

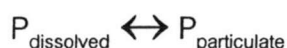
CHAPTER II

PHOSPHORUS PARTITIONING IN SEDIMENT

2.1 Forms of phosphorus in sediment

Forms of phosphorus in sediment result from the combination of physical, biological and geochemical processes during sediment transport, deposit and early diagenesis that are controlled by the physico-chemical parameters in aquatic environment (Mortimer, 1971; Bostrom, 1984; De Groot and Golterman, 1990). Processes involving in the formation of sedimentary phosphorus speciation are: (1) adsorption/desorption (2) uptake and release by living organisms (3) precipitation (3) remineralization (4) resuspension and (5) remobilization (Froelich, 1988; Lebo, 1991; Berner et al. 1993; Ruttenberg and Berner, 1993; Horne and Goldman, 1994; Spivakov et al., 1999; Compton et al., 2000). These processes are illustrated in Figure 2.1.

Phosphorus, derived from rock weathering and erosion processes and anthropogenic activities, is introduced into the aquatic environment in a number of different chemical forms (dissolved inorganic phosphorus, DIP; dissolved organic phosphorus, DOP; particulate inorganic phosphorus, PIP and particulate organic phosphorus, POP) and has been described in general as being present in the aqueous phase as a small portion of the total and in solid phase as a large portion of the total. Each portion is made up of a large number of different components. Most of these chemical forms may change between their dissolved or particulate phase according to a dynamic equilibrium by adsorption or desorption processes.



This dynamic equilibrium is called "the phosphate buffering mechanism" (Froelich, 1988; Lebo, 1991 ; Spivakov et al., 1999).

The most significant form of DIP is orthophosphate (H_2PO_4^- , HPO_4^{2-} and PO_4^{3-}) under the pH conditions normally encountered in natural waters. Seawater (pH = 8) consists of 12 % PO_4^{3-} , 87 % HPO_4^{2-} and 1% H_2PO_4^- (cited in Stumm and Morgan, 1996). Orthophosphate is readily available for assimilation by living organisms. It plays an irreplaceable role as structure link in the genetic materials RNA and DNA. In adenosine triphosphate (ATP), phosphate is involved as short-term energy carrier in biochemical reactions, and it is a component in the phospholipid membranes of cell walls. It can be released together with DOP by organism excretion and bacterial decomposition (rem mineralization) of dead organisms (Horne and Goldman, 1994).

Particulate inorganic phosphorus (PIP) consists of detrital phosphorus and adsorbed phosphorus on suspended inorganic particle such as clays and other minerals (Compton et al., 2000). Detrital phosphorus (e.g. fluorapatite) is covalently bounded in mineral lattice of some silicate minerals and not available for uptake by the biosphere or by adsorption until released by chemical weathering. The amount of detrital phosphorus can be represented by the natural origin of phosphorus. Adsorbed phosphorus occurs on mineral particles such as clay minerals and Fe-Mn oxides or organic matter coated on clay minerals in fresh water and releases from particles with increased salinity or estuarine mixing zone (Carritt and Goodgal, 1954 ; Froelich, 1982; Froelich, 1988; Lebo, 1991). Adsorbed phosphorus is potentially available to the biosphere through reversible exchanges.

Phosphorus is removed from the dissolved phase by biological uptake or sedimentation of dead biota or chemically formed precipitates with Fe^{3+} , Al^{3+} and Ca^{2+} with increased salinity. The substantial evidences indicated that most Fe, Mn, Al, and P flocculate with organic matter (DOM) in estuaries (Sholkovitz, 1976) while most riverborne particulate organic matter (POM) and associated POP are removed into estuarine sediment before reaching coastal zone or shelf (Wallast, 1983). It was found that hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$) occurs upon settling of CaCO_3 particles (cited in Berner et al., 1993; Spivakov et al., 1999).

Many studies (e.g. Palmer, 1985; Fox 1989; Berner et al, 1993) have shown that sorption to Fe oxides and oxyhydroxides (poorly crystalline) is the major process responsible for binding phosphate at short time scales in oxidized marine surface sediments. A substantial part of this Fe bound P is released again when the Fe(III)-phases are buried into the reduced sediment zone or upon changes in redox conditions in the sediment or in the overlying water, e.g. as a result of an increased loading of organic material. On the other hand, Fe oxides and oxyhydroxides with a high affinity for phosphate may be formed freshly upon oxidation of dissolved Fe(II) diffusing upward across the redox boundary in the sediment. As a consequence, an intense cycling of phosphate between sorptive Fe(III) surfaces and the pore water can occur at the Fe(III)-Fe(II) redox interface, showing up as sharp peaks in both dissolved phosphate and Fe(II) at that depth in the sediment. Therefore, Fe(III) phosphates or Fe(III) complexes, which absorbed phosphorus, most obviously plays important roles in the cycling of phosphorus in the environment.

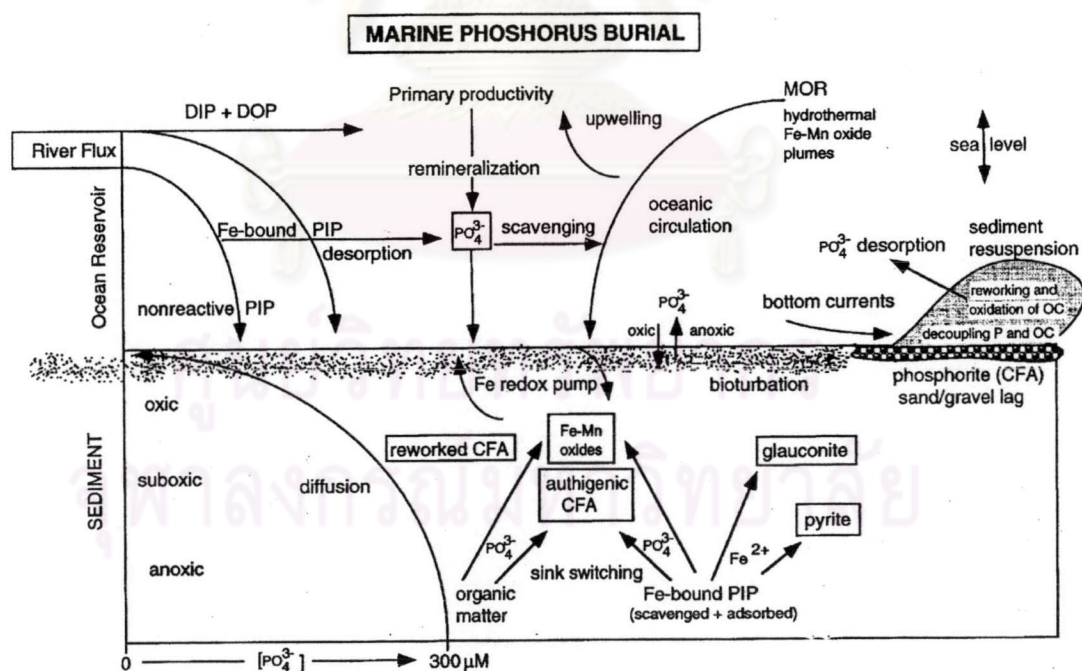


Figure 2.1 Schematic diagram of the processes involved in the burial of phosphorus in marine sediment (Compton et al., 2000).

After burial, phosphorus can be mobilized during bacterial degradation of organic matter and reduction of iron oxides. Most of this mobilized phosphate is removed from the pore water as authigenic carbonate fluorapatite (CFA or francolite) - $\text{Ca}_{x1}\text{Na}_{x2}\text{Mg}_{x3}(\text{PO}_4)_{x4}(\text{CO}_3)_{x5}(\text{F})_{x6}$ or is resorbed by iron oxide particles and does not return to the water column. This simulates the sedimentary preservation of P (see Ruttenberg and Berner, 1993; Compton et al., 2000). Diagenesis (post depositional processes) also produces a large number of well-defined chemical species, the most often encountered ones being vivianite $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ and ludlamite $(\text{Fe}, \text{Mn}, \text{Mg})_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, others such as dufrenite $\text{Fe}_3^{2+} + \text{Fe}_6^{3+} [(\text{OH})_3/\text{PO}_4]_4$, graffonite $(\text{Fe}, \text{Mn}, \text{Ca})_3(\text{PO}_4)_2$, phosphoferrite $(\text{Fe}, \text{Mn})_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$, klinostrengite $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$, variscite $\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$, and anapatite $\text{Ca}_2\text{Fe}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ are to be expected as well according to the local redox condition (Spivakov et al., 1999). Carbonate hydroxyapatite or dahllite $(\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_2(\text{CO}_3)_6)$ in fish bones, teeth or other carbonate skeletons that burial in sediments can also be slowly converted to carbonate fluorapatite due to substitution of F^- from pore water to OH^- in carbonate hydroxyapatite (Krauskopf, 1989).

When sediments are resuspended in estuaries and on the shelf by upwelling, bottom current, estuarine mixing and bioturbation, significant amount of DIP may be released into the water column by desorption (Fox et al., 1986; Sundby et al., 1992). Resuspension of sediments is predicted to result in desorption of PIP because the phosphate concentrations in pore waters are much higher than those in bottom waters.

Polyphosphate from detergents utilized in human activities can be degraded to orthophosphate by hydrolysis. Accumulation of soluble polyphosphate in sediments was found in polluted estuaries and coastal waters. Therefore, the amount of polyphosphate in estuaries and coastal waters might be a useful index of pollution from human activities (Martin, 1970).

From the context above, the phosphorus species in which the researchers have been interested can be classified by its origin and biogeochemical processes (also cited in Barbanti et al., 1994) as the followings:

- *Exchangeable or loosely bound P or labile P*, the fraction easily adsorbed and released by exchange sites. Once released, it will become available for algal growth (Chang and Jackson, 1957; Hieltjes and Lijklema, 1980; Van Eck, 1982; Psenner et al., 1988; Ruttenberg, 1992; Vink et al., 1997; Agemian, 1997);
- *Fe bound P or Fe and Al bound P*, the fraction associated with Al, Fe and Mn oxides and hydroxides (Chang and Jackson, 1957; William et al., 1967; Hieltjes and Lijklema, 1980; Golterman and Booman, 1988; Ruttenberg, 1992; Agemian, 1997);
- *Carbonate bound P*, this fraction includes authigenic carbonate fluorapatite plus biogenic carbonate hydroxyapatite (Ruttenberg, 1992);
- *Detrital P or detrital apatite*, this fraction includes fluorapatite from igneous, sedimentary and metamorphic rocks (Ruttenberg, 1992);
- *Calcium bound P*; this fraction is generally referred to as apatite P (Williams et al., 1976; Psenner et al., 1988) or *Ca-bound P* (Chang and Jackson, 1957; De Groot and Golterman, 1996; Agemian, 1997);
- *Residual P or refractory P*, this fraction includes in very resistant minerals, such as monazite and xenotime (William et al., 1980), and those in the crystal lattices of some silicate minerals (Ruttenberg, 1992);

- *Organic P*, the nature of organic pool is much more complex and less well described. Different compounds have been found: phytic acid, sugar phosphates, humic and fulvic acids, phospholipid, nucleotides and nucleic acids (cited in Barbanti et al., 1994 and Agemian, 1997). Because of the difficulty in identification and quantification of the various components, the organic fraction is usually either considered cumulatively (e.g. Aspila et al., 1976; Golterman and Booman, 1988) or divided into operational groups (labile organic components, humic and fulvic substances, acid-soluble organic components, residual organic components, phosphate esters, and phosphonates) (see Barbanti et al., 1994); and
- *Polyphosphate*, this fraction can be used as the index of pollution from human activities in estuaries and coastal waters (Martin, 1970; Psenner et al., 1988, Agemian, 1997).

2.2 Study of phosphorus partitioning in sediments

Phosphorus partitioning results can provide the information on the origin of phosphorus in sediments, the degree of pollution from anthropogenic activities, the bioavailability of phosphorus in sediments which is responsible for eutrophication, and finally, the biological and chemical processes that occur with phosphorus in aquatic environment (the same as trace metal partitioning results described by Martin et al., 1987).

To understand the origin of phosphorus, the degree of phosphorus pollution from human activities, the bioavailability of phosphorus and the biogeochemical processes (especially, burial and diagenesis) involving phosphorus in estuarine and marine sediments, it is necessary to identify, separate and quantify the various solid phases of sedimentary P. This task is difficult because of its low concentration and the fine-grained nature of estuarine and marine sediments. These two factors preclude the

uses of most standard methods for direct identification of mineral phases, such as X-ray diffraction (XRD). Independent chemical analysis on phosphatic phases, such as isotopic or trace element signatures clearly cannot be applied when discrete phases are not separable (Ruttenberg, 1992).

As a result, it is necessary to turn to indirect means to determine the identity and size of sedimentary P reservoirs. These indirect means are described as follows:

- Use of observed correlation and anti-correlation between total P (from X-ray fluorescence technique or ignition method of Aspila et al., 1976 or fusion method of Shapiro, 1967) and mineral components or major elements chemistry of bulk sediment (from X-ray fluorescence technique) to deduce the identity of sedimentary P phases has been a common approach (Baturin, 1988; Froelich et al., 1982; Moody et al., 1988, Ruttenberg, 1992). This method can only predict the likelihood of occurrences or processes involving P-bearing phases in sediments.
- Physical separation of different sedimentary fractions, usually grain size, and measurement of total P in the different fractions after each has been separately dissolved is another method for indirect identification of P-bearing phase(s) (Froelich et al. 1982). This method can easily lead to ambiguous interpretation because complete physical separation of different phases from a fine-grained sediment can rarely be achieved, and surface coatings of various sorts, potentially important in P removal to sediment can remain undetected and unidentified in such treatment (Ruttenberg, 1992).
- Use of pore-water profiles to infer the nature of chemical reactions occurring in a sediment also applied to study burial and diagenetic P. However, more than one scenario can be constructed to explain pore-water data; therefore this

approach rarely yields an unambiguous interpretation of solid-phase processes (Ruttenberg, 1992).

On the other hand, there are some direct means to identify, separate and quantify sedimentary P reservoirs. These direct means are described as follows:

- Ignition method is the simplest method to separate and quantify total P, inorganic P and organic P. This method was firstly developed by Aspila et al. (1976) and has become popular because of its simplicity; and
- Sequential extraction method is the most promising methods for separating and quantifying various P reservoirs in sediments. This method is operationally or functionally defined on the basis of reactivity of a particular phase in a given extractant. The basic assumption of selective extraction technique is that the reagent use is able to selectively destroy one phase without any solubilization of the others (Martin et al., 1987). Sediment is extracted with a series of extractants, each is chosen to selectively dissolve a single phase or group of phases of similar chemical characteristics. The sequence of extraction is designed so that the most reactive phases are removed first and the severity of the extractants increase with each step (Ruttenberg, 1992). All sequential extraction methods were developed on the basis of the study in efficiency and specificity of reagent used to be extractants with the standardization of analog phosphorus phases and the matrix effect study due to readsorption. Factors controlling sequential extraction method that need to be considered are grain size of sediment, ratio of the amount of solid matter to the volume of extractant, chemical reactivity and kinetic effect (e.g. type, strength and pH of reagents), time of contact, temperature, constant suspension of solids in extractant via shaking, extraction sequence (the sequence of reaction) and readsorption (Martin et al., 1987; Ruttenberg, 1992).

Sequential extraction methods have been widely used as an environmental tool to quantify sedimentary P reservoirs in soils (Chang and Jackson, 1957), lake sediments (Williams et al., 1976; Hieltjes and Lijklema, 1980; Golterman and Booman, 1988), river particulate matters and sediments (e.g. Chase and Sayles, 1980; Lucotte and Anglejan, 1985; Psenner et al., 1988; Lebo, 1991; Chambers et al., 1995; Conley et al., 1995) and marine sediments (Ruttenberg, 1992; Berner et al., 1993; Delaney and Anderson, 1997). The history of sedimentary phosphorus extraction procedures is described in Table 2.1.

2.3 Related researches

Sequential extraction method for phosphorus partitioning was firstly developed by Chang and Jackson (1957, 1958) for soil fertility study. The procedure uses various dilute solutions of NH_4Cl , NH_4F , NaOH , HCl , citrate-dithionite bicarbonate (CDB) and hot 1 M NaOH (at 85°C for 1 h) to recover labile phosphorus, aluminium-, iron- and calcium-bound phosphorus, and reductant-soluble and refractory phosphorus, respectively. Modifications were subsequently introduced since it was revealed that the NH_4F step extracted iron-bound phosphorus in addition to aluminium bound phosphorus, for which it was intended (Williams et al., 1967, 1980). In addition, phosphate was found to readsorbed on CaF_2 , which was by product of the reaction. Therefore, Williams et al. (1967) proposed a modification of Chang and Jackson's procedure by using citrate-dithionite bicarbonate (CDB) to extract nonapatite P; NaOH to extract Fe and Al bound P; and HCl to extract apatite P. However, CDB extraction was later omitted because the citrate component dissolves substantial organic matter, carbonates and apatites (Hieltjes and Lijklema, 1980; Williams et al., 1980). This flaw was overcome by Hieltjes and Lijklema (1980) whose procedure was widely adopted by several authors (e.g. Van Eck, 1982; Bostrom et al., 1985 and Psenner et al., 1988). In Hieltjes and Lijklema 's extraction scheme, CDB extraction was omitted, and NH_4Cl was applied for the initial extraction step to quantify labile P and prevent readsorption during the following steps; NaOH to extract iron- and aluminum-bound phosphorus; and HCl to extract calcium-bound P.

Table 2.1 History of sequential extraction schemes for phosphorus speciation in soil and sediment

Author	Extractant	Proposed fraction
Chang and Jackson(1957)	1 M NH ₄ Cl	Labile P
	0.5 M NH ₄ F pH 8.2	Al-bound P
	0.1 M NaOH	Fe-bound P
	0.5 M H ₂ SO ₄	Ca-bound P
	CDB ¹	Reductant-soluble P
	0.1 M NaOH	Refractory P
Williams et al. (1967)	CDB ¹	Non-apatite P
	1 M NaOH	Mostly Fe and Al bound P
	0.5 M HCl	Apatite P
Aspila et al. (1976)	1 M HCl	Inorganic P
	1 M HCl after ignition at 550 °C	Total P
Hieltjes and Lijklema(1980)	1 M NH ₄ Cl pH 7.0	Labile P
	0.1 M NaOH	Fe and Al bound P
	0.5 M HCl	Ca-bound P
Psenner et al. (1988)	1 M NH ₄ Cl pH 7.0 (twice)	Water soluble P
	0.11 M CDB ¹ (twice)	Reductant soluble P
	1 M NaOH(twice)	Iron- P
	0.5 M HCl(twice)	Calcium bound P
	1 M NaOH at 85 °C	Refractory P
Golterman and Booman(1988)	0.02 M Ca-NTA ² pH 8.0 + dithionite + 0.1 M TRIS-buffer ³	Fe bound P
	0.05 M Na-EDTA ⁴ , pH 8.0	Ca bound P
	6 M H ₂ SO ₄ + H ₂ O ₂ 30 % at 135 °C	Residual(organic) P
Ruttenberg(1992)	1 M MgCl ₂ pH 8.0	Exchangeable or loosely sorbed P
	CDB ¹ , pH 7.6	Easily reducible or reactive Fe bound P
		Carbonate fluorapatite, biogenic
	1 M Na-acetate in acetic acid, pH 4.0	hydroxyapatite, CaCO ₃ ⁻ bound P
	1 M HCl	Detrital fluorapatite-bound P
	1 M HCl after ignition at 550 °C	Organic P

1 CDB = citrate dithionite bicarbonate

2 NTA = nitrilo triacetic acid

3 TRIS-buffer = tris (hydroxymethyl)-aminomethane

4 EDTA = ethylene dinitrilo tetraacetic acid

Table 2.1 History of sequential extraction schemes for phosphorus speciation in soil and sediment (continued)

Author	Extractant	Proposed fraction
Vink et al. (1997)	1 M MgCl ₂ pH 8.0	Exchangeable or loosely sorbed P
	10% SDS, HCO ₃ ⁻ buffer, pH 8.57, 80 °C	Organic P
	CDB ¹ , pH 7.6	Easily reducible or reactive Fe bound P
		Carbonate fluorapatite, biogenic
	1 M Na-acetate in acetic acid, pH 4.0	hydroxyapatite, CaCO ₃ ⁻ bound P
	1 M HCl	Detrital fluorapatite-bound P
	6 M HCl after ignition at 550 °C	Residual P
Agemian (1997)	0.5 M NaCl	Loosely bound P
	Bicarbonate ditionite (BD), pH 7.0	Fe and Al bound P
	0.1 M NaOH	Polyphosphates
	0.5 M HCl	Calcium bound P
	1 M NaOH, 85 °C	Refractory P

¹ CDB = citrate dithionite bicarbonate.

Psenner et al. (1988) also introduced a five-step fractionation scheme: NH₄Cl to extract water-soluble phosphorus; citrate-dithionite-bicarbonate (CDB) to extract reductant soluble phosphorus; NaOH to extract iron-phosphorus; HCl to extract calcium bound P; and NaOH(1 M at 85°C) to extract refractory phosphorus. This scheme is based on Williams et al. (1980)'s and Hieltjes and Lijklema (1980)'s extraction schemes. However, the concentration of all extractants was modified.

The effectiveness of the sequence that starts with an extraction with NaOH for the Fe and Al bound P fraction, and then with an extraction by HCl for the Ca-bound fraction by Hieltjes and Lijklema (1980), Williams et al. (1980) and Psenner et al. (1988), was questioned by Golterman (1984) and De Groot and Golterman (1990). They suggested that readsorption and/or precipitation of phosphates with Ca compounds occurred because of high pH resulting from the NaOH leaching, and that a significant portion of organic P was hydrolyzed by the strongly alkaline or acidic solution used. This would lead to underestimating Fe-bound P and Organic P, as well as

overestimating Ca-bound P. Therefore, organic complexing agents, such as NTA and EDTA, have been introduced to selectively separate iron- and calcium-bound phosphorus (Golterman, 1984; Golterman and Booman, 1988; De Groot and Golterman, 1990). This type of extraction has the advantage of being less harsh with respect to extremes in pH. Therefore, hydrolysis of organic phosphates or the degradation of clay structures is prevented. Furthermore, since the phosphorus removed from iron hydroxides is complexed with the extractant, the possibility of readsorption on CaCO_3 is avoided. In this approach, buffered solution of Ca-NTA/dithionite (pH 8.0) was used to extract iron-bound phosphorus and was then followed by Na-EDTA extraction (pH 8.0) to isolate calcium-bound phosphorus. Since organic phosphorus compounds are not appreciably hydrolyzed, this technique can be used as an initial cleanup prior to the determination of the concentration of organic phosphorus (De Groot, 1990; De Groot and Golterman, 1993). However, Barbanti et al. (1994) suggested that this sequence is not suitable for sediment that contains high amount of detrital apatite due to the incomplete dissolution of detrital apatite by Na-EDTA. The undissolved apatite can be wrongly attributed to organic P reservoir and produced values of organic P overestimated.

In 1992, Ruttenberg presented SEDEX scheme for phosphorus partitioning in marine sediments. This scheme was developed from Chang and Jackson (1957)'s, Lucotte and D'Anglejan (1985)'s, and Aspila et al. (1976)'s extraction methods. This extraction scheme can separate the two main categories of authigenic phosphate phases, namely ferric oxyhydroxide-phosphorus and authigenic carbonate fluorapatite in marine sediments by using CDB (pH 7.6) and acetate buffer (pH 4.0), respectively. In addition, this scheme can also separate authigenic and detrital apatites by using acetate buffer and HCl extractions, respectively. Furthermore, the readsorption problem was resolved by washing the sediment residue with MgCl_2 between extraction steps. The SEDEX scheme and reaction mechanism is shown in Table 2.2.

Table 2.2 SEDEX scheme and reaction mechanism (Ruttenberg, 1992).

Step	Extractant	Phase extracted	Reaction*
I	1 M MgCl ₂	Exchangeable or loosely sorbed P	Formation of MgPO ₄ ⁻ complex and (or) mass action displacement by Cl ⁻
II	0.3 M Na ₃ -citrate 0.11 M NaHCO ₃ (pH7.6) 0.563 g of Na-dithionite in 22.5 ml of citrate bicarbonate solution	Easily reducible or reactive ferric Fe-bound	Reduction of Fe ³⁺ by dithionite and subsequent chelation by citrate
III	1 M Na-acetate buffered to pH 4 with acetic acid	Carbonate fluorapatite-biogenic hydroxyapatite-CaCO ₃ -bound	Acid dissolution at moderately low pH and (or) chelation of Ca ²⁺ by acetate
IV	1 M HCl	Detrital fluorapatite	Acid dissolution
V	Ash at 550 °C 1 M HCl	Organic P	Dry oxidation at 550 °C 1 M HCl extraction of ashed residue

* The reaction mechanism for MgCl₂ washes following steps II and III is as described for step I; H₂O washes following steps I-III displace residual solution volumetrically before the next principal extractant is added.

After SEDEX scheme was presented, it became very popular since it was thoroughly developed according to the principle of sequential extraction development. The reagents used were tested for efficiency and specificity by standardization with the widest analog phases. In addition, readsorption problem was resolved. However, it provides the underestimated organic P when compare with organic P from Aspila method (Berner et al., 1993). Barbanti et al. (1994) indicated that it might be due to organic P hydrolysis by some extractants (mostly CDB and HCl) in the successive steps before organic P extraction.

Consequently, Vink et al. (1997) modified the SEDEX scheme by changing the extraction sequence of organic P to be extracted before Fe-P in order to resolve the organic P hydrolysis, and proposed sodium dodecyl sulfate (SDS) as an organic phosphorus extractant. The other extractants are the same as those of SEDEX scheme.

MgCl₂ is also used to wash the sediment residues between the extraction steps. The analog standardization results showed that SDS can extract a small amount of inorganic phosphorus together with organic phosphorus and SDS is not suitable for sediment that contains high amount of refractory organic P. The modified SEDEX scheme and reaction mechanism is shown in Table 2.3.

Table 2.3 Modified SEDEX scheme and reaction mechanism (Vink et al., 1997).

Step	Extractant	Phase extracted	Reaction*
I	1 M MgCl ₂	Exchangeable or loosely sorbed P	Formation of MgPO ₄ ⁻ complex and (or) mass action displacement by Cl ⁻
II	10 % SDS, HCO ₃ ⁻ buffer pH 8.57, 80 °C, 2 h(x6) 0.2 M NaCl wash(x2), H ₂ O wash(x2) Ash 600 °C, 4 h, 6 M HCl 16 h	Organic P	Reduce surface tension, freshly organic matter can be extracted
III	0.3 M Na ₃ -citrate 0.11 M NaHCO ₃ (pH7.6) 0.563 g of Na-dithionite in 25 ml of citrate bicarbonate solution	Easily reducible or reactive ferric Fe-bound P	Reduction of Fe ³⁺ by dithionite and subsequent chelation by citrate
IV	1 M Na-acetate buffered to pH 4 with acetic acid	Carbonate fluorapatite- biogenic hydroxyapatite-CaCO ₃ - bound	Acid dissolution at moderately low pH and (or) chelation of Ca ²⁺ by acetate
V	1 M HCl	Detrital fluorapatite	Acid dissolution
VI	Ash at 550 °C , 6 M HCl	Residual P	Dry oxidation at 550 °C 6M HCl extraction of ashed residue

* MgCl₂ and H₂O washes are the same as described in SEDEX scheme except step II.

Agemian (1997) proposed a sequential extraction scheme that was modified from Van Eck (1982)'s, Psenner et al. (1988)'s, and Jensen and Thamdrup (1993)'s extraction schemes. In Agemian' s scheme (as described in Table 2.4), NaCl is used in extracting loosely bound P instead of NH_4Cl because it does not dissolve Fe and Al bound P, and NaCl is used in preventing readsorption of phosphorus on sediment particle between the extraction steps (Van Eck, 1982; Jensen and Thamdrup, 1993). In addition, Bicarbonate ditionite (BD) is applied to extract Fe and Al instead of CDB because BD does not resolubilize calcium bound P (Jensen and Thamdrup, 1993), and 0.1 M NaOH is used to extract polyphosphates (Jensen and Thamdrup, 1993). Calcium bound P is extracted with 0.5 M HCl (Van Eck, 1982; Psenner et al., 1988; Jensen and Thamdrup, 1993). Finally, hot 1 M NaOH is utilized to extract refractory P (Psenner et al., 1988).

Table 2.4 Sequential extraction scheme and reaction mechanism (Agemian, 1997)

Step	Extractant	Phase extracted	Reaction*
I	0.5M NaCl , 1 h	Loosely bound P	Formation of Na_2PO_4 complex and (or) mass action displacement by Cl^-
II	Bicarbonate ditionite (x2)	Fe and Al bound P	Reduction of Fe^{3+} by dithionite
III	0.1 M NaOH, 18 h	Polyphosphates	Base dissolution
IV	0.5 M HCl	Calcium bound P	Acid dissolution
V	1 M NaOH , 85 ^o C , 1 h	Refractory P	Acid dissolution

*One time of NaCl wash between step I – IV

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2.4 Selection of sequential extraction methods

In this study, SEDEX scheme of Ruttenberg (1992), Modified SEDEX scheme of Vink et al. (1997) and extraction scheme of Agemian (1997) were selected as tools in investigating the phosphorus partitioning in estuarine sediments for comparison study. The reasons for choosing these sequential extraction methods are described below:

- These extraction schemes use the same type of extractant (combination of acid-base extractants and reducing agents) in the sequential extraction scheme
- These extraction schemes inherently differ in classification of some extracted species (as described in Table 2.5), type and strength of extractants to extract the same P fraction, extract conditions (temperature, time of extraction, ratios of the amount of sediment to volume of extractant), sequences of extraction and amount of steps in extraction schemes.
- SEDEX scheme (Ruttenberg, 1992) is the most popular sequential extraction scheme utilized for studying sedimentary P reservoirs in estuarine and marine sediments.
- Modified SEDEX scheme (Vink et al., 1997) was developed to reduce the organic P hydrolysis that occurs in the successive steps before organic P extraction. The sequence of organic P was to be extracted before Fe-P, Authigenic and detrital apatite and residual P extraction by using SDS as organic P extractant. The other reagents are the same as SEDEX scheme.
- Sequential extraction scheme of Agemian (1997), having different origin from the other two, was modified from Van Eck (1982)'s, Psenner et al. (1988)'s and Jensen and Thamdrup (1993)'s scheme. All reagents, extract conditions and names of phosphorus speciation differ from SEDEX and Modified SEDEX schemes.

Table 2.5 Classification of P species based on the selected sequential extraction methods of this study

Step	Sequential extraction method		
	SEDEX method (Ruttenberg, 1992)	Modified SEDEX method (Vink et al., 1997)	Agemian (1997)
I	Loosely sorbed P	Loosely sorbed P	Loosely bound P
II	Reducible or reactive Fe bound P	Organic P	Fe and Al bound P
III	Carbonate authigenic Apatite+biogenic hydroxy apatite+ CaCO ₃ bound P	Reducible or reactive Fe bound P	Polyphosphates
IV	Detrital apatite	Carbonate authigenic Apatite+biogenic hydroxy apatite+ CaCO ₃ bound P	Calcium bound P
V	Organic P	Detrital apatite P	Refractory P
VI	-	Residual P	-

2.5 Accuracy of the selected sequential extraction methods

The accuracy of the selected sequential extraction methods cannot be directly determined because there is no certified reference material. Therefore, the accuracy of these extraction schemes can be indirectly confirmed by comparison between the sum of all P fractions and total P. The most popular method for total P is Aspila method (Aspila et al., 1976). However, total P value can be obtained through various methods such as XRF, total digestion, and ignition methods. Therefore, the outcome of this study is that the best method for total P analysis can be determined.