In summary, the design software we describe is useful for estimating device performance and creating microfluidic chip layouts; these layouts can be rapidly modified to design chips that meet performance requirements. We used the software to improve the design of a microfluidic immunoassay chip. The resulting design occupied ~25% of the area of the original chip, and the time required for electrophoretic separation was decreased by more than 50% relative to the original design, allowing for a faster assay. This process is more than 2 orders of magnitude faster than conventional design techniques and is ideally suited for design optimization of microfluidic lab-on-a-chip systems.

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References


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An Analytical Method for Size and Shape Characterization of Blood Lipoproteins, Diana Cristina Rambaldi,1 Andrea Zaltoni,1 Sonia Casolari,1 Pierluigi Reschiglian,1* Dierk Roesser-ner,2 and Christoph Johann1 (1 Department of Chemistry “G. Ciamician”, Bologna, Italy; 2 Wyatt Technology Europe GmbH, Dernbach, Germany; * address correspondence to this author at: Department of Chemistry “G. Ciamician”, Via Selmi 2, 40126 Bologna, Italy; fax 39-0-51-2099452, e-mail pierluigi.reschiglian@unibo.it)

Determination of total cholesterol (TC), HDL and LDL cholesterol, and triglycerides (TG) is used to assess lipoprotein abnormalities and coronary artery disease (CAD) risk. Furthermore, studies have demonstrated that small, dense LDL particles penetrate more easily into the arterial intima (1), exhibit increasing binding to arterial wall proteoglycans (2), and are more prone to oxidative stress (3). Small, dense LDL particles also have a prolonged plasma half-life because of their lower binding affinity for the LDL receptor (4). The presence of small, dense LDL particles in plasma is, therefore, considered to be proatherogenic.

Analytical methods for LDL size characterization are therefore expected to improve classification, diagnosis, and therapy of dyslipidemic patients. However, simple methods for routine LDL size measurement are not yet available. LDL size is mainly measured by nondenaturing PAGE (5), which is time-consuming and may be unsuitable for large numbers of samples. Easier methods for measuring LDL size have been proposed, including high-performance gel-filtration chromatography (HPGC) (6).

Both PAGE and HPGC can give accurate size estimations only if appropriate standards are available. Moreover, recent studies have shown that LDL particles may be discoidal, with diameter and height that are not significantly correlated (7). PAGE and HPGC cannot give information on particle conformation.

Flow field-flow fractionation (FlFFF) is a separation technique in which macromolecules and particles are
separated by the combined actions of an axial flow and a perpendicular cross-flow (8). No stationary phase is present, and the separation mechanism is sufficiently gentle not to alter the native structure of proteins and protein complexes (9). Proteins are fractionated according to their difference in diffusion, with retention time that is inversely proportional to the analyte diffusion coefficient. In principle, with the use of FIFFF it is possible to obtain the hydrodynamic radius ($R_h$) of proteins from their retention time values. A microchannel, prototype variant of FIFFF, the hollow-fiber (HF) FIFFF that uses a piece of porous HF as separation channel ([10] and references therein), has shown advantages not only because the channel is of reduced volume, but also because it is simple to construct and inexpensive, making it potentially disposable (11). Disposable FIFFF channels should be particularly advantageous in clinical analysis, for which sample carryover must be strictly avoided.

Since its early development stage, FIFFF has been used to fractionate lipoproteins (12). FIFFF was used to characterize LDL particles of patients with CAD (13), and HF FIFFF of LDL particles from CAD patients and from healthy donors was recently reported (14). However, no independent, uncorrelated measurements of the fractionated LDL size were used to fully characterize serum lipoproteins in size and shape.

Multiplex light scattering (MALS) allows for the absolute determination of molar mass ($M_w$) and particle size over a broad range (15). If MALS is not coupled to a size-based separation device, however, it gives only mean values. Online FIFFF-MALS makes it possible to size-sort macromolecules and particles and to online characterize values. Online FIFFF-MALS makes it possible to size-sort a size-based separation device, however, it gives only mean values. Online FIFFF-MALS makes it possible to size-sort macromolecules and particles and to online characterize the narrowly dispersed fractions without using standards. Thus, distribution of the absolute $M_w$ values can be obtained even for broadly dispersed samples if the concentration and specific refractive index increment (dn/dc) are also measured or known. The root mean square gyration radius ($R_g$) can be calculated from the angular dependence of the MALS signals alone, without information on dn/dc (15).

This study was the 1st to investigate the feasibility of FIFFF-MALS as a method for size and shape characterization of lipoproteins from whole human serum samples. Because MALS was applied to HF FIFFF for the accurate estimation of $M_w$ and size values of protein aggregates (16), we compared the performance of the method when used with either commercial FIFFF or prototype HF FIFFF instruments. The method will require further validation and verification to be applied on a routine scale.

Human blood serum samples were obtained from healthy donors who gave informed consent. Samples were stored at −20 °C until analysis. In some experiments, sera were stained before the analysis with Sudan Black B (SBB; Fluka), a dye for lipid components, which generates a specific absorption maximum at 600 nm. For these experiments, a volume of 2 μL SBB solution (1% wt/vol in ethanol) was added to 100 μL serum and 400 μL phosphate buffer (7.5 mmol/L KH₂PO₄, 7.5 mmol/L Na₂HPO₄ × 2 H₂O, pH = 7.2).

The commercial FIFFF system was the model Eclipse 2 (Wyatt Technology Europe). The channel had a trapezoidal shape, with 350-μm thickness and 175-mm length, and with breath decreasing from 18 mm (inlet end) to 3 mm (outlet end). The accumulation wall was a 30 000 Da cutoff regenerated cellulose membrane.

The prototype HF FIFFF channel was built up as described elsewhere (11, 17). A piece of polyacrylonitrile HF membrane was sheathed by 2 pieces of one-eighth inch outer diameter Teflon tube. A T connection was positioned between the 2 tubes to make the radial flow outlet. Hand-tight male fittings were positioned at the channel inlet and outlets. The HF membrane had a 30 000 $M_w$ cutoff, nominal inner radius of 0.40 mm, and a length of 240 mm (dried conditions).

The mobile phase was phosphate buffer (7.5 mmol/L KH₂PO₄, 7.5 mmol/L Na₂HPO₄ × 2 H₂O, pH = 7.2) degassed with a 1100 Series vacuum degasser (Agilent Technologies) and delivered by a 1100 HPLC iso-pump (Agilent). The injected sample volume was 10 μL (with the commercial FIFFF channel) or 4 μL (with the prototype HF FIFFF channel). With either the commercial FIFFF or the prototype HF FIFFF channel, flow rates and patterns were controlled using the Eclipse 2 separation system. The FIFFF and HF FIFFF operation conditions are reported in the Fig. 1 legend.

Fractionated components were online detected as a function of retention time, and a serum profile (fractogram) was recorded. Detection was performed by an HP1100 UV and visible diode array detector (Agilent), operated at 280 and 600 nm wavelengths, a refractive index detector model Optilab rEX (Wyatt Technology), and a MALS detector model DAWN HELEOS (Wyatt Technology). ASTRA software version 5.3.1 (Wyatt Technology) was used to handle signals from the detectors and to compute the $R_g$ values.

A serum profile obtained using the commercial FIFFF channel is shown in Fig. 1A. The concentrations were known for TC (5.05 mmol/L, 195 mg/dL) and TG (5.36 mmol/L, 473 mg/dL). Total analysis time was approximately 1 h. The scattering intensity signal shows bands not only for the main lipoprotein classes (HDL, LDL, VLDL) but also for the most abundant serum proteins (albumin, IgG). Assignment of the lipoprotein bands was then necessary. The MALS trace was compared with the UV trace at 280 nm, in which the lipoprotein bands were hardly detectable owing to their low concentration. Using SBB-stained samples, we then compared the MALS trace with the UV trace at 600 nm, in which the stained lipoproteins were the only detected species. One band ascribed to IgG (band 2) and 3 bands ascribed to the HDL corelabeled with albumin (band 1), to LDL (band 3), and to VLDL (band 4) were found. According to FIFFF theory, lipoproteins are eluted at retention times that increase with increasing $R_g$ values (12). The $R_g$ values we obtained (top x axis) were in reasonable agreement with those previously obtained by other method: 2–7 nm for HDL, 8–17 nm for LDL, and 15–40 nm for VLDL (18). From the MALS signal we also were able to evaluate the corresponding $R_g$ values as plotted in Fig. 1A. Teerlink et al. (7)
calculated the height values for model disk-like LDL particles. By applying a hydrodynamic model for disk-like particles (19), we obtained for LDL a predicted range for $R_g/R_h$ of 0.787–0.830. The experimental LDL $R_g/R_h$ values we obtained for a set of serum samples were in the range 0.38–1.38. These values are higher than those expected for either a homogeneous sphere ($R_g/R_h = 0.774$) or for the above disk-like model ($R_g/R_h = 0.787–0.830$). However, LDL particles are core shell with nonhomogeneous density (20), with the shell denser than the core. Because $R_g/R_h$ increases with increasing contribution of the shell to the LDL mass distribution, our results are compliant with a core-shell, discoidal conformation. Finally, from band intensities the relative abundance of the different lipoprotein classes can be determined: high intensity of band 4, for instance, reflects the known correlation existing between TG concentration and VLDL content.

Compared with the profile in Fig. 1A, the profile obtained with the same serum sample by use of the prototype HF FIFFF channel (Fig. 1B) shows a lower signal-to-noise ratio, likely because of the lower sample load. The lipoprotein classes (bands 1, 3, 4), however, are fractionated with comparable resolution, and the calculated $R_h$ values are also comparable. Because of the different flow conditions used in HF FIFFF, the VLDL band (band 4) is broader, but, as also plotted in Fig. 1B, the increase in $R_g$ values across the VLDL fraction indicates that band broadening is not due to a separation efficiency lower than in commercial FIFFF but to the high size-based selectivity of HF FIFFF.

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Lanthanide Phosphate Nanorods as Inorganic Fluorescent Labels in Cell Biology Research, Chitta Ranjan Patra, Resham Bhattacharya, Suijata Patra, Suijit Basu, Priyabrata Mukhopjee, and Debabrata Mukhopadhyay (Department of Biochemistry and Molecular Biology, Mayo Clinic Cancer Center, Mayo Clinic, Rochester, MN; * address correspondence to this author at: Departments of Biochemistry and Molecular Biology, and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN 55905; fax 507-284-1767, e-mail Mukhopadhyay.debabrata@mayo.edu)

One of the fundamental goals in biology is to understand the complex spatiotemporal interplay of biomolecules from the cellular to the integrative level. To study these interactions, researchers commonly use fluorescent labeling for both in vivo cellular imaging and in vitro assay detection (1, 2). In this context, one of the fastest developing and most exciting interfaces of nanotechnology is the use of inorganic quantum dots or fluorescent nanoparticles in cell biology. The unique optical properties of inorganic nanoparticles make them appealing as in vivo and in vitro fluorophores in a variety of biological investigations.

In addition, the ability to make such nanoparticles and then target these particles to specific biomolecules has led to promising applications in cellular labeling, deep-tissue imaging, and assay labeling, and also as efficient fluorescence resonance energy transfer donors.

Conventionally used fluorescent labels, such as organic dyes in cell biology, are prone to problems such as broad spectral features, short lifetime, photobleaching, and potential toxicity to the cells. Inorganic fluorescent species, especially europium (Eu) and terbium (Tb) in the lanthanide group, have several unique optical and electronic properties, such as size- and composition-tunable emission from visible to infrared wavelengths, large absorption coefficients across a wide spectral range, symmetric emission spectrum, a large Stokes shift, simultaneous excitation of multiple fluorescent colors, very high levels of brightness, and photostability (3–5). Furthermore, analysis by cell proliferation and apoptosis assay (dUTP nick-end labeling) showed that lanthanide phosphate (LnPO₄•H₂O, where Ln = Eu) and Tb nanorods synthesized by microwave (MW) were nontoxic to endothelial cells (5).

In this study, we synthesized LnPO₄•H₂O nanorods by an exclusive MW technique and used a transmission electron microscope (TEM) to investigate the internalization of LnPO₄•H₂O nanorods into 786-O cells and human umbilical vein endothelial cells (HUVECs). Confocal microscopy results showing improved fluorescence imaging (red fluorescence for Eu, green fluorescence for Tb, and no autofluorescence for controls) suggest that this technique might be used for live-cell imaging, a requisite analytical tool in most cell biology experiments and a routine procedure in neuroscience, developmental biology, pharmacology, and several other related biomedical research fields.

Eu(III) nitrate hydrate, Tb(III) nitrate hexahydrate, and ammonium dihydrogenphosphate were purchased from Aldrich and used without further purification. 786-O cells were purchased from ATCC, and HUVECs were obtained from Cambrex.

The inorganic fluorescent nanorods (LnPO₄•H₂O) were synthesized and characterized using MW techniques as we previously reported (6). Briefly, 20 mL of a 0.05 mol/L aqueous NH₄H₂PO₄ solution was added to 20 mL of a 0.05 mol/L aqueous solution of Ln(NO₃)₃ in a 100-mL round-bottomed flask (see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue11). The yield of the as-prepared products was more than 95%.

\[
\text{Ln(NO}_3\text{)}_3 + \text{NH}_4\text{H}_2\text{PO}_4 + \text{H}_2\text{O} \rightarrow \text{LnPO}_4\cdot\text{H}_2\text{O}
\]

The MW-prepared LnPO₄•H₂O products were pure and crystallized with high yield. The technique did not require high temperatures, high pressure, a catalyst, reduced pressure conditions, or preprocessing.

This method is easy to perform, fast, clean, efficient, and nontoxic. The MW technique is considered to be ecologically friendly for the following reasons: (a) the

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