Accuracy of the dimethylmethylene blue spectrophotometric assay in measuring the amount of encapsulated pentosan polysulfate into nanoparticles

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OBJECTIVES

Pentosan polysulfate (PPS), a highly sulfated semisynthetic polysaccharide, chemically and structurally resembles glycosaminoglycans (GAGs). Therefore, it was used here the dimethylmethylene blue (DMMB) binding assay, which is a rapid spectrophotometric assay that is widely used to measure sulfated GAGs (1, 2), to estimate the amount of PPS encapsulated into chitosan-based nanoparticles (NPs).

**Figure 1.** Schematic configuration of the experimental procedures used for the preparation of the NPs and the determination of PPS entrapment efficiency.

Test NPs were prepared by drop-wise addition of PPS (1 mg/ml) to chitosan (1 mg/ml) under continuous magnetic stirring. The NPs were separated by centrifugation.

The encapsulated PPS into NPs was quantified either by measuring the free PPS (indirect method) or by direct measurement of the entrapped PPS after releasing from NPs by incubation in Tris buffer followed by centrifugation. Drug entrapment efficiency of the test NPs was calculated from the ratio between the amount of encapsulated PPS into NPs and the total amount of PPS added x 100.

PPS was measured by the DMMB assay (2).

RESULTS AND DISCUSSION

PPS was detected by the DMMB dye in a linear relationship at both low and high concentrations, as shown in **Figure 2.**

**Figure 3** shows NPs entrapment efficiency estimation (direct and indirect) and the relative measurement uncertainty. The measurement uncertainty expressed by the relative standard deviation (RSD) suggests that the DMMB assay is highly reliable in measuring the PPS entrapped amount.

After spiking of 0.2, 0.4 and 0.7 mg of PPS with chitosan solution (1 mg/ml), the DMMB assay measured quantities of PPS that were very close to those nominal, as shown in **Figure 4.** This result indicates the high accuracy and precision of the DMMB assay (Table 1).

![Graph showing accuracy and precision of the DMMB assay in measuring spiked PPS](image)

**Table 1.** Accuracy and precision of the DMMB assay in measuring spiked PPS

<table>
<thead>
<tr>
<th>Spiked PPS (mg)</th>
<th>Recovered PPS (mg)</th>
<th>%Recovery</th>
<th>%RSD$^a$</th>
<th>SD of a 2 independent assays performed in duplicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.156 ± 0.006</td>
<td>78.4</td>
<td>3.7</td>
<td>2.20</td>
</tr>
<tr>
<td>0.4</td>
<td>0.394 ± 0.010</td>
<td>95.3</td>
<td>3.25</td>
<td>2.50</td>
</tr>
<tr>
<td>0.7</td>
<td>0.605 ± 0.010</td>
<td>85.0</td>
<td>2.86</td>
<td>2.86</td>
</tr>
</tbody>
</table>

$^a$ RSD difference from actual; RSD relative standard deviation; SD standard deviation of at least 2 independent assays performed in duplicate.

These results are collectively in agreement with previously published data obtained by the capillary zone electrophoresis (CZE) (3), as shown in **Table 2.** However, all the uncertainties related to results produced by the DMMB assay are much smaller (at least 30-fold) than those produced by the CZE, indicating its higher accuracy over other detection assays.

**Table 2.** Comparison between the DMMB assay and CZE in measuring PPS and determining its loading efficiency into chitosan-based NPs

<table>
<thead>
<tr>
<th>Direct method</th>
<th>%Entrapment efficiency (Indirect)</th>
<th>%Entrapment efficiency (Direct)</th>
<th>LOQ (mg/ml)</th>
<th>Linearity</th>
<th>%DFA$^b$</th>
<th>Accuracy error</th>
<th>%RSD$^c$</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMMB</td>
<td>4.2% ± 0.084</td>
<td>44.1 ± 0.10</td>
<td>2.0 ± 0.008</td>
<td>R = 0.986</td>
<td>2.0 – 2.16</td>
<td>0.004 – 0.02</td>
<td>0.10 – 0.12</td>
<td>97.14 – 98</td>
</tr>
<tr>
<td>CZE</td>
<td>82.4 ± 2.5</td>
<td>87.4 ± 2.8</td>
<td>R = 0.977</td>
<td>2.8 – 5.8</td>
<td>0.01 – 0.14</td>
<td>0.10 – 0.12</td>
<td>91.4 – 95</td>
<td></td>
</tr>
</tbody>
</table>

$^b$ DFA difference from actual; DFA relative detection limit; %DFA quantification limit CZE, capillary zone electrophoresis; NPs, nanoparticles. Values are average SD of at least 2 independent assays performed in duplicate.

CONCLUSIONS

The DMMB assay demonstrated to be able to estimate the PPS entrapment efficiency into the NPs with high accuracy both within and between the assay. Moreover, the DMMB dye showed high stability and binding specificity reflected by the absence of any interaction with nonsulfated molecules present in a mixture with the PPS.

REFERENCES