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# ESR CHARACTERIZATION OF CHITINS AND CHITOSANS

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Summary. Chitins and chitosans from crabs and shrimps as well as the chitosan-glucan complex from Aspergillus niger show an ESR singlet at 3387-3391 G and g values 2.00117-2.00354; this signal is altered by the action of oxygen from the atmosphere and from hydrogen peroxide, and by hot water.

## INTRODUCTION

Chitin and its derivatives are becoming more and more important in basic sciences as well as in applied technology (1 - 7). The physico-chemical characterization of chitin/chitosan samples of various origins is today mandatory if reproducible results and reliable processes are desired; however, scarce information has so far been obtained with the aid of ESR spectrometry, that conversely has been applied to the study of the interactions of other biopolymers with metal ions: cellulose (8), modified cellulose (9), oligopeptides (10), besides maltose and dextrose (11) and ion-exchange resins (12). Some results on chitin and chitosan have recently been offered but no indication of the origin of the polymers, of their preparation and of the treatments carried out on them was given (13).

In view of the chelating ability of chitin/chitosan and since the ESR spectrometry is a valuable tool for the study of metal complexes, we have planned a coordinated investigation on chitin/chitosan based on a number of instrumental techniques. The present paper is the preliminary background on which further results about chitin/chitosan and their metal complexes will be based.

It is a well known fact that chitins of various origins differ from each other from the physico-chemical standpoint and that the variables of the preparative process introduce further differences. Thus, we feel that

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a comparative study of some of the most representative chitins and chitosans presently being marketed or manufactured is a priority in this kind of investigation.

## MATERIALS AND METHODS

**Instrumentation.** A Varian E-104-A EPR spectrometer was used for this investigation. The usual settings were: field 3400 G, scan range 500 G, time constant 0.25 sec, scan time 4 min, modulation amplitude 10 G, modulation frequency 100 KHz, receiver gain 5000 or lower, microwave power 5 mW, microwave frequency 9.53 GHz, room temperature. The powders were 100-200 mesh, to fill the ESR tubes to an heigth of about 6 cm; however, the various powders were more or less pressed depending on their nature. The g values were determined with DPPH in benzene under the same conditions, taking g = 2.00354 from the literature. An Edwards Minifast 470 freeze-dryer was used to complete the preparation of the samples: start temperatures -40°C (p) and -45°C (c), final temperature +25°C , vacuum 0.1 T.

**Chitins and chitosans.** The following chitins and chitosans have been used: they are representative of the products now available on the market and in the research laboratories.

**Kyowa chitin and chitosan:** industrial products kindly provided by Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan, from crab shells (crabs from the Tottori, Japan, area).

**Kypro chitosans:** industrial products kindly provided by Kypro Company, Seattle, Wash., USA, from Dungeness and King crab shells (crabs from the Washington State area).

**Rousselot chitin:** industrial product kindly provided by Rousselot & Kuhlmann, Paris, France, from crabs from Norway.

**MCI chitosans:** industrial products kindly provided by Marine Commodities Intl., Ltd., Brownsville, Tx, USA, from shrimp shells (shells from the Texas coasts).

**Chesapeake chitosan:** laboratory product from selected parts of the crabs from the Chesapeake area, demineralized and deacetylated under controlled conditions.

**BDH chitin:** research product supplied by BDH, London, England.

**Polyplate chitosan:** industrial product kindly provided by Polyplate India Ltd., Calcutta, India, from shrimp shells.

**Chitosan-glucan complex:** laboratory product prepared by us from the Aspergillus niger mycelia (14).

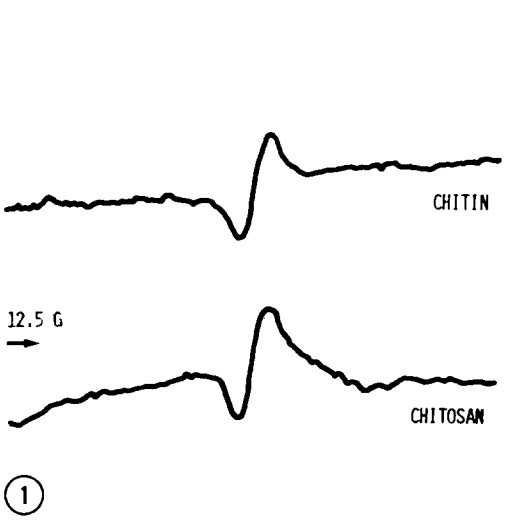
All of these samples were extracted in a Soxhlet extractor with these solvents: methanol, water, petroleum ether and acetone, in the order, for about 24 hr each. Then, they were freeze-dried just before spectrometry. For certain preparations, air was bubbled in the Soxhlet apparatus. In addition, some Rousselot chitin was deacetylated according to the Broussignac method (1) for 30, 60, 90 and 360 min (either under usual conditions or under nitrogen) and also with aqueous 40 % KOH for 6 hr. The Kypro chitosan (Dungeness) was deacetylated for 30 min.

Treatments with 100 vol hydrogen peroxide were carried out as follows on the Chesapeake chitosan and on the Rousselot chitin: **first sample**: the polymer powder (1 g) was suspended in water (100 ml) and hydrogen peroxide (2 ml) was added at 90°C over 5 min at pH about 5. **Second sample**: the polymer (0.5 g) was suspended in dilute NaOH (100 ml) and hydrogen peroxide (4 ml) was added at room temperature over 90 min, at pH about 11. **Third sample**: the polymer (0.5 g) was suspended in dilute sulfuric acid (100 ml) and hydrogen peroxide (4 ml) was added over 90 min at room temperature at pH 4.7. The samples so obtained were washed and freeze-dried.

## RESULTS AND DISCUSSION

We have first verified that all of the chitins and chitosans tested exhibit the same ESR signal. The typical signals recorded for one chitin and one chitosan are in Fig. 1; they are quite similar and represent a single absorption curve averaging about 8-10 G in width; the signal center is in a position comprised between 3387 and 3391, as shown in Table I. The MCI-HV chitosan and both the Kypro chitosans show an ESR spectrum where the signal, while still detectable, is not clearly readable because of the slope of the curve, such as in Fig. 2.

In an attempt to define the importance of the deacetylation degree on the ESR spectrum, four samples of deacetylated Rousselot chitin were



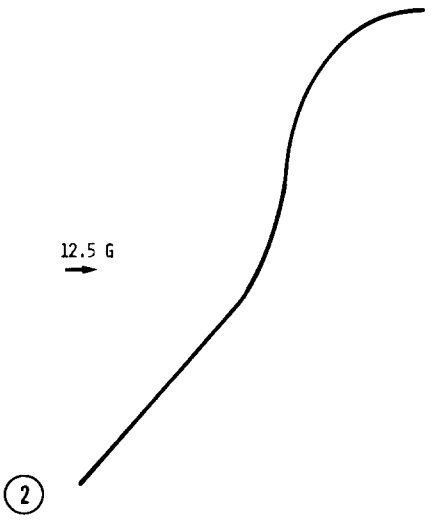




**Figure 1.** ESR spectra of freeze-dried Rousselot chitin and freeze-dried Chesapeake chitosan.

**Figure 2.** ESR spectrum of Kypro chitosans and MCI-HV chitosan.

Table I
Spectral characteristics for some chitins and chitosans after extraction and freeze-drying.

| Polymer, | g value | Width, G | Amplitude, a.u. |
|---|---|---|---|
| **CHITINS** | | | |
| Kyowa | 2.00295 | 10 | 6 |
| BDH | 2.00295 | 10 | 10 |
| **CHITOSANS** | | | |
| Kyowa | 2.00117 | 10 | 8 |
| Polyplate | 2.00354 | 8 | 21 |

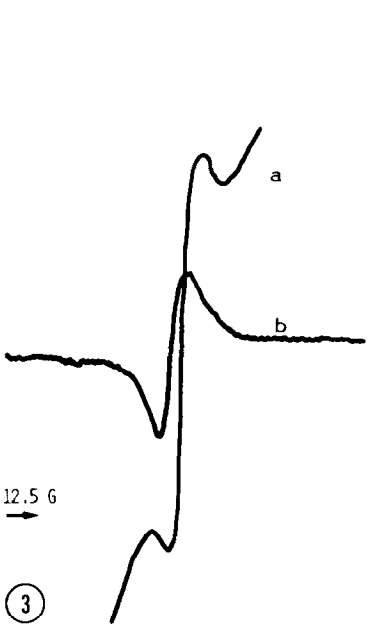
prepared after variously prolonged periods of deacetylation, but no univocal responses were observed except for the tendency to lower fields for the signal center.

After deacetylation in both aqueous and organic media, significant increases in the signal amplitude were observed for all samples, presumably due to the presence of oxygen in the deacetylation vessel and to the effect of hot water where present.

While the signals recorded on samples deacetylated and extracted under nitrogen are about constant, the signals are deeply altered when the extraction in water is carried out with simultaneous bubbling of air into the Soxhlet vessel: under these circumstances, the spectra of the Rousselot chitin/chitosan samples become similar to the Kypro chitosan spectrum in Fig. 2. The ESR signals are therefore altered by the action of oxygen and hot water on the chitosan samples, while they are not clearly correlated with the degree of deacetylation.

Fig. 3 shows the ESR spectra taken on the chitosans obtained from the Rousselot chitin, respectively in aqueous and organic KOH: while the spectrum of the first sample (from aqueous KOH) is deeply altered and the signal appears on a big shoulder, in the second sample (from KOH in ethanol + glycol) the signal appears on the base line.

Chitin and chitosan samples treated with warm hydrogen peroxide exhibit identically altered spectra, whose signal has the same center but lower amplitude (about one fourth) in the case of chitin (Fig. 4), while



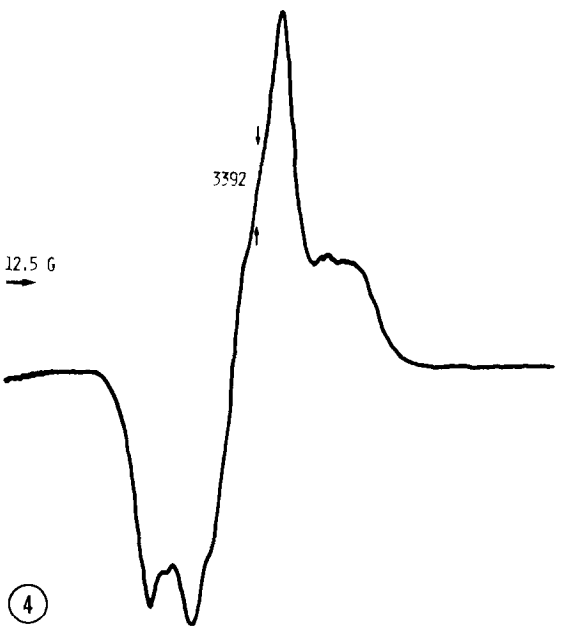




Figure 3. ESR spectra of chitosans obtained from the Rousselot chitin after 6-hr deacetylation, extraction and freeze-drying (a) with aqueous KOH, last line in Table II, and (b) according to Broussignac with KOH in ethanol+glycol, second line from bottom in Table II.

Figure 4. ESR spectrum of chitosan after treatment with warm hydrogen peroxide and freeze-drying.

no signal whatsoever was recorded with a cellulose sample. Therefore, it is believed that oxygen affects the amino groups of these polymers. Any contribution to the ESR signal from physically adsorbed oxygen can be excluded since some measurements were taken on samples under depression with the same results.

Special attention deserve the spectra taken on the chitosan-glucan complex from Aspergillus niger; this polymeric complex is the insoluble fraction left after alkaline treatment. A series of signals, possibly due to manganese, accompanies the singlet described above; manganese was found to occur at the concentration of 40 ppm and copper at the concentration of 9 ppm in the freeze-dried complex.

## CONCLUSIONS

Most of the samples of chitin/chitosan studied exhibit a typical ESR signal whose origin is possibly to be ascribed to an initial stage of

Table II

Spectral characteristics for the Rousselot chitin and chitosans and for the Chesapeake chitosan. (Conditions under Experimental).

| Treatment | Rousselot chitin | | | Chesapeake chitosan | | |
|---|---|---|---|---|---|---|
| | g value | Width, G | Amplitude, a.u. | g value | Width, G | Amplitude a.u. |
| Freeze-drying | 2.00295 | 9 | 15 | 2.00177 | 10 | 14.5 |
| Extraction + freeze-drying | very small signals | | | 2.00177 | 11 | 56-16 § |
| | Rousselot chitosans | | | | | |
| Deacetyl. 30 min | 2.00236 | 9 | 25 | | | |
| id. under $N_2$ | 2.00354 | 9 | 14 | | | |
| Deacetyl. 60 min | 2.00177 | 10 | 14 | | | |
| id. under $N_2$ | 2.00354 | 10 | 23 | | | |
| Deacetyl. 90 min | 2.00117 | 10 | 16 | | | |
| id. under $N_2$ | 2.00295 | 10 | 14 | | | |
| Deacetyl. 6 hr | 2.00117 | 10 | 23 | | | |
| id. under $N_2$ | 2.00236 | 10 | 21 | | | |
| Deacetyl. 6 hr in aqueous KOH | 2.00236 | 12 | 56 | | | |

a.u. = arbitrary units

§ for the upper part (brown) and lower part (white) in the extractor.

oxidation. Some other samples show a degenerated spectrum which is not modified after purification of the samples and which seems to be inherent to conditions and procedures adopted for their production.

While it is difficult to correlate the deacetylation degree with the ESR spectral features, it is in any case evident that chitin has a more marked resistance to oxidation than chitosan when they are treated with hydrogen peroxide. Hot water is also responsible for some deterioration of chitin/chitosan. Extended washing of chitin/chitosan with hot water at the end of the preparation can introduce alterations. Also, the use of hydrogen peroxide for bleaching and for the removal of pigments is not without collateral effects on the polymers.

The protection of chitosan during manufacture has been in the past recommended and various ways have been proposed to this end; for instance, the Broussignac mixture was empirically devised to protect the polymer from oxygen and to avoid the use of hot water: the present results substantiate those findings with instrumental evidence.

The chitosan-glucan samples obtained from Aspergillus niger have undergone a generally milder treatment than the chitin/chitosan samples of animal origin, since no acidic treatment is necessary for their production. However, the signal reported above is present in all samples obtained after an alkaline hydroxide treatment under a variety of conditions (14) and one cannot exclude that the singlet is to some extent due to the polymer itself. Our data are at variance with those recently published (13) on the absence of ESR signals with chitosan samples.

Metal-ion treated chitins and chitosans are therefore susceptible of ESR spectrometry, since their original spectra are quite simple, provided that they have been produced under controlled conditions to avoid excessive oxidative degradation.

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