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Research Article

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A sensitive and extractive spectrophotometric method for determination of trace amount of nitrite in environmental and biological samples

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ABSTRACT

A rapid, simple, selective and sensitive method for the spectrophotometric determination of nitrite in water, soil and biological samples has been developed and optimum reaction conditions along with other analytical parameters have been evaluated. Nitrite reacts with N-phenyl-benzo-hydroxamic acid N-PBHA(I), N-p-chloro-phenyl-benzo-hydroxamic acid N-PClPBHA(II) and N-p-chloro-phenyl-cinnamo-hydroxamic acid N-PClPCHA(III) in acidic medium to form pink color complex. The intensity of colour increases with substitution. At analytical wavelength the maximum absorption of the complexes were 522 nm, 517 nm and 506 nm, and beer's law is obeyed over the concentration range of 0.0002664-0.002930 ppm, 0.0002664 – 0.003729 ppm and 0.002664 - 0.01864 ppm, for N-PBHA, N-PClPBHA and N-PClPCHA, respectively. The molar absorptivities were found to be $3.55x10^5$, $2.65x10^5$ and $5.62x10^5$ L mol^{-1} cm^{-1} and the relative standard deviation 1.19%, 1.84% and 1.28%, respectively with detection limit of 0.01998, 0.01998 and 0.1332 µg/25 ml of nitrite for N-PBHA, N-PClPBHA and N-PClPCHA, respectively. The optimum reaction conditions such as pH, time and solvent, etc for complete color reaction have been evaluated with all three hydroxamic acid complexes. The method has been successfully applied for determination of nitrite in water, soil and biological samples.

Key words: Spectrophotometric determination, N-PBHA, N-PClPBHA and N-PClPCHA.

INTRODUCTION

The forms of nitrogen like nitrate, nitrite, ammonia and organic nitrogen are in greatest interest in order to decrease oxidation state. All these forms of nitrogen, as well as nitrogen gas (N_2) are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons.

Nitrite is the intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and also in the reduction of nitrate. Such oxidation and reduction may occur in waste water treatment plants, water distribution systems and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is actual biological agent of methemoglobinaemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines (RR'NH) to form nitrosamines (RR'N-NO), many of which are potentially carcinogenic. The toxicologic significance of nitrosation reaction in vivo and in the natural environment is the subject of much current concern and research.

The use of nitrite in food preservation, as fertilizers and detergent and also in the gas, coke, fertilizer, wood pulp, dye and synthetic fiber industries has caused serious pollution problems[1, 2]. Nitrite is regarded as hazardous compound[3].

Numerous methods are reported for determination of nitrite such as kinetic, spectrophotometry[4-6], flouresence[7-12], chemiluminiscence[13-15], polarography[16], chromatographic[17-18] and flow injection methods[19, 20].

In the present investigation, nitrite is determined spectrophotometrically using N-PBHA, N-PClPBHA and N-PClPCHA. The method is based on the reaction of nitrite with acidified potassium iodide to liberate iodine. The liberated iodine is reacted with the solution of hydroxamic acids in n- hexane to form stable pink to dark pink complexes which gives maximum absorptions at 522 nm, 517 nm and 506 nm for N-PBHA, N-PClPBHA and N-PClPCHA complexes, respectively.

The main advantage of the proposed method over the other methods is its higher sensitivity, short analysis time and higher the stability of complex formed. The method is also free from interference of many foreign species.

EXPERIMENTAL SECTION

Instruments

"SHIMADZU SPECTROPHOTOMTER 1700" was used for electronic spectral measurements with 10 mm matched quartz cells. A Hanna 8521 model pH meter was used for pH measurements.

Reagents

All the chemicals used were of AR grade. Double distilled water was used throughout the experiments.

Preparation of solutions

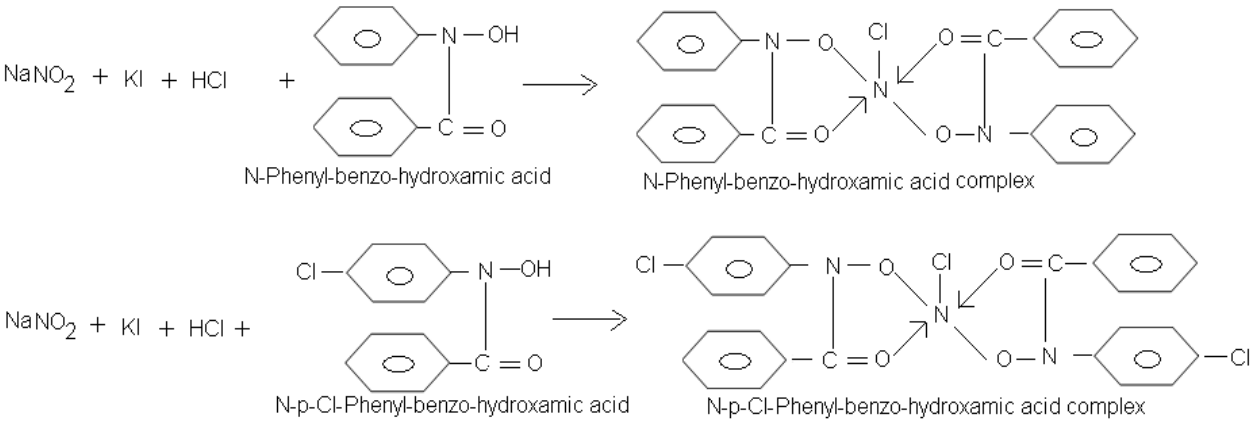
0.1g sodium nitrite was dissolved in 100 ml of double distilled water. One pellet of sodium hydroxide and 1 ml of chloroform were added to prevent nitrite decomposition and bacterial growth [21]. Working standard solutions were prepared by diluting the stock solution. 0.1% aqueous solution of potassium iodide (E.Merck) was used. 3% of EDTA solution which acts as masking agent [22] was also used. N-PBHA, N-PClPBHA and N-PClPCHA solutions were prepared by dissolving 0.1g of respective hydroxamic acid in 100 ml n-hexane. For making buffer solution 3.4g potassium phosphate was dissolved in 98 ml water and 85% phosphoric acid was added drop wise until the pH became 4.0. The solution was then diluted to the mark with distilled water. 6M hydrochloric acid solution was used.

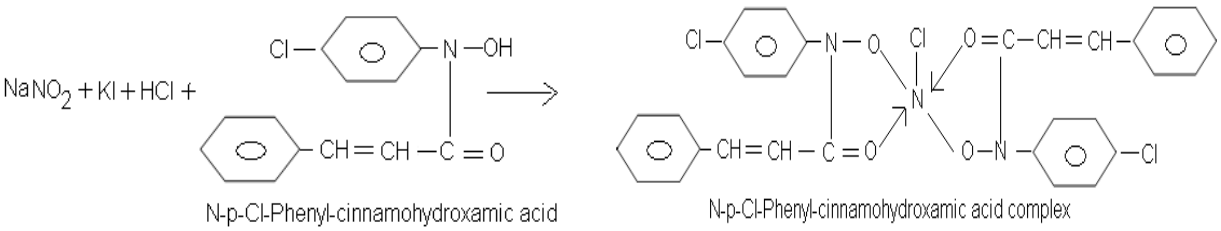
Procedure

The calibration curve was obtained by the following method:

An aliquot of the sample solution containing 0.00666 - 0.07326 µg/25 ml, 0.00666 - 0.09322 µg/25 ml and 0.0666 – 0.4662 µg/25 ml of nitrite was transferred to a series of 25 ml calibrating flask. To this 1ml of 3% EDTA solution was added as masking agent. In this solution, 1 ml of potassium iodide and 1 ml of 6M hydrochloric acid were added. Liberation of iodine is indicated by the appearance of yellow colour. Then 1 ml of all three hydroxamic acid solution were added and pH was maintained at 4.0 for N-PBHA, N-PClPBHA and N-PClPCHA with dropwise addition of buffer solution and contents were diluted to 25ml by adding n- hexane. Pink colour was obtained immediately by the formation of NO_2-ligand complex. The absorbance was measured at 522 nm, 517 nm and 506 nm, respectively against reagent blank.

Expected reaction





RESULTS AND DISCUSSION

a) Absorption spectrum and calibration curve

After reaction, complex present in organic phase was scanned from 400 nm to 600 nm against reagent blank (figure1). Maximum absorption values were observed at 522 nm, 517 nm and 506 nm for nitrite complex with N-PBHA, N-PClPBHA and N-PClPCHA, respectively. Therefore, 522 nm, 517 nm and 506 nm was selected with N-PBHA, N-PClPBHA and N-PClPCHA, respectively for the absorbance measurement throughout the experiments.

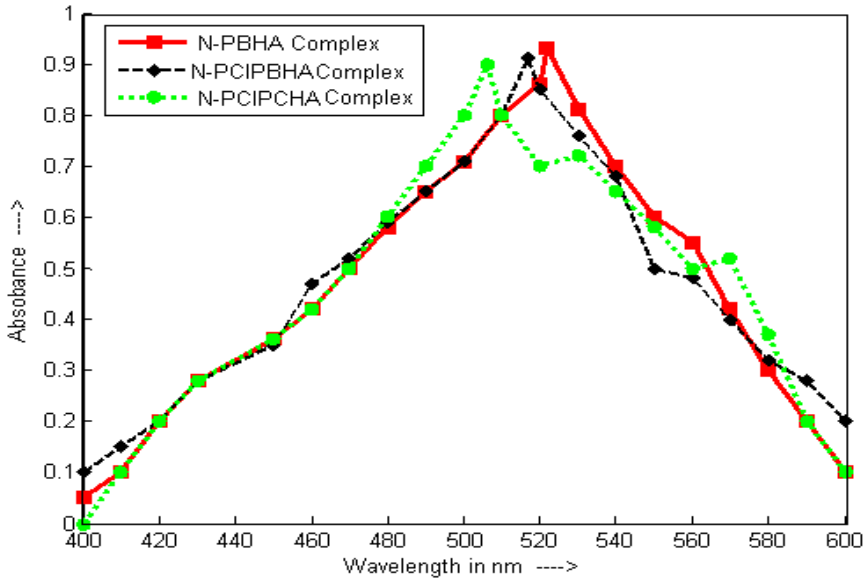


Fig. 1. Absorption spectra of nitrite complexes with hydroxamic acids

A calibration plot of absorbance against concentration of N-PBHA, N-PClPBHA and N-PClPCHA complexes at the absorption maxima gave a linear and reproducible graph in the concentration range of 0.0002664-0.002930 ppm, 0.0002664 – 0.003729 and 0.002664 - 0.01864, respectively (figure3). The beer's law is obeyed in this range.

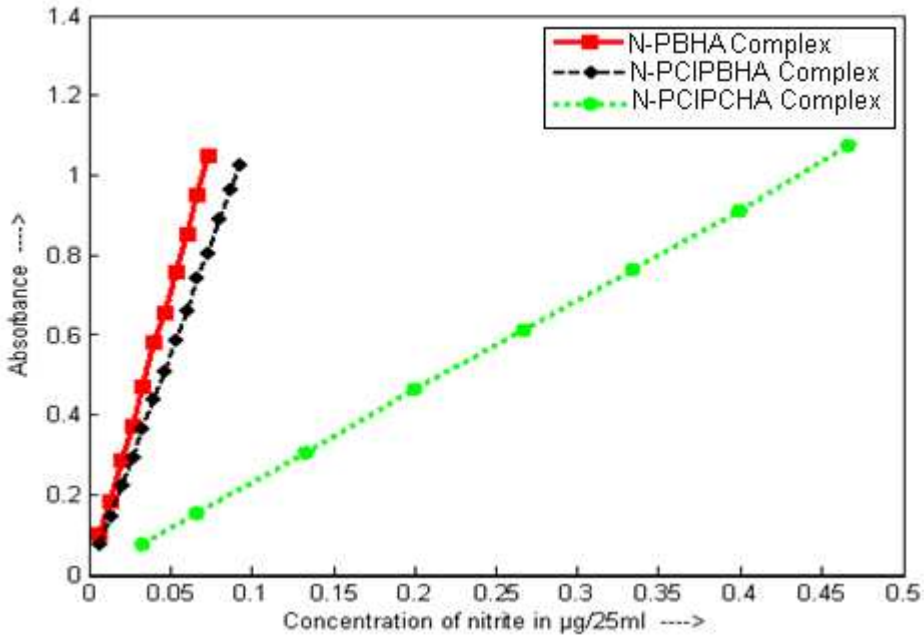


Fig.2: Calibration plot for N-PBHA, N-PClPBHA and N-PClPCHA complexes

b) Effect of reagent concentration

Optimal concentration of hydroxamic acids were investigated by varying the amount of hydroxamic acids used as reagent.To a series of 1 ml of nitrite standard solution, varying concentration of hydroxamic acids solutions were added and mixed. It was observed that by increasing the concentration of hydroxamic acid absorbance increases and with 3 ml of 0.0001% hydroxamic acid solutions it reaches maximum absorption. Then it decreases rapidly. Therefore 3 ml of 0.0001% concentration of hydroxamic acid solution was selected for all experiments.

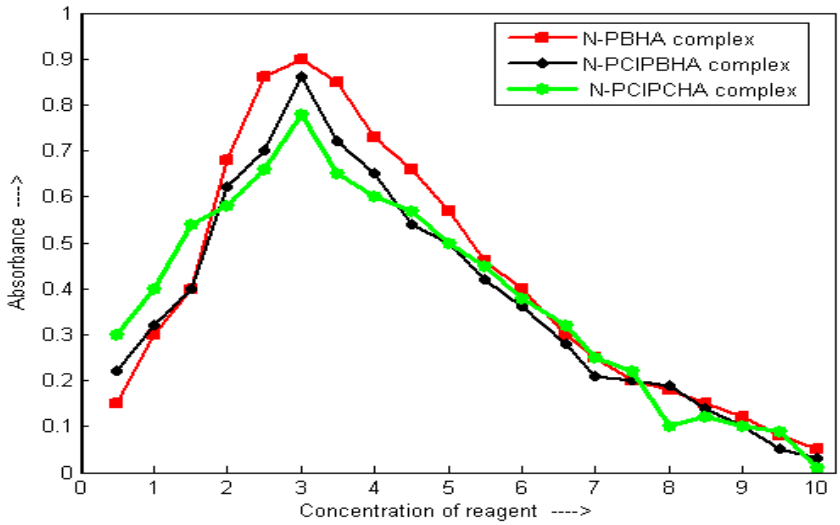


Fig.3: Effect of reagent concentration on absorbance of complexes

c) Effect of pH

In order to obtain the optimum condition for the determination of nitrite, absorbance was measured at the pH range 1.0 -10.0 (figure 2). It was observed that the absorbance increased upto 4.0 pH then it started decreasing. Therefore, pH 4.0 was used further for all experiments. The determination was done by using n-hexane as a medium.

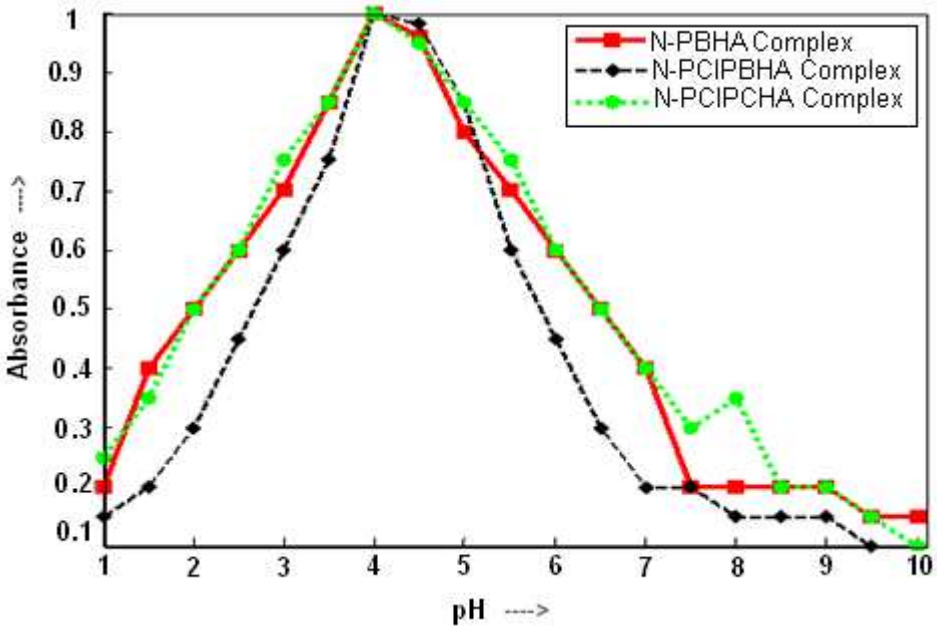


Fig. 4: Effect of pH on absorbance of complex

d) Effect of different solvent

Different organic solvents like ethanol, n-butanol, diethyl ether ethyl, methyl ketone, ethyl acetate, chloroform, toluene, n- hexane and carbon tetrachloride were used for determination. n- hexane was found to be most suitable solvent as it gave better and quick phase separation. Therefore, n- hexane was selected.

e) Effect of temperature and stability

The effect of temperature on colour stability and absorbance of the complex was studied over the temperature range of 10^0C – 50^0C. It was observed that at room temperature (25^0C) complex gives maximum and stable absorption. On increasing the temperature of reaction, stability and absorbance of the complex decreases. Therefore, all experiments were performed at the room temperature.

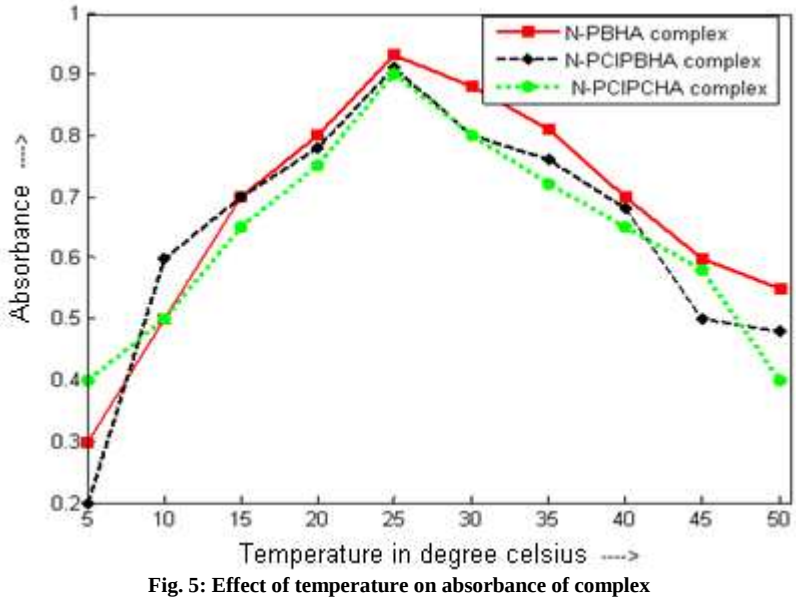


Fig. 5: Effect of temperature on absorbance of complex

f) Effect of diverse ions

The effect of various non-target species on the determination of nitrite was investigated. The tolerance limit of interfering species were established at those concentrations that do not cause more than ± 5% error in absorbance values of nitrite. The studies revealed that Ce(IV) and Hg(II) showed severe interference. However, the tolerance levels of these ions are increased by the addition of 1ml of 3% EDTA. The results are given in Table 1.

Applications

In order to evaluate the analytical applicability of the method, the procedure established was used for the determination of nitrite in water, soil and biological samples.

Analysis of water samples

For analysis of real sample some pretreatment is necessary. All suspended particles should be removed by suitable procedures. For waste water sample, precentrifugation was used. For tap water analysis, chlorine was also removed to prevent oxidation of nitrite. The samples were transferred in 25 ml calibration flask and 0.5 ml of l mol L^{-1} NaOH and 1 ml of 3% EDTA solution was added and above procedure was applied. The results (Table 2) agreed well with those given by the standard method[31].

Table 1. Effect of diverse ions on the determination of nitrite using N-PBHA, N-PClPBHA and N-PClPCHA as reagents

S No.	Diverse ions	Tolerance limit (ppm)		
		N-PBHA	N-PClPBHA	N-PClPCHA
1	Al^{3+}	300	290	285
2	Ba^{2+}	215	212	205
3	Ca^{2+}	500	498	480
4	Cd^{2+}	205	195	190
5	Ce^{4+}*	28	25	23
6	CH_3COO^-	>2025	>2020	>2000
7	citrate	810	800	790
8	Cu^{2+}*	35	30	28
9	Fe^{3+}*	30	25	20
10	Hg^{2+}*	28	24	25
11	K^+	>2030	>2020	>2000
12	Mg^{2+}	510	505	495
13	Mn^{2+}	515	505	502
14	Mo^{6+}*	35	28	25
15	Na^+	>2030	>2022	>2015
16	oxalate	798	795	780
17	Sn^{2+}*	22	20	19
18	Pb^{2+}*	29	25	26
19	tartarate	795	796	785

** Masked by EDTA*

Analysis of soil sample

Soil sample from manured garden was collected. 4 g of this soil was dried at 55^OC in an oven for 12-16 hours. The dried sample was dissolved in water, shaken thoroughly and filtered. The filtrate was subjected to centrifugation for about 15 min. The supernatant liquid was taken and mentioned procedure was applied.

Analysis of biological sample

The proposed method was applied successfully for the determination of nitrite in urine sample. The recoveries are close to 100% which indicates that the proposed method is effective for the determination of nitrite in the sample. The results of above analyses are given in Table 2.

Table 2. Determination of nitrite in water, soil and biological samples

Samples	Nitrite added (µg)	Nitrite found(µg)		% of Recovery	
		Present method	Reported method[31-32*]	Present method	Reported method[31-32*]
Polluted water (2 ml)	0.00	1.20	1.09	-------	-------
	0.50	1.69	0.48	98.0	96.0
Tap water (1ml)	0.00	0.90	0.78	-------	-------
	0.80	1.69	0.79	99.4	98.8
Soil (4g)	0.00	0.85	0.80	-------	-------
	1.00	1.84	0.99	99.4	99.2
Urine	0.00	1.85 ± 0.05	1.78 ± 0.05*	-------	--------
	0.22	2.04 ± 0.02	1.99 ± 0.03*	98.0	95.0
	0.44	2.28 ± 0.05	2.24 ± 0.08*	99.5	104.0

Table 3. Comparison of proposed method with other methods

S. No.	Reagents[Ref]	Range(ppm)	λmax	Remark
1	Sulphanilic acid + naphthylamine[23,24]	0.05-1.20	520	Cu^{+2}, Fe^{+3} and strong oxidants interfere
2	4-aminobenzoic acid[25]	0.1-1.3	519	Less sensitive
3	p-rosaniline+NEDA[26]	0.08-0.72	565	Fe^{+3}, Cr^{+6} and S^{-} severely interfere
4	o-nitroaniline+1-aminonaphthalene-2-sulphonic acid[27]	0.08-0.68	545	Less sensitive
5	Benzidine+Orcinol[28]	0.004-0.48	460	Less sensitive
6	p-aminophenyl-mercapto acetic acid[29]	0.1-1.6	495	S^{-} and Sb^{+3} interfere
7	p- Nitroaniline+phloraoglucinol[30]	0.004-0.04	420	Cu^{+2} and Fe^{+3} interfere above 75 ppm
8	Leucocrystal violet(LCV)[31]	0.00025-0.0025	590	Sensitive, rapid,extractive method
9	Nitrite -N-PBHA Nitrite-N-PClPBHA Nitrite-N-PClPCHA	0.000266 - 0.00293 0.000266 - 0.00372 0.00266 - 0.0186	522 517 506	Simple, rapid, sensitive, less tedious and stable method

CONCLUSION

A spectrophotometric method was developed for estimation of nitrite. All the hydroxamic acids were successfully used for quantitative determination of nitrite at pH 4.0. Since the reaction time is very less, the method is very quick. The colour developed is very stable. The method offers simple and rapid determination of nitrite in water, soil and biological samples.

The solution of reagent made in organic solvent is very stable and can be used for more than a year. The method has ben compaired with other previously reported method Table 3.The results show that with the increase in substitution in hydroxamic acid, the sensitivity of method decreases. Thus, the determination of nitrite with the parent hydroxamic acid, N-PBHA is most sensitive among the hydroxamic acids.

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