EVALUATION OF A FLOW SYSTEM FOR CHEMICAL SPECIATION OF METALS BY SEQUENTIAL EXTRACTION

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This study examined a flow method for sequential extraction of metals. Conventional batch methods are tedious and slow. A three-step sequential extraction scheme was used to evaluate a novel flow extraction system by analyzing calcium (Ca), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) in certified reference materials. The flow extraction system was performed in a closed chamber through which three extractants were passed sequentially to extract acid soluble, reducible and oxidizable phases. The extracts were collected in a number of fractions for subsequent flame atomic absorption measurement.

Effects of extracting conditions were studied. Results of pH change of extracts during extraction indicated that flow system ensured extraction being performed at designated pH values without any need of adjustment. In the extraction of the acid soluble fraction, varying concentrations of acetic acid in the range 0.11 to 0.52 M had no significant effect on the amounts of elements extracted except for Fe. Increasing the concentration of hydroxylamine hydrochloride in the reducible fraction step from 0.04 M to 1.0 M affected the extraction efficiency for Fe, Mn and Zn.

The flow method was compared with the conventional batch method for the amounts of metal extracted and analytical repeatability. The overall recovery rates from both batch and flow methods for soil reference material SRM 2710 were in the range 81-111% for all 5 elements determined. The results also showed that larger amounts of metals were extracted in acid soluble fraction in the flow than the batch system. Reducible and oxidizable fractions showed slight different results between the two systems for some elements. The use of extractograms from the flow method to evaluate the association between elements in samples was demonstrated.

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สุมลธา หนูคาบแก้ว : การประเมินระบบโฟลว์สำหรับการวิเคราะห์สปีชีส์ของโลหะโดยการสกัดลำดับขั้น (EVALUATION OF A FLOW SYSTEM FOR CHEMICAL SPECIATION OF METALS BY SEQUENTIAL EXTRACTION). คณะกรรมการควบคุมวิทยานิพนธ์ : ยุวดี เชี่ยววัฒนา, Ph. D., ดวงใจ นาคะปรีชา, Ph. D. 79 หน้า. ISBN 974-663-912-9

งานวิจัยนี้ได้พัฒนาระบบโฟลว์ในการสกัดลำดับขั้นเพื่อวิเคราะห์สปีชีส์ของโลหะเพราะระบบแบทช์เป็นวิธียุ่งยากและทำได้ช้า ได้ทำการประเมินระบบการสกัดแบบลำดับขั้นด้วยระบบโฟลว์ที่พัฒนาขึ้นโดยใช้ขั้นตอนการสกัด 3 ขั้นตอน และประยุกต์กับการวิเคราะห์ธาตุ 5 ชนิด คือ แคลเซียม เหล็ก แมงกานีส ทองแดงและสังกะสีในสารมาตรฐานอ้างอิง ระบบโฟลว์ที่ออกแบบขึ้นเป็นระบบปิด ประกอบด้วยภาชนะที่ใช้สกัดและระบบการส่งผ่านตัวสกัดอย่างต่อเนื่องเข้าไปในภาชนะสกัด เพื่อสกัดธาตุที่ละลายได้ในกรด ในสารรีดิวซิง และในสารออกซิไดซิง สารละลายที่สกัดได้จะเก็บเป็นแฟรกชันเพื่อวิเคราะห์หาปริมาณธาตุด้วยเทคนิคการดูดกลืนรังสีของอะตอมต่อไป

การศึกษาวัดการเปลี่ยนแปลงของ pH ของตัวสกัดในแต่ละแฟรกชันทั้งในระบบแบทช์และระบบโฟลว์พบว่า ในระบบโฟลว์ ค่า pH ของการสกัดในแฟรกชันท้ายๆเป็นค่าใกล้เคียงกับ pH ของตัวสกัดเริ่มต้นโดยไม่จำเป็นต้องมีการปรับค่า pH ในระหว่างการสกัด ในการศึกษาผลของความเข้มข้นของตัวสกัดในแฟรกชันที่ละลายด้วยกรดโดยใช้กรดอะซิติก เข้มข้น 0.11 ถึง 0.52 โมลต่อลิตร พบว่า ไม่มีผลกระทบอย่างมีนัยสำคัญต่อปริมาณธาตุที่สกัดได้ยกเว้นเหล็ก นอกจากนี้ การศึกษาการเพิ่มขึ้นของความเข้มข้นของตัวรีดิวซ์ คือ ไฮดรอกซีลามีน ไฮโดรคลอไรด์ จาก 0.04 ถึง 1.0 โมลต่อลิตร พบว่า มีผลต่อประสิทธิภาพการสกัดของเหล็ก แมงกานีสและสังกะสี

ได้เปรียบเทียบประสิทธิภาพการสกัดของระบบโฟลว์และระบบแบทช์ที่นิยมใช้กัน โดยการสกัดดินมาตรฐานอ้างอิง SRM2710 พบว่าทั้งสองวิธีให้ค่าปริมาณรวมของธาตุในทุกแฟรกชันกับค่ารับรอง มีค่าในช่วง 81-111 เปอร์เซ็นต์ สำหรับทั้ง 5 ธาตุที่ศึกษา อย่างไรก็ตาม พบว่าปริมาณธาตุที่ได้ในแฟรกชันที่ละลายด้วยกรดในระบบโฟลว์มากกว่าระบบแบทช์ ส่วนในแฟรกชันรีดิวซิเบิลและออกซิไดซิเบิลจะให้ผลที่แตกต่างกันบ้างสำหรับบางธาตุ นอกจากนี้ยังได้ใช้ extractogram ที่ได้จากระบบสกัดแบบโฟลว์ในการประเมินการเกาะจับระหว่างธาตุแต่ละชนิดในตัวอย่าง

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LIST OF ABBREVATIONS

^{o}C	Temperature in degree of Celsius
ml	Milliliter
g	Gram
mg	Milligram
μg	Microgram
mA	Milliampere
M	Molarity
v/v	Volume by volume
min	Minute
h	Hour
RSD	Relative standard deviation
RT	Room temperature
FAAS	Flame atomic absorption spectrometry

CHAPTER I

INTRODUCTION

1.1 Speciation in Soils and Sediments

The existence of an element in different chemical forms in the gaseous, solid or aqueous solution phases provides the conceptual basis for speciation in the three phases of soils. More particularly, a 'chemical species' in soil refers either to a specific molecular arrangement of the atoms of an element or, quite often, to the result of an operational process of detection and quantitation aimed at elucidating chemical form [1].

Speciation may be described by considering the type of bonding between an element and other components of soil. For example, an element in ionic form may be bound to clay minerals or organic matter by coulombic forces, whereas covalent bonds may be formed with surface ligands on hydrous oxide [1].

Speciation may also be described from the standpoint of a process, such as plant nutrient uptake ('available' and 'unavailable' forms), toxicity; and mobility ('readily leachable' and 'slowly leachable').

Metal ion associations in soil and sedimentary systems are very complex like those segments released by different chemical treatments. Thus, operationally (and not strictly chemically) defined fractionation is commonly used with the aid of sequential extraction methods. They may enable to estimate the relative strengths of the various processes leading to associations of metals and soils, e.g. chemical bonding, (co)precipitation, specific and non-specific adsorption, etc. [2].

1.1.1 General Procedure of Sequential Extraction

The solid material can be partitioned into specific fractions by selective extraction using appropriate reagents. In sequential extraction technique, chemical extractants of various types are applied successively to the sample of sediment, soil or sludge, each follow-up treatment being more drastic in chemical action or different in nature [1].

To perform sequential extraction, the dried bulk soil or sediment sample is sequentially extracted by n different solvents in n steps, yielding n extract solutions and one final solid extraction residue (Figure 1.1) [2]. The resultant extract solutions are usually analyzed by instrumental techniques suitable for the chemical multielement analysis of liquids, i.e. atomic absorption (Flame or Nonflame), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

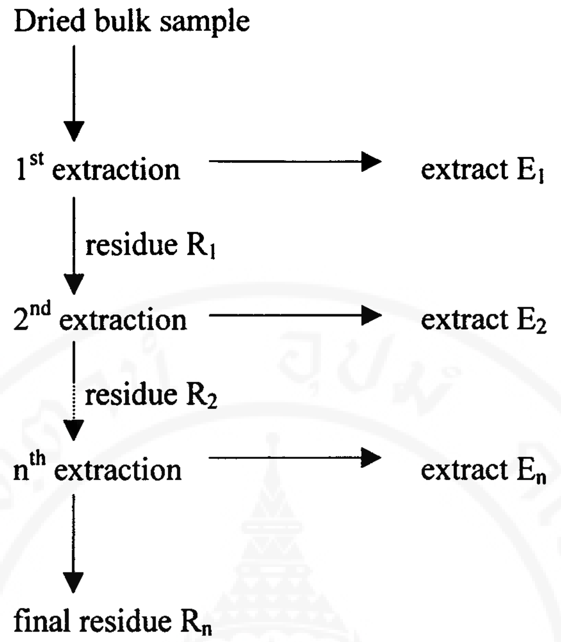

Figure 1.1 General scheme of sequential extraction of sediments and soils [2].

The extraction can be performed by shaking [3,4,5] and magnetic stirring [6]. Microwave- assisted extraction techniques can be used to speed up the extraction process [7,8]. It should be emphasized that when replacing the traditional shaking (i.e. magnetic shaking) by the microwave heating, the extraction time was reduced, including the additional advantage that the tedious control of temperature was eliminated. Pérez-Cid et el. [9] applied focused ultrasounds for sequential extraction of a sludge sample with a view to shorten the extraction time in each of the stages using the sequential extraction method proposed by the BCR.

The extractes were separated by centrifugation and decantation [4,5,10,], pipetting [3,8] or filtering through 0.45 μm [7,11] or Whatman No.42 filter paper [12,13].

1.1.2 Scheme of Sequential Extraction

During the past few decades, chemical extraction methods have been widely used on sediments and soils to determine heavy metal binding forms in the solid phase. The grouping used in descriptions of distribution patterns are somewhat arbitrary, but the fractions more commonly considered appear to be the ion-exchangeable, the weakly absorbed, organic-bound, hydrous oxide component material. The latter component has been termed as "residual", with the sum of the other fraction being "non-residual" [14]. The extractants more commonly used in sequential extraction schemes are generally applied according to the following order: unbuffered salts, weak acids, reducing agents, oxidizing agents and strong acids.

A large number of sequential extraction methods have been reported (Table 1.1). Extraction schemes may employ three to eight extractants to which the form of metal extracted is related.

Table 1.1 Sequential extraction schemes for extracting various components of soil and sediment.

Sample/ Element	Fraction	Extractant	Reference
Soils/	Exchangeable	0.05 M $CaCl_2$	[15]
Cu	Inorganic bound	2.5% CH_3COOH	
	Organic bound	1.0 M $K_2P_2O_7$	
	Metal oxides	0.175 M $(NH_4)_2C_2O_4$/ $H_2C_2O_4$	
	Residual	HF	
Sediments/	Exchangeable	1 M $MgCl_2$	[3]
Cd, Co, Cu,	Carbonates	1 M NaOAc/HOAc	
Fe, Mn, Ni,	Fe-Mn oxides	0.04 M $NH_2OH{\cdot}HCl$ / CH_3COOH	
Pb, Zn	Organic	H_2O_2/HNO_3, NH_4OAc	
	Residuals	HF/$HClO_4$	
Soils/	Exchangeable	0.5 M KNO_3 + H_2O	[16]
Cd, Cu, Ni,	Organic	0.5 M NaOH	
Pb, Zn	Carbonate	0.05 M EDTA	
	Sulfide	4 M HNO_3	
Soils/	Exchangeable	$MgCl_2$	[17]
Cd, Cu, Fe,	Amorphous Fe oxides	Oxalate	
Mn, Zn	Crystalline Fe oxides	Citrate-dithionite bicarbonate	
	Organic	NaOCl	
	Residual	HNO_3/ $HClO_4$	

Table 1.1 Sequential extraction schemes for extracting various components of soils and sediments. (Continued)

Sample/ Elament	**Fraction**	**Extractant**	**Reference**
Soil/ Cu, Fe, Mn, Zn	Exchangeable	1 M $Mg(NO_3)_2$	[18]
	Organic	0.7 M NaOCl	
	Mn oxide	0.1 M $NH_2OH{\cdot}HCl$	
	Amorphous Fe oxides	0.2 M $(NH_4)_2C_2O_4/H_2C_2O_4$	
	Crystalline Fe oxides	0.1 M ascorbic acid in 0.2 M $(NH_4)_2C_2O_4/H_2C_2O_4$	
	Residual	HF/ HNO_3/HCl	
Sediment/ Ca, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn,	Exchangeable	1 M NH_4OAc	[11]
	Carbonates	1 M NaOAc	
	Mn oxides	0.01 M $NH_2OH{\cdot}HCl$	
	Amorphous Fe oxides	0.1 M $(NH_4)_2C_2O_4/H_2C_2O_4$	
	Sulphides	H_2O_2/HNO_3, 1 M NH_4OAc	
	Crystalline minerals	HNO_3	
Sediment/ Cd, Cu, Ni, Pb, Zn	Exchangeable	0.11 M CH_3COOH	[4]
	Fe-Mn oxides	0.1M $NH_2OH{\cdot}HCl$	
	Organic	H_2O_2/HNO_3, NH_4OAc	
	Residual	HF/ HCl/ HNO_3	

Table 1.1 Sequential extraction schemes for extracting various components of soils and sediments. (Continued)

Sample/ Element	Fraction	Extractant	Reference
Soil and sediment/ As,Be,Ca,Cd, Ce,Co,Cr,Cu, Fe,La,Li,Mn, Ni,P,Pb,Ti,Tl U,V,Zn	Exchangeable	1 M NaOAc	[5]
	Amorphous Fe-Mn oxides	0.25 M $NH_2OH{\cdot}HCl/HCl$	
	Crystalline Fe-Mn oxides	1.0 M $NH_2OH{\cdot}HCl/CH_3COOH$	
	Organics and sulphides	$KClO_3$	
Soils/ Cd, Cu, Pb	Distilled water	H_2O	[19]
	Exchangeable	1 M $MgCl_2$	
	EDTA-extractable	1% NaCaHEDTA/ NH_4OAc	
	Reducible Mn	0.2% Quinol in 1 M NH_4OAc	
	Carbonates	0.5 M NaOAc/HOAc	
	Organics	H_2O_2/NaOAc/HOAc	
	Fe and Al oxides	0.175 M $(NH_4)_2C_2O_4/H_2C_2O_4$	

The water soluble fraction may be obtained by two ways, by sampling solid sample pore solution using in situ filtration, dialysis tubes or bags, or by a leaching procedure in the laboratory. This fraction is usually negligible, except in areas where evaporites are present. When this procedure is used, the pH may be indeterminate because of the low buffering capacity of the extractant and problems with readsorption occur.

To extract exchangeable fraction, an electrolyte such as salts of strong acids and bases or salts of weak acids and bases at pH 7 is normally used to prevent oxyhydroxy phases precipitation. For the carbonate bound fractions use of an acid such as acetic or buffer solution of acetic acid-sodium acetate at pH 5 is most common. These reagents are not able to attack all the carbonate content (for example dolomitic carbonates). They are not able to attack carbonate selectively and can also remove partially organically bound trace metals [6].

The fraction obtained when a reducing solution is used as extractant is mainly related to metals bound to iron and manganese oxides. Hydroxylamine in acid solution is the reducing agent most widely used to solubilise these oxides although iron oxide is not completely dissolved. Ammonium oxalate seems to be most effective when used in the dark, although some problems in heavy metals oxalate phase precipitation may occur even at low pH. The sodium dithionite/citrate/carbonate reagent dissolves the oxide and hydroxyoxides but can attack iron rich silicates. So reducing extractants are neither selective nor completely effective for iron and manganese oxides.

Other group of extractants includes oxidizing reagents that destroy organic matter and also oxidizes sulphides to sulphates. The extractants most widely used in this group are H_2O_2 and NaOCl. Hydrogen peroxide seems to be more efficient if used after the oxide extraction step.

One of the first, and most applied, sequential extraction procedure proposed is the 5 step procedure proposed by Tessier et al. [3]. This procedure has been applied and modified for use not only for soils and sediments [20,21,22,23,24], but also for atmospheric particulate matter [25], sewage sludge [26,27,28] and street dust [29].

Sequential extraction scheme developed by Ure et al. [4] has also been widely used [30,31].

1.1.3 Advantages of Sequential Extraction

Measurement of the total metal concentration of bulk samples will provide little indication of the metal's potential interactions with the biotic and abiotic components present in the environment [2]. Therefore, the determination of total concentrations of the metal in a sample is increasingly inadequate for many purposes. The total concentration alone will not reflect, for example, the toxicity of the sample. However, the determination of specific chemical species or binding forms is difficult and often hardly possible. Therefore, determinations of broader forms, e.g. so-called 'mobile' or 'carbonate-bound' forms, depending on operationally defined procedure can be good compromise to give information on environmental contamination risk [32].

Sequential extraction provides more than single extraction and has several advantages [2].

i. Extraction procedures applied are comparable to those occurring in nature. In natural environments, soils and sediments are subject to similar leaching procedures by natural and anthropogenic electrolyte solutions.

ii. The total sum of all fractions should be more-or-less 100%, so the results are self-checking.

iii. It is an essential tool in establishing element partitioning with natural samples.

iv. Chemical extraction sequences can be used for the estimation of the potential remobilization of metals under changing environmental conditions.

Other operationally defined speciation results will provide additional information on the possible mineralogical forms actually dissolved in each step [33]. Consecutive leaching techniques allow us to obtain information about the mobilities of major and trace constituents under different environmental conditions such as acidic or alkaline, oxidizing or reducing behavior, the action of chelating agents, and so on.

1.2.4 Nonselectivity of Extraction

Many schemes and reagents (Table1.1) have been used in sequential extraction and it is recognized that no extractant is totally selective. A reagent designed to dissolve one particular phase selectively (causing the liberation of associated with it) may attack other phase. Generally it is difficult to associate a given extractant reagent with a particular physicochemical phase. Instead the specificity of an extractant is operationally defined according to what it extracts, e.g. acetate buffer extracts the "acetate-buffer-extractable" phase. Although it is often believed that this particular phase includes carbonate minerals, these minerals may not be uniquely attacked by this reagent. This is the nonselectivity problem [34]. The studies on the selectivity are predominated in the major species than trace element. For example, Tessier et al. [3] measured Al, Si, Ca, S inorganic C and organic C in leachates and residues of river sediments to establish removal of major phases. A significant limitation of these studies is that one must assume that selective removal of a major phase means also selective removal of its associated trace elements. The studies of nonselectivity of reagents have been reported [6,35,36,37,38].

Pickering [35] extracted the Cu, Pb, Zn or Cd ions sorbed on $CaCO_3$ by 16 different extractants. He indicated that the extraction with mineral acid or EDTA solutions has been used to determine the non-detrital fraction (i.e. metal not present as mineral fragments), but the results showed that the reagents used retrieve variable proportions of metal ion sorbed on $CaCO_3$. Similarly, acidified ammonium oxalate has been chosen as the optimum reagent for evaluating available Cu and Pb in soils, but it can be inferred from the results that this reagent would retrieve little Pb from alkaline and calcareous soils. Whalley and Grant [36] evaluated the geochemical phase-specificity by applying the extraction to single synthetic substrates previously equilibrated with Cu, Ni and Zn-spiked artificial seawater. They observed good specificity for calcium carbonate and manganese dioxide, but varying quality of specificity for humic acid, and quite poor specificity for amorphous iron oxide.

Coetzee et al. [6] found that synthetic goethite co-precipitated with Ni was also attacked by acetic acid, releasing about 50% of Ni in acid soluble fraction. With the organic matter, they used the natural humic acid in their study. They found that Zn bounded to humic acid is primarily released in acid soluble fraction with acetic acid whereas Cu could only be released in the oxidizable fraction where the humic acid is dissolved completely. Howard and Vandenbrink [37] found the levels recovered in a particular step increase with increasing abundance of non-targeted phases.

Kim and Fergusson [38] found about 50% of cadmium associated with cadmiun-sorbed humic acid was liberated during the exchangeable and carbonate steps of the sequential extraction.

1.2 Problems in Sequential Extraction

1.2.1 Redistribution among Phases during Extraction

Readsorption or redistribution in sequential extraction is referred to the problem that metals liberated by selective dissolution during earlier steps are readsorbed and redistributed artificially amongst the remaining solid phases in the sample. This problem is a function of both the natures of the extraction scheme and the metal under investigation. For some metals it is apparently insignificant whereas for others it may be severe. A variety of approaches have been used to investigate the extent of redistribution for different metals.

Tipping et al. [39] performed the Shuman's procedure to investigate the sample naturally occurring mixture of Mn and Fe oxides. They observed the behaviors of Ca, Zn and Ba were predominantly associated with the Mn oxide since, together with Mn, they were each solubilized almost completely by treatment with hydroxylamine. In the case of Pb it appeared that about two-third was originally associated with the iron oxide phases. Electron microscopy analysis before extraction indicated that Ca was rather evenly divided between the crumpled-sheet phase (mainly Mn oxide) and the iron oxides, while Zn, Ba and Pb were predominantly in the Mn phase. The reason for the transfer of a large amount of Pb from Mn to Fe oxides was ascribed to the adsorption of Pb on the remaining Fe oxide during hydroxylamine treatment.

Belzile et al. [40] used a standard addition technique to examine the degree of readsorptions of As, Cd, Cu, Ni, Pb and Zn during various extractions performed on oxic lake sediments. Oxic sediments were chosen in their study because they contain the components (organic matter, various oxides, clays) that were suspected to be responsible for postextraction readsorption of trace metals. For all cases examined, except Pb, the trace element spikes (<100% of the amount present in control samples) were recovered within the limits given by the experimental errors. Low recoveries have been reported when high level (>100%) of trace elements were spiked to sediment during extraction of Fe oxide fraction.

Other workers [6,41,42] used the synthetic soil and sediment to evaluate redistribution of metals in sequential extraction procedure (Table 1.2).

Table 1.2 Some studies the redistribution during sequential extraction.

Model sample types	**Scheme of sequential extraction**	**Redistribution observed**	**Reference**
Synthesized model sediment	Tessier et al. [3]	Cu: StepIII → StepV Pb: StepII → StepIII	[34]
Synthesized model soils	Tessier et al. [3]	Ca: StepI → StepIII and V StepIV → StepV Mn,Ni,Pb,V,Zn: StepIII → StepIV Cu: StepI,II,III → StepV Sr: StepIV → StepV	[41]
Synthesized model sediments	Ure et al. [4]	Cu: StepI → StepIII Pb: StepI → StepII and III	[6]
Synthesized model soils	Tessier et al. [3] Ure et al. [4]	Pb: StepI → StepIII Pb: StepI → StepIII	[42]

Clearly, redistribution is still likely to be a serious issue for some metals in sequential extraction. Although model sediments or soils cannot represent real systems exactly, models allow one to assemble systems with particular compositions to test particular hypotheses, and they permit great flexibility since individual phases can be combined in any ratio mimic a wide variety of sample types.

Clearly, models cannot be expected to duplicate the complex structure and reactivity of real sample; however, models do represent a necessary limiting case. If extractions perform poorly on model system, performance on real samples is likely to be even more questionable [34].

However, only a few attempts to inhibit or counteract this problem have been reported [37,43,44]. It is well established that chelating agents form stable water-soluble complexes with heavy metals and are thus highly effective for maintaining metals in solution by limiting precipitation and sorption. Hence, a chelating agent theoretically may be utilized to prevent or minimize resorption and thereby improve the accuracy of sequential extraction procedures.

Howard and Shu [43] tried adding a trace amount of nitrilotriacetic acid (NTA) to each of the reagents used in sequential extraction analysis; the idea being that metals released in the centrifuge tube during selective dissolution would remain in solution by chemical chelation. They suggested that NTA can effectively counteract resorption during sequential extraction analysis of heavy metals in quartz-rich sediments and soils. In general, the levels of Pb, Ni and Zn recovered at the first two extraction steps (designed to target 'water-soluble' and 'exchangeable' forms) were found to significantly increase with increasing NTA concentration. They also suggested that resorption is likely to remain problem in soils and sediments containing high level of strong sorbents such as organic matter. This problem may be overcome by using higher concentrations of NTA, but this is done at the risk of dissolving non-targeted phases.

Raksasataya et al. [44] attempted to test the potential usefulness of NTA and cryptand 2.2.2 (a macrocyclic crown-like ligand) during the BCR extraction of synthetic soil models containing high concentration of the major scavenging phases, i.e. humic acid and hausmannite. They suggested that cryptand 2.2.2 and NTA, added in the conventional first extraction solution of the BCR sequential extraction scheme, was useful in counteracting Pb redistribution in five phases synthetic soil models, with Pb originally spiked on calcite. The Pb recovery appears to be more sensitive to the presence of hausmannite than humic acid.

Howard and Vandenbrink [37] evaluate the utility of NTA for counteracting resorption during sequential extraction analysis using sediment contained variable amounts (up to 20% by weight) of feldspar, calcite, Fe-oxide or organic matter. Resorption was counteracted by adding 400 mg l^{-1} of NTA to all extracting solutions used in the procedure, without causing significant dissolution of non-target phases. They commented that solving the resorption problem in sequential extraction analysis was still necessary to contend with the problems of: (1) choice of reagents and order of extraction needed for optimum selective dissolution; and (2) handling of samples from reducing environments.

1.2.2 Change of pH during Extraction

Changes of pH of the extracts can effect accuracy of extraction in both single and sequential extraction [45,46,47]. The pH change was always apparent and this meant that the pH of the final sediment or soil extract could significantly differ from that extractant added. The changes of pH on the course of extraction is dependent on sample composition, i.e. calcareous sediment will produce pH shifts greater than the siliceous ones.

1.3 Flow Sequential Extraction

The schemes of sequential extraction have been described in section 1.1.2. Practical extraction has usually been done in a batch system. The conventional batch process has many disadvantages such as time consumption and tedious procedure. Redistribution among phases during extraction has been reported in a batch system [6,34,41,42]. Hirner [2] recommended that the redistribution problem may be reduced if sequential extraction was done by dynamic extraction process in a flow cell. A flow system for the chemical fractionation of metals in soil or sediment by sequential extraction was developed in our laboratory to speed up and facilitates the chemical fractionation of metals in soil or sediment. A three-step sequential extraction scheme (acid soluble, reducible and oxidizable) was used to evaluate this novel system. In the flow system, extraction occurred in a closed chamber through which extractants were passed sequentially. In the previous study [48], online sequential extraction (continuous flow of extracts to the FAAS) was attempted but many disadvantages were observed. On the online system, the flow rate of extractant to the FAAS was not uniform and decreased with time due to gradual blockage of the filter by fine solid particles, the peak area of the extractogram could not be directly related to the amount of metal extracted. Consequently, an off-line system where the extract was collected in constant volume fractions was preferable. The fractions were subjected to subsequent determination of metal concentration. The remaining extracts can be kept for later use unlike in the on-line system where extracts are totally consumed in the FAAS measurement.

Effects of extraction temperature and sample/chamber volume ratio were investigated in the previous study [48]. The first step of extraction was done at ambient temperature for fear that other phases could be leached at elevated temperature. The extraction was kept at 55°C for stepIII since the temperature at this step could not be performed at temperature higher than 55°C in flow system because the vigorous gas evolution can caused undesirable back pressure in the system. Effect of extraction temperature in stepII was shown in the previous study. It has been found that temperature can substantially affect extractability at this step. The amounts extracted are obviously higher at higher extraction temperatures for all elements studied.

The effect of sample/chamber volume ratio was studied and extractable amount of metals obtained by summation of all fractions showed that no significant difference at varying sample/chamber volume ratios for all elements. This is obviously one of the advantages of the flow extraction system over the batch method.

One serious concern about the extraction schemes stated in Table 1.1 and other similar schemes is that the rate of extraction is slow and take several days to complete the analysis. For example, the procedure of Tessier et al. [3] needs overnights for the extraction steps to be completed whereas the procedure of Ure et al. [4] needs more than 2-3 days for sequential extraction. The flow system may be reduced the operation time in sequential extraction. The extractograms can be used to evaluate the association between metals in samples.

1.4 Aims of This Work

The objectives of this work are as follow:

I. To study the pH change of the extract during flow sequential extraction compared to the pH change of the batch sequential extraction.

II. To study the effect of extractant concentrations for stepI and stepII of the sequential extraction.

III. To compare the fractionation data between a flow system and a batch system.

IV. To evaluate the association between element in solid samples from the extractograms obtained from a flow extraction system.

CHAPTER II

EXPERIMENTAL

This chapter contains the description of instrument, chemicals and standard preparation. Sequential extraction and methods for study the effects of extraction are also described.

2.1 Instrument and Equipment

I. Extraction system set up

Flow sequential extraction was set up following Figure 2.1.

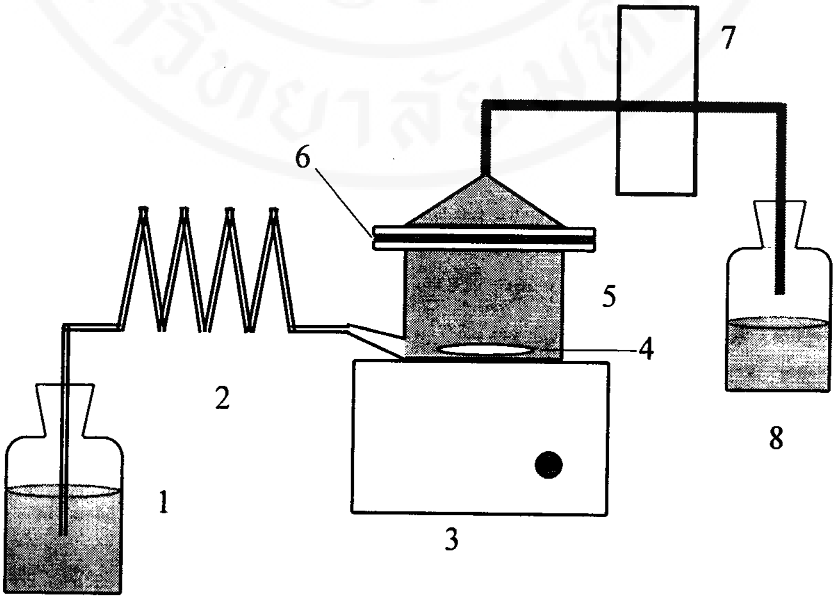

Figure 2.1 Flow extraction system in this work. 1: extractant reservoir, 2: heating tape, 3: magnetic stirrer, 4: magnetic bar, 5: glass extraction chamber, 6: glass fiber filter, 7: peristaltic pump, 8: fraction collector.

An extraction chamber was designed to allow containment and stirring of a weighed sample, and through which extractants could flow sequentially and leach metals from the target phase. Chamber and their covers (Figure 2.1) were constructed from borosilicate glass to have a capacity of approximately either 3 or 10 ml. The hot plate stirrer used to increase the temperature of extraction and to speed up flow extraction was purchased from Patterson Scientific, model B292. The outlet of the chamber was furnished with a filter (Whatman glass microfibre filter GF/B 47 mm diameter, 1 μm particle retention) to allow dissolved matter to flow through. Extractant was pumped through the chamber using a peristaltic pump (Ismatec SA, MS-CA4/620C) at its highest rate, and using tygon tubing of 1.52 mm inner diameter (flow rate for an aqueous solution is approximately 10 ml/min). Heating of extractants, when required, was carried out by passing through a glass coil approximately 1 m in length, wrapped in heating tape (Glass-Col 300 watt heating tape). The voltage of heating tape was controlled by Veriac transformer, model Y16HM (The Zenith Electric Co., Ltd.).

II. Flame atomic absorption spectrometer (FAAS)

FAAS measurements were performed using a Perkin Elmer Model 3100 equipped with deuterium background correction (Connecticut, USA), providing a background corrected results. The operating parameters for measurement are shown in Table 2.1.

Table 2.1 Operating parameters for FAAS measurement.

Element	Wavelength (nm)	Operating lamp current (mA)	Slit width (nm)	Flame type
Ca	422.7	8	0.7(high)	Air-acetylene
Fe	248.3	8	0.7(high)	Air-acetylene
Mn	279.5	5	0.7(high)	Air-acetylene
Cu	324.8	5	0.7(high)	Air-acetylene
Zn	213.9	5	0.7(high)	Air-acetylene

III. Microwave digestion system

Dissolution of sample was achieved using commercial microwave ovens model MLS-1200 MEGA Milestone (Bergamo, Italy). The operating parameters of microwave system are given in Table 2.2.

Table 2.2 Operating parameters of microwave digestion system

Step	Power (watts)	Time (min)
1	250	5
2	400	5
3	500	5
4	500	5
5	600	5
6	600	2
Vent	0	5

IV. Analytical balance

The Precisa 40 SM-200A (Zurich, Switzerland) and Mettler AJ150 were used for weighing when accurate amounts were required.

V. pH meter

A pH meter of Fisher Scientific Model 955 (USA) with a glass combination electrode was used for all pH measurements. Commercial standard buffers (Damstadt, Germany) of pH 4.00±0.01 and 7.00±0.01 were employed for the instrumental calibration.

VI. Horizontal shaker

An IKA-Horizontal shaker, model HS 250 basic (Germany), was used to speed up batch extraction.

VII. Centrifuge

Hettich Universal II centrifuge, model D 7200 (Germany), was used to separate the solid residue from the supernatant after extraction. Polypropylene copolymer centrifuge tubes (catalog number 3119-0050), Nalgene, Rochester (NY, USA), were used for extraction.

2.2 Description of Certified Reference Materials

The certified reference materials used in this study were SRM 2704 and SRM 2710. SRM 2704 is sediment from Buffalo River in the area of the Ohio Street Bridge, Buffalo, N. Y. This sediment was freeze-dried and passed through a 38 µm. SRM 2710 is a highly contaminated soil from pastureland along Silver Bow Creek in the Butte, Montana area. This soil was oven-dried, sieved and ground to pass through a 74 µm.

2.3 Chemicals and Reagents

Chemical and reagents were obtained from various suppliers, as shown in Table 2.3

Table 2.3 List of chemicals

Chemical	Supplier
Glacial acetic acid (99%w/w)	J. T. Baker (Phillipsburg, USA)
Hydroxylamine hydrochloride (99%w/w)	E. Merck (Damstadt, Germany)
Hydrogen peroxide (30%w/w)	BDH Analar (England)
Nitric acid (70%w/w)	J. T. Baker (Phililpsburg, USA)
Hydrofluoric acid (50%w/w)	Merck (Damstadt, Germany)
Ammonium acetate (98%w/w)	BDH (England)
Standard solution	
Ca standard solution 4008 μg/ml	Certified traceable to NBS Standard
Fe standard solution 1000 μg/ml	Titrisol, MERCK
Mn standard solution 1000 μg/ml	Titrisol, MERCK
Cu standard solution 1000 μg/ml	Titrisol, MERCK
Zn standard solution 1000 μg/ml	1 g of Zn metal in 25 ml of 50% HNO_3 dilute to 1000 ml with deionized water

2.4 Preparation of Reagents and Standard Solution

I. Acetic acid

6.30, 12.60 and 30.0 ml of glacial acetic acid was pipetted into 1 L volumetric flask and made up to exactly 1 L with deionized water to obtain an acetic acid concentration 0.11, 0.22 and 0.52 M, respectively.

II. Hydroxylamine hydrochloride

Approximate weight of hydroxylamine hydrochloride (Table 2.4) was dissolved in deionized water and 250 ml of glacial acetic acid was added in this solution. The solution was made up to 1 L with deionized water.

Table 2.4 Weight of hydroxylamine hydrochloride

Concentration (M)	Weight (g)
0.04	2.8
0.10	6.9
0.50	34.7
1.00	69.5

III. Hydrogen peroxide

Hydrogen peroxide was used as supplied by the manufacturer and was adjusted to pH 2.00 with diluted nitric acid. Acidified hydrogen peroxide was mixed with 0.02 M nitric acid at ratio 8:3 by volume.

IV. Ammonium acetate (3.2 M in 20%HNO_3)

24.7 g of ammonium acetate was dissolved in adequate water and 28.5 ml of distilled nitric acid was added in this solution and made up to 100 ml with further deionized water.

V. Standard solution

All standard solutions were prepared in extractant using high purity deionized water, which were obtained by passing laboratory distilled water through a Milli-Q water purification system.

All glassware, polyethylene bottles, pipette tips and other materials were carefully cleaned by washing with detergent to remove dust, particulate remnants, then soaked in 10% nitric acid at least overnight (storage vessels) and rinsed two times with deionized water (Mill-Q plus, 18.2 MΩcm^{-1} resistivity).

2.5 Procedure for Batch Sequential Extraction

Before opening any bottle of sample, it was manually shaken for a few minutes to rehomogenise the contents sample were taken with a plastic spatula.

The extraction was performed in 40 polypropylene centrifuge tubes using an end-over-end mechanical shaker operating at 300 rpm. The sequential extraction procedure is described below:

StepI: 20 ml of 0.11 M was added to 1 g of sample in a 40 ml centrifuge tube and shaken for 16 h at room temperature. The extractant was separated from the solid residue by centrifugation 5000 rpm and supernatant was pipetted into polyethylene container. The container was stoppered and stored at 4 °C for analysis. The residue was washed two times by adding 20 ml of water, manual shaking for a few minutes and finally centrifuging the resulting suspension. The supernatant was decanted and discarded, taking care not to discard any of the solid residue.

StepII: 20 ml of 0.04 M hydroxylamine hydrochloride in 25 % acetic acid was added to the residue from stepI in the centrifuge tube, The extraction was performed at 96 °C with occasional agitation for 6 h. After extraction, the extract solutions were pipetted as described above.

StepIII: 3 ml of 0.02 M nitric acid and 5 ml of 30% H_2O_2 (adjusted to pH 2.0 with HNO_3) were added to the residue from stepII. The sample was heated progressively to 85 °C, and maintained at this temperature for 2 h with occasional agitation. A second 3 ml aliquot of 30% H_2O_2 (adjusted to pH 2.0 with HNO_3) was then added, and the mixture was heated again at 85 °C for 3 h with intermittent agitation. After cooling, 5 ml of 3.2 M ammonium acetate in 20%v/v nitric acid were added, followed by dilution to a final volume of 20 ml with deionized water. The extraction was performed as described above (shaking time 30 min).

Blank extraction i.e. without sample, were carried through the complete procedure for each set of analysis and using the same reagents.

Table 2.5 Sequential extraction scheme used in this work

Step	Extractant	Fraction	Forms extracted
I	0.11 M CH_3COOH, RT	acid soluble	surface adsorbed, relatively mobile
II	0.04 M $NH_2OH{\cdot}HCl$ in 25%CH_3COOH, 80 °C	reducible	associated with hydrous oxide, slightly mobile
III	30%H_2O_2 in 0.02 M HNO_3 (8:3 v/v), 55 °C	oxidizable	associated with organic matters, slightly mobile

2.6 Procedure for Flow Sequential Extraction

A weighed sample (0.25 g) was transferred to a clean extraction chamber together with a magnetic bar. A glass microfibre filter was then placed on the outlet followed by a rubber gasket, and the chamber cover was securely clamped in position. The chamber was connected to the extractant reservoir and the collector vial using tygon tubing and placed on a magnetic stirrer. The magnetic stirrer and peristaltic pump were switched on to start the extraction. Extract from the flow extraction chamber was collected at 10-60 ml intervals in plastic vials. When a total volume of 150-180 ml was collected, the next extractant was passed through and collection of fractions was repeated until all three leaching steps were completed. In between steps II and III, pure water (ca 20 ml) was passed through the chamber to reduce the vigorous reaction between the two extractants. The extracts were subjected to FAAS measurement after all extraction steps were finished. When a hot extractant was required, an electrical voltage of 108 or 148 V was supplied to the heating tape to obtain an extractant of approximately 55 or 80 °C respectively. The three-step sequential extraction was performed using the following extractants as in Table 2.5.

The residue from flow sequential extraction was deposited at filter and was collected in centrifuge tube by rinsing with water, then centrifugation, decant the supernatant and discarded and drying at 60 °C for two days.

2.7 Procedure for Digestion of Residue

0.10xx g of dried residue from stepIII of sequential extraction was digested with 4 ml of concentrated HNO_3 (70%), 4 ml of HF (40%) and 2 ml of H_2O_2 (30%) using microwave digestion system following the program shown in Table 2.2.

2.8 Study on the pH of Extracts during Extraction

2.8.1 Measurement of pH of Extracts in Batch Method

The batch sequential extraction procedure was performed as described in section 2.5. Each step was repeated twice before the next step. The pH of extracts were measured by a pH meter. The pH of original extractants were also measured.

2.8.2 Measurement of pH of Extracts in Flow Method

The flow sequential extraction was performed as described in section 2.6. pH of extracts were measured as in batch method.

2.9 Study on Effect of Extractant Concentration

2.9.1 Concentration of Acetic Acid

Acetic acid concentration of 0.11, 0.22 and 0.52 M was used. Extractants in stepII and stepIII are 0.04 M $NH_2OH \cdot HCl$ and a mixture of 30%H_2O_2 and 0.02 M HNO_3 in a 8:3 volume ratio, respectively.

2.9.2 Concentration of Hydroxylamine Hydrochloride

Concentration of hydroxylamine hydrochloride at 0.04, 0.50 and 1.0 M was used. Extractant in stepI and stepIII are 0.11 M CH_3COOH and a mixture of 30%H_2O_2 and 0.02 M HNO_3 in a 8:3 volume ratio, respectively.

2.10 Determination of Element in Extracts and Digested Residue

The element contents of the extracts and residues were determined by flame atomic absorption spectrometry. Conditions were shown in Table 2.1. Five points calibration for each element was accomplished using multiple element extractant standard solution for extracts analysis and aqueous standard solution for digested residue, including an instrument blank of deionized water. These standards covered the linear working concentration range of up to 10, 6, 3, 2 and 1.5 μg/ml for Ca, Fe, Mn, Cu and Zn analysis. Leachates in the fraction that exceeded these concentrations were diluted by extractant for extracts analysis and by deionized water for residue analysis.

CHAPTER III

RESULTS AND DISCUSSION

Preliminary studies of flow sequential extraction were described by Tantidanai et al. in a separate report [48]. This chapter presents the results of the evaluation of the proposed flow method. Firstly, pH of extracts during extraction is studied and is discussed in section 3.1. Effect of extractant concentration is described in section 3.2. Comparison of analytical results with batch method is then discussed in section 3.3. Finally, discussion on the association between elements in solid samples is presented in section 3.4.

3.1 pH of Extracts during Sequential Extraction

The pH of extractant can affect on the amount of metal extracted in sample [45,46,47]. Exact pH value is important to give correct analytical results, i.e. pH of extracts should be similar to the pH of the extractant used. Sahuquillo et al. [45] found that pH of the final extractant in reducible metal extraction step was significantly greater than that of the extractant added (up to 1.5 unit) in batch extraction.

The operationally defined speciation results were dependent on extraction pH changing [36,38,39,49,50,51]. Lindsay and Norvell [51] studied the effect of pH of diethylenetriamine pentaacetic acid (DTPA) extracting solution on the five test soils.

There was a tendency for the pH to shift upward by 0.1 to 0.3 units during a 2 hour extraction. They also found that increasing pH from 7.0 to 7.9 had little effect on the amount of Zn and Cu extracted from the test soils. The amounts of Mn and Fe extracted decreased dramatically with increase in pH.

Rendell et al. [49] extracted surface aerobic sediments by 7 different extractants. They found that the pH change varied between 0.1 and 1.3 units for the five samples studied. Tipping et al. [39] found that the pH of the suspension increased during hydroxylamine (initial pH=3) treatment, reaching a final value of 5.2. The final pH value in hydroxylamine treatment reflects the consumption of protons during Mn (IV) reduction together with buffering by the remaining Fe oxide and detrital phases, and it will vary among samples, depending on their chemical nature and the solid/solution ratio employed. Whalley and Grant [36] found that pH of extracts from single synthetic $CaCO_3$ ranged 3-4 for acetic acid (initial pH=2.9) extracts, 2-4 for hydroxylamine hydrochloride (initial pH=2) and 2.0-2.1 for the ammonium acetate (initial pH=2) extracts. Kim and Fergusson [38] indicated that, in some cases, pH of the material extracted was found to have a marked effect on the relative proportion of Cd liberated during exchangeable fraction and bound to carbonate fraction of the sequential extraction schemes used.

It is well established that the pH of extracts can better guarantee changed during the batch extraction. Flow sequential extraction may be giving the desired pH of extracts. The investigation on pH change during a batch and a flow extraction were presented as described in sections 3.1.1 and 3.1.2.

3.1.1 pH of Extracts during Batch Sequential Extraction

The pH change of extracts was investigated by multiple batch extraction. The multiple batch extraction was done by repeated twice before the next step of batch extraction. The pH change of extracts in successive batch method is shown in Table 3.1. It can be observed that a final pH value was higher than the original extractant in first batch extraction and tend to reach the initial pH (pH of extractant) in successive batch. The results indicated that extraction pH in batch method is not strictly correct since single batch sequential extraction is usually operated. It can be seen that the pH still deviated slightly from the nominal value even in the third batch of the multiple batch process. If not measured and carefully adjusted, the extraction pH in a batch process can be substantially different from the nominal value. Some authors [52] have emphasized the need to adjust the pH of the final solution to obtain correct pH conditions for extraction.

Table 3.1 pH of extracts in a multiple batch extraction. Sample: SRM 2710; sample/extractant volume ratio: 0.50 g/20 ml (1/40).

Step	pH of extractant	pH of extracts		
		1st batch	2nd successive batch	3rd successive batch
I	3.05	3.35	3.26	3.18
II	1.88	2.11	2.02	2.00
III	1.86	2.07	2.03	2.01

3.1.2 pH of Extracts during Flow Sequential Extraction

pH of extracts in flow sequential extraction at each fraction is shown in Table 3.2. The results show the slightly increase of final pH values in first fraction and tend to reach the initial pH at the next fraction. This indicates that extraction pH in a flow method is closer to the assigned pH since the last fractions in each step of sequential extraction show closer pH values to the pH of extractant.

Table 3.2 pH of extracts in flow extraction. Sample SRM 2710; sample/extractant volume ratio: 0.25g/10ml (1/40). Fraction volume 30 ml.

Step	pH of extractant	pH of extracts				
		Fraction No.1	Fraction No.2	Fraction No.3	Fraction No.4	Fraction No.5
I	3.0	3.13	3.02	2.99	2.97	2.97
II	2.00	2.25	2.11	2.08	2.07	2.07
III	1.94	1.67	2.23	2.23	2.16	2.09

As can be observed, the flow method can provide better control of extraction pH than a batch method because pH of extracts in the end of leaching was near the initial pH of extractant. In conclusion, the flow system ensures that extraction is performed at designated pH values without any need of adjustment because the amounts of element in flow method was combined of all fraction number extracted but in the batch method, first batch sequential extraction is usually operated.

3.2 Effect of Extractant Concentration

3.2.1 Effect of Variation in Acetic Acid Concentrations

Acid soluble fraction shows the amount of each metal that would be released into the environment if conditions become more acidic. This fraction includes weakly absorbed metals retained on the soil or sediment surface by relatively weak electrostatic interaction, metals that can be released by ion-exchange process and metals that can be coprecipitated with carbonates present in many sediment or soil. Changes in the ionic composition, influencing adsorption-desorption reaction, or lowering of pH could cause remobilization of metals from this fraction [8]. Reagents which are recommended for dissolving selectively the carbonate and acid soluble fraction, in soil and sediment, are shown in Table 3.3.

Table 3.3 The extractants used for carbonates fraction

Sample/Element	Extractant	Extractable forms	References
Sediments/ Ca,Cd,Cr,Cu,Fe,Mn,Ni, Pb,Zn	1 M NaOAc	Carbonates bound	[11]
Sediments/ Cd,Cr,Cu,Ni,Pb,Zn	0.11 M HOAc	Exchangeable and carbonate	[4]
Soils/ Cu	2.5% HOAc	Metal bound by inorganic	[15]

Reagents for dissolving the carbonate fraction generally make use of sodium acetate and/or acetic acid. Ure et al.[4] and McLaren and Crawford [15] used acetic acid but with different concentrations (0.11 M and 0.44 M) for dissolving the carbonate fraction.

In flow system, CH_3COOH was used to fractionate the exchangeable and carbonate fractions and the effect of concentration of acetic acid was studied. Samples were extracted in triplicate at three different acetic acid concentrations: 0.11 M, 0.22 M and 0.52 M. 50 ml of fraction volume was collected in each step. The concentrations of each fraction can be plotted against fraction number to obtain the extraction profiles (or extractograms) as seen in Figure 3.1. The extractograms given by the three acetic acid concentrations are similar for all elements in stepI and stepII but small difference of extractogram in stepIII for Fe, Zn and Mn. This difference is due to the poor control of extraction temperature in stepIII and backs flow of extractant owing to vigorous gas formation. Poor reproducibility was obtained in this step for the above reason. The poor reproducibilities of the extractograms resulted in poor reproducibility in the amount of Fe, Mn and Zn in stepIII (Table 3.4). This problem is not obvious for Ca and Cu because low concentration of Ca and Cu in stepIII was found.

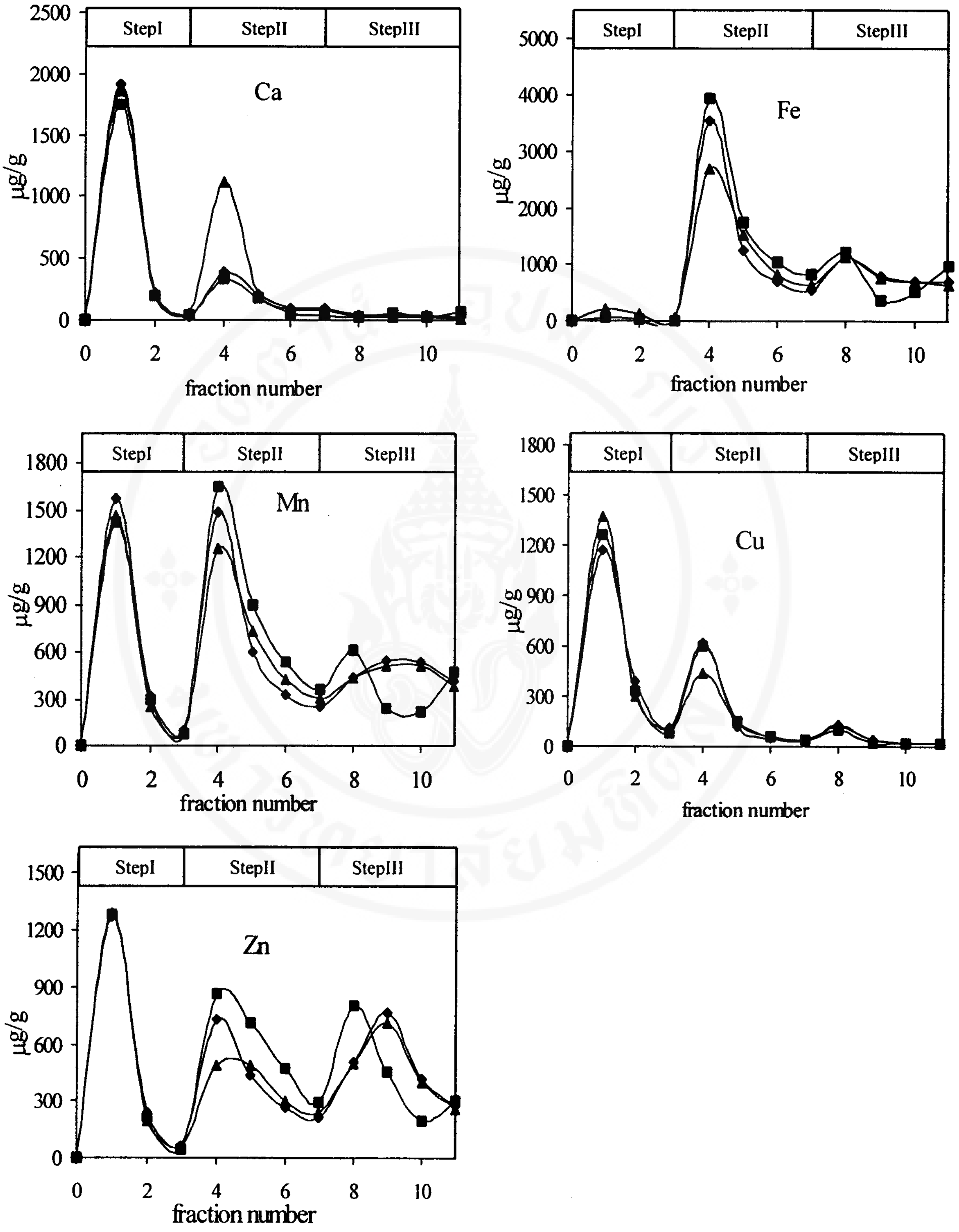

Figure 3.1 Extractogram of Ca, Fe, Mn, Cu and Zn using 0.11 M (◆), 0.22 M (■) and 0.52 M (▲) CH_3COOH in stepI. 0.04 M $NH_2OH{\cdot}HCl$ in stepII. 30%H_2O_2 : HNO_3 8:3, v/v in stepIII. Fraction volume 50 ml.

The amounts of elements extracted obtained by the summation of all fraction number in each step are shown in Table 3.4. Results of the distribution pattern of elements in each of the acetic acid concentration are also shown (Figure 3.2).

Table 3.4 Effect of varying acetic acid concentrations on extractable amounts.

Element	CH_3COOH concentration (M)	Amount extracted, µg/g (n=3)		
		StepI	stepII[a]	StepIII[b]
Ca	0.11	2000±150	640±140	89±55
	0.22	1950±21	570±86	127±110
	0.52	2090±120	1270±450	124±24
Fe	0.11	70±20	6300±770	2650±610
	0.22	120±26	6760±680	3000±610
	0.52	400±100	6020±420	2600±570
Mn	0.11	1860±190	3000±300	1410±450
	0.22	1720±140	3220±370	1630±90
	0.52	1980±300	2650±290	1470±330
Cu	0.11	1600±70	900±60	200±30
	0.22	1650±30	870±30	180±30
	0.52	1780±10	690±30	180±26
Zn	0.11	1480±80	1770±250	1740±310
	0.22	1490±70	2090±300	1840±70
	0.52	1560±27	1650±130	1740±120

[a] reducible fraction (0.04 M $NH_2OH{\cdot}HCl$ in 25%CH_3COOH)

[b] oxidizable fraction (30%H_2O_2 : 0.02 M HNO_3 8:3, v/v)

No significant increase in Ca, Mn, Cu and Zn extracted when the concentration was increased from 0.11 M to 0.22 M, but only Cu showed slight increase at 0.52 M. The increasing of Cu extracted can slightly affect the amount of Cu in reducible fraction.

For Fe, an increase in extractability in stepI was observed with increasing acetic acid concentration. But no significant decreasing in extractability was observed in stepII since small amount of Fe in stepI was present.

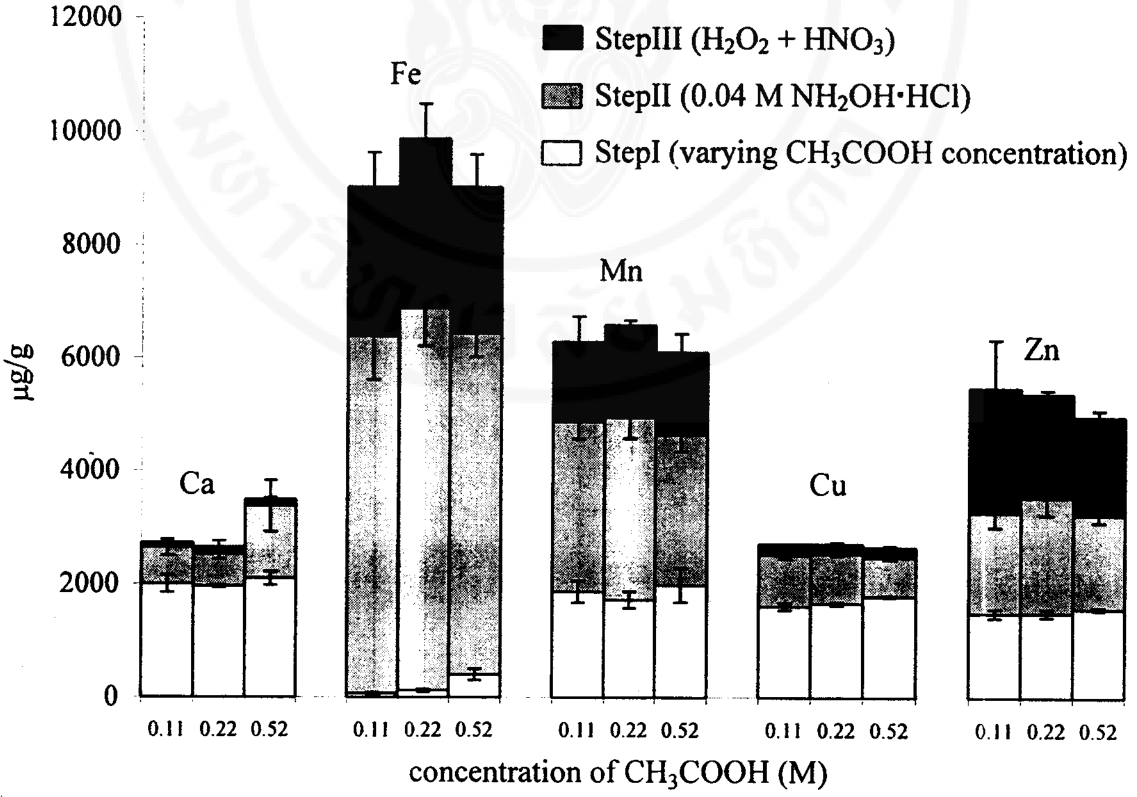

Figure 3.2 Effect of varying CH_3COOH concentration on metal distribution in soil sample (SRM 2710). Sample weights 0.25 g. Chamber volume 10 ml.

3.2.2 Effect of Variation in Hydroxylamine Hydrochloride Concentrations

Reducible fraction theoretically represents the contents of each element bound to iron and manganese oxides that would be released if the sample were subjected to more reductive conditions. Most successful methods for leaching iron and manganese oxides involve the combined action of reagents reducing these metals to their ferous and manganous forms, respectively. The most frequently used extractants in reducible fraction are shown in Table 3.5.

Table 3.5 The extractants used for reducible fraction.

Sample	Extractant	Extractable form	Reference
Sediments	0.04 M $NH_2OH{\cdot}HCl$ in 25% CH_3COOH (96°C, 6 h)	Fe-Mn oxides	[3]
Sediments	0.1 M $NH_2OH{\cdot}HCl$ (RT, 16 h)	Fe-Mn oxides	[4]
Soils	0.1 M $H_2C_2O_4$ + 0.175 M $(NH_4)_2C_2O_4$ (water bath, 2.5 h)	Metal occluded by free oxides	[15]
Sediments	0.01 M $NH_2OH{\cdot}HCl$ / 0.01 M HNO_3 (RT, overnight)	Mn oxides	[11]
	0.1 M Oxalate (RT, 24 h)	Amorphous Fe oxides	
Soils and sediments	0.25 M $NH_2OH{\cdot}HCl$ in 0.05 M HCl (60°C, 2 h)	Amorphous Fe oxide	[5]
	1.0 M $NH_2OH{\cdot}HCl$ in 25 % CH_3COOH (96°C, 6 h)	Crystalline Fe oxide	
Soils	0.1 M $NH_2OH{\cdot}HCl$ (RT, 0.5 h)	Mn oxides	[18]
	0.2 M $(NH_4)_2C_2O_4$ in 0.2 M $H_2C_2O_4$ (dark, 4 h)	Amorphous Fe oxides	
	0.1 M ascorbic in 0.2 M 0.2 $(NH_4)_2C_2O_4$ + 0.2 M $H_2C_2O_4$ (100°C, 0.5 h)	Crystalline Fe oxides	

Tessier et al.[3] and Ure et al.[4] used $NH_2OH \cdot HCl$ to extracted the Fe-Mn oxides but concentration and extraction conditions were different. The results of fractionation of SRM 2710-2711 using Tessier's scheme studied by Li et al.[33] were parallel those found by Ho and Evans [30] using Ure's scheme.

McLaren and Crawford [15] used oxalate solution for removal of metal present in Fe-Mn oxide. This extraction was exposed to ultraviolet light. Kersten and Förstner [11] differentiate reducible fraction into Mn-oxide and amorphous Fe-oxide. Extraction condition of Mn-oxide fraction in this scheme was similar to Fe-Mn oxides fraction in the Ure's scheme. Hall et al. [5] fractionated metal in amorphous Fe-oxide and crystalline Fe-oxide by increasing concentration of $NH_2OH \cdot HCl$ and extraction temperature.

Shuman et al. [18] fractionated reducible fraction in the following fractions: Mn oxide, amorphous Fe oxide and crystalline Fe oxide using $NH_2OH \cdot HCl$, oxalate buffer and ascorbic acid and oxalate buffer respectively.

It can thus be concluded that fractionation schemes have not been standardized and each researcher uses their schemes or a modification of the one developed earlier. The main modification suggested by subsequent researchers was to differentiate the Fe and Mn oxides-bound species into three distinct species viz., easily reducible metal oxide, amorphous metal oxide and crystalline metal oxide.

The ability of hydroxylamine hydrochloride to dissolve iron oxides and/or manganese oxides in soils and sediments was investigated by Chao [46]. He studied the effect of concentration of the $NH_2OH \cdot HCl$ solution (in 0.1 M HNO_3) and time of shaking on dissolution of manganese and iron from two sediment samples. No significant difference in manganese release was observed, regardless of the concentration of the $NH_2OH \cdot HCl$ solution varying from 0.025 to 0.25 M. The amounts of iron dissolution increased steadily with greater concentration of the $NH_2OH \cdot HCl$ solution.

In the present work, $NH_2OH \cdot HCl$ in 25% CH_3COOH was used in this fraction. Different concentrations of extractant were studied to evaluate the effect of reagent concentration. Triplicates of the residues remaining at the end of stepI of flow sequential extraction were extracted at three different hydroxylamine hydrochloride concentrations: 0.04 M, 0.50 M and 1.0 M. All concentrations used were performed in 25% CH_3COOH. Figure 3.3 shows the extractograms of this investigation. No significant differences were found between the extractograms in stepII of all elements at the three extractant concentrations but changes in extractograms in stepIII of Mn and Zn were observed. This is the same problem as described in section 3.2.1.

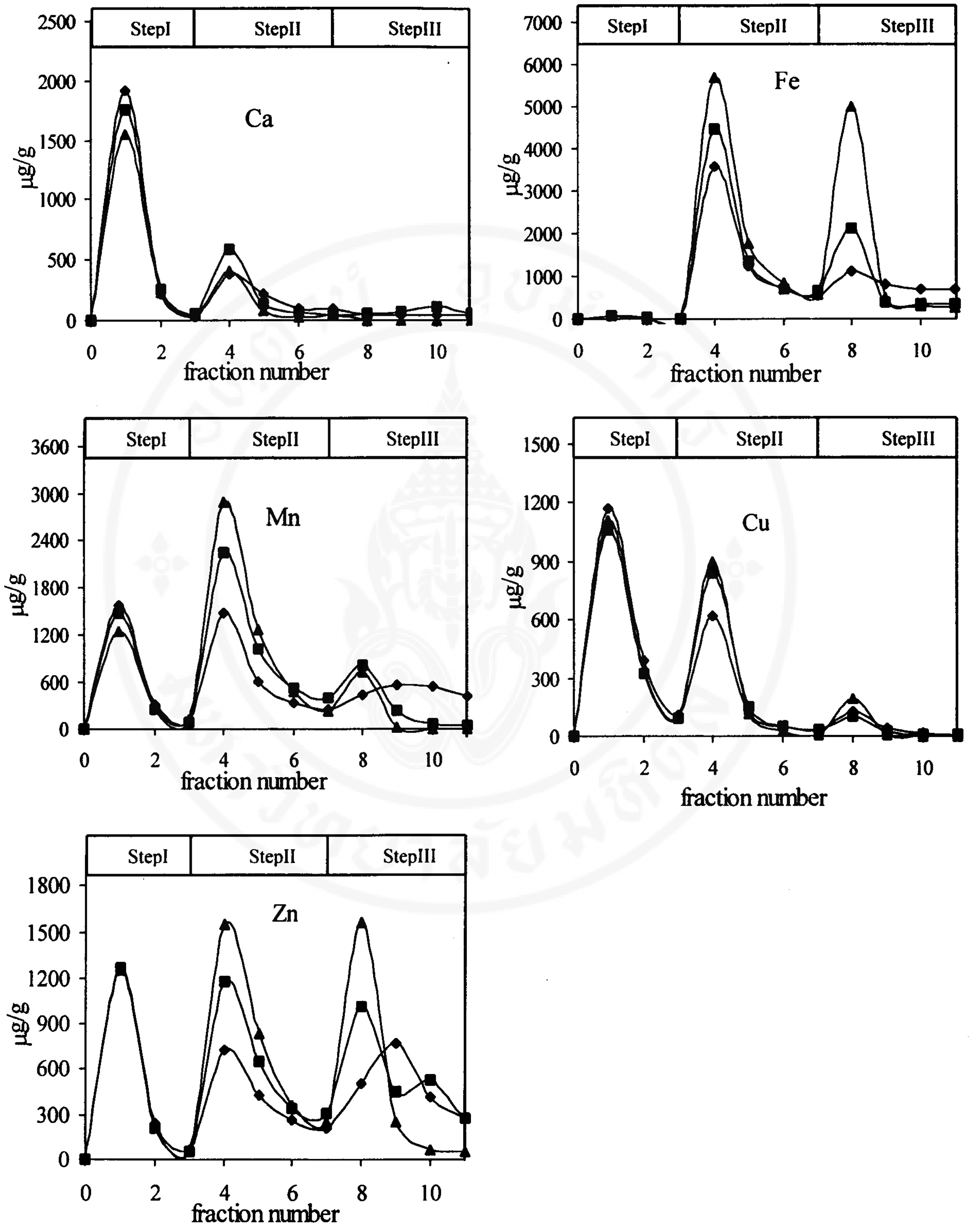

Figure 3.3 Extractogram of Ca, Fe, Mn, Cu and Zn using 0.04 M (◆), 0.50 M (■) and 1.0 M (▲) $NH_2OH{\cdot}HCl$ in stepII. 0.11 M CH_3COOH in stepI. 30%H_2O_2 + HNO_3 in stepIII. Fraction volume 50 ml.

Table 3.6 shows the amount of element extracted. Distribution patterns of elements at each of the hydroxylamine hydrochloride concentration are shown in Figure 3.4(A). The results indicated no significant difference between either the amounts of Ca extracted, or the standard deviations in the amounts, under the three sets of extractant concentrations. Greater amounts of Mn, Cu and Zn were extracted when the concentration was increased to 1.0 M. The use of a higher concentration of hydroxylamine hydrochloride did influence the decreasing of the Mn extracted in stepIII. This effect did not significantly influence the amounts of Cu and Zn extracted in stepIII.

For Fe, no significant increase was observed when the concentration of hydroxylamine hydrochloride was increased to 0.5 M, but an increase in extractability at 1.0 M was observed. It should be noted that the use of a more concentrated reductant (hydroxylamine hydrochloride) also resulted in an increased amount of Fe extracted in stepIII. Similar observation was reported by Sahuquillo et al. [45]. There is no clear explanation of this increasing. However, vigorous reaction in the initial of stepIII was noticed. The vigorous reaction was reduced if residue from stepII were washed by deionized water and discarded before flow the oxidizing agent in stepIII (Figure 3.4(B)). As a result, the decreasing in amounts of Fe extracted in stepIII were obtained.

Table 3.6 Effect of variation in hydroxylamine hydrochloride concentrations on extractable amounts.

Element	$NH_2OH.HCl$ concentration (M)	amount extracted, µg/g (n=3)		
		stepI[a]	stepII	stepIII[b]
Ca	0.04	2000±150	640±140	89±55
	0.50	2010±130	680±240	180±160
	1.0	1940±100	690±130	n.d.[c]
Fe	0.04	70±20	6300±770	2650±610
	0.50	90±10	7040±210	4280±1280
	1.0	89±10	8460±610	5710±1230
Mn	0.04	1860±190	3000±300	1410±450
	0.50	1690±100	4200±40	1220±66
	1.0	1590±170	4860±100	740±89
Cu	0.04	1600±67	900±63	200±33
	0.50	1470±50	1070±10	160±10
	1.0	1520±40	1090±47	170±42
Zn	0.04	1480±81	1770±250	1740±310
	0.50	1510±28	2400±140	1940±310
	1.0	1520±10	3050±90	1890±60

[a] acid soluble fraction (0.11 M CH_3COOH)

[b] oxidizable fraction (30%H_2O_2 : 0.02 M HNO_3 , 8:3 v/v)

[c] not detectable

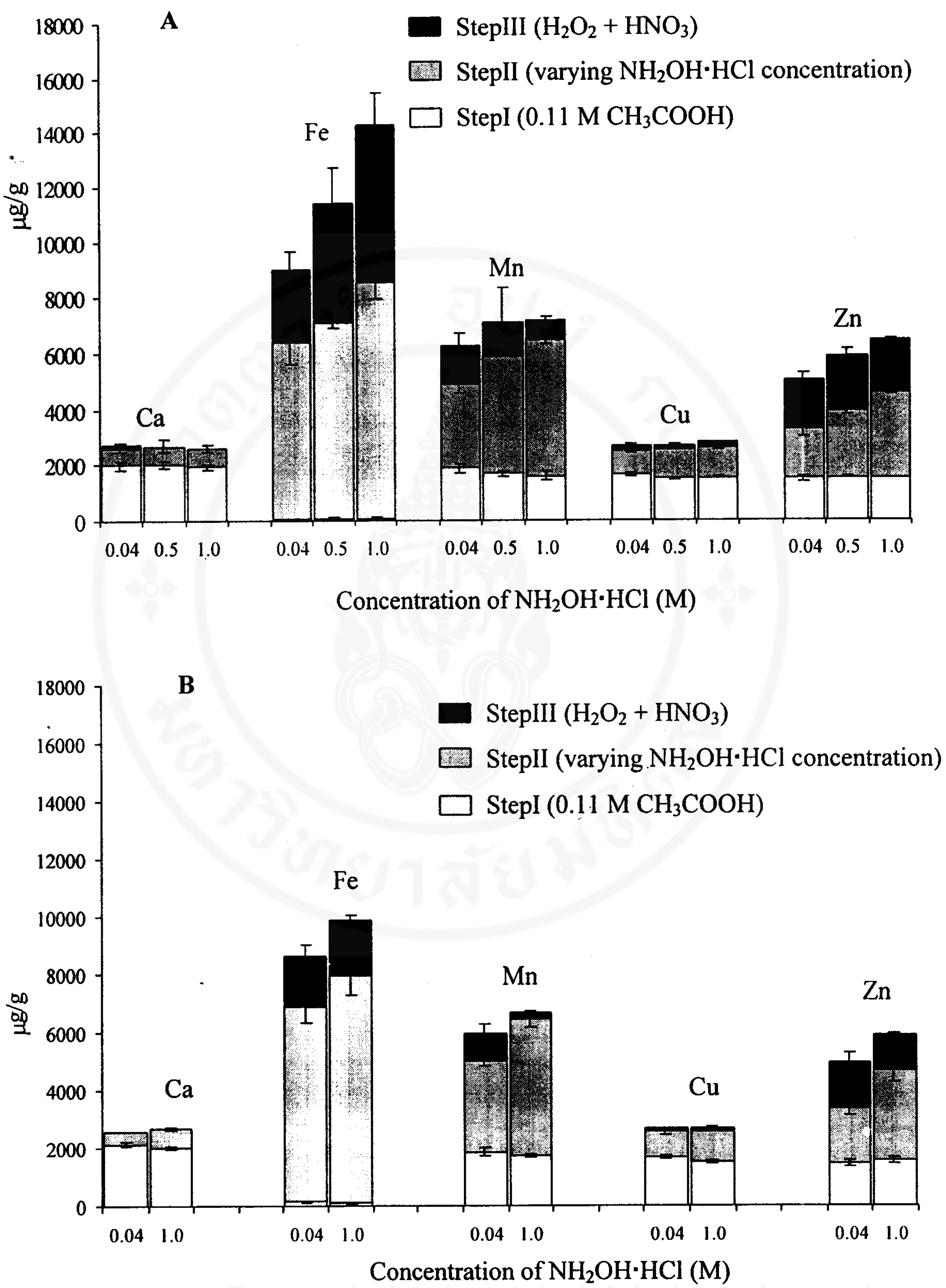

Figure 3.4 Effect of varying $NH_2OH{\cdot}HCl$ concentration on metal distribution in soil sample (SRM 2710). A: No washing residue; B: Washing residue after stepII by 20 ml deionized water.

The major function of the acidified $NH_2OH\cdot HCl$ solution is both reduction and dissolution [46]. The increase in amounts of elements extracted in reducible fraction at higher extractant concentrations may be owing to more effective attack on the crystalline oxide as well as amorphous oxide.

3.3 Comparison between the Batch and the Flow Using Certified Reference Material

The accuracy of sequential extraction methods is much more difficult to estimate than that of a total metal concentration due to the many extraction steps involved. In order to assess the overall accuracy of the sequential extraction procedure, two methods can be employed [8].

The first more rigorous method is the use of certified reference materials (CRMs) and comparison with certified data at each step. Since the results obtained are operationally defined, in other words, the 'forms' of metals are defined by the determination of extractable elements, using a given procedure and, therefore the significance of the analytical results is highly dependent on the extraction procedure used.

The lack of suitable certified reference materials for this type of studies did not enable the quality of the measurements to be controlled. With the purpose to overcome this problem, the Community Bureau of Reference (BCR), now Standard, Measurement and Testing Programme has launched projects for the improvement of the quality of determinations of extractable trace metal contents in soil and sediment [53]. Sequential extraction scheme was proposed which includes three steps: acetic acid, hydroxylamine hydrochloride or a reducing reagent and hydrogen peroxide or an oxidizing reagent. Quevauviller et al. [32] use the BCR scheme for preparation and certification of sediment reference material (CRM 601). This sediment reference material has been recently certified for five metals, Cd, Cr, Ni, Pb and Zn in the first step, Cd, Ni and Zn in the second step and Cd, Ni and Pb in the third step by BCR sequential extraction. Interlaboratory comparison was used for certification this sediment. The techniques used to determine metal concentration in extracts were generally FAAS or ETAAS. ICP-AES and ICP-MS were also used by some laboratories. Not all the elements were certified because the lack of reproducibility attributable to non-adherence to the protocol, in the acceptance of too large tolerance in the conditions specified in it or in the existence of critical aspects in the procedure referred mainly to the second step.

In the second method, the sum of the concentrations from all extraction steps is compared with the total measured concentration or the total certified value. In this work, the second method will be used for accuracy evaluation.

3.3.1 Accuracy and Repeatability of the Batch and Flow Methods

Comparison of the summed result with recommended values provides some estimate of overall accuracy. Table 3.7 shows the results found by applying flow and batch methods to the soil samples (SRM 2710). In both cases the determination of the metal contents in the residual phases was also performed. The recoveries of the metals, expressed as percentage of the total concentration, are also reported.

Table 3.7 Comparison the analytical results of the flow and batch sequential extraction method.

Sample: SRM 2710 0.25 g, Chamber volume: 10 ml. Fraction volume: 50 ml.

Elements	System (n=6)	Concentration of metal in µg/g (mean ± S.D)				Total	Total certified value	%Recovery
		StepI	StepII	StepIII	Residue			
Ca	Batch	990±90	210±160	n.d.	10300±220	11500±190	12500±300	92.0
	Flow	2100±100	480±60	n.d.	8430±700	11010±700		88.1
Fe	Batch	14±4	6500±420	960±80	27600±1100	35070±900	33800±1000	104
	Flow	130±20	6800±700	1780±340	23800±240	32500±1800		96.1
Mn	Batch	1030±70	4600±370	340±10	2290±110	8260±330	10100±400	81.8
	Flow	1750±150	3320±420	940±320	4370±600	10400±520		103
Cu	Batch	710±60	510±170	890±140	430±40	2540±90	2950±130	86.1
	Flow	1630±70	910±80	160±30	250±40	2950±60		100
Zn	Batch	1000±90	2660±460	370±30	1980±10	6010±170	6952±91	86.4
	Flow	1470±80	2200±570	1630±190	2440±210	7740±270		111

To summarize, Figure 3.5 shows the average values of the total concentrations of metals extracted in each step with the batch and flow methods. In previous study of soil and sediment, it was possible to obtain recoveries in the range 89-111% for Cu, Mn and Zn [54]. Li et al. [33] have reported recoveries of 80-120% for fifteen elements isolated from standard reference materials by a five sequential extraction. Other workers have reported recoveries in the range 80-177% for Cu, 71-146% for Mn and 110-151% for Zn isolated from industrially contaminated land [55].

In the present work, the over-all recovery rates from the batch and flow methods for SRM 2710 are in the range 80-108% for all of 5 elements determined (Table 3.7). As can be observed, the overall extraction efficiencies of both sequential extraction methods attempted are virtually the same for Fe and Ca. For Mn, Cu and Zn, the overall extraction efficiencies are slightly lower in batch method. The high over-all recovery rates (>80%) indicate acceptable accuracy by this two methods for Ca, Fe, Mn, Cu and Zn.

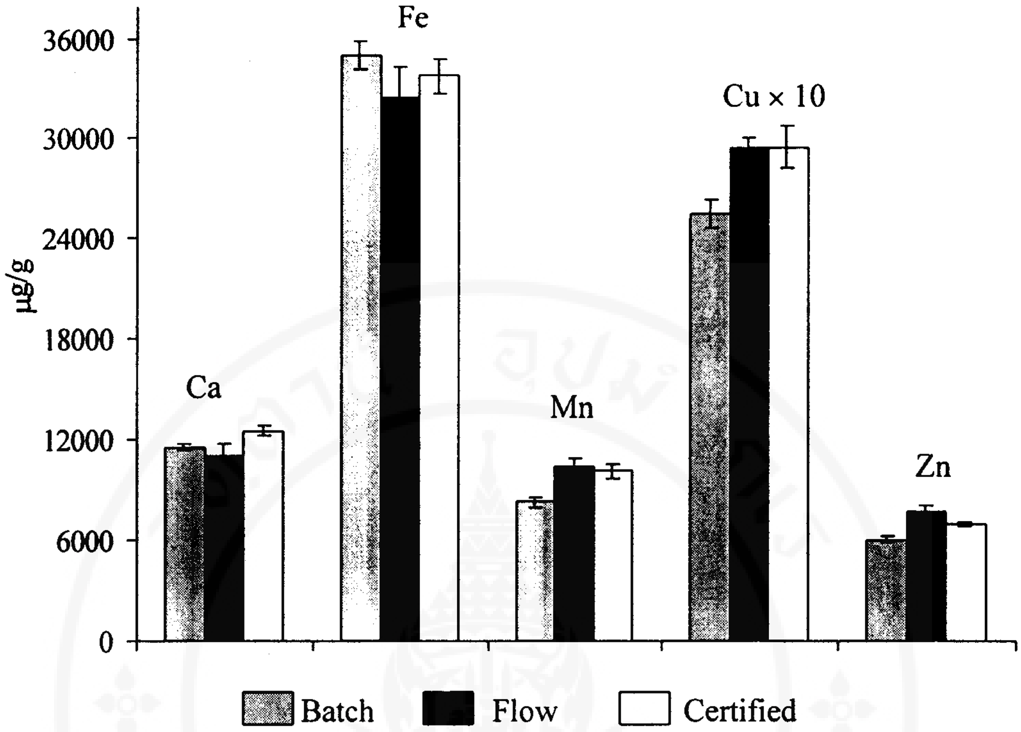

Figure 3.5 Average values of the total concentrations of elements (µg/g) extracted with the flow and batch methods compared to the certified total value.

To assess the repeatability of the flow method compared to the batch method, extraction of two soil subsamples (Set A) were performed simultaneously using the flow extraction procedure. This was repeated twice (Set B and C). Batch method was done in three soil subsamples in the two sets (Set A and B). The %RSD of 'in-set' and 'between-set' of flow and batch methods is shown in Table 3.8.

Table 3.8 Repeatability of the batch and flow sequential extraction methods (%RSD).

Element	Step	%RSD in batch method			%RSD in flow method			
		Set A[a] (n=3)	Set B (n=3)	N=6	Set A (n=2)	Set B (n=2)	Set C (n=2)	N=6
Ca	I	2.21	6.60	**9.09**	1.48	1.84	8.06	**4.76**
	II	1.79	4.44	**76.2**	16.7	2.32	5.77	**12.5**
	III	n.d.[b]	3.20	**n.d.**	n.d.	n.d.	n.d.	**n.d.**
Fe	I	14.9	11.8	**28.6**	16.0	4.00	18.2	**15.4**
	II	1.11	3.45	**6.46**	3.68	1.27	7.21	**10.3**
	III	8.64	3.53	**8.25**	13.5	30.9	11.0	**19.1**
Mn	I	1.15	10.3	**6.80**	3.49	10.0	0.12	**8.57**
	II	0.96	3.09	**8.04**	0.68	0.31	9.21	**12.6**
	III	5.00	2.30	**2.94**	38.5	52.7	23.2	**34.0**
Cu	I	0.81	9.96	**8.45**	8.75	2.41	0.61	**4.29**
	II	14.6	20.8	**33.3**	14.3	5.26	5.26	**8.79**
	III	13.2	7.91	**15.7**	14.3	10.5	10.7	**18.7**
Zn	I	8.65	7.91	**9.00**	0.19	6.38	4.14	**5.44**
	II	0.65	6.58	**17.3**	8.05	2.47	20.2	**25.9**
	III	5.71	4.31	**8.11**	22.0	24.8	7.42	**27.0**

[a] 'Set' represents the simultaneous sequential extraction of three (batch) or two (flow) soil subsamples.

[b] not detectable.

The results obtained by the flow and the batch methods in each fraction will be discussed.

3.3.1.1 Acid Soluble Fraction

Harrison et al. [29] have suggested that mobility and bioavailability of the metals decrease approximately in the order of the extraction sequence. By these criteria, the acid soluble fraction may indicate which metals are most available for plant uptake and other releases into the environment.

Figure 3.6 shows the levels solubilized with the flow method are considerably higher than those obtained with the batch method because, in flow method, the equilibrium was not required but the fresh extractant was flowing through the suspended sample until the extraction was completed as can be seen from the signal lowering to baseline levels. But in the batch extraction procedure, the extraction was performed until the equilibrium was reached for metal ions between the solid sample and the extracting solution. The amount extracted was dependent on the solubility limits that different metal ions have in equilibrium with other ions in the extracting solution. In such a system, the metal extracted may not represent the total amount extractable. The 'between-set' variance was generally larger than 'in-set' variance in batch methods. Acceptable precision (RSD values < 15%) could be obtained for all elements in acid soluble fraction, except Fe. The greatest values in the standard deviations are obtained in the Fe acid soluble (28% and 15% in batch and flow methods respectively) because they are near the limits of detection of the analytical methods used. This step was performed at room temperature, no problem in fluctuation of temperature of extraction, therefore good reproducibility was obtained. Reproducibility of the flow method was better than the batch method in both 'in-set' and 'between-set'. The results obtained indicated that the batch extraction may have been vulnerable to experimental errors, i.e. shaking time or centrifugation conditions and personal procedural errors.

3.3.1.2 Reducible Fraction

The concentrations of each metal in reducible fraction of the flow method (with the evident exception of Mn and Cu) is in good agreement with the concentrations found in reducible fraction of the batch method. For Mn, a significant higher concentration in the reducible fraction of the batch method has been found (Figure 3.6). For Cu, a significant higher concentration in the reducible fraction of the flow method has been found. The Set A results obtained in batch method for Ca and Cu in this fraction was disparate from those of the Set B leading to a degradation in the overall precision (>30%). Similar variability between replicate sequential extraction was observed in the study by Davidson et al. [54]. For Cu, the difference between-set results obtained may be due to the redistribution of Cu in stepII [41]. The poor repeatability of some elements (e.g. Zn) in both methods is probably due to the experimental errors.

This step was performed at 96°C and 80°C in batch and flow methods respectively, the difference in temperature in each set can affect to the variance obtained. The %RSD of flow was higher than batch method in all elements. The 'between-set' variance was generally larger than the 'in-set' variance in both batch and flow methods. This may be due to the temperature used in each set differed slightly.

3.3.1.3 Oxidizable Fraction

Trace metals in soil and sediment can be bound to organic substances, which have various binding mechanisms such as adsorption, complexation and chelation [56]. Hydrogen peroxide in an acidic medium is generally used to oxidize organic matter in soil and sediment analysis, eventhough oxidation of all forms of organic matter may not be complete. Similarly to in stepII, the extraction was performed at 85°C and 55°C for batch and flow methods respectively. The same reason as discussed in stepII can be applied, the 'between-set' variance was larger than 'in-set' variance, except Fe and Mn (poor reproducibility was also obtained in 'in-set' experiment).

The concentration of each metal in the oxidizable fraction of the flow and batch methods indicated significant difference between these two methods except for Ca. Significant higher Fe, Mn and Zn concentrations were found in the flow method. For Cu, a significant higher concentration was found in the batch method. The 'in-set' variance in this step was significant higher than stepI and stepII. Greater flow relative standard deviation values than batch for all elements were obtained at this step. This is due to the elevated temperature used in stepIII can not be reproducibly controlled and back flow of extractant. Temperature used in stepIII, 'in-set' experiment, cannot be controlled to give similar condition. This problem will be subjected to further improvement.

3.3.2 Fraction Distribution of Elements between the Batch and Flow Methods

Comparison of fraction distribution of each metal was made between the batch and flow methods. Figure 3.6 shows the percentages as average values.

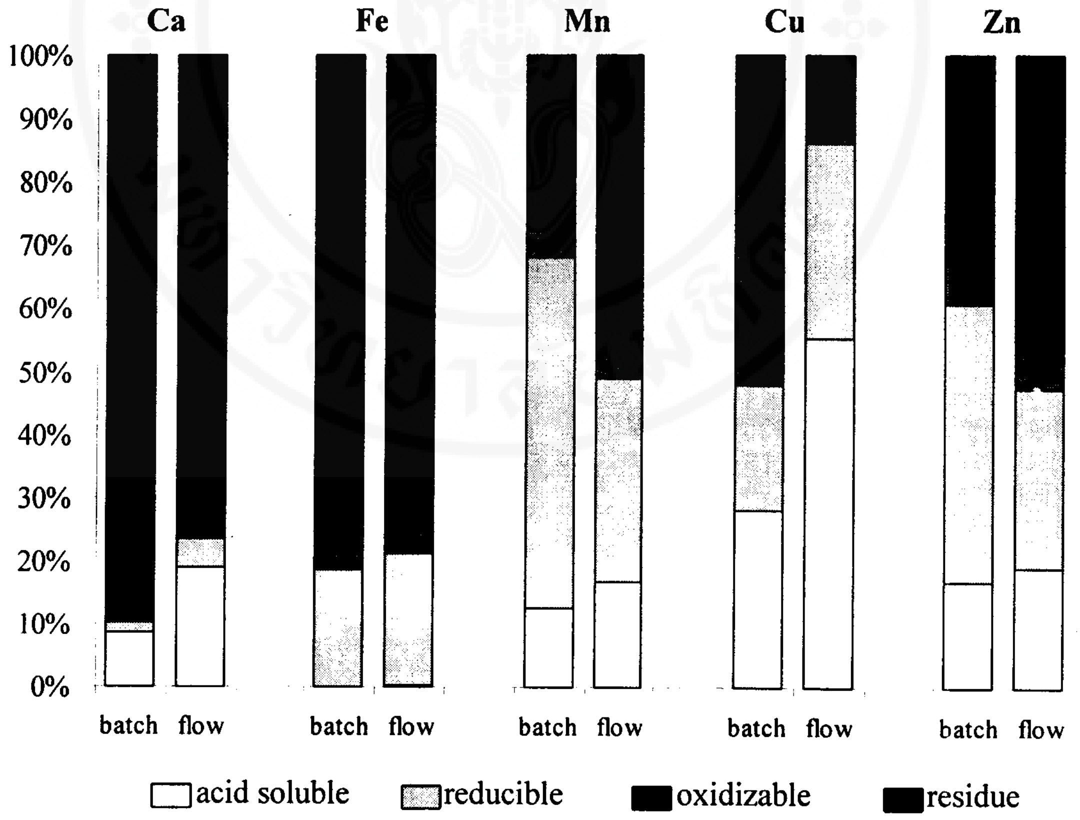

Figure 3.6 Comparison of fraction distribution (mean value of duplicate extraction) for the different fractions.

Flow method: sample (soil SRM2710) 0.25 g, chamber volume 10 ml.

Batch method: sample (soil SRM 2710) 1.0 g, extractant volume 20 ml.

Taking into account Table 3.5 in Figure 3.6, the following comments can be made for each element analyzed.

3.3.2.1 Calcium

About 80% of Ca were present in the residual fraction in both methods. This may indicate that most of Ca is in silicate form in the sample. Ca extracted in stepIII by two methods was below detection limit.

3.3.2.2 Iron

Iron was present mostly at 79% and 73% in the residual fraction as analyzed by the batch and flow methods respectively. 18% and 20% were found in the reducible fractions by the two methods. Among all metals studied, Fe is the one with lowest percentage associated with the acid soluble fraction, which indicates the presence of crystalline Fe oxides and Fe in silicates. The Fe-Mn oxide form is the second most important phase for Fe, which probably represents the amorphous Fe oxides in soils.

3.3.2.3 Manganese

The results obtained by batch method for this metal are different from those found with the flow method. The percentage extracted by the batch method in the reducible fraction (50%) is clearly higher than those from the flow method (30%).

3.3.2.4 Copper

This metal was mainly associated with the non-residual fraction (stepI+stepII+stepIII) in both methods (80% and 90% for batch and flow respectively). The results obtained for this metal for the batch and flow methods in each fraction are different. The most abundant phase in the flow method is the acid soluble fraction (53%), and the least abundant phase is the oxidizable and residual phase. With the batch method, the greatest percentages of Cu are obtained in the oxidizable (38%) and the lowest percentages of Cu are obtained in reducible fraction (14%). It seems that readsorption of Cu occurred at stepII in batch method as previously reported by Xiao-Quan and Bin [6,41]. As far as the differences of Cu between the batch and flow methods are concerned, part of the reducible Cu at stepII was readsorbed onto the organic phase. When the organic phase wre decomposed by the hydrogen peroxide, the readsorbed Cu was again transferred into solution. Therefore, greater batch values than flow for Cu were observed at stepIII.

3.3.2.5 Zinc

Zn was mainly associated with the non-residual fractions. Zn was distributed among all the fractions in both sequential extraction methods but was mainly associated with stepII of batch method and almost similar %distribution in the stepI (20%), stepII (30%) and stepIII (20%).

3.4 Extractogram as a Tool for Evaluation of the Association between Element in Solid Samples

The amount of each element in a particular phase was obtained by addition of the amount of all fractions of the corresponding step. On the other hand, a plot of concentration versus fraction number can provide an extractograms of each element giving additional useful information about elemental association. Figure 3.7 and 3.8 present extractograms of 5 elements for a soil and sediment SRM samples respectively. The peak shape of the extractogram can be useful for further evaluation.

Table 3.5 showed some extractant used in the reducible fraction. This fraction was defined and called in different metal oxides, i.e. Fe-Mn oxides, amorphous Fe oxide and crystalline Fe oxides. In this work, it may be expected that the amorphous and crystalline Fe-Mn oxides was observed (Figure 3.7)

In soil sample, double peaks were evident for Fe, Mn, small first peak of Zn and small second peak of Cu (whereas a single peak for Ca) in the reducible phase. The double peaks obtained may be ascribed as being originated from the amorphous and crystalline phases of the Fe-Mn oxides respectively. The existence of Cu with the reducible phase can be seen in Figure 3.7 as associated with amorphous Fe-Mn oxides rather than crystalline Fe-Mn oxides. Zn is thought to be associated with the crystalline of Fe-Mn oxides rather than the amorphous of Fe-Mn oxides. In oxidizable phase, the behaviors of the association of each element are similar, except Zn.

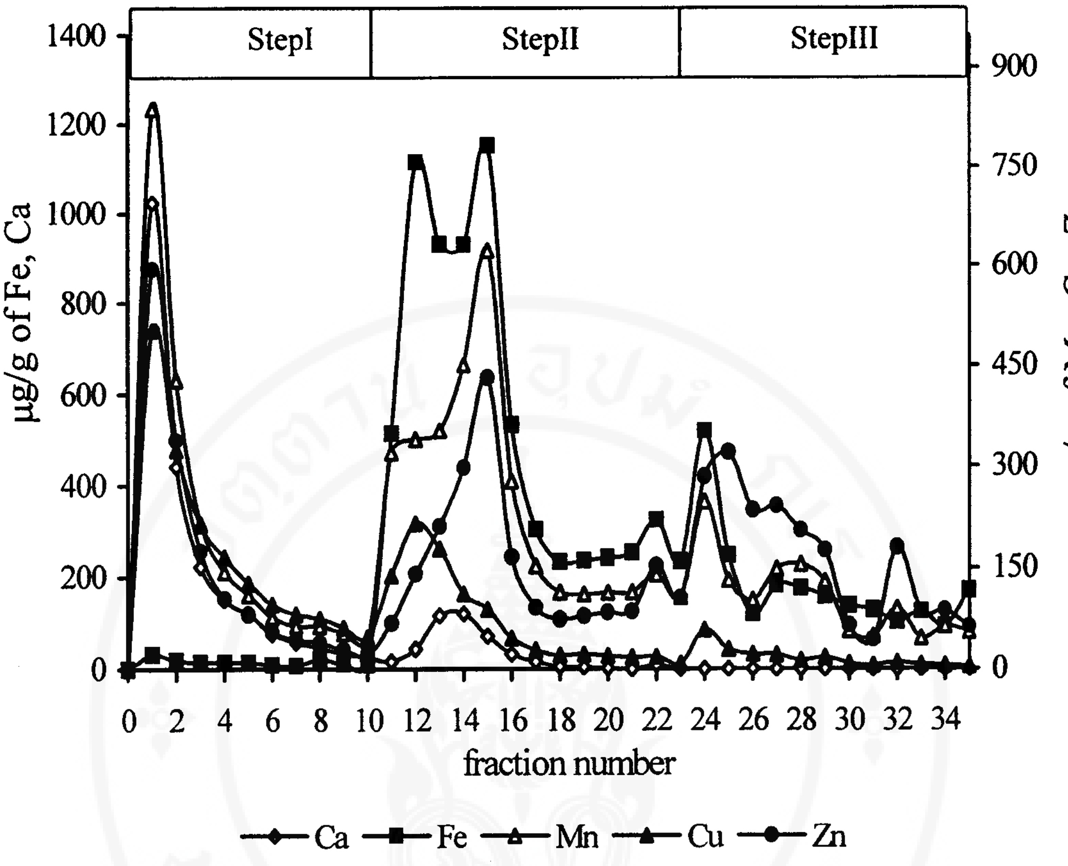

Figure 3.7 Extractograms of flow sequential extraction of Ca, Fe, Mn, Cu and Zn from soil. Sample: soil (SRM 2710) 0.25 g, chamber volume 10 ml, fraction volume 15 ml.

In sediment sample, single peak was observed for Ca and Cu (Figure 3.8). This indicated that Ca and Cu was probably associated with the amorphous Fe-Mn oxides. Double peaks were evident for Fe, Mn and Zn but small peak of the second peak was obtained in these elements. This indicated that Zn was probably associated with the amorphous and crystalline Fe-Mn oxides.

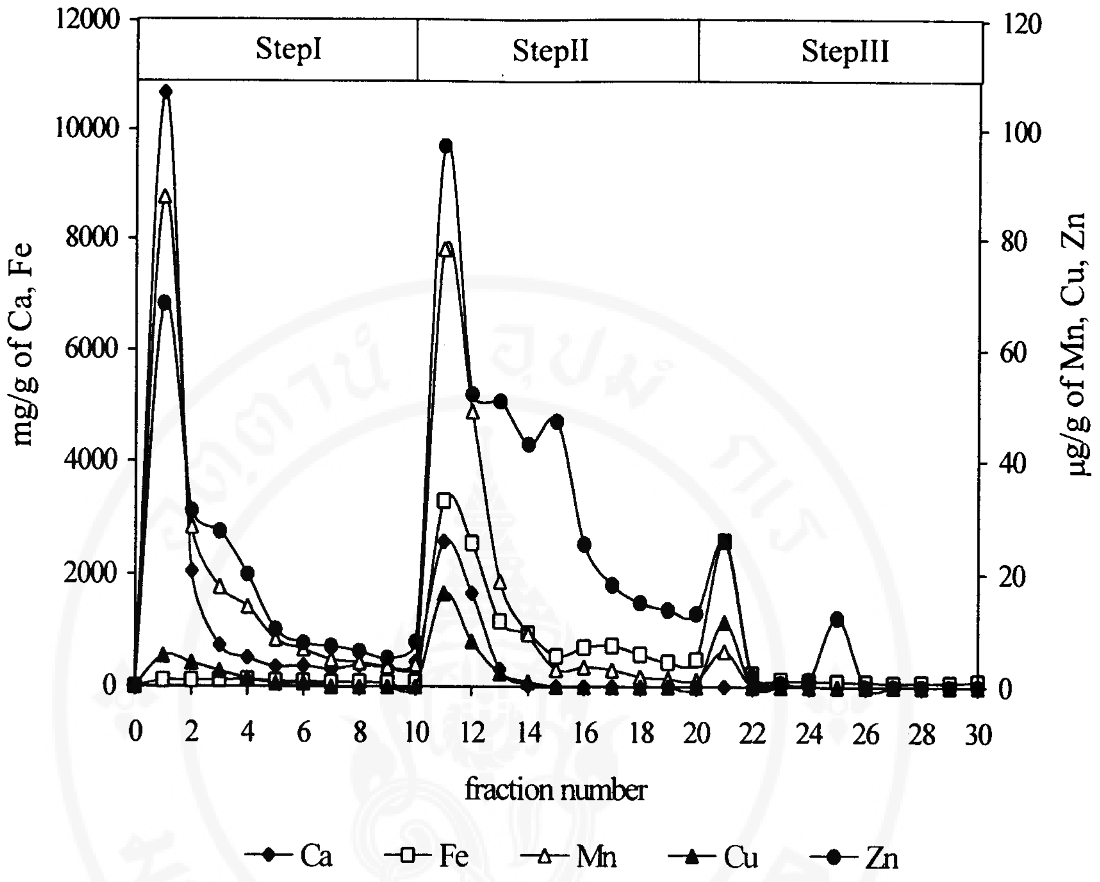

Figure 3.8 Extractograms of flow sequential extraction of Ca, Fe, Mn, Cu and Zn from soil. Sample: soil (SRM 2704) 0.5 g, chamber volume 3 ml, fraction volume 15 ml.

To confirm this assumption, model soil could be proved this assumption, i.e. preparing model soils by combined in equal proportions quantities of both amorphous Fe-Mn oxides and crystalline Fe-Mn oxides. This synthesized model soil should be applied to the flow sequential extraction for obtain the extractogram.

CHAPTER IV

CONCLUSION

The flow sequential was developed to speed up, facilitate and improve the accuracy of the chemical fractionation of metals in solid materials. The design and optimal condition of extraction have been studied. In this work, the flow system was evaluated by 3-step sequential extraction of some soil and sediment certified reference materials. A modified Tessier's scheme was used to extract acid soluble, reducible and oxidizable phases sequentially.

In the batch method, extraction pH was not strictly correct, since pH of extracts were differed from pH of extractant. In the flow method, pH of extracts at the beginning of extraction can effect by the acid-base of sample but at the end of the leaching, extraction is carried out at the designated pH.

The concentrations of extractants were also studied. Three concentrations of CH_3COOH (0.11, 0.22, 0.52 M) were studied. Extractograms of five elements were not differed in the three sets of CH_3COOH concentrations, except Zn in stepIII. No significant increase in extracted amount of Ca, Mn, Cu and Zn was observed when the concentration was increased to 0.22 M, but only Cu showed an increase at 0.52 M CH_3COOH. For Fe, an increase in extractability in stepI was observed with increasing CH_3COOH concentrations.

The effect of $NH_2OH{\cdot}HCl$ concentration was studied at the three different concentrations (0.04, 0.50, 1.0 M). No significant differences were found between the extractograms of all elements at the three $NH_2OH{\cdot}HCl$ concentrations. Greater amounts of Mn, Cu and Zn were extracted when the concentration of $NH_2OH{\cdot}HCl$ was increased to 1.0 M. The use of a higher concentration of $NH_2OH{\cdot}HCl$ decreased the Mn extracted in stepIII. For Fe and Cu, no significant increase in extractability when the concentration of $NH_2OH{\cdot}HCl$ was increased to 0.50 M, but Fe showed an increase at 1.0 M. The use of a more concentrated reductant also increased the amounts of Fe in stepIII. The amounts of Fe in stepIII were similar at all reductant concentration studied when the residues were washed after stepII. The increase in amounts of elements extracted in reducible fraction at higher $NH_2OH{\cdot}HCl$ concentration may be owing to a more effective attack on the more refractory crystalline form of oxyhydroxides in the sample.

The sum of all fractions of the batch and flow extractions was compared with total certified value. The overall recovery rates from the batch and flow method for soil certified reference materials SRM 2710 are in the range 81-111% for all 5 elements determined. The recovery rates at this range can be considered acceptable because many steps are involved in the analytical procedures.

The repeatability of extractions was studied in six replicates of extraction divided into 2 and 3 sets of experiment for the batch and flow respectively. The %RSD is in the range 2.94-76.2 and 4.29-34.0 for the batch and flow methods respectively. Reproducibility of the flow method in stepI was better than the batch method in both 'in-set' and 'between-set'. Both methods have a good reproducibility in stepI except Fe. Poor reproducibility of some elements in stepII and StepIII were observed because the elevated temperature used in these two steps can not be reproducibly controlled.

The fraction distribution of the flow method differed from that of the batch method. This may be due to redistribution which is more likely in the batch system than in a flow system.

The associations between element in soil and sediment samples were evaluated using the extractograms. Associations of elements in soil (SRM 2710) and sediment (SRM 2704) were described. The reducible fraction of amorphous and crystalline phases of Fe-Mn oxides can be distinguished from the extractograms. The peak appearance of the extractograms in soil SRM 2710 showed that Cu was associated with the amorphous Fe-Mn oxides more than crystalline Fe-Mn oxide phases. Zn was considered to be associated crystalline Fe-Mn oxides phases more than the amorphous Fe-Mn oxide phases.

The peak appearance of the extractogram in sediment SRM 2704 showed that Cu was associated with the amorphous Fe-Mn oxide phases while Zn was associated with both amorphous and crystalline Fe-Mn oxide phases.

Based on the present study some advantages of the flow extraction system can be drawn:

1. Simple and easy to perform
2. Tedious procedures such as solid-liquid phase separation by centrifugation and filtration are not required.
3. Less risk of contamination from the experimental environment and personal procedual errors.
4. Rapid, because no equilibrium has to be established between the solid-liquid phases. (A 3-step extraction can be carried out within 6-7 h compared to 32 h for a batch method.)
5. Washing between steps can be simply done by flowing pure water between extraction steps.
6. Extraction is performed at the designated pH conditions without any need of pH adjustment during extraction.
7. Extractant concentration did not affect metal extraction for stepI but affected some metals in stepII.
8. The extractograms for each element can be used for evaluation of the chemical association between elements in samples.
9. The problem of redistribution can be reduced in a flow extraction system because extracts are rapidly removed from contact with the solid phase, therefore allowing less time for readsorption to take place.

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