Characterisation of Chitosan Molecular Weight Distribution by Multidetection Asymmetric Field-Flow Fractionation and SEC-MALS

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Molecular weight determination of chitosan is commonly conducted using techniques such as intrinsic viscosity measurements, light scattering techniques and/or size exclusion chromatography (GPC or SEC)[1]. All of these techniques require the complete dissolution of the polymer chains by the solvent (normally an acidic aqueous solution) to avoid inaccurate estimation of molecular weight (MW) due to the presence of intermolecular aggregates present even at low polymer concentrations (~1 mg/mL), as has been highlighted previously by several research works and which mechanism of self-association continues being debated[2]. A few recent studies have reported the use of asymmetric flow field flow fractionation (AF4) to characterise the MW distribution of chitosan[3-5]. However, the experimental conditions used, such as the type of separation membrane, carrier liquid and flow separation parameters, all of which can influence the outcome, have vaguely been reported, thus making the methods all the more difficult to reproduce. We present here a recently developed method for determination of the MW of chitosans by AF4 coupled with MALS and DRI detectors. The method highlights the power of the technique allowing the separation of molecularly dispersed polymer chains from that fraction of intermolecular aggregates typically found in solutions of chitosan, thus making possible the determination of molecular weight distribution of the polymer. We also evaluated the effect of different experimental conditions on the results obtained and compared them with those obtained from SEC-MALS-DRI under identical solvent conditions as for AF4. We have analysed a set of chitosan samples (both commercial and own-manufactured) from different biological sources (crustaceans, squid pen and fungi) and of varying degree of acetylation (DA ~1 to ~50%). AF4-MALS-DRI results revealed that although aggregates are significantly minimised when using a good solvent (acetic acid 0.3 M/sodium acetate 0.2 M), a minor fraction of the smaller size still remains, and it is only completely eliminated by filtration through small pore sizes (~0.2 μm). This can represent a limitation for the characterisation of high MW chitosan, as filtration of this type of samples can lead to considerably high amount of the material being retained in the filter. In this respect, the method described here has the advantage of not only allowing us to identify and separate the aggregates present in solution, but also to accurately determine the MW distribution for a wide range of chitosans of varying type.

References