American Journal of Analytical Chemistry, 2011, 2, 776-782
doi:10.4236/ajac.2011.27089 Published Online November 2011 (http://www.SciRP.org/journal/ajac)

Hydralazine Hydrochloride: An Alternative Complexometric Reagent for Total Iron Spectrophotometric Determination

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Received July 14, 2011; *revised August* 22, 2011; *accepted September* 3, 2011

Abstract

An alternative spectrophotometric method has been developed for total iron determination using flow injection analysis (FIA). The procedure is based on the coordination reaction between hydralazine and Fe^{2+} ions, which results in the formation of a purple complex monitored at 538 nm. For determination of total iron, Fe^{3+} ions were reduced using ascorbic acid. Under optimized conditions, a linear calibration graph (0.1 - 6.0 $\mu g \cdot ml^{-1}$; $n = 6$) was obtained. The method allows LOD (3σ of blank/slope = 0.06 $\mu g \cdot ml^{-1}$) and LOQ (10σ of blank/slope = 0.22 $\mu g \cdot ml^{-1}$). The RSD ($(s/\bar{x}) \times 100$) for a mixed standard containing 0.60 $\mu g \cdot ml^{-1}$ Fe^{2+} and Fe^{3+} was 0.10% ($n = 10$). Recoveries of spiked samples were 94.3% - 106.0%. The analytical frequency was 60 h^{-1}. The effect of possible interferences has been studied. The procedure was successfully applied for analysis of environmental samples. The real samples results were comparable with those obtained by the official method considering a paired *t*-test and 95% of confidence level.

Keywords: Hydralazine, Total Iron Determination, Spectrophotometry, Flow Injection Analysis

1. Introduction

The iron element (Fe) is the fourth most abundant chemical specie of the planet and is present in nature in the following oxidation states: Fe^{2+} and Fe^{3+} [1].

Nowadays the determination of iron in some real samples cannot be considered an analytical challenge but the introduction of alternative complexing agents for this purpose it is very attractive. Some analytical procedures already employed commercial drugs to determine iron, for example: the antibiotics chlortetracycline and norfloxacin were used by Ruengsitagoon and Pojanagaroon respectively to quantify Fe^{3+} in real samples [1,2]. Using the same strategy, this work comes demonstrate the use of hydralazine hydrochloride for total iron determination in real samples. A list of some complexometric organic reagents not usual used for iron determination employing spectrophotometry is shown in **Table 1**.

Hydralazine hydrochloride (**Figure 1**) is an antihypertensive drug that acts as a potent arteriolar dilator and

Table 1. Some complexometric organic reagents not usual used for iron determination employing spectrophotometry.

Organic Reagent	Type of Iron	λ_{MAX} (nm)	Ref.
DPQH[a]	Fe^{2+}	504	[3]
1,10-Phenantroline	Total[b]	510	[4]
Thioglycolic acid	Fe^{3+}	535	[5]
DPPH[a]	Fe^{2+} and Fe^{3+}[c]	535	[6]
5-Br-PSAA[a]	Total[b]	558	[7]
Nitro-PAPS[a]	Total[b]	582	[8]
Tiron[a]	Total[d] and Fe^{3+}	635	[9]
DPKBH[a]	Total[b]	686	[10]
DPFTH[a]	Total[b]	738	[11]
TLCR[a]	Fe^{2+}	741	[12]

[a]See list of acronyms in section 10 of this paper; [b]Total iron expressed as Fe^{2+} after a reduction of Fe^{3+}; [c]Conversion of Fe^{3+} to Fe^{2+} with a reduction agent; [d]Total iron expressed as Fe^{3+} after an oxidation of Fe^{2+}.

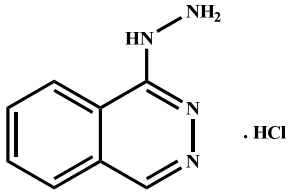

Figure 1. Chemical structure of hydralazine hydrochloride (1-hydrazinophthalazine monohydrochloride).

also is used in the treatment of congestive heart failure [13]. It is a white powder, soluble in water with a pKa value of 7.3 [14]. This drug demonstrates redox properties (acting as a reducing agent) [14], antioxidant activity [15] and coordination capacity toward some metal cations [16]. Up to now and at the best of our knowledge, no report of spectrophotometric procedure using hydralazine as the chromogenic reagent for determination of metal ions has been available in literature.

Before the facts stated, this paper describes the development of a simple, rapid and sensitive flow injection method for total iron determination using hydralazine as an alternative chromogenic reagent. The proposed procedure is based on the spectrophotometric detection of the purple complex formed by the coordination reaction between hydralazine and Fe^{2+} ions in neutral media. The resulting complex is monitored at 538 nm. The developed method was successfully applied for determination of total iron, after the reduction of Fe^{3+} into Fe^{2+} ions using ascorbic acid, in samples of drinking, tap and lagoon waters, besides lagoon sediments.

2. Equipments

An UV/Vis spectrophotometer (Femto®, model 700 Plus, Brazil) equipped with a 20 mm "U" glass flow cell was used as a detector in all FIA experiments, and the absorbance signal was obtained directly from the instrument. A multichannel peristaltic pump (Watson Marlow®, model 400 Sci-Q, United States), a manual injector (made of acrylic, with two fixed sidebars and a sliding central bar), pump tubes (Tygon®, model R-3603, 1.02 mm i.d.) and polyethylene tubes (1.0 mm i.d.) were also used in the proposed flow injection system.

3. Reagents and Solutions

All chemicals were of analytical reagent grade and were used without further purifications. Hydralazine hydrochloride was acquired from Sigma (St. Louis, USA). Deionized water from a Milli-Q system (resistivity 18 MΩcm) was used for preparation of reagents and buffers. A stock solution containing 5.0 mmol·l^{-1} HCl, prepared from concentrate hydrochloric acid (Vetec, Rio de Janeiro) was used for preparation of all standard solutions, and for dissolution and dilution of all real samples.

A stock solution containing 0.01 mol·l^{-1} hydralazine hydrochloride was prepared by dissolving 0.1000 g of $C_8H_8N_4$.HCl in 50 ml of deionized water.

A stock solution containing 0.15 mol·l^{-1} buffer NaH_2PO_4/Na_2HPO_4 (pH 7.0) was prepared by dissolving 5.2000 g of $NaH_2PO_4.H_2O$ (Vetec, Rio de Janeiro) and 6.7000 g of $Na_2HPO_4.2H_2O$ (Vetec, Rio de Janeiro) in 250 ml of deionized water. After that, the buffer working solution was prepared by dilution of 50 ml of the stock solution in 100 ml of deionized water with pH previously adjusted to 7.0 with 1.0 mol·l^{-1} HCl solution using a pHmeter.

Finally, the reagent solution (chromogenic reagent) was prepared by mixing 10 ml of the hydralazine stock solution and 20 ml of the buffer working solution into 100 ml of deionized water. So, the final concentration of the hydralazine at this solution was 1.0 mmol·l^{-1}.

A stock solution containing ascorbic acid 1.0% w·v^{-1} was prepared by dissolving 0.5000 g of this reagent (Sigma, St Louis) in 50 ml of deionized water. So, the ascorbic acid 0.1% w·v^{-1} working solution was obtained by appropriate dilution of the stock solution in deionized water. The ascorbic acid stock solution was prepared every week and was kept in dark bottles and under refrigeration.

A stock solution containing 100 μg·ml^{-1} Fe^{2+} was prepared by dissolving 0.4980 g of $FeSO_4.7H_2O$ (Vetec, Rio de Janeiro) in 1000 ml of HCl 5.0 mmol·l^{-1}. In the same way, a stock solution containing 100 μg·ml^{-1} Fe^{3+} was also prepared by dissolving 0.2904 g of $FeCl_3$ (Vetec, Rio de Janeiro) in 1000 ml of HCl 5.0 mmol·l^{-1}. The standard working solutions were prepared by appropriate dilution of these stock solutions in HCl 5.0 mmol·l^{-1}. All the stock solutions were prepared every week and the working solutions every day.

4. Flow Injection Manifold and Procedure

The flow injection manifold used for total iron determination can be seen in **Figure 2**. At this flow system, the

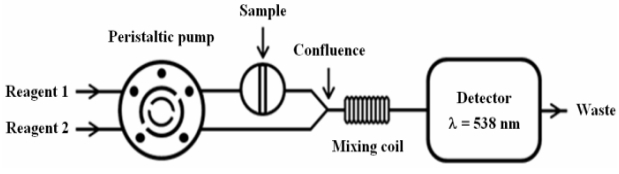

Figure 2. Flow injection manifold for total iron determination. Experimental conditions: reagent 1 (hydralazine 1.0 mmol·l^{-1} in buffer NaH_2PO_4/Na_2HPO_4 pH 7.0), reagent 2 (ascorbic acid 0.1% w·v^{-1}), peristaltic pump (0.9 ml·min^{-1}), sample volume (200 μl) and mixing coil (30 cm).

chromogenic reagent acts as the proper carrier solution. The peristaltic pump drives the solutions forward, at the same time, the reagent 1 (hydralazine 1.0 mmol·l^{-1} in buffer NaH_2PO_4/Na_2HPO_4 pH 7.0) into the injection valve and the reagent 2 (ascorbic acid 0.1% w·v^{-1}) into the confluence. After this, an aliquot of the standard or the real sample is injected into the carrier stream and meets the reagent 2 in the confluence before the mixing coil. From this point, the resulting stream follows to detector and the analytical signal generated by the Fe^{2+}-hydralazine complex is finally observed. After attaining the signal maximum, the central bar of the injection valve is moved back to the sampling position to start another measurement cycle.

5. Samples Preparation

Drinking water was acquired in local markets. Tap water was collected in some points of the Federal University of Alagoas (Maceió, Alagoas, Brazil). Samples of water and sediments were from Mundaú Lagoon (Maceió, Alagoas, Brazil) and were collected in different points of this estuary. Before the analysis of the water samples, all of them were previously acidified and preserved during 24 h with HCl 1.0 mol·l^{-1}. In this procedure, the water sample was initially filtered through a 45 μm glass fiber membrane filter. In a 100 ml volumetric flask the water sample was acidified with 500 μl of HCl 1.0 mol·l^{-1}, and the final volume was completed with the real sample. Some of these samples were adequately diluted before the determination of total iron.

Approximately 1.20 g of dry sediment of the Mundaú lagoon was treated with HCl 0.1 mol·l^{-1}. The resulting acid solution was shaken during 2 hours at 200 rpm, and filtered through a 45 μm glass fiber membrane filter into a 50 ml volumetric flask, which final volume was completed with deionized water.

6. Results and Discussion

6.1. Absorption Spectra and Metal:Ligand Ratio

The absorption spectrum of the purple complex obtained by coordination reaction between Fe^{2+} ions and hydralazine was measured over the range of 400 - 600 nm using a spectrophotometer. The absorption maximum of the complex was 538 nm. In order to achieve the greatest sensitivity, measurements were made at this wavelength.

The metal-to-ligand ratio (Fe^{2+}:hydralazine) was determined by the Job's method (continuous variation method) and was found to be 1:2 at pH 7.0, as shown in **Figure 3**. The stoichiometry was verify remained the plus of concentrations of the metal and ligand constant, however each plus differ one each other ($C_{Fe} + C_{hid}$ = 0.4 mmol·l^{-1}; $C_{Fe} + C_{hid}$ = 0.3 mmol·l^{-1}; $C_{Fe} + C_{hid}$ = 0.2 mmol·l^{-1}; $C_{Fe} + C_{hid}$ = 0.1 mmol·l^{-1}).

6.2. Optimization of the Flow Injection System

The physical parameters optimization of the flow injection system was conducted employing standard solutions of Fe^{2+} (1.0 μg·ml^{-1}), since the proposed method determines total iron by reduction of Fe^{3+} to Fe^{2+}. In these studies, the effect of sample volume and mixing coil length were evaluated to reach the best conditions of analysis. Different sample loop lengths were tested: 10, 15, 20, 25, 30 and 40 cm with volumes of 100, 150, 200, 250, 300 and 400 μL, respectively. Various mixing coil tubing lengths: 20, 30, 40, 50 and 70 cm was also studied. The parameters were compared in terms of peak height and precision. Among the evaluated parameters the most suitable injection loop volume was 200 μl, and because of the higher signal the mixing coil of 30 cm was also chosen for next studies.

The following chemical parameters of the flow injection system were also studied: hydrochloric acid concentration, hydralazine concentration, ascorbic acid concentration, effect of the Fe^{3+} concentration in the reduction step and pH of the reaction media. In all of these cases, the chosen of the concentration and the pH was made considering the studied value that produced the maximum absorbance signal (in order to obtain greatest sensitivity) and the lowest Schlieren effect. The **Table 2** summarizes the optimum conditions of all studied parameters in the proposed flow injection method for total iron determination.

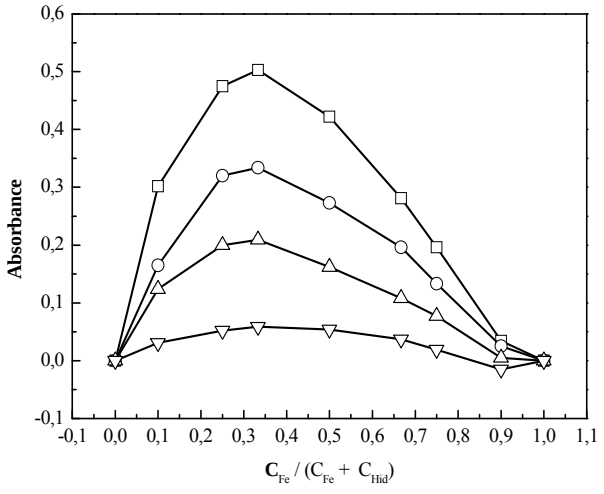

Figure 3. Job's method for the study of complexation of hydralazine and Fe^{2+} ions at pH 7.0. ($C_{Fe} + C_{hid} = k$) with k = 0.4 mmol·l^{-1} (□); k = 0.3 mmol·l^{-1} (○); k = 0.2 mmol·l^{-1} (△); k = 0.1 mmol·l^{-1} (▽).

Table 2. Physical and chemical parameters for flow injection determination of total iron.

Parameters[a]	Studied Range	Optimum Value
Physical:		
Wavelength (nm)	400 - 600	538
Mixing coil length (cm)	20 - 70	30
Sample injection volume (μl)	100 - 400	200
Chemical:		
Hydralazine concentration ($mmol \cdot l^{-1}$)	0.5 - 8.0	1.0
Ascorbic acid concentration (% $w \cdot v^{-1}$)	0.01 - 0.2	0.1
HCl concentration ($mmol \cdot l^{-1}$)	1.0 - 100	5.0
pH (NaH_2PO_4/Na_2HPO_4 buffer)	6.2 - 8.2	7.0

[a]Studied only at room temperature (25˚C).

7. Method Validation

The calibration curve was obtained employing mixed standards containing the same amounts of Fe^{2+} and Fe^{3+}. Under the optimum conditions, the graph was found to be linear over the range of 0.1 - 6.0 $\mu g \cdot ml^{-1}$. This short range is due to the previously discussed reduction step. To determine total iron in real samples, six mixed standards were employed, containing 0.05, 0.1, 0.2, 0.4, 0.7 and 1.0 $\mu g \cdot ml^{-1}$ of Fe^{2+} and Fe^{3+}, so leading to concentrations of 0.1, 0.2, 0.4, 0.8, 1.4 and 2.0 $\mu g \cdot ml^{-1}$ of total iron. The regression plot obtained for total iron determination fitted the equation: $A = 0.0325\ C - 0.0013$ ($r = 0.9990$, $n = 6$), where A is the signal (in absorbance) and C the concentration of total iron ($\mu g \cdot ml^{-1}$). The limit of detection (LOD) obtained by the proposed method defined as $3\sigma/0.0325$ and the limit of quantification (LOQ) defined as $10\sigma/0.0325$ was 0.06 and 0.22 $\mu g \cdot ml^{-1}$ respectively, where σ is the standard deviation of the blank signal ($n = 10$) and 0.0325 is the slope of the calibration curve [17].

The relative standard deviation (peak height in absorbance; $(s/\bar{x}) \times 100$) calculated from 10 replicate injections of a mixed standard containing 0.60 $\mu g \cdot ml^{-1}$ Fe^{2+} and Fe^{3+} was 0.10%. Considering the optimum conditions of the proposed flow injection system, a maximum sample throughput of 60 h^{-1} was obtained without any carryover effect.

7.1. Interference Studies

The effects of potential interfering ions were examined by using solutions containing 1.0 $\mu g \cdot ml^{-1}$ Fe^{2+} and the ionic species evaluated at different concentrations. The ions selected for this study were those usually found in water samples besides some metal cations. The tolerable concentration of each different ion was taken as a highest concentration causing a relative error of ±5.0%. The results were summarized in **Table 3**. Most of the ions examined did not interfere with the determination of iron. Copper was found to seriously interfere in the determination of iron when its concentration reaches 0.5 $\mu g\ ml^{-1}$. The positive interference observed when Cu^{2+} is present in samples is probably due to the formation of a complex between this metal cation and hydralazine that also absorbs electromagnetic radiation next to 538 nm.

To eliminate the Cu^{2+} interference in analyses was used a liquid-liquid extraction method developed by Faquim and Munita [18]. Adapting this procedure for the purposes of this work, an organic solution of dithizone (diphenylthiocarbazone) was used to extract selectively Cu^{2+} ions from aqueous standard solutions containing Fe^{2+}, Fe^{3+} and Cu^{2+} ions. The procedure consisted in treat, previously of analyses, 50.0 ml of the aqueous standard solutions with 5.0 ml of an extraction solution containing 1.0 $mmol^{-1}$ of dithizone in chloroform. The standard solutions were prepared always containing 1.0 $\mu g \cdot ml^{-1}$ Fe^{2+} e Fe^{3+} and different concentrations of Cu^{2+} (1.0, 2.0, 3.0 e 4.0 $\mu g \cdot ml^{-1}$). In treatment, the aliquots of the extraction solution were added to the standards and then,

Table 3. Effect of chosen ions on the peak height of 1.0 μg ml^{-1} Fe^{2+} standard solution ($n = 3$).

Ionic Species (concentration in $\mu g \cdot ml^{-1}$)	Relative Percentage of Peak Height (%)
None	100.0
Cations:	
Na^+ (150)	94.2
K^+ (150)	102.8
Mg^{2+} (300)	98.5
Ca^{2+} (25)	102.0
Cu^{2+} (0.5)	105.4
Cd^{2+} (5)	103.5
Pb^{2+} (10)	102.7
Anions:	
Cl^- (50)	98.9
NO_2^- (10)	102.8
NO_3^- (50)	101.5
CO_3^{2-} (10)	101.0
SO_4^{2-} (5)	102.4
PO_4^{3-} (25)	98.3

the resulting mixture remained under vigorous agitation during 5 minutes. Finalized the time, waited the organic phase decant and then, the aqueous phase was collected with a syringe to be analyzed in the proposed flow system. Comparing the spectrophotometric signals in analyses of the standards before and after the treatment with dithizone was verified that the procedure was efficient to eliminate or reduce the interference of Cu^{2+} ions present until the concentration of 3.0 $\mu g \cdot ml^{-1}$, a fact verified by checking the relative percentage of peak height of the standards containing 1.0 (100%), 2.0 (101.2%), 3.0 (104.8%) and 4.0 (108.6%) $\mu g \cdot ml^{-1}$ of Cu^{2+} ions.

7.2. Analysis of Total Iron in Real Samples

The proposed flow injection method was applied to tap and drinking water and also natural water besides sediments from Mundaú lagoon. When the obtained results, showed in **Table 4**, were compared with those obtained by using 1,10-phenanthroline spectrophotometric method [19], it was seen that the proposed procedure provide good results, and no statistical difference was found considering the paired *t*-test at the 95% confidence level [17].

The good agreement between the results of the concentration of total iron measured as Fe^{2+} using the standard and proposed methods is showed in **Figure 4**. The value obtained from linear regression showed that the intercept including 0 (0.002 ± 0.02) and the slope including 1 (0.98 ± 0.02) [17]. The proposed method using hydralazine as chromogenic reagent was efficient for the determination of tap and lagoon water.

Table 4. Determination of total iron by the proposed flow injection method and standard method.

Samples[a]	Total Iron Found ($\mu g \cdot ml^{-1}$ or $g \cdot kg^{-1}$)	
	Proposed FI Method	Standard Method[b]
Tap water 1($w \cdot v^{-1}$)	1.09 ± 0	1.07 ± 0.02
Tap water 2 ($w \cdot v^{-1}$)	0.75 ± 0	0.73 ± 0
Tap water 3 ($w \cdot v^{-1}$)	1.27 ± 0	1.28 ± 0
Tap water 4 ($w \cdot v^{-1}$)	1.67 ± 0	1.62 ± 0
Lagoon water 1 ($w \cdot v^{-1}$)	1.26 ± 0.02	1.22 ± 0.02
Lagoon water 2 ($w \cdot v^{-1}$)	0.83 ± 0	0.82 ± 0.01
Lagoon water 3 ($w \cdot v^{-1}$)	0.70 ± 0.02	0.68 ± 0.01
Lagoon water 4 ($w \cdot v^{-1}$)	0.98 ± 0.04	0.95 ± 0
Lagoon sediment 1 ($w \cdot w^{-1}$)	42.5 ± 0.83	44.0 ± 0.52
Lagoon sediment 2 ($w \cdot w^{-1}$)	70.8 ± 1.02	68.5 ± 0.95
Lagoon sediment 3 ($w \cdot w^{-1}$)	65.8 ± 0.67	66.0 ± 0.80
Lagoon sediment 4 ($w \cdot w^{-1}$)	48.9 ± 1.61	47.0 ± 0.72

7.3. Recovery Studies

In order to estimate the accuracy of the procedure, different amounts of Fe^{2+} and Fe^{3+} were spiked in samples of drinking and tap water. Samples of water and sediments from Mundaú Lagoon (Maceió, Alagoas, Brazil) were also investigated. The results are given in **Table 5**. A good agreement was obtained between the added and measured analyte amounts. The average recoveries of total iron for added standards were superior to 99%, thus confirming the accuracy of the proposed procedure. Recoveries above and below 105% and 95%, respectively, may be due to interference from other elements in the samples, since complex matrices were analyzed.

8. Conclusions

A spectrophotometric method using FIA for total iron determination employing hydralazine was developed.

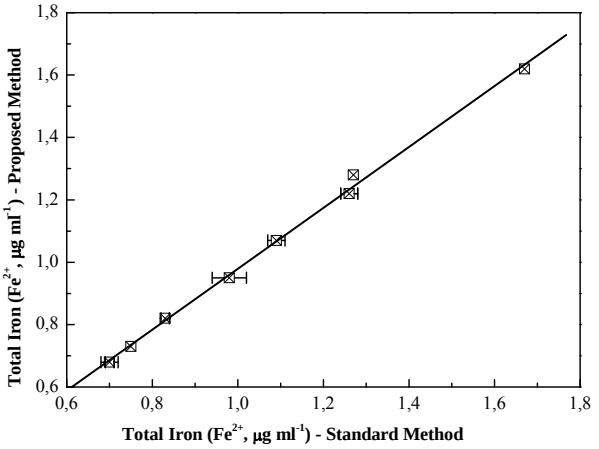

Figure 4. Correlation between the proposed and standard methods for total iron determination in water samples.

Table 5. Recoveries of total iron from drinking, tap and lagoon water and extracts of lagoon sediments.

Samples	Added ($\mu g \cdot ml^{-1}$) Fe^{2+}	Fe^{3+}	Found[a] ($\mu g \cdot ml^{-1}$)	Recovery (%)
Drinking water 1	1.00	0.80	1.88	104.4
Drinking water 2	0.80	1.00	1.83	101.7
Drinking water 3	0.80	0.80	1.65	103.1
Tap water 1	0.60	0.80	1.32	94.3
Tap water 2	0.50	0.50	1.06	106.0
Lagoon water 1	0.60	0.40	1.05	105.0
Lagoon water 2	0.20	0.40	0.58	96.7
Extract of lagoon sediment 1	0.50	0.50	1.06	106.0

The procedure showed to be accurate, precise and sensitive for the analyte determination in some environmental samples. Disadvantages of the proposed method were the difficult to make the speciation between Fe^{2+} and Fe^{3+} and the significant interference of Cu^{2+} ions when it is present up to 0.5 $\mu g \cdot ml^{-1}$ in samples. Despite these disadvantages, the use of hydralazine as an alternative chromogenic reagent, a commercial drug of low cost and easily acquisition, still ensures the applicability of the procedure in chemical analysis of total iron.

9. Acknowledgements

Financial support from CNPq, CNPq/CTHIDRO, CAPES and FAPEAL (Brazil) is gratefully acknowledged. A DTI/CNPq fellowship for C. X. Galhardo is acknowledged.

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Acronyms

The **Table 6** shows a list of acronyms and its respective meanings used in this paper.

Table 6. List of acronyms used in this paper.

Acronyms	Meanings
DPQH	2,2'-Dipyridyl-2-quinolylhydrazone
DPPH	2,2'-Dipyridyl-2-pyridylhydrazone
5-Br-PSAA	2-(5-Bromo-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sul-fopropyl)-amino]aniline
Nitro-PAPS	2-(5-Nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sul-fopropyl)-amino]-phenol disodium salt dihydrate
Tiron	4,5-Dihydroxy-1,3-benzenedisulfonic acid disodium salt
DPKBH	Di-2-pyridyl ketone benzoylhydrazone
DPFTH	2,2'-Dipyridyl-2-furancarbothiohydrazone
TLCR	4-(2-Thiazolylazo)-6-chlororesorcinol

