



Quantitative and Qualitative Analysis of RAMEB in Soil

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Abstract

An HPLC method has been developed for determination of RAMEB in soil samples contaminated with hydrocarbons. The extraction efficiency of RAMEB from soils was found to depend on the soil properties. The extraction method elaborated results in a good recovery from sandy and loamy soils, but only a poor recovery was obtained from soils of high clay content.

A SPE method was worked out to remove the components disturbing the HPLC analysis, and a one-peak method has been developed for the quantitative analyses. The isomer distribution of RAMEB was characterized by fingerprint chromatograms. The new methods have been applied for determination of RAMEB content in soil samples from field treatment.

Introduction

Most of the soil contaminants, e.g., polycyclic aromatic hydrocarbons (PAHs) [1], chlorinated hydrocarbons [2], chlorinated biphenyls (PCBs) [3], explosives [4] can be solubilized by inclusion complex formation. The different CD derivatives have different solubilizing effect. The best solubilizer for PAHs was found to be the randomly methylated β -cyclodextrin (RAMEB) [5], which solubilized more trichloroethylene [6] and trinitrotoluene [7] than hydroxypropyl β CD. Besides solubilizing the contaminants RAMEB has some other effects on soil: it can mobilize the contaminants by its surface activity, and as a result droplets of the polluting liquid can get emulsified in the washing aqueous solution [6]; the volatility of PAHs is decreased by complex formation [8]; the pore structure of soil clay minerals is strongly influenced by the presence of RAMEB [9]; stimulates the proliferation of soil microflora both in contaminated and uncontaminated soil [10, 11]; enhances the biotransformations in soils, e.g., dechlorination of PCBs [11] and acetylation of trinitrotoluene metabolites [7]; etc.

As the cyclodextrins have been recently found to be effective additives in remediation of polluted soils either by soil washing (pump and treat) [12] or by bioremediation [11, 13], appropriate analytical methods are needed for their qualitative and quantitative determination in soil samples. A lot of unusual problems can arise because of the complexity of soils, the soil/pollutant, pollutant/CD and soil/CD interactions.

In biological samples, in which the CD derivatives should be quantitatively determined, size exclusion chromatography is used, where all isomers are – on purpose – compressed into one peak [14, 15]. The main difficulties in the HPLC analysis of CDs are caused by limitations of the detection system. The CDs have no chromophore and consist of a mixture of structurally related compounds. We have not found any literature on determination of CDs in environmental samples.

Our aim was to work out analytical methods for determination of RAMEB to be able to follow the distribution, adsorption and biodegradation of this additive in soil.

Experimental

Three uncontaminated soils were used for the method development. A soil contaminated with motor oil was also studied. The physico-chemical properties of the soils measured by standard methods are listed in Table 1.

Randomly methylated β -cyclodextrin (RAMEB), degree of substitution (DS):12.6 (CAVAMAX W 7), was used and RAMEB 50% aqueous solution (RAMEB50s) (RAMEB content: 48.1% (w/w), CAVASOL W7 MTL), obtained from Wacker-Chemie as the reference and working standards.

Test samples were obtained by aqueous extraction of soils containing RAMEB.

Methanol and acetonitrile of chromatography grade were purchased from Merck (Darmstadt, Germany), water (for HPLC) was prepared with Milli-Q system (Millipore, USA).

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Table 1. Physico-chemical characteristics of soils

Type of the soil	pH _{H₂O}	Humus content % (w/w)	Nitrogen content g/kg	Phosphor. content g/kg	Org. carbon g/kg	Mechanical composition		
						Sand	Silt	Clay
						% (w/w)	% (w/w)	% (w/w)
Sandy	5.12	0.45	0.49	0.299	2.65	87.12	9.60	3.28
Loamy	7.30	4.18	2.10	0.462	26.15	18.98	56.31	24.71
Clay	7.40	3.91	1.81	0.326	23.01	4.33	46.80	48.87
Contaminated sandy loam	8.42	8.04**	1.17	3.678	40.66**	n.m.	n.m.	<20*

n.m. not measured.

*The sedimentation of the soil is changed by the contamination, therefore the clay content can not be measured directly.

**These values are high because of the carbon content of the oil pollution.

Table 2. RAMEB extracted at the end of a 3 week-biodegradation experiment from different soils (average of three determinations, contamination: 30 000 ppm Diesel or transformer oil)

RAMEB added (w/w %)	RAMEB recovered (related to the initially added amount, %)						
	Sandy soil			Humic soil			Clay soil cont. with Diesel oil
	Control	Contaminated with Diesel oil Transf. oil		Control	Contaminated with Diesel oil Transf. oil		
0.3	95	52	71	41	20	31	<10
0.7	79	77	80	42	24	47	<10

HPLC methods

Two methods have been worked out: size/exclusion chromatography (one-peak method) for the quantitative analysis and a fingerprint method for the characterization of the isomer distribution of RAMEB.

Hewlett-Packard 1050 Pumping System equipped with Hewlett-Packard 1050 Series Autosampler, Hewlett-Packard 1050 Variable Wavelength Detector and ERC-7515B RI detector was used. The data analysis was performed with Hewlett-Packard 1050 ChemStation, Version No: A.06.03.

In both methods column temperature was 30 °C, the flow rate 1.0 ml/min, UV detector wavelength: 200 nm, RI detector temperature: 40 °C (Fast mode).

In size exclusion chromatography TSK-GEL G 2000SW silicagel based column with guard column TSK-GEL G SW (75 × 7.5 mm) (TosoHaas) was applied. The mobile phase consisted of methanol and water (10:90).

In fingerprint method a Nucleosil NH2 7 µm (100 × 4 mm I.D) column, Macherey-Nagel, Duren Germany, was applied. The mobile phase consisted of acetonitrile and water (90:10). Beside of purification of samples the pre-treatment of soil sample extracts serves also for the change of the matrix needed for subsequent HPLC analysis with RI detection.

Since the fingerprint method developed could offer characteristic chromatogram only over 0.5 mg injected RAMEB, enrichment of RAMEB by the SPE procedure was also needed.

Solid Phase Extraction procedure was carried out using a Vac Elut SPS 24 vacuum manifold and RP18 solid

phase extraction columns, (LiChrolut^R 200mg, Merck). After column conditioning with methanol and water, the aqueous soil extract was added onto column. The columns were washed with water, then dried under vacuum for 5 min. Samples were eluted with methanol/water (9: 1) mixture.

The solvent was evaporated to dryness under stream of nitrogen using a sample concentrator at about 60 °C. The residue was dissolved in 1.0 ml of mobile phase, and 20 µl and 100 µl were injected onto the HPLC system in case of the size-exclusion and fingerprint chromatography, respectively.

The specificity and the linearity (in the range of 0.5–50 µg injected RAMEB) of the elaborated one peak HPLC method were proved, (correlation was >0.999), the recovery of the SPE pre-treatment was 100 ± 5%. The precision of the method was acceptable, detection limit was 0.2 µg injected RAMEB.

Results and discussion

Nearly 90% of RAMEB is recovered when solid RAMEB is added to the loamy soil, mixed and re-extracted with water. After a few days aging, however, the recoverable amount decreases to about 40–45%. During this time RAMEB interacts with some soil components. When RAMEB is applied in aqueous solution or dissolved in methanol, the recoverable amount decreases to 35–38% and to about 20%, respectively. In the latter case probably some soil components will be dissolved temporarily, and the possibility of complex formation and/or adsorption increases.

Table 3. RAMEB extracted by diluted hydrochloric acid expressed in percentage of the initially added amount (average of three determinations extraction after 1 week of RAMEB addition)

pH	Recovery (%)	
	Loamy soil	Clay soil
2	88	17
3	91	26
4	107	23

Samples of the technological experiments (aimed to get information on the effect of soil properties and RAMEB level on biodegradation of the hydrocarbon contaminant) were extracted with water (Table 2). RAMEB being slowly biodegradable, was not expected to be biodegraded during the 3 weeks of the experiment. The recovery of RAMEB was found to depend rather on the soil composition than on the presence and nature of the contaminant.

The best recovery was achieved in case of the acidic sandy soil, therefore the pH of the extracting aqueous solution was shifted towards acidic region. The effect of pH of the extracting hydrochloric acid solutions was found to influence the extraction efficacy dramatically with a pH optimum between 3-4 especially when loamy soil was extracted (Table 3). In case of clay soil, however, the alkaline pH was more successful: with NaOH solution of pH 12 the recovery was 44%. Raising the temperature (extracting with boiling water for 1 h) resulted in 56% recovery. The microwave extraction and dispersion of clay soil in aqueous potassium pyrophosphate solution into small grains were also unsuccessful. These results suggest that RAMEB strongly interacts with clay. The RAMEB – clay interaction seems to be pH-dependent suggesting as if RAMEB could interact with the pH-dependent surfaces of soils. This hypothesis can be further studied yet.

Application of the method

Soil samples from a field experiment, studying the effect of RAMEB on the biodegradation of motor oil in soil were analysed for their RAMEB content. The clay content of the soil was lower than 20%, so the acidic extraction was used. As it is shown in Figure 1, a decreasing tendency was observed in the summer–autumn period (0–15 weeks) when the temperature was favourable for the activity of microbes, while a plateau can be observed in winter period (15–30 weeks) when the microbes are much less active. The 20–25% decrease during the first 15 weeks of the experiment is in good agreement with the data of the manufacturer stating that 8% of RAMEB was biodegraded in 4 weeks in soil in a standard biodegradation test. The decrease in the RAMEB content is probably the result of biodegradation, but other processes in the soil (adsorption, coagulation, fixation, etc) might have contributed to this phenomenon, too.

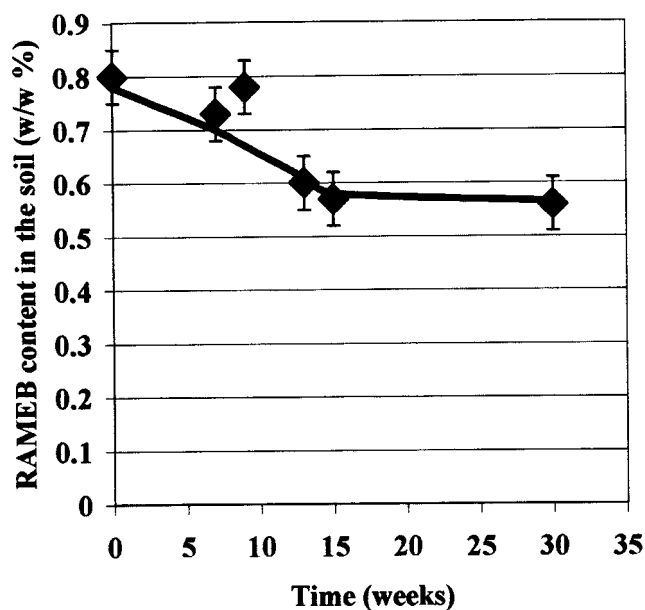


Figure 1. RAMEB content in soil contaminated with motor oil in the time course of biodegradation (there was a winter period between 15–30 weeks).

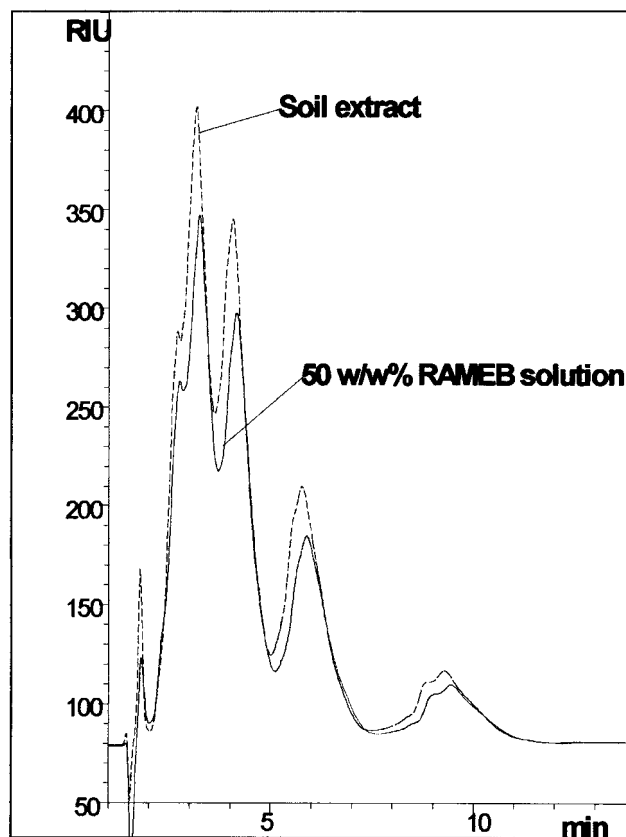


Figure 2. Fingerprint chromatograms of 50 w/w% aqueous RAMEB solution used for the treatment of a soil, and of a soil extract after 30 week-treatment.

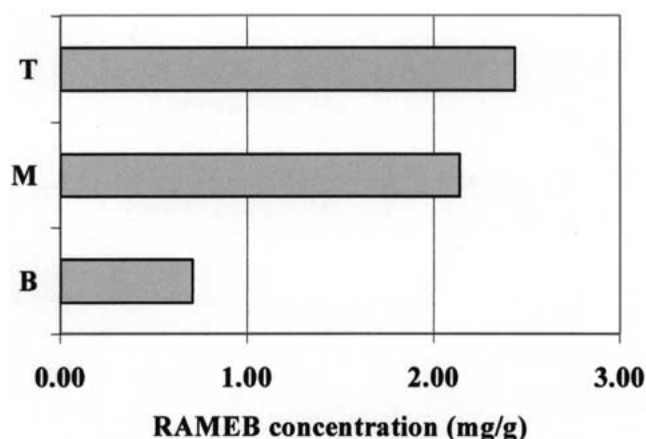


Figure 3. RAMEB content in soil column segments T: top (25% of the soil), M: medium (50% of the soil), B: bottom (25% of the soil).

Comparing the fingerprint chromatograms of 50% w/w aqueous RAMEB solution used for the treatment of soil and of a soil extract after 30 week-treatment no difference in the component distribution could be detected (Figure 2).

To demonstrate the adsorption of RAMEB to soil a model experiment was performed: a glass tube was filled with dry loamy soil and aqueous RAMEB solution was let through this column from the top. No more solution was added when the bottom of the column became wet. The vertical segments of the soil column were separately analysed for RAMEB content (Figure 3). The moisture content of the segments were practically identical (30, 30 and 28 w/w% for the top, medium and bottom layer, respectively), but significant difference was found in the RAMEB concentration showing the adsorption of RAMEB in the upper layers of the soil.

The new analytical methods provide useful data on the fate (distribution and biodegradation) of RAMEB used for the bioremediation of soils contaminated with hydrocarbons.

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