Analytica Chimica Acta 882 (2015) 83–89

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Single-step, paper-based concentration and detection of a malaria biomarker

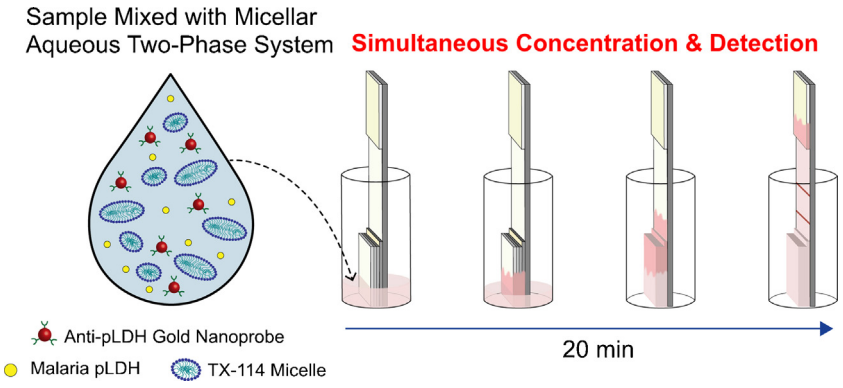
David Y. Pereira [a], Ricky Y.T. Chiu [a], Samantha C.L. Zhang [a], Benjamin M. Wu [a,b], Daniel T. Kamei [a,*]

[a] Department of Bioengineering, University of California, 420 Westwood Plaza, 5121J Engineering V, P.O. Box 951600, Los Angeles, CA 90095-1600, USA
[b] Division of Advanced Prosthodontics & Weintraub Center for Reconstructive Biotechnology, UCLA School of Dentistry, Los Angeles, CA 90095-1600, USA

HIGHLIGHTS

- Micellar two-phase systems rapidly separated and formed within fiber-glass paper.
- Two-phase systems concentrated biomarkers for use in the lateral-flow assay.
- A 3-D paper-based device was used to detect malaria pLDH in serum solutions.
- Concurrent pLDH concentration and detection were achieved in the paper-based device.
- A 10-fold improvement in the LFA detection limit for pLDH was achieved in 20 min.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form 15 April 2015
Accepted 20 April 2015
Available online 21 April 2015

Keywords:
Lateral-flow immunoassay
Malaria
Micellar aqueous two-phase system
Triton X-114
Diagnostic

ABSTRACT

The lateral-flow immunoassay (LFA) is an inexpensive and rapid paper-based assay that can potentially detect infectious disease biomarkers in resource-poor settings. Despite its many advantages that make it suitable for point-of-care diagnosis, LFA is limited by its inferior sensitivity relative to sophisticated laboratory-based assays. Our group previously introduced the use of a micellar aqueous two-phase system (ATPS), comprised of the nonionic Triton X-114 surfactant, to concentrate biomarkers in a sample and enhance their detection with LFA. However, achieving complete phase separation and target concentration using the Triton X-114 system required many hours, and the concentrated sample needed to be manually extracted and applied to LFA. Here, we successfully integrated the concentration and detection steps into a single step that occurs entirely within a portable paper-based diagnostic strip. In a novel approach, we applied the micellar ATPS to a 3-D paper design and effectively reduced the macroscopic phase separation time from 8 h to approximately 3 min. The 3-D design was integrated with LFA to simultaneously concentrate and detect *Plasmodium* lactate dehydrogenase (pLDH), a malaria biomarker, in both phosphate-buffered saline and fetal bovine serum within 20 min at room temperature. Compared to a conventional LFA setup with a pLDH detection limit of 10 ng μL^{-1}, our single-step diagnostic successfully detected pLDH at 1.0 ng μL^{-1}, demonstrating a 10-fold detection limit improvement and resulting in a sensitive and user-friendly assay that can be used at the point-of-care. The integration of a micellar ATPS and

* Corresponding author. Tel.: +1 310 206 4826.
E-mail address: kamei@seas.ucla.edu (D.T. Kamei).

http://dx.doi.org/10.1016/j.aca.2015.04.040
0003-2670/

LFA represents a new platform that can improve and promote the use of paper-based diagnostic assays for malaria and other diseases within resource-poor settings.

1. Introduction

Over 95% of deaths related to major infectious diseases (including malaria, acute respiratory infections, HIV, and tuberculosis) occur in resource-poor countries that have limited access to electricity, laboratory equipment, and trained personnel [1]. Developing reliable diagnostic tests that can be used at the point-of-care can result in earlier disease diagnosis, improved patient treatment, and more efficient outbreak prevention. An ideal point-of-care diagnostic test would need to be robust, rapid, inexpensive, equipment-free, and simple to use and interpret. One potential assay that satisfies these criteria is the lateral-flow immunoassay (LFA), a paper-based, antibody-based test that has received commercial success as over-the-counter pregnancy tests and recreational drug screening tests. However, due to its low sensitivity relative to laboratory-based assays, such as the enzyme-linked immunosorbent assay (ELISA) and the polymerase chain reaction (PCR), the LFA has seen limited use in infectious disease detection.

In order to improve the sensitivity of LFA, our group previously demonstrated the novel use of an aqueous two-phase system (ATPS) to concentrate a target biomarker into a smaller volume prior to its application to LFA. More specifically, the Triton X-114 micellar ATPS was used to achieve a 10-fold improvement in the LFA detection limit for a model virus bacteriophage M13 and a model protein transferrin (Tf) [2,3]. To detect proteins, such as Tf, that are smaller than viruses, gold nanoprobes (GNPs) decorated with specific antibodies were directly added into the sample solution in order to enhance the partitioning, and therefore, the concentration of the protein target. However, the point-of-care feasibility of this approach was limited by two prominent factors: (i) the many hours required for phase separation to occur within the Triton X-114 ATPS [4] and (ii) the additional user interaction necessary for extracting the GNP solution between the concentration and detection steps.

More recently, our group achieved a significant reduction in the macroscopic phase separation time of a polyethylene glycol (PEG)-phosphate salt ATPS by applying it directly to a paper-based device [5]. Through our newly discovered phenomenon, a well-mixed homogenous solution was shown to rapidly separate into its two phases as it flowed through the paper membrane, allowing for paper-based concentration of biomolecules in a predictive manner. Our work in combining LFA with ATPS represents a novel contribution to this field of paper-based diagnostics that recently has had other advances in concentrating molecules [6,7] and improving analytical detection [8–13].

Here, we applied the Triton X-114 ATPS to a 3-D paper architecture to determine if the paper set-up could also enhance the separation process of this micellar system. The Triton X-114 micellar system phase separates much more slowly than the PEG-phosphate salt ATPS, and so we witnessed an even greater reduction in phase separation time, from at least 8 h to about 3 min, extending the applicability of this phase separation phenomenon in paper to a micellar ATPS for the first time. We then integrated the paper-based design with the LFA to form an all-in-one paper-based diagnostic strip that simultaneously concentrates and detects a disease biomarker without user intervention. To demonstrate this, we used the diagnostic strip to detect the malaria biomarker *Plasmodium falciparum* lactate dehydrogenase (pLDH) in solutions of phosphate-buffered saline (PBS) and undiluted fetal bovine serum (FBS). The robust, one-step automated diagnostic strip concentrated and detected pLDH within 20 min, demonstrating a 10-fold improvement in the pLDH detection limit when compared to a traditional LFA set-up. This platform technology overcomes the abovementioned limitations and can be used to transform the current state of diagnostic assays for malaria and other diseases within resource-poor settings.

2. Materials and methods

2.1. Preparing gold nanoprobes (anti-pLDH GNPs)

The gold nanoparticles were prepared according to Frens with slight modifications [14]. Using this method, a solution of gold nanoparticles with an average hydrodynamic diameter of 24 nm was obtained, which appeared as a dark cherry-colored solution. The size of the gold nanoparticles was obtained by dynamic light scattering measurements using a Zetasizer Nano ZS particle analyzer (Malvern Instruments Inc., Westborough, Massachusetts).

After forming the nanoparticles, the pH of a 1 mL gold nanoparticle solution was first adjusted to pH 9 using 1.5 N NaOH. Subsequently, 16 μL of mouse monoclonal anti-*P. falciparum*/*P. vivax* LDH antibodies (BBI Solutions, Cardiff, UK) at a concentration of 0.5 mg mL^{-1} were added to the colloidal gold solution and mixed for 30 min on a shaker. To prevent nonspecific binding of other proteins to the surfaces of the colloidal gold nanoparticles, 200 μL of a 10% w/v bovine serum albumin (BSA) solution were added to the mixture and mixed for 20 min on a shaker. To remove free, unbound antibodies, the mixture was centrifuged for 30 min at 4 °C and 12,000 rpm; the pellet of colloidal gold nanoparticles was then resuspended in 200 μL of a 1% w/v BSA solution. The centrifugation and resuspension steps were repeated two more times, and after the third centrifugation, the pellet of gold nanoparticles was resuspended in 100 μL of 0.1 M sodium borate buffer at pH 9.0. The gold nanoparticles functionalized with anti-pLDH antibodies will henceforth be referred to as anti-pLDH gold nanoprobes (anti-pLDH GNPs). The BSA-coated gold nanoparticles not functionalized with antibodies were used for visualization purposes and will henceforth be referred to as BSA-GNs.

2.2. Preparation and vsualization of Triton X-114 ATPS

Equilibrium volume ratios (the volume of the top phase divided by the volume of the bottom phase) of the Triton X-114 ATPS were found by varying the initial w/w concentration of Triton X-114 (Sigma–Aldrich, St. Louis, MO) in solutions of Dulbecco's phosphate-buffered saline (PBS; Invitrogen, Grand Island, NY, pH 7.4, containing 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 137.92 mM NaCl, 2.67 mM KCl, and 0.49 mM $MgCl_2$) and FBS (Invitrogen, Grand Island, NY). These solutions were allowed to phase separate and reach equilibrium at 25 °C in a temperature-controlled water bath. BSA-GNs in Triton X-114/PBS solutions exhibited favorable partitioning to the top phase, while BSA-GNs in Triton X-114/FBS solutions partitioned favorably to the bottom phase. Consequently, the conditions for a 1:9 volume ratio (*i.e.*, volume of the top phase divided by that of the bottom) for Triton X-114/PBS and a 9:1 volume ratio in Triton X-114/FBS were found and used for further experiments. These volume ratios allowed for a 10-fold concentration of the nanoparticles.

In order to visualize the two phases of the micellar ATPS in PBS, 100 μL of BSA-GNs and 4 μL of Brilliant Blue FCF dye (The Kroger