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Note

Increased sensitivity of chitosan determination by a dye binding method

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Abstract—Chitosan is a topic of current research in pharmaceutics, medicine, biotechnology, and beyond. This note describes an improved quantification of chitosan using the dye Cibacron Brilliant Red 3B-A. The method is sensitive and of a good reproducibility and linearity in the range of 10–80 μg/mL.

Keywords: Chitosan; Cibacron Brilliant Red 3B-A

Chitosan, which is the partially deacetylated product of chitin, is a polycationic biodegradable substance. Recently, it has become more and more interesting as a matrix of micro- or nanoscaled drug delivery devices for conventional drugs,[1] non-viral gene transfer,[2,3] and vaccination.[4,5] Chitosan can also be used for the cationic surface modification of particles from poly(lactic-co-glycolic acid).[6,7] From the regulatory point of view, it is necessary to quantify the residual amount of such surface modifiers in pharmaceutical formulations.

For the determination of chitosan, different methods are known, for example, the formation of a complex with copper ions, or the application of an enzyme-linked immunosorbent assay (ELISA).[8] However, the drawbacks of these methods are the need of large sample volumes, the lack in sensitivity, or the time-consuming procedure. Therefore, Muzzarelli introduced a dye-binding assay employing Cibacron Brilliant Red 3B-A (synonym Reactive Red 4).[9] Due to the reaction of the dye with chitosan a bathochromic shift occurs. When the absorbance of this mixture is measured against a pure dye solution, a sharp peak can be found at about 575 nm.

Although this method was described to be quite sensitive, we found that the results were not reproducible for the determination of chitosan at concentrations <100 μg/mL. By changing the ratio of chitosan and dye solution and by modifying the reagent composition the sensitivity of the chitosan assay can be increased (Fig. 1). In this way chitosan can be quantified in the 10–80 μg/mL range.

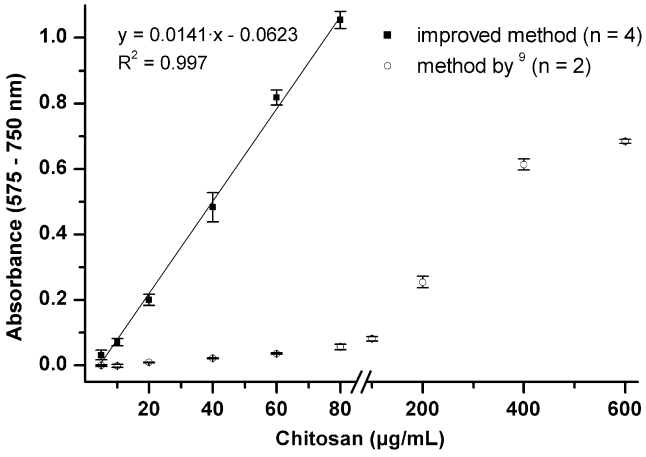

Figure 1. Determination of chitosan with Cibacron Brilliant Red 3B-A either by the method of Muzzarelli[9] or by the improved one (median and range).

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Overall, the improved chitosan detection with Cibacron Brilliant Red 3B-A showed a high sensitivity and a good linearity and reproducibility. The assay might be applicable in several fields, since chitosan is a topic of current research in pharmaceutics, medicine, biotechnology, and other areas.

1. Experimental

According to the improved method five parts of a chitosan solution (90/200/A1, Lot 0504-4, degree of deacetylation 90.5%, viscosity 170 cps, Heppe GmbH, Queis, Germany) were vortexed with one part of the dye reagent in a glass tube (Muzzarelli:[9] one part of the sample + 10 parts of the dye solution). The dye reagent was more concentrated and contained 0.9 mg/mL Cibacron Brilliant Red 3B-A (Aldrich, Taufkirchen, Germany) in a 0.3 M glycine·HCl buffer with a pH of 3.2. This procedure changed the final concentration of the dye from 68.2 μg/mL (method of Muzzarelli[9]) to 150 μg/mL (improved method) and of the glycine buffer from 0.07 M to 0.05 M. The absorption difference between 575 and 750 nm was measured immediately against a mixture of water and dye reagent in semimicro cells (UV-2101 PC, Shimadzu Scientific Instruments, Columbia, MD, USA).

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