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INVESTIGATION AND ALLEVIATION OF MUTUAL INTERFERENCE OF Fe^{2+}AND PO_4^{3-} IONS BY PROTONATION

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ABSTRACT

Mutual interference of iron and phosphates in the spectrophotometric estimation process is a result of the strong bond in the iron phosphate complex. The interference of these two ions can be successfully overcome by the isolation of the two ions. The present investigation was carried to study the mutual interferences of iron and phosphates in the spectrophotometric analysis. Complexing both Fe and PO_4 ions in the form of Fe-P complex were done for isolating the Iron and phosphate radicals from other interferences. Whereas to avoid the interference of Fe in PO_4 estimation, AlPO4 complex was formed. This triggered the idea to pre-concentrate the two ions by protonation using a strong acid. The results obtained justified the need for isolation of Fe^{2+} and PO_4^{3-} .This study provides an exact estimation of Fe^{2+}at pH 1.5. The results obtained were statistically correlated by Pearson's correlation coefficient and the results were statistically significant at p value 0.01.

Keywords: Iron, Phosphates, Protonation, Dual complexation, Fe-P complex, $AlPO_4$ complex, Interferences.

INTRODUCTION

When radicals like iron and phosphates having immense bonding capacity come in contact with various other elements they thereby lead to the formation of various complexes by the natural environmental process. Researchers are putting efforts by working on various ferrites[1-2] which are utilized for the remediation of water as iron and phosphates bind together forming different complexes which may turn out to be harmful. Like, it is observed that Phosphates readily form complexes with many elements like with calcium it forms calcium phosphate complex which is the vital component in milk as well as bones. [3] Iron like Phosphorous by the virtue of its property, has a tendency to form complexes with organic as well as inorganic materials. When Iron is in its complexed form it is found to be more stable than in the un-complexed form.[4] In addition, Phosphate readily combines with iron to form iron phosphate (Fe-P) complex.[5] The coordination of PO_4^{3-} ions with Fe^{3+} ions produces a binuclear complex of Fe - O - P (O_2) - O - Fe and the infrared spectroscopic techniques give fruitful evidence of phosphate adsorption on surfaces of Goethite, Hematite, Lepidocrocite, β- ferric hydroxide.[6] The ionic structure in which these cations are coordinated (usually octahedral) by water molecules and oxygen atoms belonging to the PO_4^{3-} ion are often represented as coordination polyhedral as in the figure No.1 This α-quartz structure of aluminium phosphate ($AlPO_4$) is representative for Fe-P complex which too bears α-quartz structure.

These hydrated mineral phosphates of Iron are mainly used in steel industries as a protective film as they exhibit the property of antirust. They are also used in dye industries as they form a good base for paint adhesion. [7] When the effluent from such industries make an entry in the river, lake and ground water it is surely supposed to contaminate that specific source and on the contrary, if this type of contaminated water with high phosphate content is consumed, it turns out to be fatal for lives because of phosphate toxicity. [8] Accordingly, they limit the crop production due to phosphate deficiency as bonds formed by the oxides of Iron are too strong and breaking of these bonds is difficult.[9] Hence this difficulty of breaking the strong bonds of these complexes also makes the estimation of individual radical challenging because of the interference pattern of iron in phosphate in their respective estimation process. Many researchers have

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studied interferences of various ions encountered in iron estimation process.[10-13] Likewise, phosphate estimation too is hindered by many contaminants.[14-17] S.R. Kuchekar et. al. have studied the Physico-chemical parameters in Khadakwasala reservoir where it is noted that the phosphate concentrations ranged from 0-0.47mg/L when iron concentrations were 0.04 mg/L to 1.53 mg/L hence it can certainly be related that if the iron concentration is more it imparts to affect the phosphate estimation.[18]

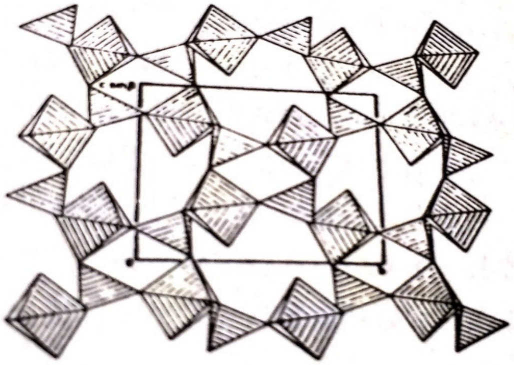

Fig.-1: Metavariscite $AlPO_4$. $2H_2O$

The Patalganga River is surrounded by many industries and its pH ranges from 6.5 to 7.0 whereas Jian-Feng Peng et.al. have clearly stated that the maximum adsorption of phosphorus takes place at pH 7.0 which has a significant impact on the binding capacity of Fe-P. [19]This beheld three different surface complexes which dominate the surface coordination between phosphate and Iron metal. This complex has two bridging bi-dentate complexes both of them being protonated and non-protonated and a non-protonated monodentate complex. The non-protonated, binuclear bi-dentate $\equiv Fe_2O_2PO_2$ surface complex is the dominant adsorbed phosphate species at neutral pH value. At this pH, phosphate exists primarily as dihydrogen phosphate (H_2PO_4) and hydrogen phosphate (HPO_4) which can readily combine with iron oxides. It may, however, be protonated at low pH values.[20]This validates the fact that the phosphate anions affect the superficial layer of the Fe cations thereby leading to the masking of Fe cations. The Patalganga river water sample showed a similar masking when the phosphate content was higher than iron in the water. This is mainly due to the affinity of Phosphates to combine with Fe metals. Chemically, Fe–P being very stable will not dissociate unless in the presence of concentrated acid, which is not present in natural surroundings. Ferric phosphate as such is not harmful to human health and is used as an intravenous treatment in human beings.[21]The stability of the Fe-P bond is not affected when the concentrations are in equivalence however if both the radicals start acting variable the activity of mutual interference of the both radicals in the estimation proceeds. It has been a long time since researchers have been working on the presence of interfering ions in the determination of Fe^{2+} and PO_4. Marija Bralic worked on the estimation of iron by Fluoride selective ion using flow injection analysis to overcome these interferences. [22] Likewise use of ion chromatography to avoid the interference of chlorides, sulphates, chlorates and carbonates by alkaline per sulphate digestion results in the conversion of all forms of phosphates to orthophosphate which is another technique that takes care of interfering radicals in the estimation of phosphates. These diversified estimation techniques selected to estimate iron and phosphates gave the evidence of interfering radicals during the estimation process hence making it obligatory to find a technique that takes care of the mutual iron and phosphate interference in their respective estimations. The current research article presents a study of mutual interference of iron and phosphate in the spectrophotometric estimation of Fe^{2+} and PO_4^{3-} ions. The prime objective of this study is to present a detailed investigational report of mutual interference of iron and phosphates when they are strongly bound in the form of Fe -P complex. The complete study was carried out by keeping the ratio of iron and phosphate concentrations equal as the 21 month study of the river water samples displayed that when the concentrations of iron and phosphates are equivalent they start interfering in the estimation of the either ions. The conventional interference controlling measures in the spectrophotometric analysis are effective on dilution and even a heavily polluted water sample can be analyzed however the mutual interferences of iron and phosphates in the either estimation in spectrophotometric estimation needs to be visualized by reforming a technique which absolutely unbinds the Fe-P bond . The first part of the experiment deals with the isolation of the two radicals in iron phosphate complex for the elimination of interference and the second part deals with the estimation process and finally, a method to treat real samples.

EXPERIMENTAL

All the reagents used were AR grade whereas the stock solutions of the Iron (M/s Thomas Baker) and Phosphate (M/s S.D. Fine Chem Ltd.) standards used for the preparation of a synthetic solution of Fe-P were prepared by dissolving known volumes and quantities of reagents in double distilled water. The ratio of Iron to phosphate for the preparation of iron phosphate complex was kept at equivalence that is 1:1. Whereas for the preparation of aluminium phosphate complex aluminium sulphate (M/s Thomas Baker) was used.

Statistical Study

The estimated results of Fe^{2+} and PO_4^{3-} ions obtained after the dual complexation method and the pre-treatment by protonation were studied statistically in order to confirm whether the ratio of iron and phosphates before and after complex formation by the two new estimation methods correlated well. A correlation study of Fe and PO_4 standards synthesized after dual complexation and protonation were done by Pearson Correlation coefficient at p value 0.01.

Procedure

(A) Dual Complexation process to eliminate interferences in Fe^{2+} and PO_4^{3-} estimation

Various standards of Iron and Phosphates were prepared in 1:1 ratio to get synthetic mixtures of Fe and P standards. These Fe-P standards were treated to isolate iron and phosphates ions to avoid their mutual interference in the estimation process. Hence the Iron Phosphate mixture was dissolved in concentrated HCl to dissociate the Fe^{3+} ions in the form of ferric chloride and the PO_4^{3-} ions in the form of phosphoric acid (H_3PO_4). The PO_4 ions in form of phosphoric acid were again complexed using aluminium sulphate to isolate phosphate ions from the ferric ions yielding a white $AlPO_4$ complex. This aluminium phosphate complex was filtered using what man filter paper. The filtrate so obtained contained ferric ions whereas the residue on the filter paper contained the Phosphate ions in the form of $AlPO_4$. This $AlPO_4$ complex was then dissolved in concentrated sulphuric acid. The Fe^{2+} and PO_4^{3-} ions thus obtained simultaneously were free from all the interferences.

Detection Methods

(B) Estimation process by preconcentration of Fe^{2+} and PO_4^{3-} by protonation using HCl and H_2SO_4. (Pre-Treatment)

In the second part of the analysis, various concentrations of HCl and H_2SO_4 were prepared to find out the exact volume of acid required for the preconcentration of the respective ions in the bi -dentate = $Fe_2O_2PO_2$ complex. For Fe^{2+} estimation, the various Fe-P synthetic mixtures were taken in known volumes and estimation was done by making up the total volume of the solution to 25ml in an std. volumetric flask then protonating it by 10ml of different concentrations of hydrochloric acid solutions. These solutions were further subjected to acid digestion till the volume of the solution was reduced to half and iron was completely in the +2 oxidation state. The reduced solutions were cooled to room temperature and the pH of the solutions was adjusted to 1.5 after studying the maximum yield at various pH. The estimation was further carried out by 1-10 phenanthroline method.

The protonation of the sample for PO_4 estimation was done using 10 ml Sulphuric acid solutions of variable concentrations. The preconcentrated samples of PO_4^{3-} ions in the known quantity of standard phosphate solution were marked up to 25 ml in the std. volumetric flasks. The pH of the solution was adjusted to 8.The estimation was further carried out by molybdenum blue method.

(C) Real Sample Treatment (Any complicated water or effluent to be tested)
The method (B) can be used to estimate iron and phosphates in any real water samples. The experimental water sample in the current paper is from Patalganga River where in iron and phosphates were estimated by method (B) to eliminate the mutual interference of the either ions.

RESULTS AND DISCUSSION

To suppress the interfering radicals a novel method to extract Fe^{2+}ions in the form of Prussian blue ferric Ferro cyanide complex was developed.[23] Hence, an interference study was carried out which revealed that Phosphate was the main interfering radical in the estimation of Iron.[17] The novel method for extraction of Iron brought into light the actual figures of Iron concentration in the Patalganga River water. The interference study revealed that the Phosphate present in the River water was in equivalent proportion to the Iron content.[17] The Fig.-4 presents the complete trend of iron and phosphate in the Patalganga river water. The iron and phosphate concentrations in the fourth, fifth, sixth and seventh month show a gradual increment in the phosphate concentration making it difficult to estimate Fe^{2+} ions as the phosphate anions completely affect the superficial layer of the Fe thereby leading to the masking of Fe cations. When iron and phosphate are in equal concentrations masking is maximum consequently the phosphate interference in iron at this juncture is extreme. As the Fe-P bond formed by the action of PO_4 bears α-Quartz structure [24] which is formed by the sorption of phosphates on the Fe metal ions specifically by an inner sphere sorption mechanism, therefore, being too strong enough to be dissociated.

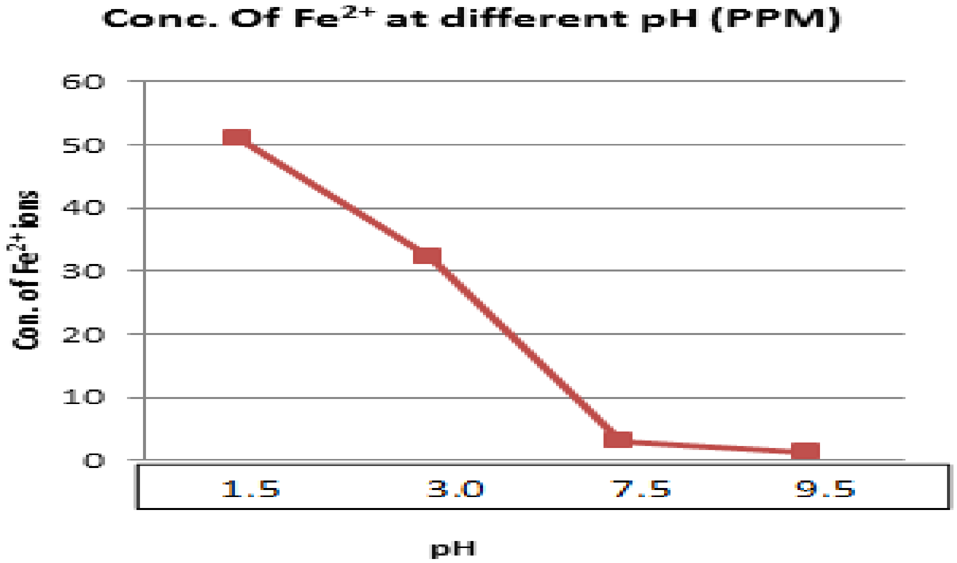

Fig.-2: Graphical representation of Conc. Of Fe^{2+} at different pH (PPM)

As seen in the Fig.-4 and Table-7 from the ninth month the iron content in the river water raised gradually and in the 18th month, the rise in iron content is substantial. This growth in iron content in the river water completely changed the estimation pattern. It was observed that when the values of iron content are larger than phosphate they start interfering in the phosphate estimation. Hence to overcome this mutual interference pattern of iron and phosphates the isolation of iron and phosphates in two separate complexes was indispensable. Accordingly, for the isolation of iron and phosphates in the water during the estimation process, a two-way complexation process was designed in the laboratory. Thus the formation of Fe-P complex in the sample was the first step to make it free from other metal impurities like Zn, Cd, Pb, Cu, Ni, Al, Mn, Cr, Na, and K which are also present in the river water. During scrutinization of the

Fe-P complex, it was comprehended that the complete estimation of PO_4 in the Fe –P complex is difficult as the Fe ions in the complex are known to interfere positively in the investigation. Sten O. Engblom has postulated the interference of iron in the determination of phosphate using a cobalt electrode in soil extract [25]. This positive interference is due to the blue color of iron which interferes at more than 100 mg/L [26] and the Patalganga river water contains iron more than 100 mg/L. Hence to isolate the PO_4 ions from the Fe ions the Phosphate ions were complexed using aluminium sulphate to obtain $AlPO_4$ complex which was free from interfering Fe ions. This dual complex formation increased the accuracy of estimation of two such ions which readily interfere in the estimation of either process. If iron and phosphates are present in the sample in such concentrations that hinder the estimation process in such a way that the presence of one confounds the estimation of other. The utmost advantage of dual complexation method is that the Fe-P complex formed in the first step is free from any other metal impurity and interferences. The Second achievement of the dual complexation method is the separation of phosphate anions from the Fe cations making the PO_4 estimation process free from the positive interference of Fe ions. The ratios of the actual standard concentrations of Fe-P were assimilated after dual complexation. This confirms the validity of the method. The results of the concentrations were found to be statistically significant at P 0.01 on correlation by Pearson's correlation coefficient. Table-1 shows a strong positive correlation of R=0.999 correlating the values of Fe^{2+} obtained after preconcentration by protonation with the HCl solution and PO4 ion from the $AlPO_4$ complex after extraction with the help of sulphuric acid.

Table-1: Correlations of various standards Fe^{2+}after complexation and PO_4 ions after complexation by Dual complexation method.

		Iron in complex	Phosphate in complex
Iron complex	Pearson Correlation	1	0.999**
	Sig. (2-tailed)		0.000
	N	5	5
Phosphate complex	Pearson Correlation	0.999**	1
	Sig. (2-tailed)	0.000	
	N	5	5

**Correlation is significant at the 0.01 level (2-tailed).

The dual estimation procedure is only one of its kinds. This method gives a clear perception of the activities of Fe^{2+} ions and PO_4 ions when they escort each other. The isolation of the Fe and PO_4 radicals by the dual complexation entirely demonstrates the fact that an interference free technique can unpack the true results in the estimation of real samples as real samples are mysterious and nothing is known about them. This unavoidable manifestation of the two ions directed to find out a technique that would curtail the efforts in dual complexation. Hence a study of the estimation process was done whereby the preconcentration of Fe and PO_4 ions was carried out by the protonation of the samples using various concentrations of HCl and sulphuric acid. The use of sulphuric acid and hydrochloric acid in the estimation of PO_4 and Fe respectively is traditional [27-33] but our experiments confirmed that the concentration of acids used in the conventional methods is insufficient to break the strong Fe-O-P bond as hydrochloric acid used is mostly for acid hydrolysis in the estimation of iron. Fe from various sources like plant body, the human body, drinking water or any other source is estimated by using HCl for the process of acid hydrolysis. At this point, it must be clearly stated that either the concentration of HCl used in estimation is less or the volume of the acid used is insufficient for estimation of iron, for example, Mehrotra and Gupta have used 1N HCl for iron estimation which yields Fe up to 10 µg [34], Md. Rafiqul Islam & Md. Mamun Hossain has used 2ml concentrated hydrochloric acid yielding maximum 6.4 PPM iron [35] hence the estimation process presented in the current studies gives a new insight into the estimation process. As the acid used for the breaking the menacing Fe-P bond was highly concentrated whereas the volume consumed was sufficiently large enough for the dissociation of the strong bond hence the accuracy of estimating Fe^{2+} and PO_4 ion simplified subsequently even if both the ions were present in the equivalent Fe-P ratio of concentration. Table No.5 shows that 10ml of Con. HCl is the most precise volume for the estimation of iron. Using Con. HCl more than 10 ml may too yield erroneous results. Later the acid digestion of the sample was done till the volume was reduced to half of the total volume resulting in changes of the oxidation states from +3 to +2. In the study carried out for the Patalganga River Fe and P are found in equal concentrations in the 5th month as seen in Table-7 that is Fe^{2+} 507.98 mg/L and PO_4 515.17 mg/L leading to interference in the estimation. Hence to evaluate the actual interferences and to check the precision and accuracy of the method various standards of Fe-P such as 10ppm, 20ppm, 30ppm, 40ppm and 50ppm were prepared. The results obtained after the protonation of the ions showed that the results were statistically significant at P 0.01 and exhibited a strong positive correlation of iron and phosphate at r = 0.997 as displayed in Table-2.

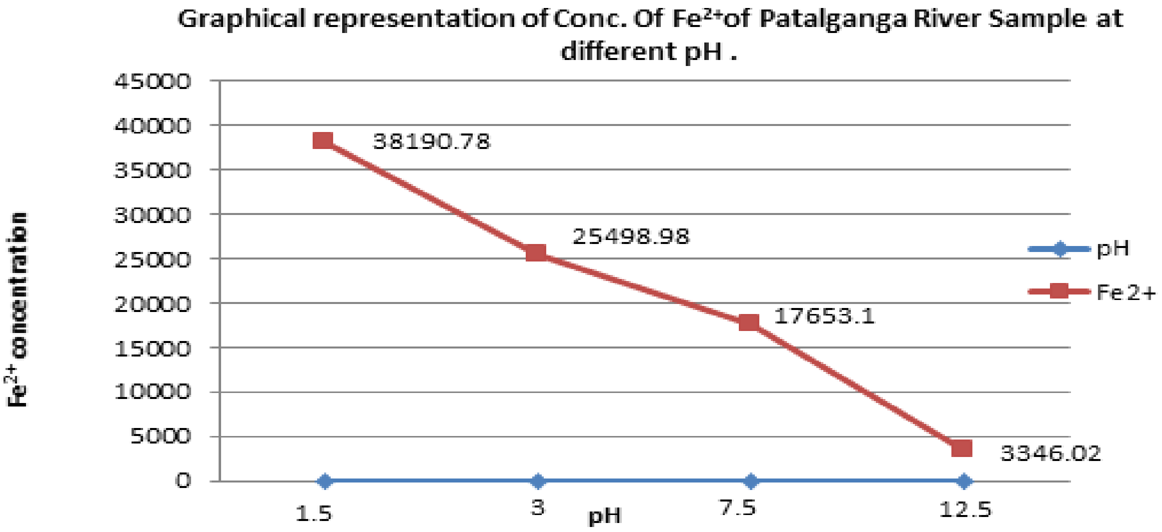

Fig.-3: Graphical representation of Conc. Of Fe^{2+}in Patalganga River Sample at different pH.

The estimation of Fe^{2+} ions was carried out at pH 1.5 as the estimation is highest at this value. Table-3 displays results of Fe^{2+} ions estimated at different pH by pre-treatment (B) estimation process of iron. Table No. 3verifies that 90.39% of Fe^{2+} ions were estimated at pH 1.5 whereas only 2.3% Fe^{2+} ions were estimated when the pH was alkaline that is 9.5. Figure-2 evidently pictures the verity of the pH study. On application of the pH study to the Patalganga River water sample, the happenings corroborated, exposing maximum achievement of Fe^{2+} ions at pH 1.5 the results of which are staged in Table-4 whereas Fig.-3 clearly displays the maximum estimation of Fe^{2+}ions at pH 1.5. The results of Fe^{2+} ions at different pH in the original river sample ascertained that the pretreatment method (B) has a great scope of estimation and follows the trend that was established on the various standards of Fe-P.

Table-2: Correlations of various standards Fe^{2+} and PO_4 ions by modified Fe^{2+} and PO_4estimation method

		Iron	Phosphate
Iron	Pearson Correlation	1	0.997**
	Sig. (2-tailed)		0.000
	N	5	5
Phosphate	Pearson Correlation	0.997**	1
	Sig. (2-tailed)	0.000	
	N	5	5

**Correlation is significant at the 0.01 level (2-tailed)

Like iron, Table-6 presents the accurate volume needed for preconcentration of phosphates in the samples. 10ml of concentrated sulphuric acid can efficiently take care of any type of interference in the phosphate estimation. Adding to excess volume of sulphuric acid does not change the results. Subsequently, it was noted that diluted concentrations of sulphuric acid could not extract complete PO_4 from the 10ppm standard as seen in Table-6. Use of nitric acid in place of sulphuric acid for extraction of phosphate ions fails to yield exact amount of PO_4 ions.[36, 37] The estimation of PO_4 ions was done by adjusting the pH to 8.0 by molybdenum blue method of estimation.

Table-3: Conc. of Std. Fe^{2+} at different pH

S. No.	Actual Conc. Of Fe^{2+}(PPM) A	pH	Conc. Of Fe^{2+} at different pH (PPM) B	% estimation of Fe^{2+} at different pH (B/A)*100
1.	56.2	1.5	50.8	90.39
2.	56.2	3.0	32.2	57.29
3.	56.2	7.5	2.9	5.1
4.	56.2	9.5	1.3	2.3

Table-4: Conc. of Fe^{2+} at different pH in Patalganga River water sample

S. No.	pH	Conc. Of Fe^{2+} at different pH (PPM)
1.	1.5	38190.78
2.	3.0	25498.98
3.	7.5	17653.1
4.	12.5	3346.02

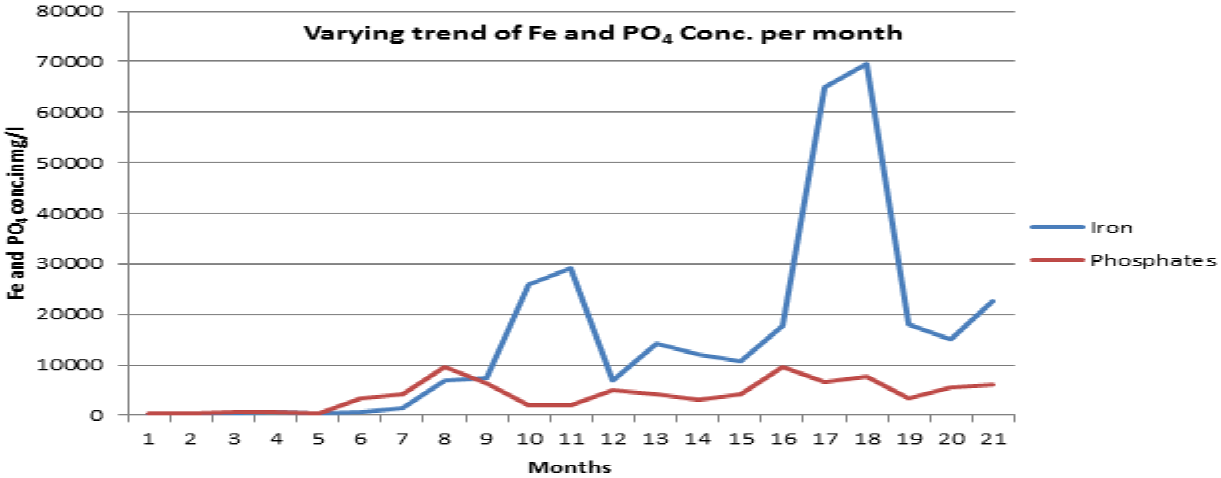

Fig.-4: the Varying trend of Fe and PO_4 Conc. per month for 21 months.

CONCLUSION

Iron and phosphate are present in water as a result of digenesis over thousands of years in a variety of complex combinations which make their estimation a grueling job. The present explorations are designed precisely to unearth distinctive approaches which in an undemanding way investigate the mutual interference of Fe^{2+} and PO_4 ions estimation methodologies. Hence, binding of Iron and phosphate together in the form of a complex proved to take care of two extremely menacing radicals concurrently. The dual complexation method presented here provides an affluent technique in the estimation of Iron and Phosphate simultaneously. This approach readily takes care of the either interferences caused by both the radicals mutually as iron positively interferes in the estimation of phosphates whereas phosphates completely mask the Fe^{2+} ions thus triggering a negative interference, in the present investigation $AlPO_4$ complex plays a significant role in eliminating the negative interference of the PO_4 ions in the estimation of Fe^{2+} ions.

Table-5: Fe^{2+} estimated by using different concentrations of HCl in 10ppm Fe-P standard sample

S. No.	0.1N HCl	0.5NHCl	1.0N HCl	Conc. HCl	Conc. HCl	Conc. HCl
Vol. of HCl	10ml	10ml	10ml	2ml	10ml	15ml
Fe^{2+} estimated (ppm)	1.7	0.17	0.28	0.63	10.4	0.26

Table-6: PO_4 estimated at by using different concentrations of H_2SO_4 in 10ppm Fe-P standard samples.

S. No.	5.0NH_2SO_4	10.0NH_2SO_4	Conc.H_2SO_4	Conc.H_2SO_4	Conc. H_2SO_4
Vol. of H_2SO_4	10ml	10ml	1ml	10ml	15ml
PO_4 estimated (ppm)	1.4	2.1	1.2	10.19	10.28

Table-7: Concentrations of Fe and PO_4 in Patalganga River Water (Real Samples)

	M1	M2	M3	M4	M5	M6	M7
Fe	167.44	237.88	365.91	507.98	247.59	509.21	1567.25
PO_4	251.17	356.82	548.87	515.17	373.73	3226.82	4200.95

	M8	M9	M10	M11	M12	M13	M14
Fe	6768.96	7341.46	25883.85	29083.77	6805.82	14103.31	12017.56
PO_4	9582.10	6398.82	2106.13	2106.13	4873.24	4289.47	3218.33

	M15	M16	M17	M18	M19	M20	M21
Fe	10686.38	17761.28	64848.78	69476.09	18078.02	15130.50	22635.57
PO_4	4045.47	9467.64	6652.52	7811.34	3356.55	5649.40	6087.03

The results acquired after dual complexing had a strong positive correlation, r 0.999 at p 0.01. The estimation of phosphate ions in the $AlPO_4$ complex produced significant results which confirmed the interference of iron in the estimation process was successfully controlled by isolating Fe and PO_4 ions. The reformed protonation method to control the mutual interference of iron and phosphate, in turn, provides a simple technique that eliminates all the steps of complex formation moreover enhancing the accuracy of estimation. The accuracy of the method is dependent on the volume of the acid used for the extraction of the two ions separately for spectrophotometric estimation. There was a strong positive correlation of r 0.997 at p 0.01proving the efficiency of the method. The pre-treatment estimation method (B) justifies that concentration of the acid used to break the strong bi-dentate Fe-P bond is of utmost importance. This paper presents an optimistic study of Fe^{2+} ions at various pH verifying 90.39% estimation at pH 1.5. Besides this, it confirms that estimation of Fe^{2+} ions in neutral or alkaline conditions yielded inaccurate results. The scope of estimation range provided by the pre-concentration method (B) of iron and phosphate estimation method is extensive.

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