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CHELATING RESIN VERSUS ION-EXCHANGE RESIN FOR HEAVY METAL REMOVAL IN TOXICITY FRACTIONATION

C. N. Mazidji*, B. Koopman** and G. Bitton**

** Sirrine Environmental Consultants, Greenville, SC 29616, USA*
*** Dept. of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611, USA*

ABSTRACT

A chelating resin (Chelex 50-100) and ion-exchange resin (Dowex 50W-X8) were evaluated for removal of heavy metals in toxicity fractionation. Microtox and ß-galactosidase activity were employed as toxicity endpoints. The resins were packed into 4 mL glass Pasteur pipettes for use. Chelating resin provided complete removal of toxicity due to polyvalent heavy metal cations (Cd, Cu, Hg, Pb, Zn). Ion-exchange resin was ineffective in removing mercury toxicity. Neither resin provided complete removal of Ag^+ toxicity. Toxicity of organic compounds was, at most, partially removed. Performance of the ion-exchange and chelating resins was insensitive to hardness and pH. Based on these results, chelating resin is recommended for heavy metal removal as part of a toxicity fractionation procedure.

KEYWORDS

Chelating resin, ion-exchange resin, toxicity, heavy metals, toxicity fractionation

INTRODUCTION

The choice of remediation or treatment technology for toxic pollution depends on the nature of the chemicals present. This is particularly true of heavy metals versus organic compounds. It is therefore advantageous to distinguish between heavy metal and organic toxicity in environmental or wastewater samples that are being screened for toxic pollution. Walsh and Garnas (1983) were the first to propose a fractionation scheme for identifying the classes of chemicals causing toxicity in water samples. Their technique utilized XAD resin for the removal of organics, followed by passage of the XAD column effluent through cation and anion exchange resins. Another technique, chelation with EDTA, has been used to neutralize heavy metal toxicity in algal bioassays (Couture *et al.*, 1985) and the EPA phase I fractionation procedures (Mount and Anderson-Carnahan, 1987). Most heavy metals exist in solution as polyvalent cations and are thus susceptible to chelation by EDTA. A major disadvantage of the EDTA chelation technique is that EDTA is toxic at high concentrations, so that a large number of dosages must be tested in order to find the one that is sufficiently high to neutralize heavy metal toxicity but low enough so that, after reaction with metals, residual EDTA is not toxic to the test organism.

Ion-exchange and chelating resins are alternatives to EDTA for removal of heavy metal toxicity from samples. Ion-exchange resins commonly consist of a styrene-divinyl benzene copolymer matrix on which ionogenic groups are attached. Cation-exchange resins contain fixed anionic groups such as $-SO_3^-$, $-COO^-$, $-PO_3^{2-}$ and $-AsO_3^{2-}$. The retention of metals by cation exchange resins occurs through the exchange, by the ionogenic groups, of loosely bound hydrogen or sodium ions (counterions) for the metal ions. The selectivity scale for Dowex 50-X8 in dilute HCl was reported by Korkisch (1989) as:

$$Mn > Zn > Fe > Mg > Co > Ni > Cu$$

Chelating ion exchangers are resins that carry functional groups able to form complex (chelate) compounds with selected ions. The most common available resin is the iminodiacetate chelating resin commercially available as Chelex 100 (Sigma) or Dowex A-1 (Korkisch, 1989) (functional group = N[phenylmethyl]-iminodiacetic acid). It consists of a divinylbenzene skeleton on which are attached iminodiacetic acid chelating groups. Iminodiacetate chelating resins have been reported to result in complete adsorption of Cu, Pb, Ni, Co, Cd and Zn at pH 4 to 8. The adsorption mechanism consists of ion exchange at low pH, chelation at high pH and both ion exchange and chelation at intermediate pH. The order of selectivity of Chelex 100 for divalent metals was reported as (Korkisch, 1989):

$$Hg > Cu > Pb > Ni > Cd > Zn > Co > Fe > Mn > Be > Ca > Mg > Sr > Ba$$

Use of chelating or ion-exchange resins can simplify fractionation of heavy metal toxicity. As long as the resin leaves no residual toxic material in the sample, the necessity of testing numerous fractions is eliminated. The work reported in this paper compares the effectiveness of the two types of resin in removing heavy metal toxicity and examines the effects of pH and hardness on their performance. In addition, the extent to which organic toxicity is removed by the resins is discussed. Toxicity was measured by Microtox and β-galactosidase activity.

MATERIALS AND METHODS

Model Toxicants

The model toxicants included eight heavy metals: cadmium ($CdSO_4$), copper ($CuSO_4$ $5H_2O$), zinc ($ZnSO_4$), lead ($PbNO_3$), mercury ($HgSO_4$), silver ($AgNO_3$), arsenic (Na_2HAsO_4 $7H_2O$), chromium ($K_2Cr_2O_7$) and seven organics: pentachlorophenol (PCP), sodium dodecyl sulfate (SDS), tetrachloroethylene, benzene, phenol, formaldehyde, and lindane. All chemicals were ACS grade or higher.

The stock solutions of PCP, benzene, tetrachloroethylene, and lindane (hexacyclochlorohexane) were prepared with methanol. Stock solutions of the other chemicals were prepared with MilliQ water (Millipore Corp., New Bedford, MA). Working solutions of the chemicals (except for silver) used in the tests were prepared in moderately hard reconstituted water (Peltier and Weber, 1985) with pH adjusted to a range of 7.0 to 7.5. Working solutions of silver were prepared using MilliQ water to avoid precipitation. Final methanol concentrations in the PCP, tetrachloroethylene, benzene, and lindane working solutions were 1% or less, within the nontoxic range for Microtox and ß-galactosidase activity.

Column Preparation and Treatment

Flint glass Pasteur pipettes (Fisher) with a volume of 4 mL were packed with 1.6 g of ion-exchange resin (Dowex 50W-X8, Baker) in the acidic form or 1.6 g chelating resin (Chelex 50-100, Sigma). Resin mass was as supplied by the manufacturers. The ion-exchange resin had a moisture content

of 50% whereas the chelating resin had a moisture content of 70%. The ion-exchange columns were preconditioned by passing 25 mL of MilliQ water (10 bed volumes) through the columns followed by 250 mL 1M NaCl, with a final rinsing with 25 mL MilliQ water. The preconditioning procedure for the chelating columns was the same except that 25 mL 1M NaCl was used. The pH of the MilliQ water was adjusted to the range of 7.0 to 7.5 before use. Samples were run through duplicate columns.

Column Capacity

The cation exchange capacity of the Dowex 50W-X8 resin after preconditioning was determined by passing a solution of 100 g m^{-3} Mg^{2+} (from $MgCl_2$) through the column until saturation was reached. The capacity of preconditioned chelating resin was determined in a similar fashion, except that a concentration of 15 g m^{-3} Mg^{2+} was used. Magnesium hardness was measured according to the EDTA titration method described in *Standard Methods*, method 314 B (APHA *et al.*, 1985). ManVer (Hach, Ames, IA) indicator reagents were used during the titration process.

ß-Galactosidase Activity

The reagent o-nitrophenylgalactopyranoside (ONPG; Sigma) was prepared at a concentration of 0.4% in MilliQ water and sterilized by filtration through 0.2 μm membrane filter (Gelman, Acrodisc). Sodium phosphate buffer (pH 7.0) contained 1.61% w/v Na_2HPO_4 $7H_2O$ and 0.55% w/v NaH_2PO_4 H_2O. Other reagents employed were 0.1% w/v sodium dodecyl sulfate and 1M Na_2CO_3.

Assays were conducted using an *E. coli* (EW1b) strain obtained from the Yale Univ. collection. This strain was maintained in 40% glycerol at -15 °C. It was grown by inoculating 25 mL broth medium containing lactose and buffered with phosphate (Bacto tryptone, 5% w/v; Bacto yeast extract, 2.5% w/v; NaCl, 5% w/v; K_2HPO_4, 0.8% w/v; KH_2PO_4, 0.3% w/v; lactose, 1% w/v) in a 125 mL Erlenmeyer flask with 50 μL of glycerol culture. The bacteria were incubated at 35 °C overnight, then diluted with fresh medium to an absorbance (A_{550}, 1 cm) of 0.3 to 0.4. Growth was continued to an A_{550} of 0.6 to 0.7. A 10 mL volume of cells was then centrifuged, washed with MilliQ water and resuspended in Reinhartz media [trehalose, 112.5 g/L; NaCl 3.5g/L; $MgCl_2$ 0.6 g/L; MOPS 2.62 g/L (Reinhartz *et al.*, 1987)].

A volume of 0.1 mL prepared cells was exposed to 0.9 mL sample for 30 minutes, then a 1 mL solution composed of phosphate buffer, SDS and ONPG at a ratio of 8:1:2 was added to the incubated sample and color was allowed to develop for approximately 60 minutes. The intensity of the yellow color (*o*-nitrophenol) was proportional to ß-galactosidase activity. The reaction was stopped after 15 minutes by adding 1 mL of 1M Na_2CO_3.

Reconstituted, moderately hard water (Peltier and Weber, 1985) was used for controls. The *o*-nitrophenol was quantitated by its absorbance at 420 nm using a spectrophotometer (Spec 21, Milton Roy Co, Rochester, NY). All incubations were at 35 °C. The degree of inhibition was determined on the basis of measured absorbance values, considering the control to represent 0% inhibition. Blanks with MilliQ water substituted for ONPG were run with each sample. Six replicates of the controls were tested in each experiment. Column influent and effluent fractions were tested in triplicate.

Microtox

Lyophilized *Photobacterium phosphoreum* (Microbics) were reconstituted for use in the tests. All assays were carried out at 15 °C with a 15-minute contact period. Microtox assays of the influent and

effluent from each chelation column were run in duplicate. Data were tabulated and reduced according to the Microtox Operating Manual (Microbics, 1982).

RESULTS AND DISCUSSION

Cation Adsorption Capacity of Resins

One of the factors influencing the suitability of a resin for use in removing heavy metal toxicity is its cation adsorption capacity. We determined the capacity of both resins under dynamic (flow-through) conditions using Mg^{2+} solutions. The quantity of Mg^{2+} removed up to the point of break through (> 0 g m^{-3} Mg^{2+} in column effluent) is termed the useful capacity whereas the total quantity of Mg^{2+} removed to the point of saturation is termed the total capacity. The useful capacity of the ion-exchange resin was 1.5 meq g^{-1} resin, compared to 0.33 meq g^{-1} resin for the chelating resin. The total capacities of the ion-exchange and chelating resins were 3.5 and 0.49 meq g^{-1} resin, respectively. The value found for the total capacity of the ion-exchange resin is consistent with values from the literature. The equilibrium capacity of the Dowex 50W-X8 ion-exchange resin reported by the manufacturer was 5 meq g^{-1} resin, whereas resins most widely used in ion-exchange chromatography have capacities in the range of 3 to 5 meq g^{-1} resin (Shpigun and Zolotov, 1988). We could not find in the literature a value for the cation adsorption capacity of chelating resin.

Background Toxicity

Another important factor is the potential contribution by the resin of toxic chemicals to water samples. This is one of the reasons for preconditioning the columns. Background toxicity was investigated by passing reconstituted water at pH 7 through preconditioned ion-exchange and chelating columns. Successive effluent fraction volumes of 5, 5, 20, 20 and 40 mL were collected. The maximum inhibition of bioluminescence in the Microtox assay by any of the effluent fractions was 8%, whereas the maximum inhibition of β-galactosidase activity was 4%.

Effect of Chelating Column Treatment on Organic Toxicity

In the proposed toxicity fractionation procedure, detoxification of a water sample by passage through an ion-exchange or chelating column would be considered evidence that heavy metals are the cause of toxicity in the untreated sample. It is thus important that the columns not remove organic toxicants, at least to a significant extent. We tested the effect of column treatment on several toxic organic compounds including benzene (175 g m^{-3}), formaldehyde (20 g m^{-3}), lindane (8 g m^{-3}), pentachlorophenol (PCP) (12 g m^{-3}), phenol (100 g m^{-3}), SDS (10 g m^{-3}), and tetrachloroethylene (100 g m^{-3}). As shown in Table 1, benzene and tetrachloroethylene toxicity were partially removed by both resins. The chelating resin, in addition, partially removed lindane toxicity. The toxicities of formaldehyde and phenol were not affected by column treatment.

Removal of Heavy Metal Toxicity

Considering that some organic toxicants will be partially removed by the ion-exchange or chelating resins, it is important for the resins to remove completely heavy metal toxicity to provide a clear distinction between the two classes of chemical toxicity. We tested the ability of the resins to detoxify solutions of the following heavy metals: silver (1 g m^{-3}), cadmium (1.2 g m^{-3}), copper (3.5 g m^{-3}), mercury (0.25 g m^{-3}), and zinc (6 g m^{-3}). The selection of heavy metal concentrations was made so as to have significant toxicity ($\geq$ 50% inhibition, if possible) without exceeding the higher range of concentrations typically found in municipal wastewater.

TABLE 1. Effect of Treatment by Ion-exchange and Chelating Columns on Organic Toxicity

Column			Microtox inhibition, %					
Resin	Toxicant	Loading μmole/g resin	Infl.	Effluent fraction*				
				1	2	3	4	5
Ion-exchange	Benzene	112	71	30	34	70	48	44
	Formaldehyde	33	43	39	46	45	45	46
	PCP	2.2	91	91	92	92	90	89
	Phenol	53	78	72	77	80	78	79
	Lindane	1.4	34	19	35	30	35	36
	SDS	1.7	92	86	95	96	95	94
	Tetrachloro-ethylene	30	62	32	35	40	47	46
Chelating	Benzene	112	71	18	30	69	45	49
	Formaldehyde	33	41	36	37	47	46	46
	PCP	2.2	91	76	86	87	87	86
	Phenol	53	78	38	64	75	75	78
	Lindane	1.4	34	17	2	8	15	25
	SDS	1.7	92	78	89	94	93	92
	Tetrachloro-ethylene	30	62	26	26	23	35	38

* Fractions 1, 2, 3, 4, and 5 correspond to successive effluent volumes of 5, 5, 10, 20, and 40 mL

TABLE 2. Effect of Treatment by Ion-exchange and Chelating Columns on Heavy Metal Toxicity

Column			% inhibition						Toxicity test
Resin	Toxicant	Loading μeq/ g resin	Infl.	Effluent fraction*					
				1	2	3	4	5	
Ion-exch.	Ag^{2+}	0.46	100	19	12	22	19	25	β-gal
	Cd^{2+}	1.1	100	11	15	10	15	19	β-gal
	Cu^{2+}	5.5	98	1	-2	1	3	10	Mtox
	Hg^{2+}	0.13	100	33	41	82	100	100	β-gal
	Zn^{2+}	9.2	81	8	13	3	8	9	β-gal
Chela-ting	Ag^{2+}	0.46	58	26	24	35	37	42	Mtox
	Cd^{2+}	1.1	72	-1	-3	-8	6	-1	β-gal
	Cu^{2+}	5.5	100	1	3	3	3	19	Mtox
	Hg^{2+}	0.13	100	9	7	6	8	12	Mtox
	Zn^{2+}	9.2	51	-4	0	9	9	10	Mtox
	Pb^{2+}	2.4	28	7	3	1	13	30	Mtox
	$HAsO_4^{2-}$	11	55	52	55	57	55	55	Mtox
	$Cr_2O_7^{2-}$	41	34	25	26	10	13	17	Mtox

* Fractions 1, 2, 3, 4, and 5 correspond to successive effluent volumes of 5, 5, 10, 20, and 40 mL

Neither of the resins was effective in removing silver toxicity (Table 2). Mercury toxicity was almost completely removed by the chelating resin, whereas the ion-exchange resin was also ineffective for this metal. Toxicities of cadmium, copper, and zinc were effectively removed by both resins.

We tested a few additional metals in the chelating columns, as shown at the bottom of Table 2. These included lead, arsenic and chromium. Lead was almost completely removed. Arsenic and chromium(VI) exist as anions in water. As expected, these forms were not effectively removed by the chelating resin. We have observed similar results for chelation of As and Cr(VI) by EDTA (Mazidji, Koopman and Bitton, unpublished data).

Effect of Hardness and pH on Removal of Copper Toxicity

Divalent cations such as Ca^{2+} and Mg^{2+}, which are important minerals in water, will compete with heavy metals for active sites on the ion-exchange and chelating resins. The performance of the resins over a range of water hardness is therefore of concern.

We investigated the effect of hardness ranging between 0 and 250 g m^{-3} as $CaCO_3$ on the ability of the resins to remove copper toxicity. This range includes waters considered as very soft through very hard. As shown in Table 3, the ion-exchange resin performed well over the entire range of hardness investigated. The chelating resin was effective up to a hardness of 195 g m^{-3}. There was a modest leakage of toxicity (7 to 16% inhibition) by the chelating column at an influent hardness of 254 g m^{-3}.

TABLE 3. Effect of Hardness on Removal of Copper Toxicity*

Column		Microtox inhibition, %					
Resin	Influent hardness, g m^{-3} as $CaCO_3$	Infl.	Effluent fraction**				
			1	2	3	4	5
Ion-exchange	0	99	4	4	4	8	-6
	98	100	3	3	5	5	12
	195	99	12	1	3	8	-1
	254	100	3	3	6	5	12
Chelating	0	99	4	5	6	-1	34
	98	98	2	2	3	2	7
	195	99	17	5	3	-1	-2
	254	100	14	16	12	10	7

* Cu^{2+} concentration = 3.5 g m^{-3}; total Cu^{2+} loading = 6.9 meq g^{-1} resin
** Fractions 1, 2, 3, 4, and 5 correspond to successive effluent volumes of 20 mL each

The effect of pH on the performance of the resins is also of interest. Obviously, the larger the range of pH over which the resins perform adequately, the more flexible will be their use in toxicity fractionation. In general, it is desirable to avoid changing the pH of the sample, particularly under field conditions. We investigated a pH range of 4 to 8 on the removal of copper toxicity by the resins. pH values higher than 8 resulted in precipitation of copper and were therefore not tested. As shown in Table 4, both resins were effective at influent pH values of 5 through 8. Modest leakage was observed at pH 4.

TABLE 4. Effect of pH on Removal of Copper Toxicity*

Column		Microtox inhibition, %					
Resin	Influent pH	Infl.	Effluent fraction**				
			1	2	3	4	5
Ion-exchange	4	100	17	13	9	9	12
	5	100	2	5	0	8	9
	6	99	5	5	4	-3	11
	7	98	1	-2	1	2	10
	8	99	1	0	1	2	9
Chelating	4	100	13	10	4	5	7
	5	100	2	5	0	8	9
	6	99	5	5	4	-3	11
	7	98	1	-2	1	2	10
	8	99	3	3	2	2	9

* Cu^{2+} concentration = 3.5 g m^{-3}; total Cu^{2+} loading = 6.9 meq g^{-1} resin
** Fractions 1, 2, 3, 4, and 5 correspond to successive effluent volumes of 20 mL each

SUMMARY AND CONCLUSIONS

The results obtained from this research indicate that either ion-exchange or chelating resins can be useful for identifying heavy metal toxicity in toxicity fractionation schemes. Both resins were compatible with Microtox and β-galactosidase as toxicity endpoints and neither contributed toxicity to the samples tested. The resins were also insensitive to influent hardness and pH. Neither resin was able to remove silver toxicity, but this is not a major deficiency since silver can be readily detoxified in a fractionation procedure by adding a chloride salt to the sample. The ion-exchange resin was not effective in removing mercury toxicity, whereas the chelating resin was. For this reason, the chelating resin is recommended for use in toxicity fractionation.

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