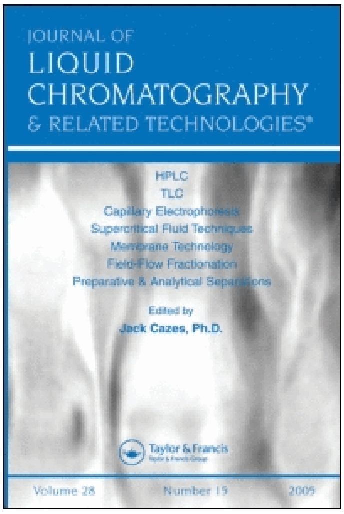

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C. A. Rodrigues[a]; F. Reynaud[a]; E. Stadler[b]; V. Drago[b]
[a] Universidade do Vale do Itajaí, Itajai, SC, U.S.A. [b] Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, SC, U.S.A.

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PREPARATION, CHARACTERIZATION, AND CHROMATOGRAPHY PROPERTIES OF CHITIN MODIFIED WITH $FeCl_3$

C. A. Rodrigues,[1,]* F. Reynaud,[1] E. Stadler,[2] V. Drago[3]

[1] NIQFAR- Núcleo de Investigações Químico-Farmacêuticas-FAQFAR
Universidade do Vale do Itajaí
CEP 88.302-202
Itajai, SC, Brazil

[2] Departamento de Química
Universidade Federal de Santa Catarina
Cx. P. 476, CEP 88.040-900
Florianópolis, SC, Brazil

[3] Departamento de Física
Universidade Federal de Santa Catarina
Cx. P. 476, CEP 88.040-900
Florianópolis, SC, Brazil

ABSTRACT

We report some details of the $FeCl_3$ immobilized in chitin. The polymer was characterized by infrared and Mössbauer spectroscopy. Chitin modified with iron (CH-Fe) was used as a support for thin layer chromatography and column chromatography for separation of phenolic compounds. The results show that CH-Fe can be used as an alternative support for immobilized metal affinity chromatography (IMAC).

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INTRODUCTION

Immobilized metal affinity chromatography (IMAC) was introduced by Porath et al in 1975[1] and since then has been adapted for purification of many proteins. In all cases, the selectivity of IMAC was very high. Selectivity and retention on those adsorbents are adjusted by changing the type of the metal and the properties of the compounds to be separated.[2] Almost without exception, commercially available IMAC supports utilize iminadiacetic acid (IDA) as the metal - chelating group.[2]

Fe(III)-IMAC has found application in the separation of phosphorylated macromolecules.[3] IMAC - IDA-Cu (II) has been used to adsorb human, serum albumine,[4] cellulose - based chelating support, Chelat-Granecel-Cu (II), and exhibit high affinity to human growth hormone.[1]

Chitin is a natural biopolymer with a physical and chemical similarity to cellulose which is often used in chromatography.[5,6] Free amino groups on the surface of chitin have chelating properties[7] and, therefore, may be a good stationary phase for the separation of the metals capable of formation of coordinated bonds. The stationary phases chitin modified by Cu^{2+} ions may be used for separation of amino acids.[8]

Phenolic compounds have received attention because of their ubiquity in nature and due to the many different functions they perform within the plant and its environment. Phenolics are widely present in food plants and in many popular beverages; therefore, there is a current interest in them and in the effects on the human body. There has also been a renewed interest in the use of phenolics, and especially flavonoids, as medical drugs.[10]

Silica gel has been widely used for separation of phenolic non polar,[10] mixtures of dihydroxy and trihydroxybenzoic acid which are separated by microcrystalline cellulose.[9] Isoflavonoids are separated on polyamide.[10] Glycosylflavone mixture can be resolved by cellulose.[10] In this paper, we describe the preparation, characterization, and chromatographic properties of chitin modified by iron ions, for separation phenolic compounds.

MATERIALS AND METHODS

Preparation and Characterization of Support

Chitin (CH) (85% N-acetylated) was obtained according to the literature method from shrimp shells.[11] Chitin-Fe (CH-Fe) complex was prepared by stirring chitin power overnight in 1.0 M $FeCl_3$ (Sigma Aldrich) at pH 1. The solid was subsequently filtered and exhaustively washed with distilled water, methanol and acetone, to remove the excess of $FeCl_3$ and dried under vacuum at room temperature. The quantity of iron adsorbed on the polymer surface was determined using a Hitachi model Z-8000 polarized Zeeman atomic absorption spectrophotometer. The infrared spectra were obtained as KBr disk on a IR-FT Bomen MB-100 spectrophotometer.

Mössbauer spectroscopy measurements were obtained from solid samples of complexes with Wissel equipment operating at constant acceleration, using a radioactive source of $^{5-7}$ Co on the (Rh) matrix with initial intensity of 10 mCi and 0.26 mm/s of line width, when measured against a sodium nitroprussate absorber as a standard. Isomeric shift is given relative to α-iron, at the measured temperature.

Thin Layer Chromatography

The methods used to coat a glass plate (2,5 x5 cm) with a thin layer sorbent CH and CH-Fe was a spreading, and the calcium sulfate (20 %) was used as a binder. TLC was also performed on plates precoated with 0.25 mm layer of silica gel 60 HF_{254} from Merck.

A solution containing pyrogalol, cathechol and mixtures of two compounds were stopped on the TLC plates and eluted with methanol:chloroform (85:15). The compounds were detected by spraying $FeCl_3$ (2% in ethanol) solution, or visualized under UV light (254 nm).

Column Chromatography

The mixture of 150 mg of pyrogalol and 150 mg of catechol was eluted in chromatographic columns (150 x 10 mm) containing 5 g of CH, 5.7 g of CH-Fe and 8,7 g of silica gel. The mixed compounds were eluted with methanol:chloroform (85:15) and fractions of 5 mL eluent were collected. The compounds were monitored for TLC using silica gel 60 plates and detected according to methods shown above.

RESULTS AND DISCUSSION

Preparation and Characterization of Support

The quantity of iron in the solid was 9.6 $mg.g^{-1}$ determined by atomic absorption spectroscopy. This result showed that the chitin possesses little

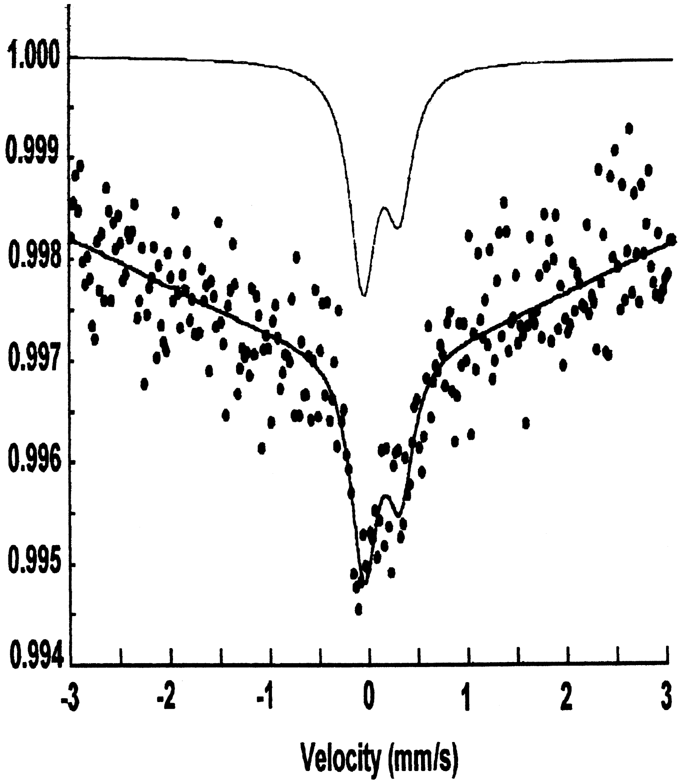

Figure 1. Mössbauer spectrum of CH-Fe.

chelating capacity due to the low quantity of amino group in polymeric chain (15%). The Mössbauer spectrum of CH-Fe complex, Figure 1, exhibits an isomer shift (δ) of 0.19 mm.s^{-1}. This is typical of low spin Fe(II) complex with σ- donor ligand. Furthermore, the asymmetric doublet with quadrupolar splitting Δ= 0.36 mm.s^{-1}, indicates the existence of a distorted octahedric complex produced by the presence of the ligand of a different nature.

The Mössbauer spectroscopy parameters are characteristic of the low spin Fe(II) species, indicating the reduction of $FeCl_3$ by chitin.[12] Another conclusion is that Fe(II) is coordinated with two amino group of chitin.[13]

The IR spectrum of CH-Fe (Figure 2(A)) exhibits characteristic absorption band at 1650, 1555, and 1320 cm^{-1} corresponding to the amide I, II and III bands.[13] The spectrum of CH-Fe exhibits the same bands of chitin, Figure 2(B) and can be attributed to low amount of iron on the surface of the chitin.

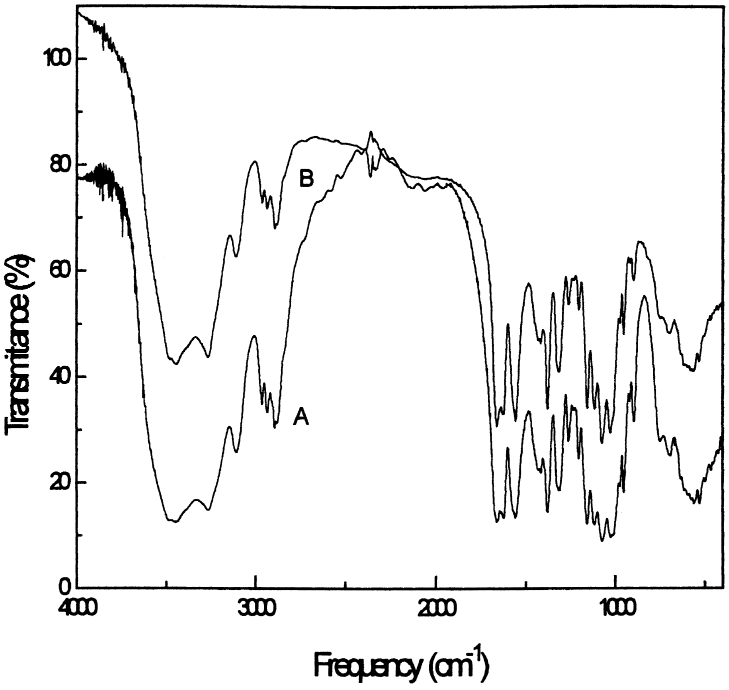

Figure 2. Infra-red spectra: A) CH-Fe; B) CH.

Table 1

Difference Between R_F Value of Phenolics, Pure and the Mixture, on the Studied Support

	R_F (cm±s) Pure Compound		**R_F (cm±s) Mixture**		
Support	**Cathecol**	**Pyrogallol**	**Cathecol**	**Pyrogallol**	**ΔR_F**
Silica gel	0.78±0.03	0.55±0.04	0.76±0.03	0.53±0.05	0.23±0.06
CH	0.82±0.02	0.47±0.05	0.79±0.03	0.43±0.04	0.36±0.05
CH-Fe	0.77±0.02	042±0.06	0.73±0.03	0.41±0.06	0.32±0.06

$\Delta R_F = R_{Fcath.} - R_{Fpyr}$ (in mixture). S = standard deviation for n=3.

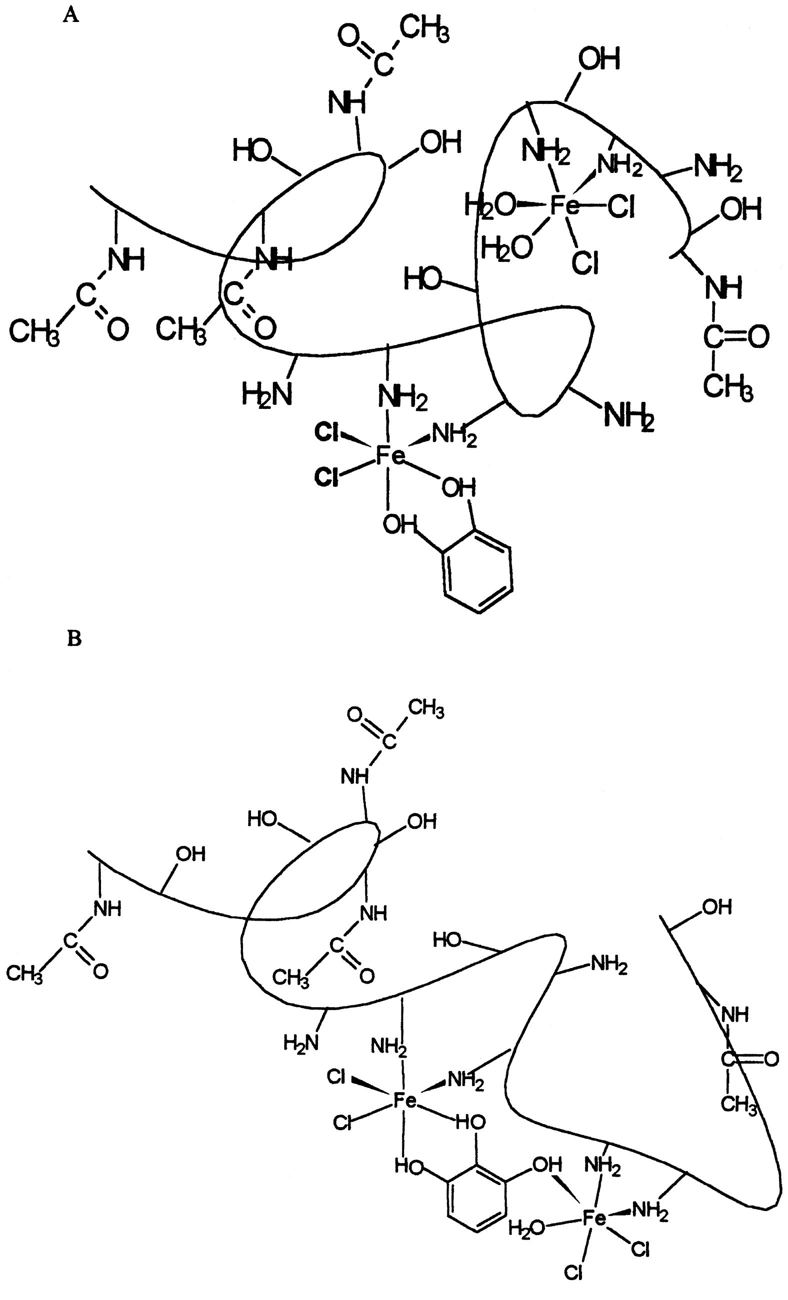

Figure 3. Possible interaction of CH-Fe with catechol A; CH-Fe with pyrogallol B.

Table 2

Efficiency of Different Supports Studied in the Separation of Phenolic Mixtures

Support	**Cathecol** **mg**[1]	 **%**	**Pyrogallol** **mg**[1]	 **%**	**Catechol + Pyrogallol** **mg**[2]	 **%**	**Recovered Initial Mixture** **%**
Silica gel	89	59	86	57	116	38	97
CH	57	38	55	37	50	18	60
CH-Fe	108	72	100	67	43	14	82

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[1] Pure compounds.
[2] Impure compounds.

Chromatographic Behaviour

The retention parameter R_F, for the investigated pure compounds and ΔR_F, mixture compounds, in three adsorbents, are report in Table 1. Catechol is better separated than pyrogallol on CH and CH-Fe than of silica gel.

On CH and silica gel beds, the migration distance is longer for compounds than with a surface modified with iron. In silica gel the separation between phenolics was not satisfactory.

The retention of phenolic compounds in the chitin surface occurred due to interaction by hydrogen bonding with variety of functional groups on the surface (OH-group, free or acetylated amino group).[8]

The adsorption of catechol and pyrogallol on CH-Fe involves interaction of the compound with iron while displaying one or more of the weakly bound ligands such as water and chloride ions.

In the support CH-Fe, the higher ΔR_F between catechol and pyrogallol can be attributed to interaction of catechol with one iron ion, while with pyrogallol, the interaction occurs within the two iron ions, Figure 3.

The separation of the mixture of phenolics in the liquid chromatography experiment was performed using the same parameter: amount of sample, height column, flow-rate, and polarity of the solvent in order to compare the efficiency of three supports.

The results are shown in Table 2. The results were in good agreement with those obtained for TLC experiments, i.e., supports with high ΔR_F (CH and CH-Fe) were more efficient.

Another interesting observation is that the lowest yield of pure compounds and initial mixture recovered was when CH was used with the support. This fact can be attributed to strong interaction between amino groups that are free of CH (15%) and OH in the phenolic compounds, resulting in retention of large amounts of compounds in the column. On the other hand, when silica gel was used with the support, all of the initial mixture was recovered. However, a large proportion was of the impure compounds.

The results of this investigation demonstrate that CH-Fe can be utililized with alternative supports for IMAC for the separation of phenolic compounds as flavonoids and biflavonoids.

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