Chitosan nanoparticles: correlation of *in situ* metrology with interactions at biological interfaces

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**ABSTRACT SUMMARY**

In *in situ* metrology showed chitosan nanoparticles to exhibit significantly different mean size and zeta potential in a range of biologically relevant media. These measurements do not reflect data obtained in the formulation vehicle. Correlation of the *in situ* measurements to cytotoxicity data obtained from human colon and liver cells suggests that particle size has a greater influence on the cell modulating activities of chitosan nanoparticles than zeta potential.

**INTRODUCTION**

Chitosan is one of the most extensively studied materials in biomedical research and a highly regarded material of choice in drug delivery applications, where it is often formulated into nanoparticles for the loading of therapeutic cargo. However, while the chitosan parent polymer is generally regarded to be safe, biodegradable and biocompatible, it is not unusual to find evidence of unique cell modulating activities, including cytotoxicity, when chitosan is presented to cells as nanoparticles. Such activities at the biological interfaces have been widely attributed to the positive zeta potential of the chitosan nanoparticles, without regard that the zeta potential was measured in a formulation vehicle that is compositionally different to the vehicle used to present the nanoparticles to the cells.

In this paper, we measured the size and zeta potential of chitosan nanoparticles in biologically relevant media, and correlated these properties to the biological data obtained for the nanoparticles in surrogate human intestinal and hepatic cell models. The chitosan nanoparticles were prepared by a novel spinning disc technology, which yielded small and monodispersed nanoparticles (mean size 20 ± 3 nm). The very narrow size distribution allowed for a more meaningful correlation of the biological activities to particle size not possible in earlier studies that employed polydispersed chitosan nanoparticle samples.

**EXPERIMENTAL METHODS**

Commercial chitosan was depolymerized and purified to 202 kDa and 79% degree of deacetylation. Chitosan nanoparticles (NP) were produced by simultaneously feeding 0.25% w/v chitosan in 0.1M CH₃COOH and 0.1% w/v sodium tripolyphosphate in water onto a spinning disc processor operating at 1000 rpm. NP after manufacture was purified by dialysis against water and lyophilized.

NP size and zeta potential were measured immediately after manufacture in water and in 1M CH₃COOH using dynamic light scattering and laser Doppler anemometry techniques, respectively. Size and zeta potential were again measured after the lyophilized NP were reconstituted in phosphate buffered saline (PBS), Hank’s balanced salt solution (HBSS), supplemented Earle’s Minimum Essential Medium (EMEM) and supplemented William E’s medium, at pH 6 and pH 7.4.

NP-mediated cytotoxicity against the Caco-2 cells and human liver BHAL cells were established by quantifying the cellular mitochondrial dehydrogenase activity at 4, 24, 48 and 72 h after exposure. For the Caco-2 cells, data at 4 and 24 h exposures were obtained using HBSS as vehicle, while data at 48 and 72 h were obtained using EMEM. NP was presented to the BHAL cells in William’s E medium.

**RESULTS AND DISCUSSION**

Vehicle composition has an important influence on NP metrology, the particles exhibiting widely differing mean sizes (20 – >1000 nm) and zeta potentials (-7.5 to 53.3 mV).
Table 1: Size and zeta potential of chitosan nanoparticles dispersed at 0.1% w/v in biologically relevant media.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Size (nm, mean±SD)</th>
<th>Zeta potential (mV, mean±SD)</th>
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<tbody>
<tr>
<td>NP prior to lyophilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>409 ± 47</td>
<td>16.0 ± 3.5</td>
</tr>
<tr>
<td>0.1M HAc</td>
<td>20 ± 3</td>
<td>53.3 ± 4.3</td>
</tr>
<tr>
<td>Lyophilized NP following reconstitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7.4</td>
<td>pH 6.0</td>
<td>pH 7.4</td>
</tr>
<tr>
<td>PBS</td>
<td>*</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>HBSS</td>
<td>333 ± 43</td>
<td>25 ± 7</td>
</tr>
<tr>
<td>William’s E</td>
<td>65 ± 4</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>EMEM</td>
<td>149 ± 16</td>
<td>26 ± 2</td>
</tr>
</tbody>
</table>

(* not determined)

Despite having opposing surface charges, NP dispersed in HBSS and EMEM at 0.1% w/v at pH 6.0 were both observed to reduce the Caco-2 cell viability by more than 60% (Fig 1). Moreover, the greater innocuity of the NP at pH 7.4 compared to pH 6.0 in HBSS was not underscored by a difference in zeta potential, but by a significant increase in particle size at pH 7.4. Similar observations were seen in the BHAL cells.

CONCLUSION

Correlation of the in vitro cytotoxicity data with the in situ metrology of chitosan nanoparticles suggest that particle size plays an important role in the interactions and associated toxic manifestations of the particles in the Caco-2 and BHAL cells, with zeta potential having a relatively minor influence.

REFERENCES


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