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Spectrophotometric Determination of Calcium with 1-(2-Pyridylazo)-2-Napthol Sensitized By Tween 80 Application to Various Samples

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Spectrophotometric Determination of Calcium with 1-(2-Pyridylazo)-2-Napthol Sensitized By Tween 80 Application to **Various Samples**

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Abstract: Spectrophotometric method for determination of Ca in aqueous solution using 1-(2pyridylazo)-2-naphthol (PAN) as colour reagent has been developed. The method was based on the formation of Ca-PAN complex at pH 12 and measuring absorbance at 550nm, with molar absorptivity of 1.70x10⁴ L.mol⁻¹cm⁻¹, average recovery is 101.72 and precision (RSD) is better than $\pm 2.8\%$ indicating that the method is relatively sensitive, accurate and precise. The binary system of Ca(PAN)2 and conditional stability constant of 1.467x10¹⁰ L².mol⁻² adherence of Beer's law over concentration range 0.08-2ppm. The method was applied for the determination of Ca in natural water, milk, yogurt, human serum, grip and tomato juice satisfactory. In presence of 1% tween 80 and composite masking agents of pH 11.5 the absorbance measurements were made at 540nm giving molar absorptivity of 2.7×10^4 L.mol⁻¹ cm⁻¹, average recovery of 101.2 and precision (RSD) better than ±3% indicating that the Ca(PAN)2 system is sensitive, accurate, and precise. In addition it is considered to be simple, rapid and do not need heating or solvent extraction steps, moreover it can be applied successfully for the assay of Ca in various samples over concentration range of 0.0008-0.8ppm.

1. Introduction

1-(2-Pyridylazo)-2-naphthol (PAN) reacts with wide variety of metal ions to form colored waterinsoluble chelates. In the present work PAN (L) at PH~12 which has a red color react with Ca but not with Mg to from colored alcoholic water soluble chelates which absorb at 550nm.PAM behave as tetra dentate ligand to from MLX-type mixed ligand chelate (X: unidentate ligand) with metal ions of Coordination number 4, and ML₂-type normal chelate with coordination number 6 such as calcium as it is reported in this work. PAN is a well-defined stable organic molecule [1].



The structure of 1-(2-pyridylazo)-2-naphthol(PAN)

The Proton dissociation scheme can be written as below:



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The pka₁ values in 20% dioxin are reported as $pka_1=1.90$ and pk2 = 12.2 [2].

Generally it is a non-selective reagent, the selectivity has to be improved by the selection of extraction and masking agents. It also used as a metal indicator in chelatometry with several metal ions [2]. Other uses of PAN are, as a detecting reagents for metal ions in solution or on ion-exchange resin beads [3]. After the early mentioned review, we have not been slow to realize the importance of the sensitive chromogenic reagent (PAN), and its highly attractive features, particularly those concerning the spectrophotometric analysis. Therefore, we have explored the valuable analytical potentialities of the red complex for the assay of calcium. Up to our knowledge the system Ca-PAN has not been studied before. On the basis of the above ideas, the new developed Ca-PAN system was fully studied in an attempt to allow applications to various samples.

The most widely used methods for the Spectrophotometric determination of elements based on using (PAN) as chelating agent are of the binary chelates type. However, the system Pb-PAN-CTAB, is managed to assay traces of lead in aqueous solution, which needs no extraction step [4]. These observations and because a significant increase in the sensitivity, stability or /and selectivity are obtained by introduction a third component into the system, we have paid very serious attention to the idea of utilising the formation of ternary complex. For this purpose the effect of the presence of surfactants on the colored system, the complex Ca-PAN is examined. CTAB (cationic surfactant), SDS (anionic surfactants), gelatin and tween 80 (non-ionic surfactants) are tested for the purpose. The best result were obtained with tween 80 relative to all other types examined.

2. Experimental

2.1. Reagents

Chemicals used are the analytical grade, analar or the highest purity available. All glass wares are rinsed with a solution of the tetrasodium salt of EDTA (0.001M) and then with double distilled water thoroughly, just before use [5].

2.1.1. Stock calcium (100ppm) solution: This solutions is prepared by dissolving 0.2775gm of calcium chloride anhydrous (*AnalarBDH*) in double distilled water, 5ml of 1:1 HCL was added to prevent plating out. The solution is completed to 1000ml volumetric flask. This solution is standardized. The working calcium (9.98ppm) solution is (2.495x10⁴M) is prepared by diluting 10ml of the above solution is then standardized using standard titrimetric EDTA method [6].

2.1.2. *1-(2-pyridylazo)-2-naphthol reagent (4.99x10-4M):* The solution is prepared by dissolving 0.03lgm of the compound (Hopkinand Williams/Ltd) in absolute ethanol and the volume is made to250ml.with absolute ethanol in a volumetric flask. This solution is kept in a dark bottle.

2. 2. Instruments

Spectral and absorbance measurements are carried out using UNICAM, He λ OS β UV-Vis computerized single beam spectrophotometer. Connected with 6 Jet laser printer. The pH measurements were made using both PHILIPS PW 9421 pH meter and HANNA HI 9321 microprocessor based Bench pH/mv/C meter. Atomic absorption measurements were made using PYE UNICAM SP 8100 AAS. The balance Mettler H 31AR, max. of 160gm and d=0.1 mg used for weighing.

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3. Results and Discussion

3. 1. Study of the Optimum Reactions Condition

The effect of various parameters on the absorption intensity of the colored complex are studied and the reaction conditions have been optimized.

3. 2. Effect of pH

The effect of pH on color intensity is examined using and (NaOH-KCI) buffer is selected for the subsequent experiments because of good sensitivity. The shift of the optimum pH range of 11.7-12.1 towards lower values 11.42-11.78 in the presence of tween 80 surfactant suggests that the presence of the latter increases the ionization power of the colorimetric reagent [4,7].

3. 3. Effect of the reagent amount:

The effect of the PAN reagent concentration on the formation of the complex Ca(11)-PAN has been carried out at pH 12 and 5ml reagent shows maximum intensity without surfactant however in the presence of tween 80 nonionic surfactant the formation of the complex carried out at pH 11.6 and 6.0 ml of 4.99×10^{-1} M PAN solution gives maximum absorbance.

3. 4. The effect of surfactants (protective colloids):

Concentration effect of different types of surfactants, CTAB (cationic), SDS (anionic), gelatin and tween 80 (nonionic), on the intensity of the colored complex is tested. The results reveal that maximum absorbance can be achieved using 1 ml of 1% tween 80 solution which is used for the subsequence experiment.

3. 5. Effect of the masking agents:

From the experimental data obtained, and in order to enhance the selectivity of PAN reagent, a mixture of composite masking agents solution is prepared. This mixture is at pH 12 and has 0.01M concentration of NaF, 1,10-phenanthroline hydrate, NH₂OH.HCl, tartaric acid, NaCN, Ascorbic acid and 5-SSA. The effect of different amounts of this solution on the determination of $30\mu g$ of Ca is tested. The composite masking agents reduce the sensitivity but we expect to enhance the selectivity. 0.5Ml of the composite masking agents solution is recommended.

3. 6. Order of the addition of reagents:

The metal ion (M), the reagent (R), the buffer solution (B) the surfactant (S) and the composite masking agent solution (MC) are mixed in different modes, to test the effect of order of the addition of the reagents on the absorbance. The results show that there is no significant effect, so order I (M+R+S+B+MC) is selected for the subsequent experiments.

3. 7. kinetics of complex formation:

The effect of the time on the development and stability of the colored system is investigated under the optimum experimental conditions described above. The formation of the colored complex is completed after 5minute and remained stable up to at least 30 minutes for two component system and up to 90 minute for three components system.

Based on the improvement obtained above, it is concluded that using tween-80 enhanced the sensitivity and extended the stability of the complex from 30 min to about 90 min. therefore, it is decided to be incorporated in the recommended procedure.

4. Final Absorption Spectra:

Under the optimum condition the absorption spectra of the colored complex and that of the corresponding reagent blank are shown in the figure (1), The spectrum of the red colored complex showed maximum at 550nm. The reagent blank shows maximum absorption at 465nm. A clear difference in the colour (with $\Delta\lambda$ =85nm). Low absorption of the reagent blank was observed at 550nm. Therefore the absorbance measurements were made against reagent blank.

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Figure 1. Absorption spectra of 30µg Ca/25ml measured against blank, distilled water and blank against distilled water.

Under the above established condition, absorption spectra were made on a solution containing 30 μ Ca, 6 ml of (4.99x10⁻⁴M) PAN, I ml of 1% tween 80, 1.5 ml of pH 12 buffer and 0.5 ml of composite masking agent, and then diluted to 25 ml with distilled water. The absorption spectra for both the complex system and the reagent blank were recorded after 5 minute against distilled water. Figure (2) shows maximum absorption at 495 and 475nm respectively. The colored complex showed maximum absorption at 540nm against reagent blank. The wavelength was used in the subsequent work.



Figure 2. Absorption spectra of 30µg Ca/25m1 with the presence of 1% tween 80.

5. Recommended Procedure and Calibration Graph:

Two calibration graphs have been constructed;

a- Having thus established the optimum condition, Increasing volumes of working Calcium (II) solution are transferred to a series of 25ml volumetric flasks to cover the range of (0-100μg) of calcium, 5ml of (4.99x10⁻⁴M) PAN reagent solution and 1.5ml of buffer pH 12 are then

added, and the solutions are made to the mark with distilled water, the reaction mixture is mixed and the absorbance is measured at 550nm after 5 min.

Figure 3 shows that Beers law is obeyed over the range (0.08-2ppm). The molar absorptivity being (1.27×10^4) l. mol⁻¹.cm⁻¹ and sandell sensitivity index being 0.0032 µg.cm⁻².



Figure 3. Calibration graph for determination of calcium in aqueous solution using PAN reagent.

Figure 4. Calibration graph for determination of calcium in aqueous solution using PAN reagent in presence of 1% tween-80.

b- Another calibration graph is conducted by transferring increasing volume of the 10 ppm Ca solution into 25 ml volumetric flasks to cover the range 0.2-30 mg. Then 6 ml (4.99 x 10⁻⁴M) PAN, 1 ml of 1% tween 80, 1.5 ml of pH 12 buffer solution and 0.5 ml of composite masking agent solution are added, and the volume are made up to the mark with distilled water. The absorbance measured against the reagent blank at 540nm after 5minute.

Figure 4 shows a straight line calibration curve indicating that beer's law has been followed in the concentration range 0.2-20 mg Ca in 25 ml (0.008-0.8ppm), the molar absorptivity is 2.7×10^4 L.mol⁻¹ cm⁻¹ and sandell sensitivity index is 0.0015µg.cm⁻², in the presence of the surfactant tween 80, while in it's absence $\mathcal{E}=1.7 \times 10^4$ L.mol⁻¹ cm⁻¹, and S.I.=0.0024 µg. cm⁻².

6. Accuracy and Precision of the method

The accuracy and precision of the recommended procedure were investigated by determining the recovery and standard deviation of three different concentrations of calcium, (5 determinations for each concentration). The results described in the Table 1 indicate that the method is accurate and precise.

Calcium	$(\mu g/25ml)$	Recovery (%)	Error (%)	SD	RSD (%)
Added	Founded				
4	3.29	98.00	-2.0	0.0837	2.13
20	19.68	98.40	-1.6	0.1304	0.66
50	50.72	101.44	+1.44	0.3347	0.66

Table 1. Accuracy and precision of the method

7. Nature of the complex[8,9]

The Calcium (II) to PAN ratio was studied under the established working conditions by Jobs continuous variation, the mole ratio and the slope ratio methods respectively. The molar ratio of Ca(II) to PAN was found to be 1:2 from the three methods, and this is in agreement with ML_2 -type normal chelate of PAN with metal ions of coordination number 8[1-10]. The explanation of this is that Ca is coordinated to the nitrogen of the pyridyl, oxygen of the hydroxyl (naphthol) and azo group from the two PAN molecules. Thus, the suggested structure of the complex can be written as follows:

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The structure of Ca(II)-PAN complex

8. The Stability Constant of the complex.

The apparent stability constant for the complex, 1:2 Ca-PAN is subsequently estimated using the following formula,

$$K = \frac{1 - \alpha}{4c^2 \alpha^3} \tag{1}$$

Where *K*=stability constant

c=final concentration of metal ion.

 α =degree of dissociation

The results of estimation of the stability constant (*K*) using the above equation gave the mean value of $K=2.826 \times 10^9 \text{ L}^2$.mol⁻², indicates that the complex is stable.

The same structure for the complex is indicated with the presence of tween 80. The stability constant (mean of three values) is found to be $7.6 \times 10^4 \text{ L}^2$.mol⁻² indicating that the complex is of relatively lower stability.

9. Effects of Foreign Species

Synthetic solution containing a fixed amount of Ca ($30\mu g/25ml$), various amount of foreign species were prepared, and recommended procedure for the determination of Ca was followed. The relative error of ± 5 was considered tolerable.

10. Analytical Applications

The determination of Calcium which is carried out by the present method and the developed method in presence of 1% tween 80 and composite masking agents in aqueous solution has been applied to the analysis of calcium in various samples. In order to check the validity of the present methods, other methods such as EDTA titrimetric standard method [6] and atomic absorption spectrophotometric (AAS) method are also applied for assay of calcium in the samples.

10. 1. Determination of Calcium in water:

Calcium content may range from zero to several hundred ppm. Concentration of calcium in normal portable ground water generally range between 10 and 100ppm [6,11]. Calcium contributes to the hardness of water with the bicarbonate forming temporary or carbonate hardness, and with the sulfate, chloride and nitrates permanent or non-carbonate hardness. Three samples (0.1-1ml) from each of Duhok dam, well, Tigre river and Tap water from Duhok city are analyzed. The results are shown in Table 2.

Water Sample	Recovery %						
-	*µg Ca/ml	present method	EDTA std.	method	AAS method		
Duhok dam(BD)	87.36		100.64	103.02			
Well(W8)	106.42		99.64		94.90		
Tigre(AT)	3	6.83	105.6	2	97.74		
Tap Water	50.77		101.00		97.10		

Table 2. Determination of calcium in natural waters

^(*) mean of three determinations

10. 2. Determination of Calcium in blood serum

The total body calcium depends on that absorbed from the dietary intake and that lost from the body. The mean blood serum calcium concentration in healthy subjects is about (8.5-10.3)mg/dl[12]. Calcium Present in plasma in two mean forms that bound to proteins, mainly albumin. Quantitative measurements for ionized Calcium in blood serum help diagnoses disease in human patients [13]. The results are shown in Table 3.

Table 3. Determination of Calcium in blood serum

/lethod	present	EDTA	AAS	Healthy range
Calcium content	11.4	12.03	11.6	8.5-10.3
(µg/ml)				

^(*)Mean of three Determination

10. 3. Determination of calcium in milk and yogurt :

It is well established that Ca and Mg ion in milk and its products play an important role as nutrients in the human diet. This explains fact that methods to determine these ions continue to appear in the literature. Although a great variety of instrumental methods are available for the determination of Ca in milk and its product mainly based on the use of techniques such as, molecular absorption spectrometry and AAS [14]. The results are shown in Table 3 indicating reasonable comparisons.

Milk sample		Recover	y%	
	*µg Ca/ml	EDTA std.	AAS	µg Ca/ml
	Present	method	method	Certified
Guicose-2(baby)	6615	99.76	99.47	6500
(N&N) China powder	7350	100.89	102.72	
ICIM (Ulker) liquid	1040	100.29	100.96	1120
Natural Baffalo milk	1670	102.99	100.60	
Natural yogurt	1247	95.35	97.59	

Table 4. Determination of calcium in milk.

(*)Mean of three determinations.

10. 4. Determination of calcium in grape and tomato juice:

Calcium is one of the abundant elements in soil and also is among the most commonly analyzed, because Ca is essential element for plant growth [15]. Calcium is essential for membrane permeability, solute transport and maintenance of cell integrity. Normal concentration ranges for Ca in plant tissue (dry basis) are 0.1-10% [16], while the fruits and vegetables juice (99%water) are of much less calcium concentrations. The results are shown in Table 5 indicating reasonable comparisons.

	Recovery%			
Sample	*µg Ca/ml present	EDTA	AAS	
	method	std. method	method	
Grape	116	97.85	97.41	
Tomato	43	95.56	97.67	

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						01			5	

(*)Mean of three determinations.

10. 5. The standard addition method

Standard addition method is particularly useful for complex samples in which likelihood of matrix effects are substantial and also used when the amount of the sample is limited. Standard additions can be carried out by successive introductions of increments of the standard to a single measured volume of the unknown. Measurements are made on the standard solution and on the sample plus the standard after each addition [17], the method is applied for determination of Ca in sodium chloride.

10. 5. 1. Procedure: Following the recommended procedure a calibration graph was constructed using 1, 2, 5, 7, $10\mu g$ of Ca from standard solution of calcium. A 1 ml of NaCl solution was taken in five volumetric flasks of 25 ml, followed by adding 1,2,5,7 $10\mu g$ Ca, then continued as in the recommended procedure. Absorbance measured at 550nm. The Ca content determined from the graph as shown in Figure 5.



Figure 5. An example of the standard addition for determination of Ca in sodium chloride.

10. 6. determination of Calcium in various samples in the presence of tween 80

The developed method for determination of Ca in presence of % tween 80, and composite masking agents has been applied to various samples satisfactorily. The results obtained by the present method and those obtained by AAS and titrimetric method arc given in the Table 5 indicating reasonable accuracy and comparison.

Sample	Present method	EDTA method	AAS method
	ppm	Recovery%	Recovery%
Water (Tigris)	104	101.96	97.12
Grape juice	102	102.45	101.47
Serum	85	103.53	105.88
Milk (Guigoz)	6430	101.40	102.64
Milk (Ulker)	940	98.40	101.06
Yogurt	1026	96.49	97.47

Table 6. Determination of calcium in various samples in presence of tween 80.

10. 6. 1. Validity of the method [18]: In order to test the results for the existence of a systematic error, the "t"-test was used [19,20]. The results of the present spectrophotometric methods compared with AAS method for five samples. Five standard solution of calcium were analyzed by the two methods and the recoveries were measured. As shown in Table 7 for the concentration of Ca between (0.2-1.6ppm), the t_{exp} was (1.677). This value is smaller than the value of table "t"-test (t=2.78)at 95% confidence limit for 5 degrees of freedom. This indicates that the difference between the two methods is not significant, which also means that the proposed method is as good as AAS method.

ug Ca(taken)	Present N	lethod	Reference Method Differen		
	found	R%	found	R%	
5	5.12	104.4	4.92	98.4	0.2
10	9.88	98.8	9.51	95.10	0.37
20	20.31	101.6	20.15	100.80	0.16
30	30.28	10.9	30.54	101.8	-0.26
40	39.65	99.1	40.48	101.2	-0.82
Mean	0.36				
sd	0.490				
t_{exp}	1.643				

Table 7. Comparison of the accuracy obtained by the present method and the reference AAS method.

The t_{exp} was calculated using the following equation:

$$t_{exp} = \frac{\bar{d}}{sd}\sqrt{n} \tag{2}$$

 \overline{d} is the mean differences, *sd* is the standard deviation of the differences, *n* is the number of measurements. It is concluded from Table 7 that the accuracy of the present method compared favorably with the standard AAS reference method.

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11. Conclusion

A spectrophotometric method for the determination of calcium in aqueous solution was developed. This method is based on the formation of Ca-PAN complex in aqueous solution of pH 12 and measuring absorbance at 550nm. Microgram amount up to 2ppm can be determined. The molar absorptivity 1.704×10^4 L.mol⁻¹.cm⁻¹, accuracy (average recovery) is 101.72 and precision (RCD)in better than ± 2.8 %indicating that the method is relatively sensitive, accurate and precise. The binary system of Ca(PAN)2 and conditional stability constant of 2.826×10^9 L².mol⁻², adheres beers law over the concentration range of 0.08-2ppm.in the presence of mixed complexing solution, the method has been applied to the determination of calcium and to enhance the selectivity. In the present of 1% tween 80 as nonionic surfactant and composite masking agents, the absorbance measured at 540nm. Microgram amounts up to 0.8ppm can be determined.

The present methods are considered to be simple because it does not need heating and solvent extraction steps. Moreover, the proposed methods can be successfully applied for determination calcium in various samples. The results obtained compared favorably with AAS and titrimetric EDTA standard method However, the present spectrophotometric method compared statistically with standard EDTA and AAS method using "t-test". The calculated "t"-test is found to be less than the theoretical "t"-test indicating that the present methods has good validity.

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