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Chitosans carrying the methoxyphenyl functions typical of lignin

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Methoxyphenyl aldehydes vanillin, *o*-vanillin, syringaldehyde and veratraldehyde were found to react with chitosan under normal and reducing conditions and to impart insolubility and other characteristics to chitosan; for instance, *o*-vanillin yielded a bright yellow product exhibiting novel bands in the FTIR spectrum at 1630, 1460, 1250, 950 and 730 cm^{-1}, and peaks at 5·52 and 20·12 2θ values in the X-ray diffractogram. The films obtained from veratraldehyde were insoluble, biodegradable and mechanically resistant. Suspensions of Kraft lignin and chitosan yielded very thick pastes within minutes upon addition of an organic acid and, upon drying at 50°C, 3 mm thick sheets harder than plywood.

INTRODUCTION

The filmogenic ability of chitosan has so far been exploited to a very limited extent due to the fact that films dissolve in slightly acidic aqueous media. The same can be said for chitosan filaments, despite efforts to upgrade such products (Motosugi *et al.*, 1986).

The new modified chitosans having filmogenic ability are difficult to exploit commercially because they are too expensive to produce. Among the chitosan derivatives so far described in the literature, those most pertinent to the present work are the ones from simple aldehydes (Hirano, 1979), aldehydoacids (Muzzarelli *et al.*, 1982), ketoacids (Muzzarelli, 1986), aldoses (Hall & Yalpani, 1984) and salicylaldehyde (Hall & Yalpani, 1984; Roberts, 1985; Levdik *et al.*, 1986; Yang & Vigee, 1991). To prevent dissolution, modified chitosans have been crosslinked (Koshugi, 1983) or combined with other polymers such as hyaluronic acid (Brode, 1988; Takayama *et al.*, 1990), polyacrylic acid (Takahashi *et al.*, 1990) and starch (Kume & Takehisa, 1984). So far, no attempt has been made to modify chitosan with the functions typical of lignin nor with lignin itself.

Certain aldehydes are industrially obtained from the chemical degradation of lignin (Zimmermann, 1989), whose functionality mainly includes the syringyl and guaiacyl groups corresponding to 3,5-dimethoxy-4-hydroxyphenyl and to 4-hydroxy-3-methoxypphenyl groups, respectively. Vanillin, syringaldehyde and veratraldehyde are the major products from hardwood lignins. *o*-Vanillin would also appear to be very suitable for the purpose of modifying chitosan. All of these aldehydes are distinguished by the fact that they carry methoxyphenyl groups, as shown below.

Aromatic ring position in 3-methoxyphenylaldehydes

2	4	5	Name
–H	$–OCH_3$	–H	Veratraldehyde
–H	–OH	$–OCH_3$	Syringaldehyde
–H	–OH	–H	Vanillin
–OH	–H	–H	*o*-Vanillin

The present work was therefore undertaken to explore the possibility of providing a class of well characterized modified chitosans carrying the methoxyphenyl functions typical of lignin, as well as an association of chitosan and lignin.

pt > MATERIALS AND METHODS

Chemicals

Chitosan was purchased from Protan (Redmond, OR, USA) stock no. 027-572-02. The degree of deacetylation was 0·81 and the average molecular weight was 410 000 Da; the grain size was 180–350 μm. The aldehydes were purchased from Aldrich Chimica (Milan, Italy). Kraft lignin was made available by Sandoz Chimie (Basel, Switzerland).

Collection of aldehydes on chitosan powder

Water–ethanol solutions were used for determining the collection of aldehydes on chitosan powder. The quantities in reciprocal presence were in all cases 150 mg of chitosan and 150 mg of aldehyde. Each aldehyde (0·10 g) was dissolved in ethanol (3 ml): these solutions were further diluted for analytical purposes to 75 ml with a water–ethanol (97:3) mixture.

Data were obtained by UV spectrophotometry by measuring at the characteristic absorption bands for each phenolic aldehyde: vanillin, 203, 229 (max.), 278, 308; *o*-vanillin, 217 (max.), 263, 340; syringaldehyde, 213 (max.), 305; dihydroxybenzaldehyde, 205 (max.), 229, 278, 309 nm. The instrument was a Kontron Uvikon 680. Reference curves were obtained by using the same water–ethanol solvent.

X-ray diffraction spectrometry

The reaction products in the form of powders were submitted to X-ray diffraction spectrometry by using a Rigaku RU 300 instrument with a powder diffractometer.

FTIR spectrometry

Films and powders were analysed with a Nicolet Magna IR-500 (Madison, WI) equipped with HATR accessory. Typically, the instrument was set for 64 sample scans, resolution 4·000, number of scan points 8480.

Film casting

Films could be cast from crude non-hydrogenated solutions and from degassed solutions already submitted to hydrogenation, by evaporation in polystyrene Petri dishes at 25°C (3 days) and at 50°C (overnight). The preparation of films from veratraldehyde is described here. The other aldehydes were used in a similar way.

Chitosan was dissolved in 1·0% acetic acid to make a 1·7% solution; veratraldehyde was dissolved in the minimum amount of glacial acetic acid. After mixing the two solutions, aliquots (50–100 g) were evaporated and the films obtained were allowed to equilibrate with room humidity (70–75%) before removal from the dishes.

The films were then dipped into a bath containing sodium borohydride (1·0 g), water (450 g) and ethanol (250 g); the latter was added to slow down the hydrogen release. The films were kept submerged in this bath for 10 min with occasional stirring. At higher borohydride concentrations the films had a tendency to roll up. After drying at 25°C between filter paper sheets, the films showed an attractive silver tone.

For analytical purposes, films were also cast from solutions submitted to hydrogenation after pH adjustment (4·2) and addition of sodium borohydride to reach pH 6·0. These solutions were dialysed against demineralized water for 3 days in tubes with cutoff at 15 000.

RESULTS

The reaction of aldehydes carrying the methoxyphenyl function turned out to be a straightforward way to introduce into chitosan the typical functionality present in lignin. Vanillin, *o*-vanillin, syringaldehyde and veratraldehyde [3, 4-dimethoxybenzaldehyde], as well as 3,4-dihydroxybenzaldehyde and salicylaldehyde [2-hydroxybenzaldehyde] easily react with chitosan by forming aldimines that can be optionally hydrogenated to secondary amines.

The aldehydes considered here are soluble in ethanol and in ether; vanillin, syringaldehyde and veratraldehyde are also soluble in acetic acid, the preferred acid for the dissolution of chitosan: reaction can therefore be carried out under homogeneous conditions, i.e. in dilute acetic acid.

The results for methoxyphenyl aldehydes, shown in Table 1, indicate that *o*-vanillin is particularly reactive towards chitosan, because it is collected to a remarkable extent (35%) within the first 30 min of contact. At equilibrium (3 days), more than one half is collected (54%), which means that one weight of chitosan reacts with more than one half the weight of *o*-vanillin. For the aldimine obtained from *o*-vanillin it was estimated that about one half of the available amino groups in chitosan underwent the Schiff reaction.

All of the above aldimines were insoluble in water and in alcohol. Those from syringaldehyde and vanillin

Table 1. Collection percent of methoxyphenyl aldehydes by chitosan powder (150 mg, 180–350 μm) at 25°C, pH 5·0, from water–ethanol (3%) solutions

Aldehyde	10 min	30 min	1 h	4 h	3 days
o-Vanillin	23	35	40	45	54
Vanillin	15	25	27	30	38
Syringaldehyde	7	17	25	27	43
Veratraldehyde	15	24	28	33	40
3,4-Dihydroxybenzaldehyde	7	13	14	15	20

were soluble in acetic acid, but the others were not. The aldimines were treated with a dilute aqueous solution of sodium borohydride and thus hydrogenated to yield insoluble products.

X-ray diffraction spectrometry

The diffractograms obtained on the powders prepared as indicated above, showed the presence of diffraction peaks in all samples, for the aldimines and their reduction products. The data are collected in Table 2.

It should be noted that the chitosan used was deprived of crystallinity. In general, chitosans exhibit two main diffraction peaks centered around 9 and 19 2θ values, indicative of their structural and conformational features (020 and 110 reflections, respectively). They are known to be strongly reduced by harsh chemical treatments and the 020 crystallographic plan can be totally destroyed by mechanical treatments.

In the present case, aldimine formation led to crystalline products having one of the reflections at 19·76–20·08 2θ values (Fig. 1). For *o*-vanillin, preparations were made under homogeneous conditions and enhanced crystallinity was observed (4·22, 14·22 and 20·22 2θ values) as shown in Fig. 2. The hydrogenation of the aldimine led to crystallinity increase in the case of *o*-vanillin and syringaldehyde for which novel reflections were detected at 5·62 and 8·08 2θ values, respectively.

The reflections at the highest 2θ values lie close to the values observed for chitosans and therefore it could be speculated that these reactions on the amorphous chitosan restore in part the macromolecular order; however, the reflections at lower 2θ values were far from the values found in chitosan and were representative of a novel structure which accommodated the bulky substituents.

The FTIR spectra taken on the modified chitosans obtained from the methoxyphenyl aldehydes studied, showed all of the corresponding bands found in the aromatic moiety. In particular, for the *o*-vanillin and chitosan reaction product, novel bands not found in chitosan and corresponding to spectral features of *o*-vanillin were in the region 700–800 and at 1480 cm^{-1}, while the 1550 band was no longer evident and the one at 1650 cm^{-1} was strengthened (Fig. 3). On the basis of the second derivative FTIR spectra, the main difference between the aldimine and the hydrogenated product was at 1530 cm^{-1}, as expected for secondary amine formation.

For the modified chitosans from vanillin, syringaldehyde and dihydroxybenzaldehyde, the FTIR spectra

Table 2. X-ray diffraction data for the chitosan derivatives obtained from methoxyphenyl by Schiff reaction, 2-theta values

Aldehyde	Aldimine		Hydrogenated product			Filmogenicity
o-Vanillin	20·08 f	yellow	5·62 S	20·07 f	yellow	Poor (brittle film)
Vanillin	19·76 s	yellow		19·66 s	pink	Good
Syringaldehyde	19·84	yellowish	8·08 f	20·00 b	silver	Fair
Veratraldehyde		yellowish			silver	Very good
3,4-Dihydroxybenzaldehyde	19·86	yellowish		20·02	yellowish	Poor

Heterogeneous conditions: f, faint; m, medium; s, strong; b, broad.

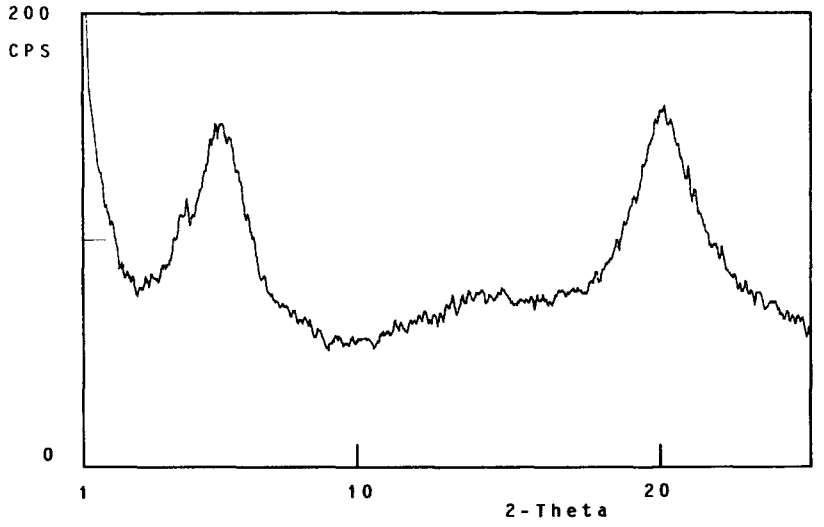

Fig. 1. X-ray diffractogram of the product obtained as follows: a solution of *o*-vanillin (0·3 g) in ethanol (6 ml) was added to a suspension of chitosan (0·15 g) in water (194 ml). After 1 week, borohydride was added. The powder was washed and dried. The degree of substitution was 0·40.

showed the presence of bands corresponding to the aromatic moieties, minor differences being evident between the aldimines and the respective reduction products. Bands at 1260–1220 cm^{-1} typical for phenols and aromatic ethers were clearly visible.

All of the aldimines were insoluble in common aqueous and organic media, with the exception of those from vanillin and syringaldehyde, soluble in acetic acid.

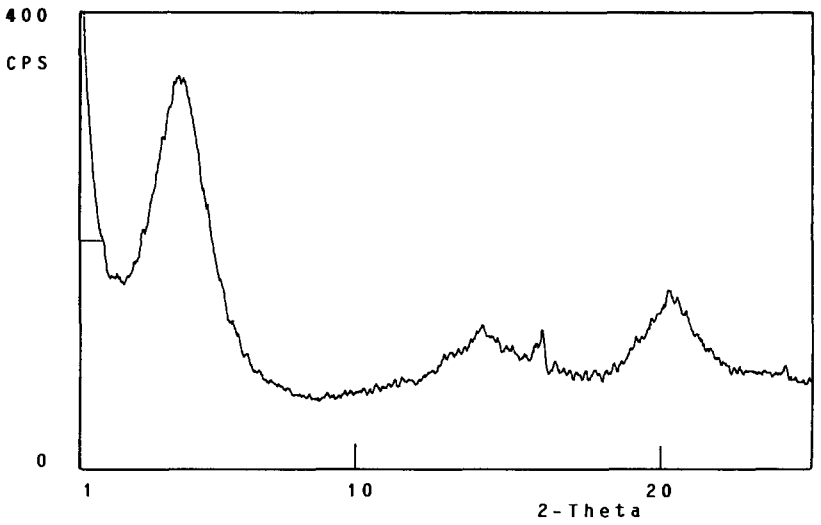

Fig. 2. X-ray diffractogram of the product obtained as follows: a solution of *o*-vanillin (1 g) in ethanol (20 ml) was added to a solution of chitosan (60 ml, 1·0%, acetic acid 1·0%) which provided a rigid gel within 5 min. The gel was washed and freeze-dried.

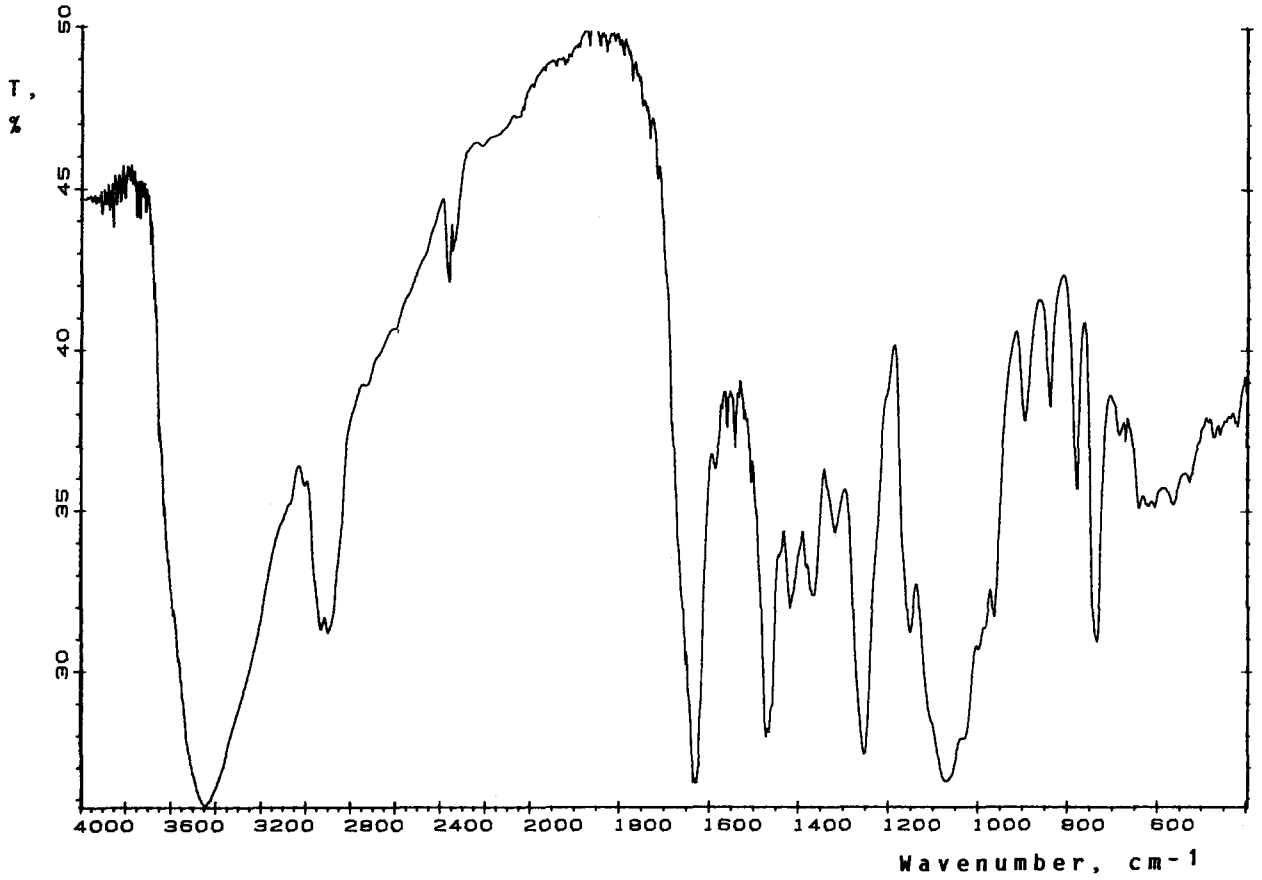

Fig. 3. Infrared spectrum of the *o*-vanillin chitosan aldimine showing bands at 1630, 1460, 1250, 950 and 730 cm^{-1} assigned to the aromatic moiety. The chitosan amide II band at 1553 seems to be depressed. The second derivative spectrum indicated that differences from the hydrogenated product are in the 1600–1650 and in the 1200–1250 cm^{-1} regions, as expected for the reduction of a substituted imine to secondary amine.

Table 3. Characteristics of veratraldehyde–chitosan films, according to ASTM 882

Film	Thickness (μm)	Tensile strength (kg)	Elongation (%)
Veratraldehyde chitosan	50	9·8	9
	25–30	4·2	6
Low density polyethylene[a]	50	1·5	300
Polypropylene[a]	30–50	10·5	30
Polystyrene[a]	30–50	10 ca.	10 ca.

[a] Values provided by Grace Co.

The one from veratraldehyde showed contact angle 110° for water, high mechanical resistance, brilliant silver tone, absence of shrinking and moderate possibility of thermoforming. The veratraldehyde–chitosan films exhibited mechanical characteristics similar to those of polypropylene and polystyrene, and were superior to low-density polyethylene films as shown in Table 3.

In general, the veratraldehyde–chitosan films had even better mechanical characteristics when obtained from solutions not submitted to dialysis, thus it would seem that excess veratraldehyde would irreversibly react in an unknown way when the concentration becomes very high.

The films obtained from hydrogenated products or from the aldimine films upon hydrogenation, once dry were found to be insoluble in all aqueous media tested, including dilute solutions of hydrochloric acid, sulphuric acid and sodium hydroxide.

Veratraldehyde–chitosan films made with a Brabender mixer

In a Brabender variable speed mixer, the chitosan (17 g) soaked with water (38 g) was stirred at 100 rev/min for 20 min to ensure complete swelling. Upon addition of glacial acetic acid (17 g) chitosan dissolution took place and was complete 1 h later, as revealed by the optical microscope. The veratraldehyde solution was then added (11 g veratraldehyde and 5 g glacial acetic acid). At the chamber temperature set at 50°C, the reaction of veratraldehyde with chitosan was fast: the product extruded 20 min after mixing was put in an oven at 120°C under vacuum and a transparent and somewhat elastic film was obtained. The films, once completely dry, did not smell of acetic acid and were water insoluble. Further treatments were deemed unnecessary, nevertheless it was noted that the films in a 4% aqueous sodium borohydride solution became opaque and were endowed with good mechanical characteristics.

Biodegradability tests

Preliminary examination of the biodegradability of the products was done by adding lysozyme (10 mg) to a 1·4% solution (100 ml) at pH 4·7, and recording the viscosity drop during 50 min. The films were further found to be biodegradable to at least 50% over 60 days according to the Sturm test or with respect to ASTM G21.

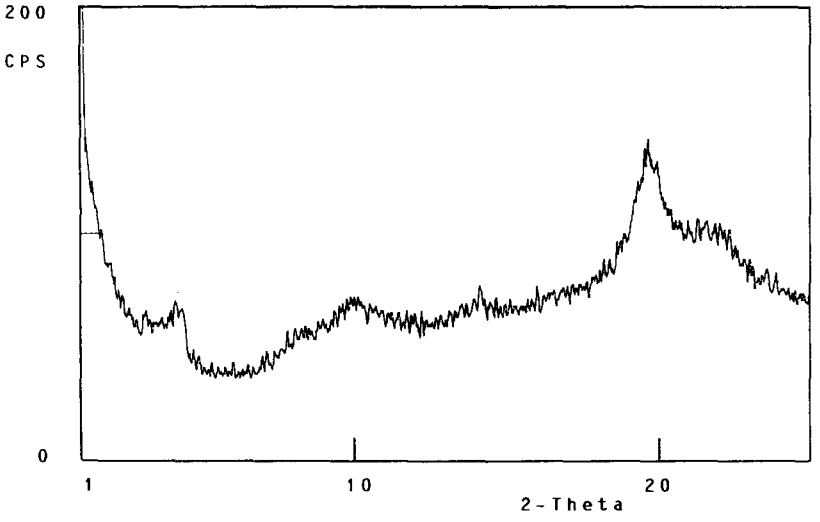

Fig. 4. X-ray diffractogram of the product obtained as follows: to a suspension of lignin (32 g) and chitosan (32 g) in water (160 g) under stirring, lactic acid (6 g) was added. The resulting paste was dried at 50°C. The peak at 21·88 indicates a novel supramolecular order.

Lignin and chitosan

Lignin (30 g) was first dispersed in water (150 g) with the aid of a homogenizer to disrupt the aggregates, then chitosan powder (30 g) was added and stirring continued for at least 30 min, finally an acid (malic, 6 g in 10 ml water) was added under vigorous stirring (pH 7·6). A very viscous paste was obtained in a matter of minutes; it was spread on a perforated aluminum board and dried at 50°C in a ventilated oven, to yield an extremely hard material. In spite of the fact that both chitosan and lignin separately were amorphous, X-ray analysis revealed that a certain degree of crystallinity was achieved upon mixing and drying (Fig. 4).

CONCLUSIONS

By taking advantage of a group of methoxyphenyl aldehydes, the typical functions of lignin can be introduced into chitosan: hybrid compounds possessing the typical insolubility of lignin and the filmogenic nature of chitosan can be obtained in the case of veratraldehyde.

All of the modified chitosans possess a certain degree of crystallinity. In particular, *o*-vanillin is collected to the greatest extent on chitosan, thus introducing the most evident changes in the FTIR spectrum and in the X-ray diffractograms. It leads to fairly insoluble materials, hydrogenated or not; the aldimine is itself a bright yellow insoluble and crystalline product. When added to chitosan solutions, alcoholic solutions of *o*-vanillin promote gelation and yield rigid self sustaining gels stable for long.

This class of aldehydes leads to insoluble or sparingly soluble modified chitosans, as a point of difference from unsubstituted aliphatic (lower *n*-alkyl aldehydes) and aromatic (benzaldehyde) aldehydes. There is a striking difference in characteristics between the vanillin–chitosan and the *o*-vanillin–chitosan, especially in terms of filmogenicity: the *o*-isomer depresses filmogenicity while the *p*-isomer does not.

In this work we have identified veratraldehyde as a suitable and convenient compound for the production of fully biodegradable films of technological interest: under suitable shear, the dissolution of chitosan can be accelerated in a minimum quantity of water and the reaction with veratraldehyde can be completed in minutes at temperatures slightly higher than room temperature.

Finally, a novel material made of chitosan and lignin has been made available, suitable for the preparation of biodegradable and mechanically resistant shaped objects.

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