3 Analysis of Polysaccharide Structures

A broad variety of specific methods for the structure analysis of polysaccharides, their interaction with different compounds such as solvents or inorganic salts, and the superstructures both in solid state and in solution have been established. An overview of methods and results for the superstructural behaviour of polysaccharides is given in [19]. The aim of this chapter is to present a review of the techniques that can be performed on commercially available equipment to elucidate the primary structure of polysaccharides.

It is an essential prerequisite to analyse the polysaccharides before modification as comprehensively as possible to monitor all types of structural changes of the polymer backbone during the conversion to a derivative. One should always keep in mind that purification beyond the removal of low molecular mass impurities is not reasonable. The basic RU of the polysaccharides described in the book are given in Chap. 2. Nevertheless, analysis of the polysaccharide in question is always recommended because the chemical structure, including branching, sequences of sugar units, oxidised moieties in the chain (e.g. aldehyde-, keto-, and carboxylic groups in polyglucans), and the residual amount of naturally occurring impurities vary for a given type of polysaccharide, especially for fungal and plant polymers, and may significantly influence the properties and reactivity.

A number of basic chemical methods have been developed for the structure analysis and the determination of the purity of polysaccharides. Most of these chemical analyses are colour reactions, which can be quantified by UV/Vis spectroscopy. A list of methods and the features determined is shown in Table 3.1.

In addition, for ionic polymers such as alginates or chitosan salt, titration can be exploited to obtain information about the number of functional groups within the polymer. Linear potentiometric titration is used for the determination of free amino functions in chitinous materials [45].

A value that should be analysed carefully before conversion of a polysaccharide to an ester is the amount of absorbed water in the starting polymer. This is possible by thermogravimetry or by amperometric titration with Karl Fischer reagent after water extraction. In the case of cellulose extraction, the most suitable extractants are DMF, acetonitrile and isobutanol [46].

Table 3.1. Summary of chemical methods used for structure determination of polysaccharides

Test	Method	λ_{max} (nm)	Detected structure	Ref.
Anthron	Anthron in H_2SO_4	620	Free and bound hexose on polysaccharides Blue: hexose, Green: other sugars	[41]
Oricinol	Oricinol in EtOH and $FeCl_3$ in HCl	665	Free and bound pentoses on polysaccharides Green to blue: pentose produce green to blue coloration	[40]
Phenol/ H_2SO_4	Phenol and H_2SO_4	485	Free and bound sugars in soluble and insoluble polysaccharides	[41]
Biphenylol	Hydroxybiphenylol in NaOH and borax in H_2SO_4	520	Free and bound uronic acid on polysaccharides (red to blue)	[42]
Cystein/ H_2SO_4	Cystein-HCl in H_2O and H_2SO_4	380, 396, 427	Free and bound 6-desoxyhexose on polysaccharides	[40]
PAHBAH	PAHBAH in HCl and NaOH	410	Reducing sugars	[43]
Updegraff	AcOH/H_2O/ HNO_3 (8:2:1)		Cellulose	[44]

3.1 Optical Spectroscopy

Besides the above-mentioned analysis of polysaccharides with UV/Vis spectroscopy after chemical treatment, optical spectroscopy is used for some semi-quantitative methods for the determination of the amount of functional groups (NH-CO-CH_3, COOH). The DDA value in chitinous material can be determined via UV/Vis measurements at 210 nm after dissolution in 85% phosphoric acid under thermal-controlled sonication [47].

Optical spectroscopy can be used to determine the conformation of structural features of pure polysaccharides and to easily monitor structural changes during modification. FTIR spectroscopy yields "fingerprint" spectra usable as structural evidence. The most common way for FTIR measurements is the preparation of KBr pellets. To obtain well-resolved spectra, it is necessary to apply a ball mill to guarantee homogeneous mixtures of KBr and the macromolecule. Usually, samples containing about 1–2% (w/w) polymer are prepared. Common "non-polymer" signals observed by means of FTIR spectroscopy are adsorbed water at about 1630–1640 cm^{-1} and CO_2 at about 2340–2350 cm^{-1}. A number of FTIR spectra obtained for the glucanes cellulose, starch, dextran and scleroglucan are shown in Fig. 3.1. The general assignment is given in Table 3.2.

Alginates show additional signals for the C=O moiety of the carboxylate at 1620–1630 and 1410–1420 cm^{-1} or at 1730 cm^{-1}, if the alginate is transferred to