A flow-assisted point-of-care testing device based on gravitational field-flow fractionation for analysis on biological fluids

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ABSTRACT

The "Point-Of-Care Testing" (POCT) approach is based on the development of portable analytical platforms suitable to perform analysis directly in any required place. To meet analytical and diagnostic requirements, a POCT device should combine portability, minimum sample pre-treatment, and should also comprise an integrated module for on-line sample pre-analytical treatment and/or clean-up to achieve high sensitivity and specificity even in complex matrices. With this respect, field-flow fractionation (FFF) techniques, a family of flow-assisted separation techniques which can separate analytes based on their morphologic characteristics can be exploited. In this work, we propose the implementation of GrFFF as a pre-analytical module of a POCT device, thus providing a selectively enriched fraction for the analysis with an increase of overall analytical output. As example, we present the use of GrFFF to prepare whole blood samples for the automatic on-line analysis of alkaline phosphatase activity in serum.

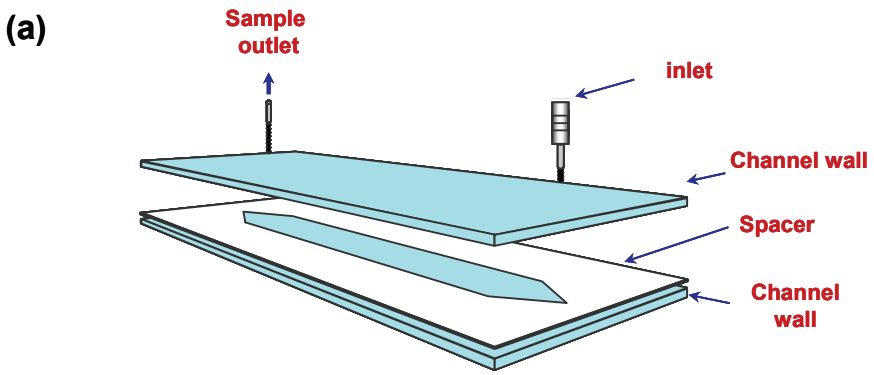
Keywords: point-of-care testing (POCT), portable device, miniaturization, sample preparation, bioanalytical assays.

1 INTRODUCTION

The "Point-Of-Care Testing" (POCT) approach is based on the development of portable analytical platforms suitable to perform analysis directly in any required place [1]. To meet analytical and diagnostic requirements, a POCT device should combine portability, minimum sample pre-treatment, and the possibility to perform highly sensitive simultaneous detection of several biomarkers (multiplexing) in a short assay time. Particularly advantageous would be the possibility to concurrently perform in the same device assays of different nature (e.g., immunological or nucleic acid hybridization assays and simple clinical chemistry enzymatic blood tests). Microfluidic integrated systems based on the use of biospecific recognition reactions and ultrasensitive bio-chemiluminescence detection techniques represent one of the most promising options. An integrated device suitable for the analysis on a complex biological sample (such as blood, urine, or saliva) should also comprise an on-line sample pre-treatment and/or clean-up component, thus eliminating the need for a separated pre-analytical sample preparation procedure.

With this respect, field-flow fractionation (FFF) techniques, a family of flow-assisted separation techniques which can separate analytes based on their morphologic characteristics can be exploited. Thanks to its "soft" separation mechanism, FFF has been successfully applied to a wide range of bioanalytes, from relatively small biomolecules to living cells in complex biological samples, which after separation keep their native characteristics such as the enzymatic activity, cell vitality, or quaternary protein structure [2,3]. In FFF separation is achieved within a capillary empty channel in which a laminar flow of mobile phase sweeps sample components down the channel. A field is applied perpendicularly to the parabolic flow to make the analytes be driven into different laminar flows due to their differences in physical properties such as molar mass/size, density, and surface properties, resulting in different retention times. Depending on the type of the perpendicular field, different variants of FFF can be realized. Gravitational FFF (GrFFF), exploiting the Earth gravitational field to structure the separation, is the humblest FFF techniques which appears to be particularly suited for its implementation in POCT devices, thanks to the simplicity of its separative device, amenable to miniaturization, and the potential easy integration with specific analytical modules. We have recently demonstrated the potential analytical applications of GrFFF coupled with chemiluminescence detection for the development of rapid and ultrasensitive bioanalytical assays [4] and the possibility to increase its selectivity by derivatizing the fractionation device walls with biospecific reagents [5].

In this work, we propose to exploit the potentialities of GrFFF towards the development of a pre-analytical module of a POCT device, able to fractionate the sample components thus providing a selectively enriched fraction for the analysis with an increase of overall analytical output. Sample components after fractionation can be transferred to different analytical modules where analytes are quantified. As a first approach, in this work we present the use of GrFFF to prepare whole blood samples for the automatic on-line analysis of alkaline phosphatase (AP) activity in serum, as a biomarker of obstructive liver diseases and bone disorders. A schematic instrumental set-up of this POCT basedc on GrFFF is reported in Figure 1.



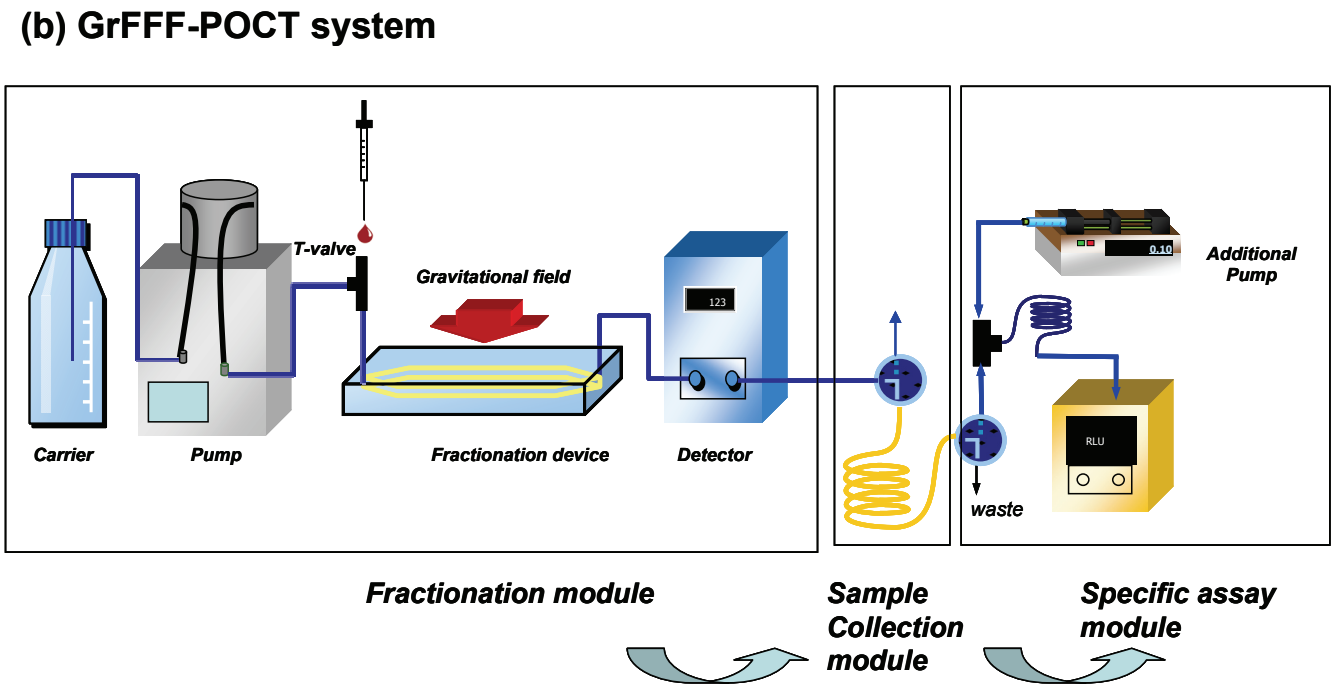


Figure 1. (a) A GrFFF channel. (b) Schematic view of a POCT system based on GrFFF: fractionation module where crude sample can be fractionated; sample collection module: after fractionation the selected faction can be collected or addressed to different analytical modules; specific assay module: one or more specific analytical module can be coupled on-line and they can be composed by additional pump to reagents addition and specific detector to reveal the analytical output.

2 GrFFF-POCT: ENZYMATIC ACTIVITY DETERMINATION

Serum alkaline phosphatase is commonly assayed by employing spectrophotometric techniques, which are limited by interferences from absorbing molecules in serum (e.g., bilirubin, haemoglobin) and require preliminary blood sample centrifugation to obtain serum. This approach is not compatible with POCT system where fast, simple and portable devices are required. A GrFFF device allows to overcome these issues because of its simple use, versatility, potential disposable use and easy integration with analytical module. As a consequence, GrFFF open interesting perspectives in its use to separate serum/plasma from micrometer-sized cells.

After the injection of heparin-treated whole blood in the GrFFF device at a floe rate of 1 mL/min of a physiologic solution, a typical fractografic profile is obtained, as reported in Figure 2.

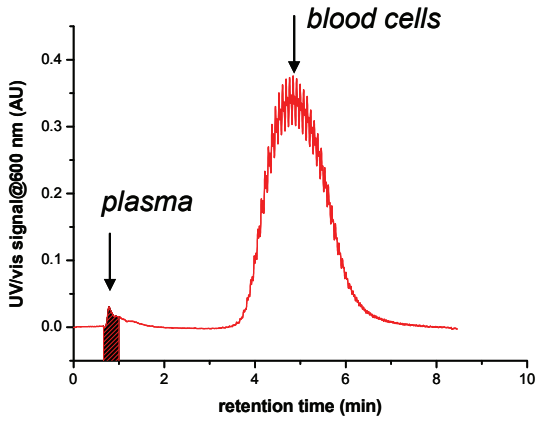


Figure 2. GrFFF Uv-vis fractografic profile for blood injection

Due to the fractionation principle, serum/plasma which contains smaller anlytes such as proteins, enzyme, antibodies, amminoacds and molecules, elutes earlier with the void channel volume; while cells can be retained at specific retention times from the GrFFF device. Serum/plasma is separated from cells by collecting the void volume which can be then addressed by means of a microfluidic system and valves to the analytical module.
Specifically, in the developed method plasma was collected in the interval time (40-100) s. For the enzymatic determination of AP, a chemiluminescent (CL) substrate was chosen. The CL substrate was added on-line on the plasma fraction by means of a syringe pump and the CL mixture was addressed to the detection system consisting of an on-line flow-through luminometer. The CL signal on plasma obtained after centrifugation was compared with those obtained after GrFFF fractionation. The enzymatic kinetic was reported in Figure 3.

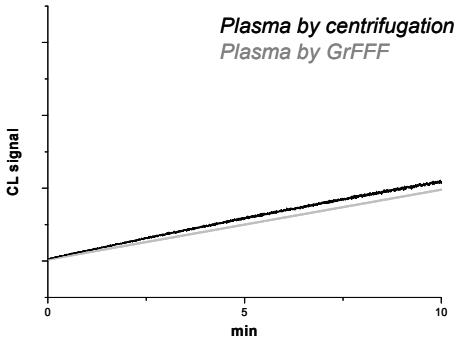


Figure 3. Enzymatic kinetics of plasma obtained after cebtrifugation and GrFFF fractionation.

The result reported in Figure 3 confirms that the GrFFF system can be used instead of centrifugation to separate plasma from blood sample and the enzyme activity is preserved during the fractionation process.

Finally, the test was applied to different blood dilution ranging from 1/5 to 1/100.
The diagnostic test developed based on GrFFF device gave quantitative results with low sample (0,5 µL of whole blood) and reagents consumption, short analysis time (10 minutes) and high reproducibility (both run-to-run and day-to-day %CV were always <10%).

3 PORTABLE GrFFF DEVICES FOR POCT

Further work is being performed to obtain compact and miniaturized GrFFF devices. In particular, with respect to conventional GrFFF rectangular channel, original curvilinear geometries were designed and their influence on fractionation performances were evaluated by employing chemiluminescent imaging accomplished by placing the GrFFF transparent channel in a dark box and by acquiring, through a highly-sensitive, back-illuminated, double Peltier-cooled CCD camera, the CL emission of fractionated analytes at fixed time intervals during the elution.

By using this system, a 75% reduction of the overall size of the device was accomplished, without loss of resolution and recovery. On the basis of these results, further studies are being conducted on a miniaturized GrFFF channel, characterized by total volume of 50 µL, as compared to 1,5 mL of a conventional channel.

The use of an array of FFF devices will be considered to increase enrichment productivity and to allow the development of multiplexed systems based on the same separative principle.

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