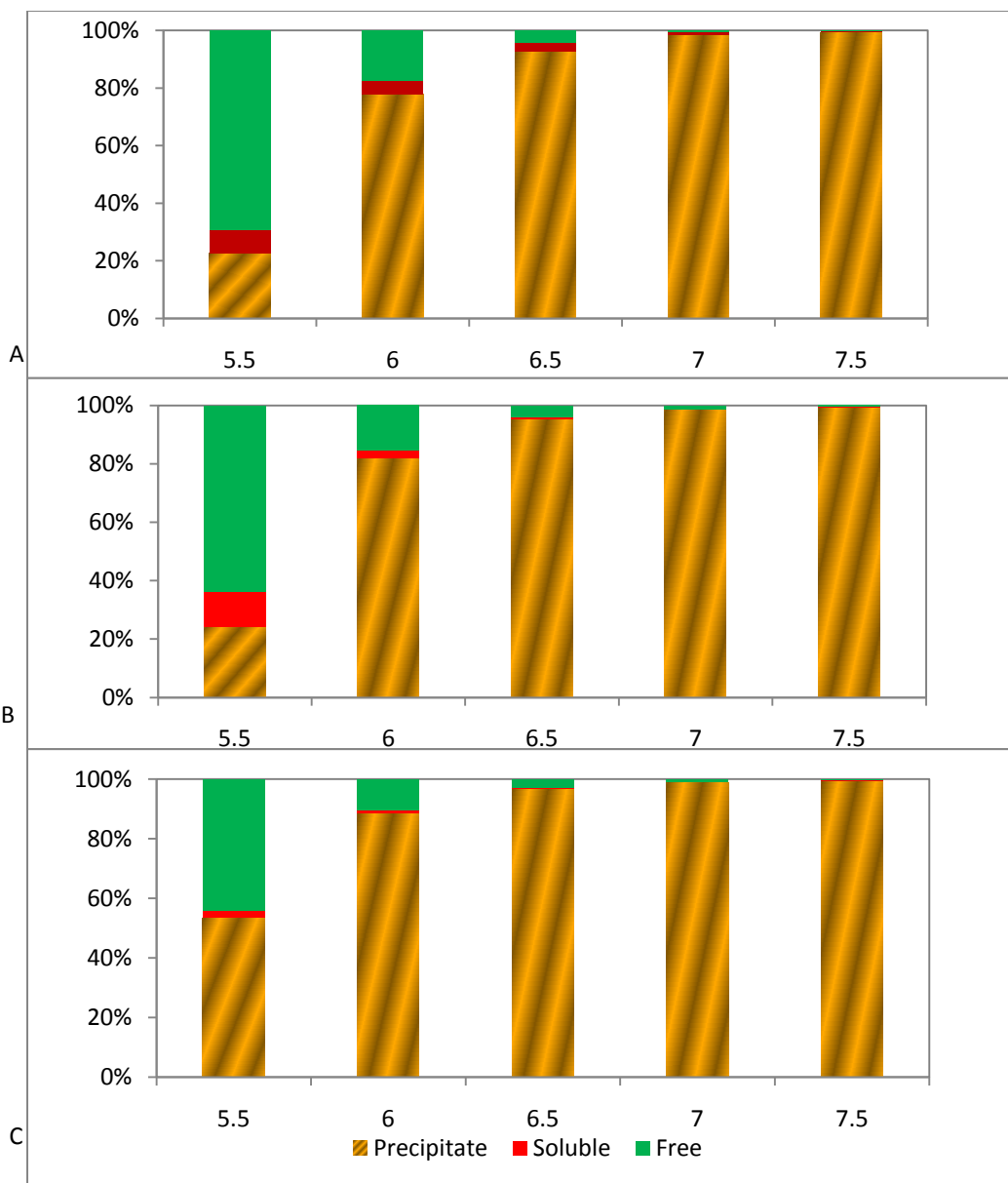


Figure 2-5 Distribution of free, soluble and precipitate forms of metals in inorganic artificial saliva at various pH values; A: Copper (Cu²⁺); B: Iron (Fe²⁺); C: Zinc (Zn²⁺)

Table 2-5 demonstrates the chemical speciation of 10 mg/L of metals in inorganic artificial saliva including the species of precipitates. Cupric phosphate, malachite and tenorite are the three major precipitates; therefore, PO₄³⁻, OH⁻ and CO₃²⁻ have high affinity for copper. Their percentages in total precipitates vary with pH because pH controls the species distribution of phosphate and carbonate. The proportion of total soluble has the reverse trend with precipitate,

with a decreasing percentage of free copper in it. The same is found for ferrous and zinc, in which PO_4^{3-} played important roles in chemical speciation.

Table 2-5 Chemical Speciation of metals in Inorganic Artificial Saliva.

Species	% of total metal				
	pH 5.5	pH 6	pH 6.5	pH 7	pH 7.5
Free Cu^{2+}	69.4	17.5	4.2	0.44	0.04
Total soluble	77.2	22.1	7.3	1.6	0.7
$\text{Cu}_3(\text{PO}_4)_2$	22.8	77.9	30.1	0	0
Malachite ($\text{Cu}_2(\text{OH})_2\text{CO}_3$)	0	0	62.6	0	0
Tenorite (CuO)	0	0	0	98.4	99.3
Free Fe^{2+}	63.4	15.5	3.8	1.1	0.37
$\text{Fe}_3(\text{PO}_4)_2$	24.2	81.9	95.6	98.8	99.6
FeH_2PO_4	10.1	2.04	0.41	0.078	0.013
FeSO_4	1.4	0.356	0.08	0.024	0.008
FeCl^+	0.9	0.199	0.05	0.014	0.005
Free Zn^{2+}	43.6	10.3	2.5	0.709	0.244
$\text{Zn}_3(\text{PO}_4)_2$	53.8	88.7	97	99	99.6
ZnSO_4	1.3	0.31	0.076	0.021	0.072
ZnHPO_4	0.60	0.39	0.251	0.150	0.080
Others	0.70	0.29	0.173	0.120	0.003

3.2 Comparison between measured and MINEQL+ modeled soluble copper in saliva.

Measured free copper ion in 1:10 diluted artificial saliva with only inorganic components was measured by ion selective electrode and compared with concentrations modeled in MINEQL+ (in Figure 2-6). They both have a decreasing trend with increasing pH. Although measured free copper ion results were always greater than those calculated by MINEQL+, with a difference from less than 1% to 30% of total copper (10 mg/L), a paired t-test indicated no statistical differences ($p=0.12$). Rachou et al. investigated the reliability of cupric ISE in pure water without metal buffer, in the presence of acetate, and in the presence of citrate. They found a very good concordance between experimental measurement and predicted values by

MINEQL+ in the range of 10^{-7} to 10^{-12} M; but measured values were a little greater than predicted when the concentration of copper was greater than 10^{-7} , which agreed with the results in this study.

The discrepancy between measured and predicted may be caused by the measurement error from the cupric ISE or inaccuracies in the thermodynamic constants used in MINEQL+. Despite the slight differences existing between these two sets of data, both approaches showed the significant changes (from 8 mg/L to 0.005 mg/L) in free copper, which has essential impact on metallic flavor of copper, at pH values of 5.5 to 7.5.

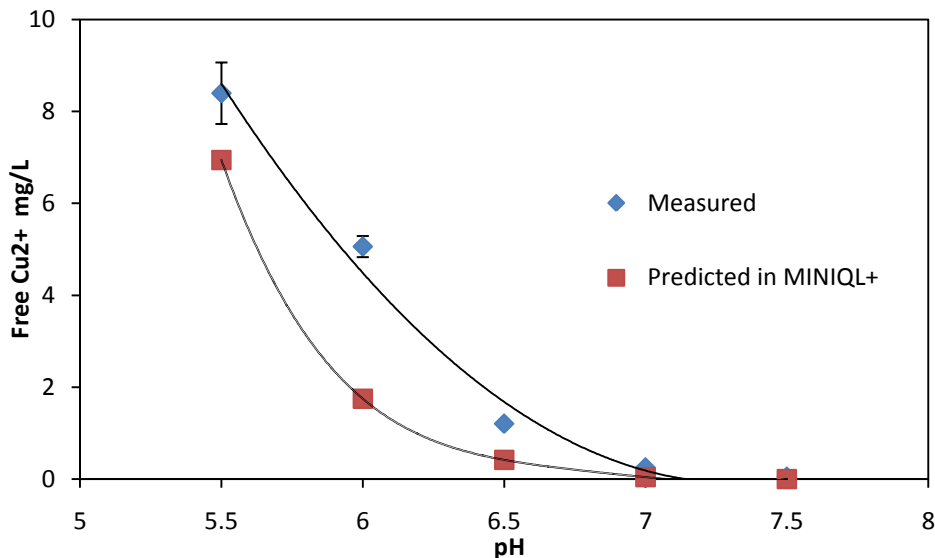


Figure 2-6 Comparison in free copper concentration between measured by copper-ISE (Ion Selective Electrode) and modeled by MINEQL+ in 10 mg/L copper in artificial saliva with only inorganic components; measured data is based on 3 replicates.

3.3 The role of proteins in copper speciation

Organic components of saliva also play an important role in the perception of metallic flavor (Hong 2009). Proteins complex with copper ion to reduce bioavailable copper, or taste receptors bind with copper and transport the signals to brain resulting in the perception of metallic taste (Riera et al. 2007). In order to understand what the roles of proteins in the

perception of metallic taste and how they impact the free copper concentration in oral cavity, three major proteins of human saliva- α -amylase, mucin and lactoferrin - were studied in this section.

Due to the lack of available constants for proteins, MINEQL+ cannot be used to model the environments containing organic compounds.

α -amylase

Free copper concentrations in the samples with only inorganic components, α -amylase plus inorganic components and only α -amylase at three different copper treatments were demonstrated in Figure 2-7. The different plots illustrate the results in samples treated with different concentrations of copper. The concentration of α -amylase in all the samples is 200, 000 unit/L (5.38 g/L) (Hong et al. 2006).

α -amylase significantly impacted the free copper concentration at low pH in small concentration of total copper. With the increase of total copper from 2.5 mg/L to 10 mg/L, the influence of α -amylase in chemical speciation of copper declined gradually. At pH of 6.5, α -amylase tended to increase the free copper concentration, compared with that in saliva with only inorganic components. These results suggested that α -amylase has limited binding capacity with copper; it controlled the chemical speciation of copper at low concentration of copper, but when more copper exists in the samples, the chemical distribution of the excess portion of copper beyond the capacity of α -amylase is still controlled by the inorganic components. Compared with its high content in human saliva, where 40% to 50% of the total salivary gland-produced protein is α -amylase (Tenovuo 1989), the influence of α -amylase on chemical speciation of copper is slight.

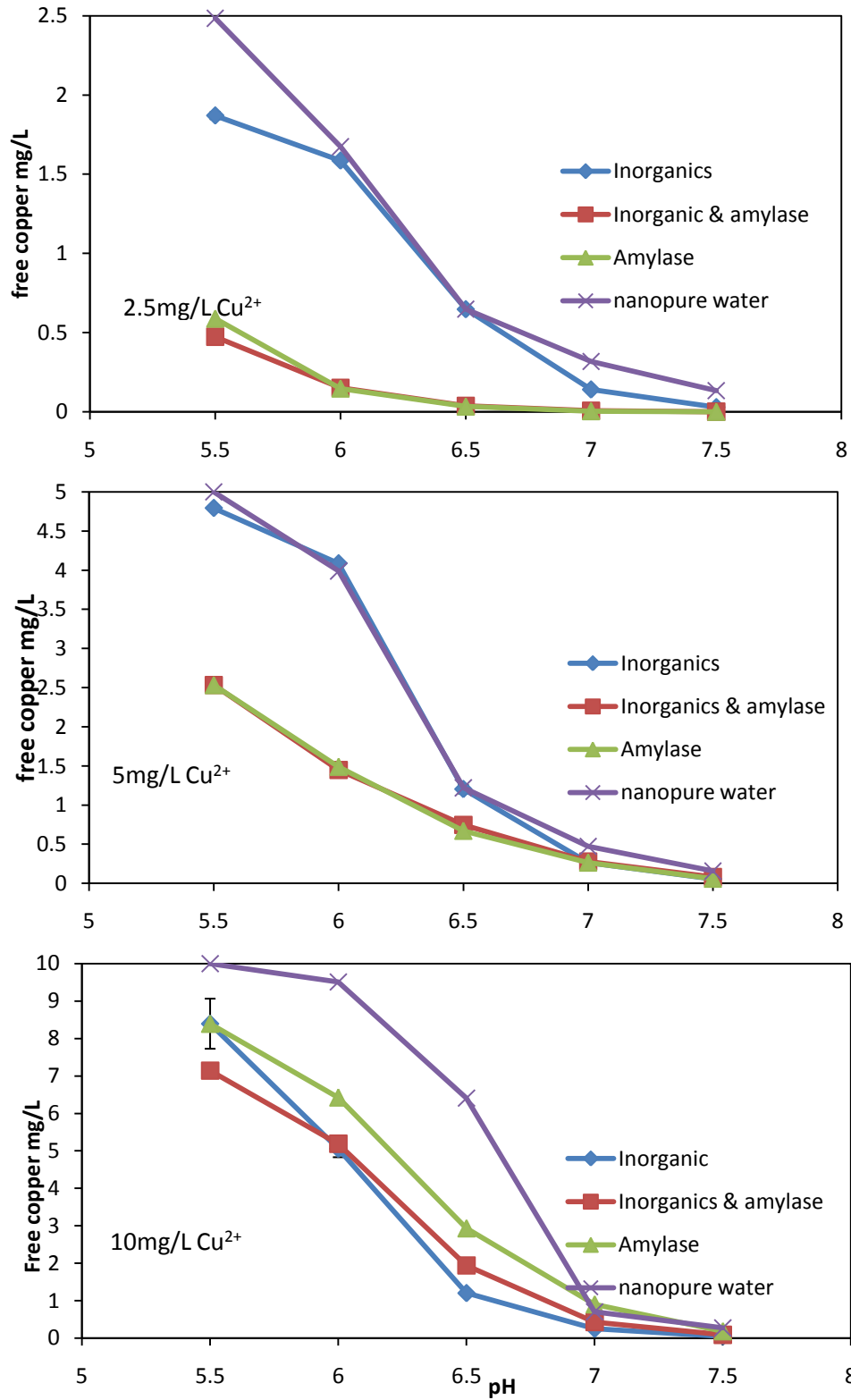


Figure 2-7 Free copper in artificial saliva with alpha-amylase

Mucin

Mucin is another major component of human saliva. Mucins, high-molecular-weight glycoproteins account for 7-26% of total salivary proteins (Slomiany, Murty et al. 1996). The same experimental and analytical method was used for mucin to investigate its effects on the chemical speciation of copper in diluted artificial saliva. Figure 2-8 demonstrated the comparison in free copper among three kind of artificial saliva in three different concentrations of total copper. Only a small amount of copper (less than 5% of total copper) was in free form at the existence of mucin, no matter what the total concentration of copper and the pH was. This suggested that mucin had very high affinity to copper in the given mucin concentration (2.16 g/L) and pH range, which was consist with Mueller's results: binding of copper to glycoprotein was as high as 5.0M Cu/M protein (Mueller 1983). Although free ion concentrations in artificial saliva with mucin were at low levels, a decreasing tendency could be found with the increase of pH in all samples.

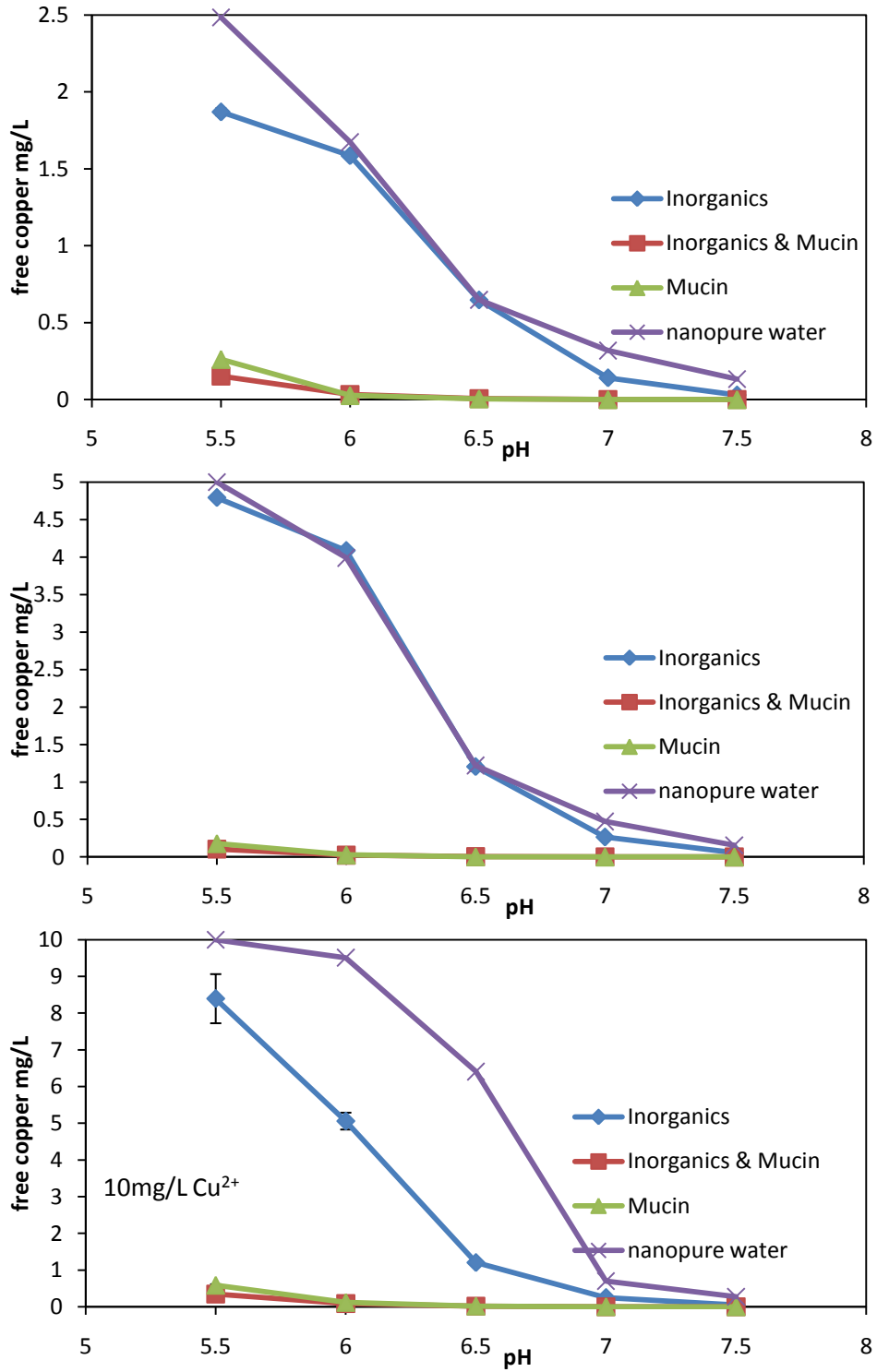


Figure 2-8 Free copper in artificial saliva with mucin

Due to the high binding capacity between copper and mucin, it is difficult to find out the influence of mucin on copper speciation at different pH. Therefore, experiments were repeated in

saliva with diluted mucin. Figure 2-9 showed the results of free copper in artificial saliva with different concentrations of mucin at total copper of 10 mg/L. The free ion concentrations were significantly influenced by the levels of mucin in artificial saliva, especially at low pH. With the increase of mucin from 10% to 100%, the free ions dropped gradually at each pH. However, the line without mucin is not totally above the lines with mucin on the graph, which meant mucin could increase the free copper level at certain concentrations of mucin and certain pH, such as 10% of mucin at pH of 6. This may be due to the interaction between mucin and other inorganic components those also complex with copper. This result could be one of the explanations for the various threshold values among individuals because different people have different pH and concentrations of mucin, and these factors could results in various levels of free copper.

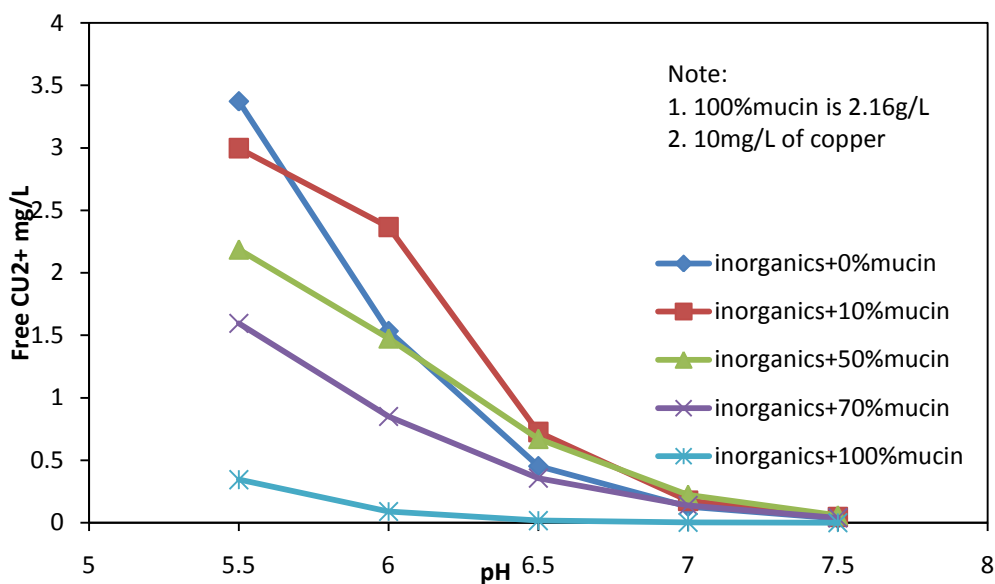


Figure 2-9 The impacts of mucin concentration on the free copper concentration in artificial saliva

Lactoferrin

Lactoferrin consists of two domains that each bind one Fe^{3+} reversibly but tightly ($K = 10^{20}$). It was also found to accommodate other transition metal ions such as Cr^{3+} , Mn^{3+} , Al^{3+} , Co^{3+} , Cu^{2+} , and Zn^{2+} with less binding affinity than that to Fe^{3+} . The binding between lactoferrin

and other metal ions would change the structure of lactoferrin and impacts its binding ability to ferric ion (Baker, Anderson et al. 1990). Although this suggests the possibility of copper-lactoferrin interaction, there has been no previous study on lactoferrin-copper binding in human saliva.

This section mainly studied the binding capacity of lactoferrin to copper and investigate its effects on the perception of metallic flavor of copper. The same experiment plan was designed like α -amylase and mucin as discussed above. By comparing the free copper in artificial saliva with and without lactoferrin, the slight changes between the sets of data in figure 12 suggested that there was some interaction between copper and lactoferrin but the binding capacity between them is very weak at a concentration of 1 mg/L of lactoferrin concentration.

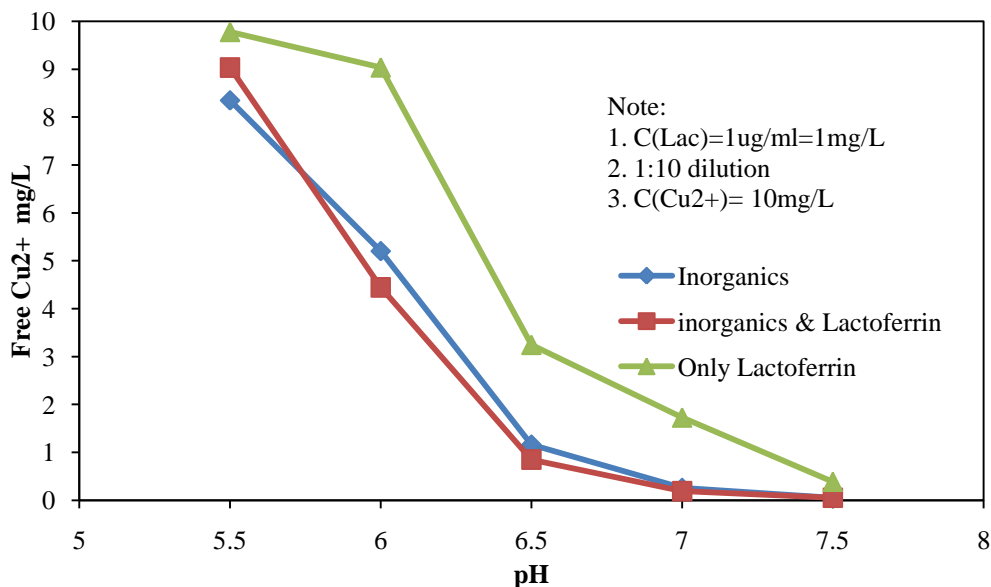


Figure 2-10 The impacts of lactoferrin on the free copper concentration in artificial saliva; data points represent mean of triplicate measurement; error bars are shown but are not visible

3.4 Binding capacity of proteins with copper

Figure 2-8 and 2-9 demonstrated the influence of amylase and mucin on the free copper concentration, while Table 2-5 shows the approximate binding capacity of these two proteins to copper at different pH. The binding capacity was calculated by the following equation,

$$\text{Binding capacity (mg Cu/g protein)} = (C_t - C_w - C_p) / M_p$$

where

C_t is the total concentration of copper;

C_w is the non-free copper concentration in the nanopure water with corresponding total concentration of copper, so $C_t - C_w$ represented the available copper to bind protein;

C_p is the free copper concentration in the artificial saliva with only protein;

M_p is the protein concentration.

This estimation ignored the interaction between proteins and other inorganic or organic components in saliva and provided us the approximate information on the copper-protein binding capacity. At pH of 5.5, the binding capacity of amylase to copper did not change too much with various copper concentrations but at higher pH there was significant difference in different copper concentrations. Amylase showed the strongest binding capacity to copper at pH of 6.5 and copper concentration of 10 mg/L, which suggested that pH and total metal concentration both influenced the interaction between amylase and copper.

Mucin generally had greater (about 10 times) binding capacity with copper than amylase from Table 2-6. With the decrease of total copper concentration and the increase of pH, the binding capacity dropped gradually. The maximum point was at total copper concentration of 10 and pH of 5.5, indicating that mucin may have greater binding capacity with copper at higher copper concentration and lower pH.

Table 2-6 Binding capacity of amylase and mucin to copper at different pH

	Cu ²⁺ (mg/L)	Binding capacity (mg Cu/g protein)			
		pH=5.5	pH=6	pH=6.5	pH=7
Amylase	2.5	3.53	2.84	1.14	0.58
	5	4.59	4.65	1.02	0.39
	10	2.99	5.74	6.47	0
Mucin	2.5	10.29	7.62	2.98	1.47
	5	22.33	18.34	5.63	2.19
	10	43.58	43.48	29.58	3.24

Each data point represents a mean for triplicate measurements; the coefficient of variation was less than 5% for all triplicates

3.5 Changes of pH and free copper in saliva with time.

Considering the kinetic aspects of the reaction between copper and saliva components, the changes of free copper with time in inorganic saliva was studied in this section. Figure 2-11 clearly showed the descending trend in free copper with time; this may imply the kinetic transforming of copper species in artificial saliva, resulting in the changes in free copper as well as other species of copper. This accords with Dietrich et al.'s finding that formation of malachite was observed within 3 min after initiating a reaction, but reached equilibrium after 96 h (Dietrich et al. 2005). This also seems to be able to explain the difference between experimental data and predicted data in MINEQL+, which models the environment in equilibrium. However, the pH of the sample also changed with time, which was shown in Figure 2-11. The increase of pH could not be due to the reaction of forming precipitates, because all the processes of precipitate formation of malachite, copper phosphate and tenorite generate H⁺ and reduce the pH. One possible reason of the increase in pH and decrease in free copper was the diffusion of carbon dioxide from artificial saliva to the air.

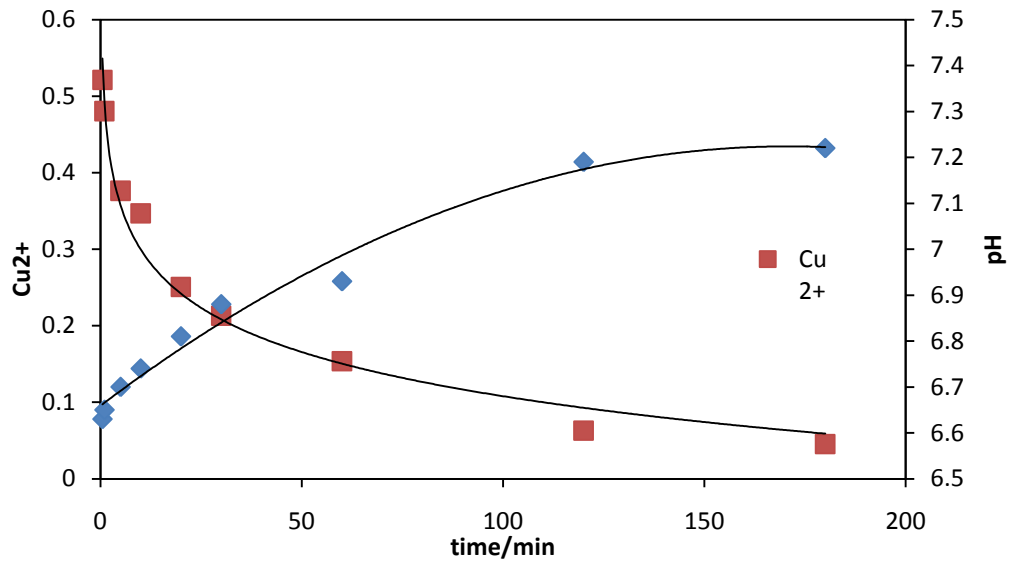


Figure 2-11 Changes in free copper concentration and pH of inorganic artificial saliva with time in 2.5 mg/L of total copper

4. Conclusions and Suggestions

Chemical speciations of copper, iron and zinc were modeled by MINEQL+ and main species including the free portion and precipitates were analyzed and compared at different pH. By comparing experimental data with predicted data in MINEQL+, the practicability and limitation of cupric ISE and MINEQL+ used in understanding the chemical speciation of metals in saliva were investigated. Although the two methods were not accurate enough to reflect the real situation perfectly, we can still use them to know the probable concentrations of each species and trends of those species varying with pH, concentration of saliva components and time. Inorganic components, typically phosphate, carbonate and hydroxide combined with copper and greatly controlled the levels of free copper when water was ingested in oral cavity. Proteins such as α -amylase, mucin and lactoferrin also impacted the chemical speciation of copper. In the three major proteins studied, mucin had the greatest affinity with copper, followed by α -amylase, and lactoferrin had very weak binding capacity with copper at the concentrations considered in this study. Inorganic components dominated in the chemical speciation of copper when no levels of mucin existed in artificial saliva. When the concentration of mucin was raised to 0.216 g/L (approximately 10% of total mucin in saliva), free copper is controlled by the concentration of mucin. The monitoring of free copper with time was conducted to study the kinetic processes that happen when copper was ingested into oral cavity. Free copper decreased with time but pH also increased at the meantime, which made it difficult to explain what caused the reducing of free copper: pH or the chemical reactions involving copper.

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Appendix

Abbreviation

PE	Polyethylene
NOM	Natural Organic Matter
PEX	Cross-linked polyethylene
HDPE	High density polyethylene
OIT	Oxidation induction time
Kow	Octanol water partition coefficient
MTBE	Methyl tert-butyl ether
Sw	Water solubility
Mv	Molecular volume
Vocs	Volatile organic compounds
ISE	Ion selective electrode
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SAGF	Artificial Saliva Gal Fovet
HPLC	High Performance Liquid Chromatography
TBARS	Thiobarbituric Acid Reactive Substances

Data for regression model

Appendix A-1 Contaminant Characteristics at 25 °C

Contaminant Name	Contaminant Property					
	μ , Debye	M_m , g/mol	ρ , g/cm ³	M_v , cm ³	S_w , mg/L	Log K_{ow}
<i>Polar Contaminants</i>						
Acetonitrile	3.92	41.05	0.786	53.3	Miscible	-0.34
2-Propanone	2.88	58.08	0.789	73.1	Miscible	-0.24
Benzaldehyde	2.80	106.12	1.041	120.5	3000	1.48
2-Butanone	2.76	72.11	0.805	91.4	223000	0.29
Water	1.85	18.01	1.000	19.1	Miscible	-
Benzyl Alcohol	1.71	108.14	1.041	125.1	40000	1.10
1-Butanol	1.66	74.12	0.809	96.2	74000	-0.30
2-Propanol	1.56	60.10	0.789	77.8	Miscible	0.05
<i>Nonpolar Contaminants</i>						
Dichloromethane	1.60	84.93	1.326	60.6	13030	1.25
Methyl <i>t</i> -butyl ether	1.36	88.15	0.740	119.1	51000	1.24
Trichloromethane	1.01	119.37	1.492	74.4	7290	1.97
Toluene	0.36	92.14	0.867	117.7	526	2.73
<i>m</i> -Xylene	0.30	106.16	0.864	135.9	161	3.20
<i>p</i> -Xylene	0.00	106.16	0.867	135.8	162	3.15

μ = Dipole moment; δ = Solubility parameter; M_v = Molar volume; M_m = Molar mass; S_w = Water solubility; ρ = Density; K_{ow} = Octanol-water partition coefficient; M_m , S_w , Log K_{ow} , and ρ values obtained from *CHEMFATE* (Syracuse Research Corporation 2009).

Appendix A-2 Bulk Characteristics of Polyethylene Materials

Property	Type of Polyethylene ¹					
	HDPE Resin	Monomodal HDPE Pipe	Bimodal HDPE Pipe	PEX-A Pipe (MDPE)	PEX-B1 Pipe (HDPE)	PEX-B2 Pipe (HDPE)
Thickness, mm.	1.65 ± 0.02	2.61 ± 0.05	2.59 ± 0.08	2.60 ± 0.02	2.64 ± 0.03	2.63 ± 0.03
<i>Composition</i>						
Polymer	99.9%	97.8%	97.7%	99.8%	99.4%	99.4%
Char & Carbon Black	0%	2.1%	2.0%	0%	0.1%	0.1%
Volatile Loss at 150 °C	0.1%	0.1%	0.2%	0.2%	0.5%	0.5%
Ash	0%	0%	0.1%	0%	0%	0%
<i>Oxidative Resistance and Morphology</i>						
Tdeg _{onset} , °C	431	459	465	460	462	461
Tdeg, °C	465	479	485	482	484	484
Tm _{onset} , °C	127.0 ± 0.6	121.7 ± 0.2	123.4 ± 0.5	120.3 ± 1.2	119.8 ± 1.1	119.5 ± 0.6
Tm, °C	135.1 ± 0.5	128.7 ± 0.5	130.2 ± 0.1	130.1 ± 0.4	130.0 ± 0.3	129.7 ± 0.2
OIT (min.)	22.4 ± 2.4	92.5 ± 1.3	119.6 ± 1.1	33.6 ± 3.9	119.6 ± 12.3	> 295
Bulk Density, g/cm ³	0.9572 ± 0.0001	0.9494 ± 0.0002	0.9547 ± 0.0001	0.9371 ± 0.0005	0.9524 ± 0.0001	0.9510 ± 0.0001
<i>Crystallinity</i>						
by T _m , %	62.7 ± 0.6	50.9 ± 1.9	51.7 ± 2.4	45.4 ± 2.1	54.4 ± 2.7	54.9 ± 1.1
by Density, %	74.3 ± 0.1	69.3 ± 0.1	72.7 ± 0.1	61.3 ± 0.3	71.2 ± 0.0	70.4 ± 0.1
Crosslink Density, % of 100	–	–	–	76.1 ± 5.5	60.4 ± 2.2	–

1. Tdeg_{onset} = Onset Temperature of Degradation; Tdeg = Degradation Temperature; Tm_{onset} = Onset of Melting Temperature; Tm = Melting Temperature; All values represent mean and standard deviation for three replicates unless otherwise noted. Compositional analysis and Tdeg tests only represent one measurement. Thickness measurements represent mean for 41 or 42 replicates per PE type (Whelton 2009)

Appendix A-3 Contaminant Solubility of Polyethylene Materials

Contaminant Class and Name ¹	Solubility, g/cm ³					
	HDPE Resin	Monomodal HDPE Pipe	Bimodal HDPE Pipe	PEX-A Pipe (MDPE)	PEX-B1 Pipe (HDPE)	PEX-B2 Pipe (HDPE)
<i>Polar Contaminants</i>						
Acetonitrile	0.0012	0.0014	0.0018	0.0024	0.0027	0.0029
2-Propanone	0.0098	0.0101	0.0100	0.0120	0.0145	0.0143
Benzaldehyde	0.0204	0.0216	0.0214	0.0238	0.0263	0.0267
2-Butanone	0.0186	0.0190	0.0191	0.0219	0.0253	0.0237
Benzyl Alcohol	0.0051	0.0048	0.0036	0.0050	0.0063	0.0049
1-Butanol	0.0084	0.0053	0.0033	0.0053	0.0088	0.0056
2-Propanol	0.0047	0.0035	0.0031	0.0048	0.0054	0.0091
<i>Nonpolar contaminants</i>						
Dichloromethane	0.0685	0.0865	0.0854	0.0996	0.0862	0.0875
Methyl <i>t</i> -butyl ether	0.0452	0.0546	0.0524	0.0591	0.0547	0.0534
Trichloromethane	0.1107	0.1428	0.1437	0.1655	0.1375	0.1417
Toluene	0.0660	0.0853	0.0859	0.0981	0.0807	0.0804
<i>m</i> -Xylene	0.0681	0.0875	0.0887	0.1010	0.0831	0.0836
<i>p</i> -Xylene	0.0691	0.0888	0.0896	0.1023	0.0844	0.0845

1. Mean values represent three replicates. (Whelton 2009)

Appendix A-4 Contaminant Diffusivity of Polyethylene Materials

Contaminant Class and Name ¹	Diffusivity, 10 ⁻⁹ cm ² /s					
	HDPE Resin	Monomodal HDPE Pipe	Bimodal HDPE Pipe	PEX-A Pipe (MDPE)	PEX-B1 Pipe (HDPE)	PEX-B2 Pipe (HDPE)
<i>Polar Contaminants</i>						
Acetonitrile	3.76	11.5	14.2	30.0	6.38	14.2
2-Propanone	5.07	10.5	10.3	17.6	8.64	9.42
Benzaldehyde	2.65	4.77	4.02	8.11	4.29	3.92
2-Butanone	4.95	7.90	9.78	17.4	8.23	10.2
Benzyl Alcohol	3.17	5.52	7.40	4.03	3.89	6.66
1-Butanol	0.28	0.99	2.92	2.69	0.89	2.13
2-Propanol	1.07	4.97	2.78	2.99	2.42	4.82
<i>Nonpolar Contaminants</i>						
Dichloromethane	52.6	64.4	85.4	132	73.1	95.6
Methyl <i>t</i> -butyl ether	5.00	6.65	8.75	12.6	5.96	6.92
Trichloromethane	41.2	58.2	66.3	91.9	61.7	63.9
Toluene	42.5	56.9	72.4	96.7	59.6	62.3
<i>m</i> -Xylene	29.7	37.1	45.9	62.7	38.1	36.1
<i>p</i> -Xylene	45.6	54.9	70.6	95.1	58.1	60.1

1. Mean values represent three replicates (Whelton 2009)

Appendix A-5 Mechanical Properties for New and Lab Aged Polyethylene

Polymer Type	Condition	Bulk Property ¹					
		Bulk Density, g/cm ³	Crystallinity, %	Tensile Strength at Yield, MPa	Tensile Strength at Break, MPa	Elongation at Break, %	Oxidation Induction Time, min.
HDPE Resin	New	0.9578 ± 0.0001	71.5 ± 0.1	45.8 ± 0.8	26.8 ± 3.8	615 ± 199	22.4 ± 2.3
	Aged	0.9581 ± 0.0000	71.7 ± 0.0	44.2 ± 1.9	24.2 ± 6.3	529 ± 69	13.5 ± 2.2
HDPE Pipe	New	0.9503 ± 0.0004	66.4 ± 0.2	30.1 ± 0.8	21.8 ± 6.6	491 ± 174	97.1 ± 1.8
	Aged	0.9513 ± 0.0002	67.1 ± 0.1	31.5 ± 1.2	22.4 ± 5.1	491 ± 181	29.7 ± 6.7
PEX-A Pipe	New	0.9385 ± 0.0002	58.4 ± 0.1	30.6 ± 1.2	35.0 ± 2.7	569 ± 44	33.5 ± 3.9
	Aged	0.9389 ± 0.0001	58.7 ± 0.1	31.8 ± 1.9	34.8 ± 2.4	536 ± 19	27.2 ± 5.7
PEX-B Pipe	New	0.9524 ± 0.0001	67.8 ± 0.1	37.4 ± 1.9	34.9 ± 2.0	379 ± 38	119.6 ± 12.3
	Aged	0.9527 ± 0.0000	68.1 ± 0.0	36.0 ± 1.0	23.7 ± 3.8	154 ± 18	5.4 ± 0.6

1. Mean and standard deviation shown for all measurements (Whelton 2009)

Appendix A-6 Contaminant Solubility in New and Lab Aged Polyethylene

Contaminant Name ¹	Solubility, g/cm ³							
	HDPE Resin		HDPE Pipe		PEX-A Pipe		PEX-B Pipe	
	New	Aged	New	Aged	New	Aged	New	Aged
<i>Polar Contaminants</i>								
Acetonitrile	0.0012	–	0.0014	–	0.0023	–	0.0026	–
2-Propanone	0.0097	0.0098	0.0101	0.0107	0.0120	0.0126	0.0145	0.0128
Benzaldehyde	0.0204	0.0206	0.0216	0.0216	0.0237	0.0250	0.0263	0.0250
2-Butanone	0.0186	0.0185	0.0189	0.0199	0.0218	0.0232	0.0253	0.0225
Benzyl Alcohol	0.0051	0.0039	0.0048	0.0036	0.0050	0.0038	0.0063	0.0038
1-Butanol	0.0084	0.0064	0.0053	0.0045	0.0053	0.0062	0.0088	0.0063
2-Propanol	0.0047	0.0037	0.0035	0.0030	0.0048	0.0048	0.0054	0.0051
<i>Nonpolar Contaminants</i>								
Methyl <i>t</i> -butyl ether	0.0452	0.0449	0.0546	0.0575	0.0591	0.0611	0.0547	0.0634
Trichloromethane	0.1106	0.1091	0.1426	0.1425	0.1652	0.1590	0.1373	0.1372
Toluene	0.0660	0.0656	0.0852	0.0853	0.0979	0.0961	0.0806	0.0812
<i>m</i> -Xylene	0.0680	0.0685	0.0874	0.0873	0.1009	0.0984	0.0830	0.0827

1.(Whelton 2009)

Appendix A-7 Contaminant Diffusivity in New and Lab Aged Polyethylene

Contaminant Name	Diffusivity, 10^{-9} cm ² /s							
	HDPE Resin		HDPE Pipe		PEX-A Pipe		PEX-B Pipe	
	New	Aged	New	Aged	New	Aged	New	Aged
<i>Polar Contaminants</i>								
2-Propanone	5.07	9.73	10.52	19.72	17.57	26.41	8.64	16.18
Benzaldehyde	2.65	3.67	4.77	6.04	8.11	9.30	4.29	5.09
2-Butanone	4.95	7.32	7.90	12.32	17.40	18.58	8.23	11.63
Benzyl Alcohol	3.17	8.99	5.52	9.92	4.03	23.31	3.89	14.98
1-Butanol	0.28	0.58	0.99	2.61	2.69	2.15	0.89	2.05
2-Propanol	1.07	1.52	4.97	4.03	2.99	2.27	2.42	1.86
<i>Nonpolar Contaminants</i>								
Methyl <i>t</i> -butyl ether	5.00	5.58	6.65	7.15	12.64	11.76	5.96	4.71
Trichloromethane	41.22	42.60	58.20	61.42	91.92	91.02	61.73	61.11
Toluene	42.55	50.24	56.92	68.82	96.73	99.24	59.62	61.35
<i>m</i> -Xylene	29.68	32.77	37.06	44.04	62.71	66.43	38.10	39.44

(Whelton 2010)

R codes for regression model

New pipe

```
“Diffusion”
“linear regression”
x<- read.csv("matrix-new.csv")
attach(x)
lmdif <- lm(Diffusion~Mu+Mw+Rhow+Sigma+Mv+Sw+Log
Kow+Thickness+Polymer+Char+VolatileLoss+Tdegonset+Tdeg+Tmonset+Tm+OIT+BulkDensity+Crystallinity+
Crosslink)
summary(lmdif)
“residuals”

residuals(lmdif)
shapiro.test(residuals(lmdif))
mv.factor<-as.factor(Mv)
plot(residuals(lmdif)~fitted(lmdif),col=mv.factor)

“tree regression”
library(rpart)
tdif <- rpart(Diffusion ~ Mu+Mw+Rhow+Sigma+Mv+Sw+Log
Kow+Thickness+Polymer+Char+VolatileLoss+Tdegonset+Tmonset+OIT+BulkDensity+Crystallinity+Crosslink)
summary(tdif)
post(tdif, file="")

“Solubility”
“linear regression”
lmsol <- lm(Solubility ~ Mu+Mw+Rhow+Sigma+Mv+Sw+Log
Kow+Thickness+Polymer+Char+VolatileLoss+Tdegonset+Tdeg+Tmonset+Tm+OIT+BulkDensity+Crystallinity+
Crosslink)
summary(lmsol)
residuals(lmsol)
shapiro.test(residuals(lmsol))
mv.factor<-as.factor(Mv)
plot(residuals(lmsol)~fitted(lmsol),col=mv.factor)

“tree regression”
library(rpart)
tsol <- rpart(Solubility ~ Mu+Mw+Rhow+Sigma+Mv+Sw+Log
Kow+Thickness+Polymer+Char+VolatileLoss+Tdegonset+Tdeg+Tmonset+Tm+OIT+BulkDensity+Crystallinity+
Crosslink)
summary(tsol)
post(tsol, file="")
```

Aged pipe

```
“Diffusion”
“linear regression”

y<- read.csv("matrix-aged.csv")
attach(y)
lmdif <-
lm(Diffusivity~Mu+Mw+Rhow+Mv+Sw+Log Kow+Tsy+Tsb+Elongation+OIT+BulkDensity+Crystallinity)
```

```
summary(lmdif)
“residuals”
```

```
residuals(lmdif)
shapiro.test(residuals(lmdif))
mv.factor<-as.factor(Mv)
plot(residuals(lmdif)~fitted(lmdif),col=mv.factor)
```

```
“tree regression”
library(rpart)
tdif <- rpart(Diffusivity ~ Mu+Mw+Rhow+Mv+Sw+Log
Kow+Tsy+Tsb+Elongation+OIT+BulkDensity+Crystallinity)
summary(tdif)
post(tdif, file="")
```

```
“Solubility”
“linear regression”
lmsol <- lm(Solubility ~ Mu+Mw+Rhow+Mv+Sw+Log
Kow+Tsy+Tsb+Elongation+OIT+BulkDensity+Crystallinity)
summary(lmsol)
residuals(lmsol)
shapiro.test(residuals(lmsol))
mv.factor<-as.factor(Mv)
plot(residuals(lmsol)~fitted(lmsol),col=mv.factor)
```

```
“tree regression”
library(rpart)
tsol <- rpart(Solubility ~ Mu+Mw+Rhow+Mv+Sw+Log
Kow+Tsy+Tsb+Elongation+OIT+BulkDensity+Crystallinity)
summary(tsol)
post(tsol, file="")
```


Experimental data for copper speciation

Appendix A-8 Copper speciation by MINEQL+

Total Cu	pH	5.5	6	6.5	7	7.5
10mg/L	Free Cu	6.94	1.75	0.42	0.044387	0.004439
	total soluble	7.72	2.21	0.73	0.16	0.07
	Cu ₃ (PO ₄) ₂	2.28	7.79	3.01	0	0
	Malachite	0	0	6.26	0	0
	Tenorite	0	0	0	9.84	9.93
5mg/L	Free Cu	4.49	1.62	0.41	0.044387	0.004439
	total soluble	5	2.065	0.725	0.16	0.075
	Cu ₃ (PO ₄) ₂	0	2.935	1.7	0	0
	Tenorite	0	0	2.575	4.84	4.925
2.5mg/L	Free Cu	2.245	1.56	0.4075	0.045	0.004439
	total soluble	2.5	2.005	0.725	0.16	0.0725
	Cu ₃ (PO ₄) ₂	0	0.495	1.0475	0	0
	Tenorite	0	0	0.7275	2.34	2.4275

Appendix A-9 Free copper concentration in artificial saliva with different concentration of total copper and proteins; all the data are based on triplicate samples

pH	5.5	6	6.5	7	7.5
10-inorganic	8.393205	5.057785	1.203762	0.251196	0.049564
10-inorganic-amylase	7.140667	5.190403	1.942438	0.426867	0.089077
10-amylase	8.393205	6.42486	2.930565	0.899837	0.182589
5-inorganic	4.79255	4.085994	1.203762	0.264537	0.05661
5-inorganic-amylase	2.53216	1.448939	0.745992	0.278985	0.082075
5-amylase	2.53216	1.489034	0.670749	0.264537	0.058866
2.5-inorganic	1.869968	1.585567	0.647917	0.141791	0.030288
2.5-inorganic-amylase	0.474331	0.151851	0.038085	0.008154	0.001339
2.5-amylase	0.588818	0.147678	0.034144	0.004845	0.000809
10-inorganic	8.393205	5.057785	1.203762	0.251196	0.049564
10-inorganic-mucin	0.34485	0.088888	0.016654	0.002524	0.000416
10-mucin	0.58726	0.119163	0.019662	0.002524	0.000264
5-inorganic	4.79255	4.085994	1.203762	0.264537	0.05661
5-inorganic-mucin	0.104259	0.023594	0.003761	0.000634	0.000116
5-mucin	0.177547	0.027736	0.003668	0.000439	6.44E-05
2.5-inorganic	1.869968	1.585567	0.647917	0.141791	0.030288
2.5-inorganic-mucin	0.151851	0.03333	0.006563	0.00113	0.000206
2.5-mucin	0.261005	0.02675	0.005256	0.000458	0.000106

Appendix A-10 Free copper concentration in artificial saliva with different concentration of mucin

	pH	5.5	6	6.5	7	7.5
MV	inorg+0% mucin	285.5	275.5	260	244	227
	inorg+10% mucin	284	281	266	248	231
	inorg+50% mucin	280	275	265	251	234
	inorg+70% mucin	276	268	257	245	229
	inorg+100% mucin	273	260	246	229	210
	Concentration (mg/L)					
	inorganics+0% mucin	3.373643	1.534067	0.45222	0.128157	0.033567
	inorganics+10% mucin	2.997512	2.36638	0.725608	0.175648	0.046006
	inorganics+50% mucin	2.187051	1.474795	0.670621	0.222495	0.058276
	inorganics+70% mucin	1.595721	0.84948	0.357004	0.138665	0.039297
	inorganics+100% mucin	1.259739	0.45222	0.150035	0.039297	0.008792

Appendix A-11 Free copper concentration in nanopure water at different pHs

pH		5.5	6	6.5	7	7.5
10mg/l	mV	325	324	319	291	279
	C (mg/L)	10	9.51	6.41	0.702	0.272
5mg/l	mV	316	313	298	286	272
	C (mg/L)	5	3.99	1.22	0.473	0.157
2.5mg/l	mV	307	305	290	281	270
	C (mg/L)	2.48	2.12	0.649	0.319	0.134

Appendix A-12 Free copper concentration in saliva with different concentration of lactoferrin

pH		5.5	6	6.5	7	7.5
inorganic		297	291	272	253	232
Inorganic & Lac		298	289	268	249	233
only Lac		299	298	285	277	258
10 times Lac		296	296	293	279	265
pH		5.5	6	6.5	7	7.5
Inorganics		8.350108	5.204023	1.164274	0.260478	0.049778
inorganics & Lac		9.03478	4.445169	0.84948	0.190051	0.053859
Only Lac		9.775592	9.03478	3.243294	1.726564	0.386277
10 times Lac		7.717322	7.717322	6.092425	2.021313	0.670621

Appendix A-13 pH and free copper changes with time

time/min	pH	mV	C(mg/L)
0.5	6.63	260	0.521172
1	6.65	259	0.48045
5	6.7	256	0.376399
10	6.74	255	0.346989
20	6.81	251	0.250601
30	6.88	249	0.212969
60	6.93	245	0.15381
120	7.19	234	0.062852
180	7.22	230	0.045393