ABSTRACT SUMMARY
The aim of this study was the development of polymeric microspheres that release encapsulated model macromolecule without the lag phase. PLGA monodisperse particles were prepared with membrane emulsification (ME) method and blue dextran (BD) was encapsulated as a model macromolecule. The relationship between formulation conditions and particle porosity and release of the macromolecule from the polymeric particles was analyzed with Artificial Neural Network (ANN) in a number of different formulations. ANN was able to successfully predict the particle characteristics of a formulation that will deliver BD in a sustained release for three months.

EXPERIMENTAL METHODS
A total of 10 different formulations were prepared in a double emulsion method with ME. The varied particle characteristics, which were also used as inputs for ANN modeling, were: PLGA concentration in the oil phase (10, 15, 20, 25 and 30%; w/w), inner water ratio (11, 20 and 33%), PVA concentration in the external water phase (2, 4 and 6%) and the addition of 1% NaCl to the external water phase. Microspheres were characterized for size, span value (sp = (d90 – d10)/d50), porosity, LE and in vitro release profile, which were also selected as output parameters for ANN modeling.

RESULTS AND DISCUSSION
Table 1 summarizes the characteristics of the formulations prepared with different particle characteristics. Relatively monodisperse particles were prepared with low span value (<0.6). The particle size ranged between 40–45 μm. Bigger particles (>50 μm) were formed with higher viscosity of the oil phase (PLGA 25 and 30%). LE correlated to PLGA concentration and ranged from 19% (formulation 1) to 88% (formulation 5). The formulations generated particles with a smooth surface and different porosity grades. Highly porous particles were formed with 10% PLGA concentration in the oil phase (formulation 1), whereas particles with no porosity were formed with 30% of PLGA in the oil phase (formulation 5) and with the addition of 1% NaCl in the external water phase (formulation 10).

Upon incubation of formulations with porosity grade <3 (at 37°C in 150 mM phosphate buffer, pH 7.4), BD was released...
from the microspheres in a three-phasic release profile with a lag phase of 25 days followed by a complete release over 90 days (Figure 1). Particles with porosity grade 4 were characterized with a sustained release of BD over a period of 90 days, albeit with >25% of burst release.

Figure 1. Cumulative release profile of BD from formulations 2, (porosity grade 4; closed diamonds), 3 (porosity grade 2; open squares), and 5 (porosity grade 1; closed squares), and from the formulation with ANN-predicted optimal release characteristics (porosity grade 3; open diamonds).

For a formulation with high LE, low burst and sustained release of BD, ANN predicted the following formulation characteristics: 15% PLGA in the oil phase, 16% inner water volume and 3% PVA in the external water phase. This reproduced formulation resulted in a LE of 70%, with only 10% of burst release and a sustained release of BD (Figure 1).

CONCLUSION
This study shows a successfully predicted formulation of PLGA monodisperse microspheres that displayed a sustained release of the model macromolecule. Albeit with high particle porosity, the prepared formulation had a LE of 70% and a low burst release of 10%. This is a great step forward in the design of controlled release devices for macromolecular drugs, such as therapeutic proteins or other biological macromolecules.

REFERENCES

ACKNOWLEDGMENTS
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Table 1. Characteristics of BD loaded PLGA microspheres prepared with membrane emulsification method. Fields in grey indicate the parameters varied in those particular formulations.

<table>
<thead>
<tr>
<th>Formulation #</th>
<th>PLGA in the oil phase (w/w%)</th>
<th>W1 (%)</th>
<th>W2 (%)</th>
<th>TL (wt%)</th>
<th>Vol-wt mean diameter (μm)</th>
<th>Span value</th>
<th>LC (%)</th>
<th>LE (%)</th>
<th>Porosity¹</th>
<th>Burst release (24h, %)</th>
<th>Slope of the release² (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>20</td>
<td>PVA 4%</td>
<td>7.7</td>
<td>40 ±13</td>
<td>0.9</td>
<td>1.5</td>
<td>19</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>20</td>
<td>PVA 4%</td>
<td>5.1</td>
<td>45 ±14</td>
<td>0.6</td>
<td>2.9</td>
<td>58</td>
<td>4</td>
<td>25</td>
<td>1.25</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>20</td>
<td>PVA 4%</td>
<td>3.6</td>
<td>40 ±6</td>
<td>0.3</td>
<td>1.9</td>
<td>52</td>
<td>2</td>
<td>9</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>20</td>
<td>PVA 4%</td>
<td>2.8</td>
<td>47 ±7</td>
<td>0.2</td>
<td>2.0</td>
<td>71</td>
<td>3</td>
<td>5</td>
<td>0.43</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>20</td>
<td>PVA 4%</td>
<td>2.2</td>
<td>59 ±5</td>
<td>0.1</td>
<td>1.9</td>
<td>88</td>
<td>1</td>
<td>0.4</td>
<td>0</td>
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<tr>
<td>6</td>
<td>20</td>
<td>11</td>
<td>PVA 4%</td>
<td>1.9</td>
<td>55 ±23</td>
<td>1.1</td>
<td>1.0</td>
<td>55</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>33</td>
<td>PVA 4%</td>
<td>7.0</td>
<td>44 ±14</td>
<td>0.8</td>
<td>3.1</td>
<td>45</td>
<td>3</td>
<td>34</td>
<td>0.30</td>
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<tr>
<td>8</td>
<td>20</td>
<td>20</td>
<td>PVA 2%</td>
<td>3.6</td>
<td>45 ±6</td>
<td>0.2</td>
<td>1.9</td>
<td>53</td>
<td>3</td>
<td>39</td>
<td>0.50</td>
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<tr>
<td>9</td>
<td>20</td>
<td>20</td>
<td>PVA 6%</td>
<td>3.6</td>
<td>44 ±12</td>
<td>0.5</td>
<td>2.1</td>
<td>60</td>
<td>3</td>
<td>27</td>
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<tr>
<td>10</td>
<td>20</td>
<td>20</td>
<td>PVA 4% + 1% NaCl</td>
<td>3.6</td>
<td>41 ±13</td>
<td>0.6</td>
<td>2.2</td>
<td>60</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹Porosity grades were scored by three individual observers and were ranked from 0 (no pores) to 4 (presence of many small pores); ²slope of the release curve from day 1 to day 20; W1- inner water phase; W2- external water phase; TL- theoretical drug loading;; LC- loading capacity; LE- loading efficiency.