Human Low Density Lipoprotein Coated Capillaries in Electrochromatography

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ABSTRACT

Low density lipoprotein (LDL) particles were immobilized on the inner wall of a fused silica capillary and used in a study of the interactions between LDL and neutral drugs in electrochromatography. The effect of coating parameters (pH, ionic strength of the coating solution, duration of the coating procedure) on the properties and stability of the coating was examined. The stability of the coating was highest when the pH of the coating solution was under the pI value of the LDL particles. Interactions of unmodified LDL coatings with drugs were compared with those of acetylated LDL coatings. Acetylation of LDL neutralizes the positive charge on the lysine residues of the protein component of LDL particles, and acetylated LDL was used as a reference to examine the effect of the positively charged amino acids in the unmodified coating. Under similar coating conditions, acetylated LDL coating yielded stronger EOF due either to the decreased number of positive charges on LDL particles or to the worse coating ability, or both. The interactions of the unmodified and acetylated LDL coatings with steroids aldosterone, testosterone and progesterone were comparable, which indicates that the density of immobilised LDL particles is not appreciably altered by acetylation. As expected, the strength of the interactions between steroids and the LDL coating increased with hydrophobicity of the drug.
FIGURE S-1

Influence of base and acid pretreatment on the mobility of EOF in LDL-coated capillaries. Coating solution: unmodified LDL diluted 1:3 (v:v) with BGE solution. Coating conditions: 40 min rinsing (at 50 mbar) and 15 min standing with coating solution. BGE solution: phosphate, I = 20 mM, pH = 7.4. Running conditions: fused silica capillary with total length of 38.5 cm (30 cm to the detector) and i.d./o.d. 50/375 μm; capillary temperature 25°C; injection 5 s at 50 mbar; running voltage 20 kV; detection at 254 nm. Between runs the capillary was flushed for 2 min with BGE solution. DMSO was used as EOF marker. EOF measurements were carried out at 200 nm.
FIGURE S-2

Effect of reconditioning on the coating performance. Coating and running conditions as in Figure S-1.
FIGURE S-3

Effect of pH on capillary performance. BGE solution was phosphate for measurements made at pH 7.4 and 6.5 and acetate for measurements made at pH 5.5 to 4.5. At pH 5.5 the EOF mobility was determined with a capillary electrophoresis instrument by the method of Williams and Vigh.\textsuperscript{17} Coating and running conditions as in Figure S-1.
Table S-1. Influence of pH and modification on the performance of LDL coatings, measured by EOF mobility and retention factor k.

<table>
<thead>
<tr>
<th>Coating</th>
<th>EOF mobility$^a$</th>
<th>k (aldosterone)</th>
<th>k (testosterone)</th>
<th>k (progesterone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL, pH 7.4</td>
<td>2.4</td>
<td>0.02</td>
<td>0.12</td>
<td>0.98</td>
</tr>
<tr>
<td>ac-LDL, pH 7.4</td>
<td>3.2</td>
<td>0.02</td>
<td>0.11</td>
<td>0.92</td>
</tr>
</tbody>
</table>

$^a$ the dimension of the EOF mobility is $10^{-4}$ cm$^2$/Vs. Mobility is the mean of 12 successive runs.