Hydrophobic Chitosan Microparticles: Heterogeneous Phase Reaction of Chitosan with Hydrophobic Carbonyl Reagents

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Hydrophobically modified chitosan microparticles were produced by various syntheses carried out as heterogeneous phase reactions. Perfluorinated carbonyl components such as carbonic acids and acid chlorides were used as hydrophobization agents. To enhance the layer stability preceding cross-linkages between the chitosan macromolecules were introduced. These cross-linkages involve some of the chitosan amino groups. The use of a highly reactive alkylene−maleic anhydride copolymer produces cross-linkages and hydrophobizes the chitosan layer simultaneously in a one-step reaction. The heterogeneous reactions serve as model reactions for the hydrophobization of chitosan films deposited on supports.

Introduction

Chitin is the second most abundant naturally occurring polymer. The largest sources are the exosceletons of Arthropoda, especially crabs (Malacostraca) and insects (Insecta) as well as the cell walls of fungi. The highly degradation resistant and insoluble chitin is the prepolymer for the production of chitosan, which is yielded by basic deacetylation of chitin.[1] The structure of chitosan (*N*-acetamino-2-desoxy-β-D-glycopyranose) is similar to that of cellulose, but bearing one amino group in place of the hydroxyl group in the C-2 position. Its origin and the high number of amino groups along the polymer chain provide the chitosan macromolecule with exceptional properties, namely, its chitosan bioactivity and biocompatibility in combination with a high synthetic potential. Chitosan is well-known as nontoxic and biologically degradable. The bioactivity is appearent in chitosan's styptic effect in supporting the cure of wounds.[2] The excellent muco-adhesion combined with anti-allergic, anti-bacterial, and anti-viral properties make chitosan excellently suitable for use as a soft and hard tissue replacement.[3−5] Soft tissue replacements are typically applied as polymer/chitosan hydrogels, which are able to incorporate aqueous biological liquids.[6,7] The mechanical stability and the resistance to hydrolysis of such ionic and interpenetrating networks can be improved by cross-linking reactions via the chitosan's amino groups.[8] Bifunctional or multifunctional molecules such as dialdehydes, dicarbonic acids, or their carbonyl derivatives seem to be suitable for cross-linkages. Furthermore, the degree of chitosan's cross-linking as well as the hydrophilic/hydrophobic properties control the water diffusion through the tissue material. The excellent film-forming ability of chitosan can be used for the production of polyelectrolyte multilayers or membranes.[9−13] Cross-linking reactions with epichlorohydrin or dialdehydes as well as with di- or tetrafunctionalized acids are well established.[10−16] Chitosan also seems to be suitable as a sustainable adhesion promoter in coating systems, opening the way to produce surfaces with pronounced wetting or dewetting behavior.[17]

The chitosan applications mentioned above and many other developments, especially in biomaterials research, the design of drug delivery systems,[7,18] and cell culture substrates,[19] require reactions controlling the degree of cross-linking, the mechanical stability, the wetting, swelling, and adsorption behavior. However, the exclusive water solubility of chitosan at pH < 5 strongly limits the reactive potential of chitosan. Many of the modification or cross-linking agents are insoluble in water. The use of solvent/solvent compatibilizers, e.g., dextrane, increases the synthesis expenditure, and the final product becomes more expensive. Reactions on the surfaces of chitosan particles, fibers, or films immobilized on supports offer a very promising way to avoid these limitations. In many cases, the effect of such heterogeneous reactions is only pronounced on the sample surface. In this way advancing properties of the chitosan sample can be maintained, while the surfaces' properties can be intensively varied and adapted to the sample's final application.

The aim of this work is the production of stable hydrophobically modified chitosan microparticles, where the hydrophobization reactions can be considered as models for the (ultra)hydrophobization of chitosan-based coating layers.[17] In principle, the hydrophobic derivatization of chitosan is accessible by N or O substitution reactions.[11,20−23] To achieve higher conversion degrees of the chitosan's functional groups highly reactive carbonyl compounds, such as anhydrides or acyl chlorides of aliphatic carbonic acids, were employed. Zhang et al. applied fluorocarbon compounds to enhance the hydrophobization effect.[24]

Network formation is a usual way of stabilization of polymer layers. In this manner thin chitosan layers or films deposited on supports can be stabilized by cross-linkages. Cross-linking reactions with epichlorohydrin and glutaraldehyde involve the functional groups of chitosan and lower the number of potential reaction centers for further chitosan derivatization reactions. Here, we discuss an alternative procedure that combines the cross-linking and hydrophobization reaction in one reaction step. Low-molecular-weight α,ω-bifunctional perfluorinated carbonic acid derivatives are able to form inter- and intramolecular hydrophobic bridges between chitosan monomers. Alternating

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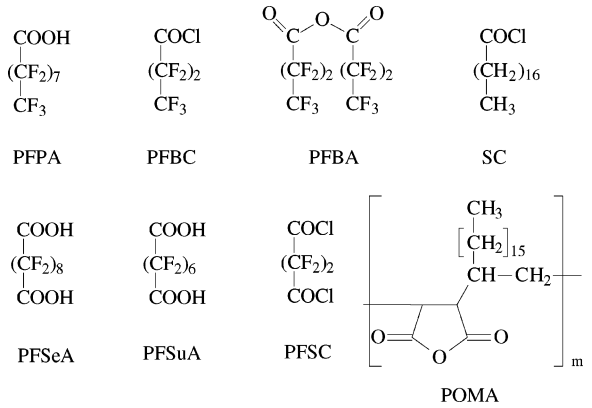
Scheme 1. Structures of Agents Employed to Hydrophobize Chitosan[a]



[a] PFPA = perfluoropelargonic acid, PFBC = perfluorobutyryl chloride, PFBA = perfluorobutyryl anhydride, SC = stearoyl chloride, PFSeA = perfluorosebacic acid, PFSuA = perfluorosuberic acid, PFSC = perfluorosuccinyl chloride, POMA = poly(octadecene-*alt*-maleic anhydride).

copolymers of long-chain alkylene units and maleic anhydride groups were also successfully used to cross-link and hydrophobize the chitosan surface.

Experimental Section

Materials. Chitosan (CHS) microparticles with diameters of 5−100 μm were kindly provided by BioLog Biotechnologie und Logistik GmbH, Halle-Queis, Germany. They were made from natural chitin by basic hydrolyzation. The degree of deacetylation was about 85%. It was used without further purification. The solvents dimethylsulfoxide (DMSO), 1,2-dichloroethane (DCE), chloroform, diethylether, dimethylformamide (DMF), tetrahydofuran (THF), pyridine, methanol, dioxan, and acetone (Ac) were purchased from Merck KgaA, Darmstadt, Germany. All solvents were purified according to conventional methods and freshly distilled before their use. Triethylamine (TEA, Merck KgaA, Darmstadt, Germany) was also distilled before its use. Formic acid (HCOOH), perfluorosebacic acid (PFSeA, or perfluorooctane-1,8-dicarbonic acid), and dimethylaminopyridine (DMAP) were purchased from Acros Organics (Geel, Belgium) and were used as received. Perfluorobutyryl chloride (PFBC), perfluorobutyryl anhydride (PFBA), perfluorosuberic acid (PFSuA, or perfluorohexane-1,8-dicarbonic acid), perfluorosuccinyl chloride (PFSC), dicyclohexylcarbodiimide (DCC), and *N*-ethylmorpholine (EM) were purchased from ABCR GmbH & Co KG, Karlsruhe, Germany, and were used as received. Perfluoropelargonic acid (PFPA, or perfluorononanoic acid), stearoyl chloride (SC, or octadecanoic acid), and poly(octadecene-*alt*-maleic anhydride) (POMA), M_n = 30 000−50 000 g/mol, were purchased from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, and were used as received. *N*-Hydroxysuccinimide (HSu), epichlorohydrin (EPC), and glutaraldehyde (GA) were purchased from Merck KgaA, Darmstadt, Germany, and were used without further purification. Scheme 1 shows the chemical structures of the hydrophobic reagents.

Cross-Linking Reaction with Epichlorohydrin. Exactly 1.000 g of CHS was suspended in a 1:1 mixture of NaOH/H_2O/Ac (V_{H_2O} = V_{Ac} = 70 mL; $n_{NaOH} = n_{CHS} + 2n_{EPC}$) and warmed to 50 °C. Under stirring the convenient mass of EPC was added dropwise to the suspension. After 2 h the reaction mixture was filtered and washed six times with a 1:1 mixture of H_2O/Ac until the filtrate was neutral. Then, the cross-linked CHS microparticles were extracted with methanol in a Soxhlet extractor for 3 days.

Cross-Linking Reaction with Glutaraldehyde. At 20 °C, 0.500 g of CHS was suspended in 10 mL of H_2O. Under stirring an aqueous GA solution (c = 0.031−0.156 mmol/mL) was added to the suspension. After 2 h the reaction mixture was filtered and washed six times with H_2O and methanol, respectively.

Reactions of CHS with Perfluorinated Carbonic Acid Derivatives. All reactions with non- and perfluorinated carbonic acid derivatives (carbonic acids, acid chlorides, and anhydrides) were performed under a dry argon atmosphere.

Reaction of CHS with PFPA in DMSO/DCE (Samples CHS-04 and CHS-37). Under stirring 0.500 g of CHS, 0.038 g of DMAP, and 1.595 g of PFPA were suspended in a mixture of 5 mL of DCE and 20 mL of DMSO. The mixture was cooled in an ice bath. Then, 0.645 g of DCC dissolved in 15 mL of DCE was added dropwise to the suspension. During the addition the suspension warmed to room temperature. After 30 h kept on room temperature the mixture was filtered. The CHS microparticles were washed several times with DCE and extracted with acetone in a Soxhlet extractor for 2 days. To prepare sample CHS-37 the appropriate amount of PFSeA instead of PFPA was used.

Reaction of CHS with PFPA in DMF (Samples CHS-07 and CHS-08). The reaction was carried out according to ref 25. The modified CHS microparticles were extracted with acetone in a Soxhlet extractor for 3 days. To prepare sample CHS-08 the appropriate amount of PFSuA instead of PFPA was used.

Reaction of CHS with PFPA in THF (Samples CHS-09 and CHS-10). Under stirring 1.000 g of CHS, 0.076 g of DMAP, and 3.000 g of PFPA were suspended in 40 mL of THF. Then, 0.5 mL of TEA was added. The mixture was cooled in an ice bath. Then, 1.290 g of DCC dissolved in 15 mL of THF were added dropwise to the suspension. After 1 h the suspension was warmed to room temperature. After 3 days the mixture was filtered. The CHS microparticles were washed several times with acetone and methanol. Then, they were extracted with acetone in a Soxhlet extractor for 2 days. To prepare sample CHS-10 the appropriate amount of PFSuA instead of PFPA was used.

Reaction of CHS with PFBC in Pyridine (Samples CHS-11 and CHS-12). Exactly 1.000 g of CHS was suspended in 100 mL of pyridine. The suspension was cooled in an ice bath. Under stirring 1.465 g of PFBC was added through a septum. After 1 h the mixture was warmed to 80 °C and kept for 1 h at this temperature. To separate the microparticles the suspension was filtered or centrifuged. The solid phase was washed several times with methanol and extracted with methanol in a Soxhlet extractor for 2 days. To prepare sample CHS-12 the appropriate amount of SC instead of PFBC was used, and the extraction was carried out with diethylether.

Reaction of CHS with PFPA in Formic Acid (Samples CHS-13, CHS-14, and CHS-15). The reaction was carried out according to ref 24. The modified CHS microparticles were extracted with methanol as well as diethylether in a Soxhlet extractor for 3 days, respectively.

Reaction of CHS with PFBA in Methanol (Sample CHS-21). Exactly 1.000 g of CHS was suspended in 20 mL of methanol. Under stirring 5.166 g of PFBA was added through a septum. The suspension was slightly cooled and stirred overnight at 20 °C. The obtained gel was diluted with some methanol and neutralized with 10 wt % NaOH. The gelatinous precipitate was filtered off and extracted with methanol as well as chloroform in a Soxhlet extractor for 2 days, respectively.

Reaction of CHS with PFSC in Dioxan (Sample CHS-22). Exactly 1.000 g of CHS was suspended in 40 mL of dioxan. Then, 0.92 mL of TEA was added. Under stirring a slightly cooled (10 °C) solution of 1.430 g of PFSC dissolved in 10 mL of dioxan was added through a septum. For 1 h the suspension was kept at 10 °C, then warmed to room temperature and stirred for another hour. The suspension was put into an ice−water mixture (thaw). The modified CHS microparticles were filtered off, washed several times with water and methanol, and extracted with methanol as well as chloroform in a Soxhlet extractor for 2 days.

Reaction of CHS with POMA in Different Solvents (Samples CHS-23, CHS-25, CHS-28, and CHS-29). Exactly 1.000 g of CHS was suspended in 20 mL of an organic liquid that is able to dissolve POMA (THF or Ac; Table 2). Then, a solution of 2.200 g of POMA dissolved in 100 mL of the organic liquid and 0.88 mL of TEA was

Table 1. Results of the Reactions of Carbonyl Reagents with Chitosan Microparticles[a]

				results				
						XPS		
chitosan sample	carbonyl reagent	suspending agent/solvent	auxiliary agents	DRIFT	NMR[b]	[N]:[C]	[F]:[C]	DF (%)
pure CHS						0.124	0.095[c]	
CHS-04	PFPA	DMSO/DCE	DCC, DMAP	F-bands, amide bands	C_{amide} at 164 ppm	0.117	0.377	20
CHS-07	PFPA	DMF	DCC, HSu, EM	F-bands, amide bands		0.118	0.160	8
CHS-08	PFSuA	DMF	DCC, HSu, EM	uncertain		0.089	0.043	3
CHS-09	PFPA	THF	DCC, DMAP, TEA	F-bands, amide bands		0.107	0.192	9
CHS-10	PFSuA	THF	DCC, DMAP, TEA	uncertain		0.106	0.026	2
CHS-11	PFBC	pyridine		uncertain		0.106	0.104	12
CHS-12	SC	pyridine		uncertain		0.082	0.035[c]	
CHS-13	PFPA	HCOOH		F-bands, amide bands	C_{amide} at 164 ppm	0.088	0.461	27
CHS-14	PFPA[d]	HCOOH		F-bands, amide bands	C_{amide} at 164 ppm	0.073	0.555	34
CHS-15	PFPA[e]	HCOOH		F-bands, amide bands	C_{amide} at 164 ppm	0.078	0.493	29
CHS-21	PFBA	methanol		uncertain		0.136	0.146	8
CHS-22	PFSC	dioxan	TEA	uncertain		0.081	0.018	3
CHS-37a	PFSeA	DMSO/DCE	DCC, DMAP		C_{amide} at 164 ppm	0.087	0.273	15
CHS-37b	PFSeA	DMSO/DCE	TEA, DCC, DMAP		C_{amide} at 164 ppm	0.105	0.177	9

[a] The reactions were performed in suspensions except for samples CHS-13, reaction on dissolved chitosan, and CHS-14 and CHS-15, reactions on strongly swollen chitosan. DF values were calculated from XPS data according to eq 1. [b] $^{13}C\{^1H\}$-CP-MAS NMR. [c] Traces of fluorine are caused by the production of the chitosan microparticles. [d] The chitosan sample was cross-linked with EPC $n_{EPC}/n_{CHS} = 2$. [e] The chitosan sample was cross-linked with EPC $n_{EPC}/n_{CHS} = 10$.

Table 2. Reaction Conditions of the Modification of Chitosan Microparticles with POMA

chitosan sample	solvent	conditions
CHS-23a	THF, not dried	20 °C, 2 h
CHS-23b	THF, not dried	50 °C, 5 h
CHS-25a	THF, dried	20 °C, 2 h
CHS-25b	THF, dried	50 °C, 2 h
CHS-28a	methanol/THF	20 °C, 4 h
CHS-28b	methanol/THF	50 °C, 4 h
CHS-29a	formamide/THF	20 °C, 20 h
CHS-29b	formamide/THF	50 °C, 5 h
CHS-29c	acetone	20 °C, 1 h
CHS-29d	acetone	20 °C, 1 h, annealing 3 h at 120 °C

added. In the cases of the solvent mixtures methanol/THF and formamide/THF the chitosan was suspended in 100 mL of methanol or formamide, respectively, and a solution of POMA in 20 mL of THF was added. The reaction suspension was stirred for 1−4 h at 20 or 50 °C. The suspension was filtered off. The modified CHS microparticles were washed several times with acetone and extracted with acetone in a Soxhlet extractor for 2 days.

Elemental Analysis. The quantitative elemental analysis (EA) was carried out as combustion analysis employing a Vario EL device, Elementar Analysensystem GmbH, Hanau, Germany.

Solid-State NMR. The solid state $^{13}C\{^1H\}$ cross-polarization magic-angle spinning (CP MAS) NMR spectra were measured on a Bruker AMX 400 spectrometer at room temperature employing 4 and 7 mm ZrO_2 rotors. ACD/Labs, version 6.00, has been used for theoretical calculations of the NMR signals with respect to possible structure elements.

Infrared Spectroscopy. Diffuse reflection infrared Fourier transform (DRIFT) spectra were recorded with an IR spectrometer (FTS 165, Biorad, Germany).

X-ray Photoelectron Spectroscopy. All X-ray photoelectron spectroscopy (XPS) studies were carried out with an Axis Ultra photoelectron spectrometer (Kratos Analytical, Manchester, U. K.). The spectrometer was equipped with a monochromatic Al Kα ($h\nu = 1486.6$ eV) X-ray source of 300 W at 15 kV. The kinetic energy of the photoelectrons was determined with a hemispheric analyzer set to a pass energy of 160 eV for wide-scan spectra and 20 eV for high-resolution spectra, respectively. During all measurements electrostatic charging of the sample was overcompensated by means of a low-energy electron source working in combination with a magnetic immersion lens. Later, all recorded peaks were shifted by the same amount, which was necessary to set the C 1s peak to 285.00 eV for saturated hydrocarbons.[26] Quantitative elemental compositions were determined from peak areas using experimentally determined sensitivity factors and the spectrometer transmission function. The spectrum background was subtracted according to Shirley.[27] The high-resolution spectra were deconvoluted by a computer routine. Free parameters of component peaks were their binding energy (BE), height, full width at half-maximum, and the Gaussian−Lorentzian ratio.

The degree of functionalization (DF) of the modified chitosan microparticles was calculated from the elemental ratio [F]:[C] obtained from the XPS wide-scan spectra. For a given fluorinated carbonyl reagent $C_aH_bF_cO_d$, where a denotes the number of carbons and c the number of fluorine atoms, DF was calculated according to eq 1

$$\mathrm{DF} = \frac{\frac{[\mathrm{F}]}{[\mathrm{C}]}\,7.41}{c - \left(\frac{[\mathrm{F}]}{[\mathrm{C}]}\,a\right)} \times 100\% \qquad (1)$$

The derivation of eq 1 is given in the Supporting Information.

Results and Discussion

Two different concepts were pursued in this work. The first approach is based on two separate steps: a cross-linking and a hydrophobization step (described in detail in the first and second parts). For the latter several carbonic acid derivatives were used. The other concept implies a one-step reaction for the cross-linking and hydrophobization using the reactive copolymer POMA (described in the third part).

Cross-Linking Reactions. Cross-linking reactions on the surfaces of chitosan microparticles were carried out in heterogeneous phases following the related procedures in refs 10 and 28. The produced materials were investigated by elemental

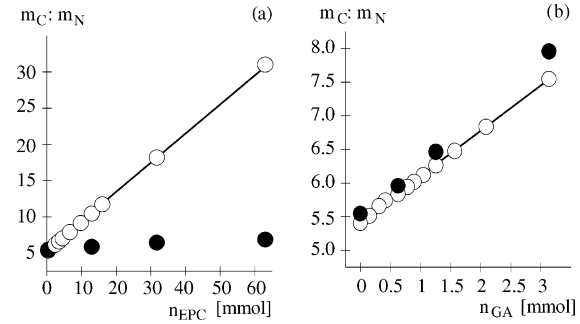


Figure 1. Mass ratios m_C/m_{N-} of chitosan microparticles after cross-linking reactions with (a) EPC and (b) GA vs the amount of the cross-linking agents. $m_{CHS} = 1$ g. Closed circles (●) show the results of the combustion elemental analysis; open circles (○) indicate the theoretically calculated values.

analysis, DRIFT spectroscopy, and $^{13}C\{^1H\}$ CP MAS NMR spectroscopy.

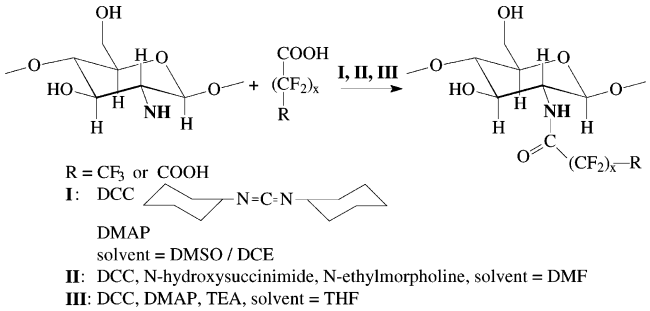
Although the ratio of the educts n_{EPC}/n_{CHS} was increased up to 10, the cross-linking with EPC only slightly increased the m_C/m_N ratio, which was determined by elemental analysis (Figure 1). The findings indicate that the cross-linking procedure with EPC does not effectively work.

However, it must be taken into account that the combustion elemental analysis analyzes the whole chitosan microparticle, while the reaction only took place on the particle surface and did not influence the particle's bulk composition. Nevertheless, the reaction of EPC does not only occur on the surface amino groups. The epoxide ring also can be opened by the acidic hydrogen of the chitosan's OH groups and the formed OH groups during the EPC's ring-opening reaction. In this way EPC forms its own homopolymer networks that are anchored on the particle surface or dissolved in the surrounding acetone/NaOH solvent. The small selectivity of the EPC cross-linking reaction seems to be disadvantageous to control the cross-linking reaction and its degree. But, later it will be shown that during the EPC's cross-linking procedure numerous amino groups will remain on the particle surface that are accessible to further derivatization reactions.

Employing glutaraldehyde (GA) to cross-link the amino groups on the chitosan microparticle surface the m_C/m_N ratio corresponds to the theoretically calculated ratios (Figure 1). The aldehyde groups of GA solely react with the amino groups of chitosan, thus forming azomethine units. Obviously, the method is more effective and selective. Furthermore, the reaction can performed in aqueous suspension without the addition of organic liquids. Water is able to penetrate the chitosan particles and introduce GA into the particle's bulk phase. Hence the cross-linking reaction is not only localized on the particle surface. High degrees of cross-linkage significantly lower the number of reactive amino groups and the possibility for further derivatization reactions. In spite of this disadvantage the cross-linking with GA seems to be suitable to easily control the degree of cross-linking by the molar ratio of the starting substances.

Reactions of the Chitosan Microparticles with Hydrophobic Carbonic Acid Derivatives. For the hydrophobic modification of chitosan several mono- and dicarbonic acid derivatives were used (Scheme 1). To enhance the hydrophobization effect most of them have perfluorinated alkyl groups. Different solvents and auxiliary agents (Table 1) were used to find effective conditions to convert the chitosan's amino groups with the acid derivatives.

Scheme 2. Reaction of Chitosan with Fluorinated Acids under Different Conditions



Quantitative XPS measurements were employed to determine the functionalization degree of chitosan microparticle surfaces. The method is extremely surface-sensitive and does not give information from more than 8 nm of the sample depth. That means that the XPS method analyses the region where the heterogeneous condensation reaction of the carbonic acid derivatives on the chitosan takes place. To support the XPS findings the modified samples were also investigated by DRIFT and $^{13}C\{^1H\}$ CP MAS NMR spectroscopies (Table 1).

High functionalization degrees were yielded in suspensions of DMSO/DCE containing DCC and DMAP as auxiliary agents (Scheme 2). Surprisingly, the carbonic acid PFPA gave the highest functionalization degree (sample CHS-04). The degrees of functionalization (DFs) obtained with the dicarbonic acids PFSuA and PFSeA were always lower. Obviously, each of the two COOH groups is able to react with an amino group. In this way numerous amino groups are involved in the derivatization reaction, but the number of introduced fluorine atoms remains rather small. The two-site reaction of the dicarbonic acids is an additional possibility for cross-linking the chitosan.

The maximum conversions during the heterogeneous condensation reactions were not higher than DF = 20%. Reactions carried out in homogeneous phases (CHS-13) or with strongly swollen cross-linked chitosan microparticles (samples CHS-14 and CHS-15) are characterized by distinctly increased reaction degrees (Table 1). The findings are not surprising because the dissolved and swollen species offer a higher number of accessible amino groups.

The utilization of activated derivatives such as anhydrides or acid chlorides for the modification of chitosan microparticles should increase the degree of functionalization. However, the results with the perfluorinated derivatives shown in Table 1 demonstrate a moderate modification effect. The reaction with stearoyl chloride (CHS-12) resulted in a hydrophobic product that was insoluble in 1% acetic acid. In this case DF was not unambiguously quantifiable. The [N]:[C] ratios obtained from XPS analysis are obviously lowered. This points to a higher functionalization as with the fluorinated chlorides.

The properties of the surface fluorinated samples correspond to their functionalization degrees. Pure chitosan is soluble in 1% acetic acid. The lesser functionalized samples CHS-08 and CHS-10 are also soluble under these conditions. In contrast samples having a higher DF (CHS-04, CHS-13, CHS-14, and CHS-15) are completely insoluble in acetic acid. The particles' shapes remain stable. Furthermore, the modification affects hydrophobic surface properties. The pure chitosan particle surface has a pronounced hydrophilic character because the outstanding amino and OH groups lead to a high surface polarity. Pure chitosan particles can be easily suspended in water. In contrast, the hydrophobized chitosan particles are not wetted by water and cannot be stably distributed in the aqueous

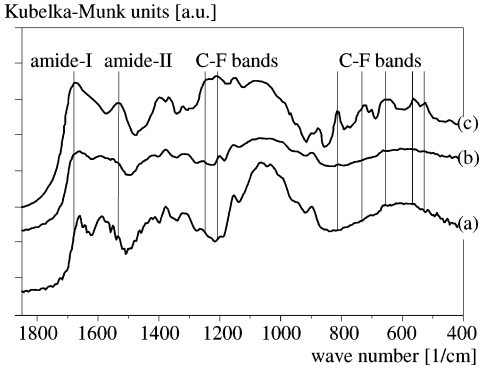


Figure 2. DRIFT spectra of (a) unmodified chitosan microparticles, (b) chitosan microparticles modified with PFSuA (sample CHS-08), and (c) chitosan microparticles modified with PFPA (sample CHS-04).

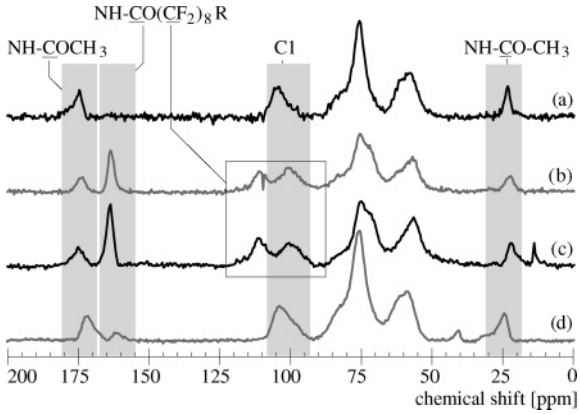


Figure 3. $^{13}C\{^1H\}$ CP MAS NMR spectra of (a) unmodified chitosan microparticles, (b and c) chitosan microparticles modified with PFPA (b, sample CHS-04; c, sample CHS-13), and (d) chitosan microparticles modified with PFSeA (sample CHS-37a). The rotational frequency was 12 kHz for all measurements.

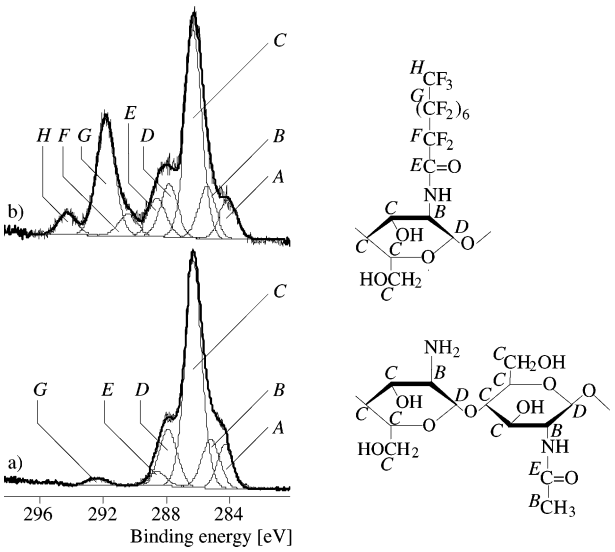


Figure 4. High-resolution C 1s spectra of (a) unmodified chitosan microparticles and (b) chitosan microparticles modified with PFPA. The assignment of the component peaks to the different carbon species is described in the text and illustrated by the structural formula.

phase. Samples with a moderate conversion of the surface amino groups (samples CHS-07, CHS-09, CHS-11, CHS-21, and CHS-22) are able to swell, and some of them dissolve partly in the 1% acetic acid. Quantitative changes in the particles' surface free energy by introduction of fluorine-containing alkyl groups will be reported in a separate communication.

Carbonic acids and their derivatives cannot only react with the amino groups of the chitosan but also with hydroxyl groups. Additional simple noncovalent electrostatic bonds of protonated amino groups and negatively charged carboxylate groups as well as strong hydrogen bridge linkages are possible. Several spectroscopic methods were employed to obtain information about the bonds between the chitosan surface and the carbonyl derivatives. The insolubility of the modified chitosan particles required us to study them as solids.

Figure 2 shows DRIFT spectra of unmodified and PFPA-modified chitosan particles (CHS-04). The primary amino group of chitosan is recognized by two bands at 1660 and 1586 cm^{-1}.[15] After the condensation reaction with PFPA the amino bands diminish and are overlaid by two new bands in this region. At 1675 cm^{-1} the amide I and at 1531 cm^{-1} the amide II band appear. Furthermore, bands at 1247 and 1208 cm^{-1} and in the region between 812 and 530 cm^{-1} (thin solid lines in Figure 2) show fluorinated aliphatic chains. Together with the intensive amide bands they prove the preferable existence of covalent bonds between the chitosan's amino groups and the carboxylic groups of the fluorinated carbonic acid.

In case of the dicarbonic acids (e.g., sample CHS-08) neither the amide bands nor the bands for the fluorinated alkyl chains were clearly detected (Figure 2b). A slightly changed shape of the bands in the region between 1500 and 1700 cm^{-1} and small peaks between 1200 and 1250 cm^{-1} indicate that the condensation reactions reached only low degrees of functionalization. These findings correlate with the observed solubility of the sample and confirm the results of XPS.

Although, carbonic acid derivatives are able to esterify alcoholic OH groups, no ester bands were found in their typically expected region between 1730 and 1770 cm^{-1}. Obviously, the amino groups are the most reactive species, and the functionalization reactions with the carbonic acid derivatives exclusively occur via the amino groups on the chitosan's particle surface.

$^{13}C\{^1H\}$ CP MAS NMR spectra of pure chitosan and recorded after its reaction with PFPA and PFSeA support the findings of the IR studies (Figure 3). After the condensation of PFPA or PFSeA the spectra show a new peak at about 160−167 ppm. It is clearly distinguishable from the acetamido carbon (appearing from N-acetylated units of chitosan) of the chitosan at 170−175 ppm. The new signal was assigned to the carbon of the amide bond, which has a fluoroalkyl group in its immediate neighborhood. In Figures 3b and 3c the corresponding fluorinated carbons are marked in the inset. Computer simulations of the expected structures showed quite similar values of the chemical shift for the amide (NH−CO−R) and possible ester carbons (O−CO−R). Hence, in the $^{13}C\{^1H\}$ CP MAS NMR spectra it is difficult to discriminate between these linkages.

As mentioned above, XPS studies on the modified chitosan samples showed the introduction of fluorine (Table 1). That can be considered as an indication of a successful reaction course. Beside the quantification of the wide-scan spectra, the analysis of the high-resolution C 1s spectra allows us also to obtain structural information about the kind and number of functional surface groups and their reaction products. Figure 4a shows the C 1s spectrum of the untreated chitosan microparticle. The shape of the spectrum is typical of that for polysaccharides. According to the structural formula of chitosan the C 1s spectrum was deconvoluted into four component peaks indicated with B, C,

D, and E. To fit the component peaks to the measured spectrum it was cogently necessary to introduce two further component peaks A and G. As shown in Figure 4a, component peaks C and D appear from the *C*−O−(H,C) bonds (C) and the acetal group (D, O−*C*−O) of the saccharide ring. The intensity ratio of the two component peaks equals [D]:[C] = 1:4, the given stoichiometric ratio. Component peak B shows the *C*−NH_2 and *C*−NH−C═O bonds remaining from nonhydrolyzed chitin species. The corresponding amide carbon atoms (C−NH−*C*═O) contribute to component peak E. Saturated hydrocarbons, which must be considered as typical surface contaminations, give component peak A. Component peak G shows that the fluorine traces found in the wide-scan spectrum (F 1s peak) are organically bonded to carbon.

The C 1s spectrum of the chitosan sample modified with PFPA (sample CHS-04) shows pronounced differences in the region of higher binding energies, while the component peak ensemble appearing from the polysaccharide remains nearly constant (Figure 4b). The fluorinated alkyl chains increase the intensity of the component peak G. The *C*F_2 groups (G) can be clearly separated from the *C*F_3 head groups (component peak H). Near the component peak of the *C*F_2 groups a new component peak F was observed. That component peak appears from the fluorinated methylene group, which is in the β-position to the amide nitrogen (F_2C−*C*O(NH)). Agreeing with the stoichiometry it has an intensity equal to that of component peak H. The presence of component peak F is a further conclusive piece of evidence that during the carbonic acid reaction amide bonds are preferably formed. The formation of amide bonds also increases the intensity of the amide component peak E. Its intensity equals the sum of the intensities of component peak E of the untreated chitosan sample plus the intensity of the *C*F_3 component peak (H) or the intensity of the methylene groups in the β-position to the amide nitrogen [F].

Reactions of the Chitosan Microparticles with POMA. The aim of the work was to control the surface properties of chitosan microparticles by hydrophobization and cross-linking reactions. Above, it was shown that fluorinated carbonic acids react with the amino groups on the chitosan surface, thus forming stable amides. The idea to hydrophobize and cross-link the chitosan microparticle surface in a one-step reaction did not successfully work because the reaction degrees with dicarbonic acids were to low to achieve changes in chitosan's surface polarity.

Maleic anhydride copolymers with hydrophobic side chains open a new synthetic way to cross-link and hydrophobize chitosan surfaces in a one-step reaction. Polymers containing maleic anhydride units can be considered as polyacid derivatives having a high reactivity to amino and OH functionalities.[29,30] The high reactivity of the maleic anhydride copolymers with amino groups was used to render textiles repellent to water and oil[31] and impregnate leather hydrophobically.[32,33] Already a small reaction degree enables a high modification effect because for the bonding of one macromolecule POMA containing a high number of alkyl groups only a few linkages to the chitosan are needed. The reaction of one maleic anhydride copolymer chain with several amino groups of chitosan can be considered as a cross-linkage stabilizing the chitosan surface and enhancing the hydrophobization effect.

We employed POMA as a suitable cross-linking and hydrophobizing copolymer. It is well-known that alkyl chains longer than 11 carbons in length significantly reduce the surface free energy.[34] In that way, it was reported that chitosan layers modified with SC showed excellent hydrophobic properties.[11]

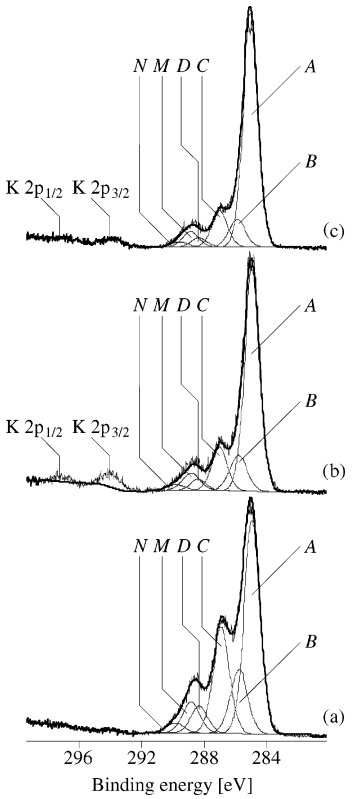


Figure 5. High-resolution C 1s spectra of chitosan microparticles modified with POMA (a, sample CHS-25b; b, sample CHS-29a; c, sample CHS-29b). The assignment of the component peaks to the different carbon species is described in the text.

To hydrophobize chitosan microparticles SC was applied, too. The produced particles showed properties related to the surface fluorinated samples. Hence, POMA was used to cross-link and hydrophobize the surfaces of chitosan microparticles in heterogeneously performed reactions. Table 2 summarizes the reaction conditions used for the modification reaction.

The reaction was carried out under different reaction conditions, but no significant differences in the final products' properties were observed. All POMA-modified chitosan microparticles show hydrophobic behavior (not wetted by water, floating on the top). Short reaction times and mild conditions seem to be sufficient to produce chitosan particles with very hydrophobic surface properties. However, after a longer storage in 1% acetic acid (ca. 1 h) the modified microparticles are gelatinously dissolved. The unmodified chitosan microparticles are more rapidly and completely dissolved in 1% acetic acid.

The hydrophobic surface properties and the gradual swelling over time indicate a successful reaction between the chitosan microparticle surface and POMA. The observed swelling in aqueous acetic acid forming a gelatinous solution demonstrates that the access of water molecules to the chitosan bulk is possible and H^+-initiated dissolution processes can take place in the interiors of the chitosan microparticles. A complete dissolution of the chitosan particle is prevented by the outer cross-linked particle layer. In this way, the POMA modification opens a way to control the swelling behavior of chitosan particles.

In the IR spectra, the POMA-modified chitosan samples do not show anhydride bands that are expected to be in the region between 1858 and 1778 cm^{-1}. The corresponding $^{13}C\{^1H\}$ CP MAS NMR spectra show a new peak at about 30 ppm that was assigned to the main part of the carbons of the octadecyl groups.

The successful reaction of POMA on the chitosan surface can be demonstrated by the C 1s XPS spectra (Figure 5).

Scheme 3. Bond Types Resulting from the Reaction of POMA and Chitosan on the Surface of the Chitosan Microparticles

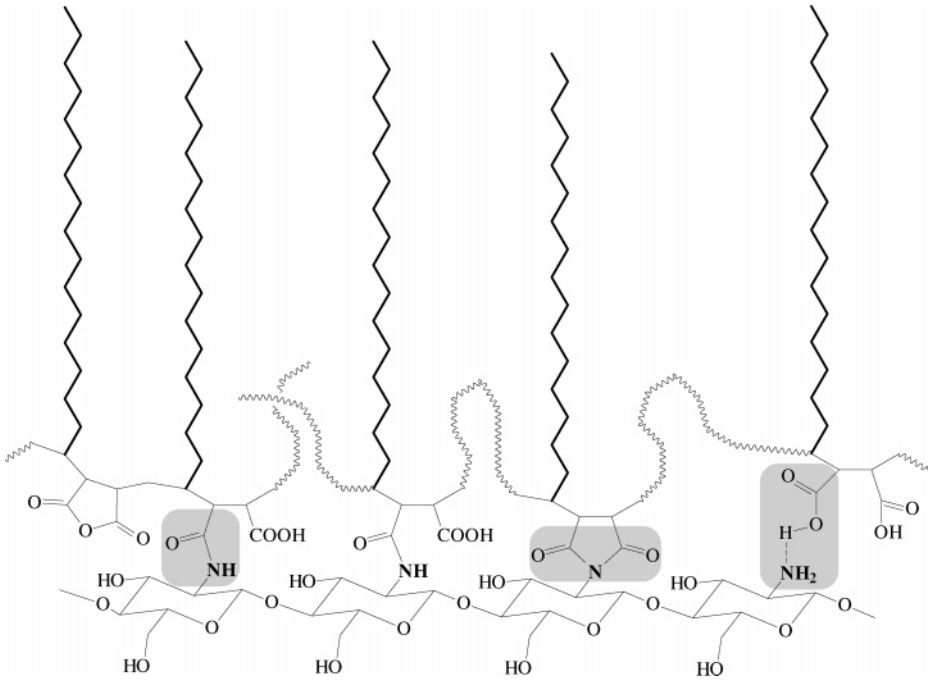


Figure 5 shows a series of C 1s XPS spectra recorded from chitosan samples modified with POMA under different conditions. Compared to the C 1s spectrum of the unmodified chitosan microparticles (Figure 4a) the high intensity of the hydrocarbon component peak A attracts attention. The increased intensity results from the octadecyl comonomer of POMA. The corresponding maleic anhydride unit, which was partly involved in the reaction with the chitosan's functional surface groups, is indicated as component peaks M and N. Component peak M was found at BEs around 288.5 eV, which is significantly too low for anhydride groups ($BE_{anhydride} \approx 289.4$ eV[26]) but typical for imide and amide carbon atoms. The latter are the expected bond types resulting from the reaction of POMA with the amino groups of the chitosan microparticles. Component peak N at BE $\approx$ 289.4 eV shows residual anhydride groups or their hydrolyzed species (dicarbonic acid and carboxylate groups). After the reaction of chitosan and POMA, component peak B is the composite of chitosan's *C*−N bonds (see also Figure 4) and the *C*H carbon atoms in the α-position to the *C*═O carbon atoms (deriving from POMA, producing component peaks M and N) (see also Scheme 3). The two component peaks C and D appearing from the chitosan structure are described above.

Figure 5 also demonstrates that the reaction conditions control the POMA's reaction degree. However, in all samples covalent amide or imide bonds between POMA and chitosan were detected. Beside these stable covalent bonds electrostatic interactions between protonated amino groups and deprotonated carboxyl groups and the formation of hydrogen bridge linkages cannot be excluded.

These analytical data are explicit hints for the covalent attachment of POMA at the surfaces of the chitosan microparticles. Scheme 3 shows the different bond types possibly accrued during the reaction of the anhydride groups of POMA with chitosan.

Both investigated methods for the hydrophobization of chitosan seem to be practicable also on chitosan layers. Especially the POMA method is a very facile and effective procedure. The application of the described methods for the hydrophobization of chitosan layers on flat supports will be presented in a subsequent paper.

Conclusions

The reaction of chitosan microparticles with several perfluorinated acid derivatives was examined in mainly heterogeneous reactions. The hydrophobic acid derivatives were linked by amide bonds onto the chitosan. This was proved by IR, $^{13}C\{^1H\}$ CP MAS NMR, and XPS analysis. The degrees of functionalization differ in dependence on the reaction conditions but do not exceed 20%. With this method it is possible to control the hydrophobicity up to a point. Hydrophobization and cross-linking is achieved in a one-step reaction by reaction of chitosan with the bifunctional copolymer POMA. This procedure is easier and more effective than the reaction with the low-molecular-weight compounds and yields highly hydrophobized particles. X-ray photoelectron spectroscopy C1s spectra indicate that the linkages between POMA and chitosan comprise amide and imide bonds.

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Supporting Information Available. Derivation of eq 1 and $^{13}C\{^1H\}$ CP MAS NMR spectrum of the POMA-modified chitosan microparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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