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## Comparison of Absorption Maxima of Glycosaminoglycan-DMMB Complexes of Urinary Glycosaminoglycans and Tissue-extracted Chondroitin Sulfate

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**Background:** Dimethyl-methylene blue (DMMB) assay is used to estimate the total glycosaminoglycan concentration in urine. Most urinary glycosaminoglycans are oligosaccharides of chain sizes from 2 DP (degree of polymerization) to 20 DP. However, high molecular weight glycosaminoglycans are routinely used as standards for urine assay.

**Objectives:** To study the difference in absorption spectra of glycosaminoglycan-DMMB complexes formed by pooled paediatric urine samples, chondroitin sulfate oligosaccharide (having 12 DP and commercially prepared using shark chondroitin sulfate), and chondroitin sulfate from bovine trachea (references) and use the observations to guide assay optimization and redevelopment efforts.

**Methods:** Ten paediatric urine samples were pooled together. Reference standards (100 µg/mL) were prepared in distilled water. DMMB dye (200 µL) was mixed with 20 µL of sample/standard and shaken for 5 minutes before the absorbance was measured. The absorption spectra of DMMB dye alone and the glycosaminoglycan-DMMB complexes were obtained by scanning the dye and the respective reaction mixtures over the wavelength range of 450-800 nm at intervals of 1 nm (at its highest spectral resolution) using a microplate spectrophotometer. Linearity of the standard curve was determined at several standard-to-dye ratios (1:3, 1:9, 1:19).

**Results:** The dye showed two peaks of absorption, at 593 and 648 nm. According to the difference spectra, urinary glycosaminoglycans formed complexes with an absorption peak at 513 nm in contrast to the complex formed with chondroitin sulfate from bovine trachea and chondroitin sulfate oligosaccharide at 520 and 518 nm, respectively. Accordingly, chondroitin sulfate oligosaccharide (reference) was used for subsequent assays as its absorption peak is reasonably close to that of urine. The standard curve was linear up to 100 µg/mL at 1:19 standard-to-dye ratio.

**Conclusions:** The maximum absorption of glycosaminoglycan-DMMB complexes formed by urinary glycosaminoglycans is significantly lower compared to those of the chondroitin sulfate references observed in the present study and other tissue extracted glycosaminoglycans reported in the literature. This finding is useful in determining the wavelength/optic filters for the DMMB assay.

**Keywords:** *Absorption spectra, Chondroitin sulfate, Dimethyl-methylene blue, Glycosaminoglycan, Maximum Absorption, Urine*

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