A POLYMER LAB-ON-A-CHIP WITH MULTIPLE SAMPLE LOADING METHOD FOR HIGH SENSITIVE IMMUNOASSAYS
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ABSTRACT
A disposable lab-on-a-chip (LOC) with the capability of multiple sample loadings for high sensitive immunoassay of thyroid stimulating hormone (TSH) has been newly proposed and realized in this work. For the multiple sample loadings on the lab chip, multiple on-chip Nitrogen generators have been developed and integrated with the lab-on-a-chip. The sensitivity of assay was linearly improved with the number of multiple sample loadings, which provides a new method for the flexibly tunable sensitivity of immunoassay.

KEYWORDS: Lab-on-a-chip (LOC), Thyroid stimulating hormone (TSH), Multiple sample loading, Point-of-care testing (POCT) diagnostics

INTRODUCTION
Many proteins are present in sample with a very low concentration, so the assay of such protein is considered as one of the most difficult tasks. For the samples such as plasma, serum or whole blood, multiple sample loading method is suitable for performing a high sensitivity of assay. Multiple sample loading method was demonstrated for 96-well platform based on microfluidic channel, using pipettes or automatic liquid handling systems [1]. The new method developed in this work has expanded the on-chip multi-sample loading technique into disposable lab-on-a-chip (LOC) for point-of-care testing (POCT) diagnostics. The on-chip reagent reservoirs, which were developed with the sample-to-answer POCT cartridge in our previous work [2], have been integrated with the disposable LOC for thyroid stimulating hormone (TSH) assay. For minimum user intervention the samples and reagents were driven into the reaction chamber using the Nitrogen gas generated by thermal dissociation of azobis-isobutyronitrile (AIBN) [3]. A huge part of the population faces medical issues due to hypo and hyperthyroidism [2, 4], so the clinical diagnosis of elevated or inhibited TSH level is greatly desired. Thus, there is a large demand for the development of analytical methods of TSH with high sensitivity for POCT diagnostics.

DESIGN AND FABRICATION
The basic concept of the multiple loading method is illustrated in Figure 1. All the stored reagents for the assay will be sequentially delivered to the spiral detection chamber, but the sample will be multiply loaded from 1 to 5 times as desired.

Figure 1. Basic concept of the multiple sample loading and sequential reagent dispensing using AIBN.
will be loaded and sequentially dispensed over the spiral reaction chamber pre-coated with the capture antibody of TSH. Each of the sample loading chambers is connected to the Nitrogen generator containing AIBN. The reagent reservoir designed with the same specification as [2] consists of a detection antibody chamber, chemiluminescent substrate chamber with a volume capacity of 16 μL, and washing buffer chamber with a volume capacity of 30 μL. They are also connected with Nitrogen generator for sequential dispensing. Figure 3(b) shows the fabricated polymer chip on cyclic olefin copolymer (COC) with on-chip micro-reservoirs for immunoassay reagents and sample wells with a dimension of 23 mm² fabricated using hot embossing method. The sample transport system, as shown in Figure 2, consists of solid AIBNs of 5 mm in diameter and heaters [3].

![Diagram of the Lab-on-a-chip](image)

**Figure 3. Lab-on-a-chip with multiple sample loading ability: (a) Schematic diagram and (b) Fabricated lab chip.**

The immunoassay protocol is similar to the sandwich immunoassay mechanism described in [2] with the variation of multiple sample loadings. TSH from diluted blood was tested for determining clinical diagnosis of hypothyroidism and hypothyroidism. The spiral reaction chamber was filled with capture antibody, and then washed and blocked with protein blocking buffer (PBS). The five sample loading chambers were filled with TSH sample and the reservoirs were filled with detection antibody, washing buffer and chemiluminescent substrate, respectively. Each heater was turned on in sequence using a control system. The solid AIBN located in the chamber on top of the heater then dissociated to produce Nitrogen gas. This Nitrogen gas drove the sample pre-loaded at the sample loading chamber into the reaction chamber. The same driving mechanism was used to drive the reagent from the chambers.

**RESULTS AND DISCUSSION**

As a demonstration vehicle of test, TSH in diluted blood was used. The chemiluminescent signal was measured using an optical reader (Synergy Hybrid Reader, BioTek). Figure 4(a) shows measured signals for the multiple sample loadings. The LOD obtained from five times (5X) sample loadings is 0.4 μIU/mL. Figure 4(b) shows the signal amplification ratio with respect to the sample loadings. The accepted range for the conventional ELISA of TSH is 0.3 - 3.0 IU/mL. However the conventional ELISA requires more reagent and sample volume as well as longer analysis time. So this conventional ELISA is hard to be adopted for POCT diagnostics. In our previous work [2] with a single sample loading (1X) the obtained LOD was 1.9 IU/mL, achieving the dynamic range of 1.9 - 55 IU/mL.

With the new concept of multiple sample loadings, the LOD was improved with more number of sample loadings. The LOD obtained from the 3X sample loadings was 0.7 μIU/mL and the LOD from 5X was 0.4 μIU/mL with the dynamic range up to 55 IU/mL. The assay results show that, as the number of sample loadings increased, the LOD was improved almost linearly due to the enhanced sensitivity. This new multiple sample loading method provides a new way to improve the LOD of most immunoassay.
CONCLUSION

The new multiple sample loading method developed in this work has successfully achieved a lower LOD, compared with the result attained from the single loading method. This work clearly demonstrates that the sensitivity of immunoassay could be improved almost proportionally to the number of multiple sampling loadings, which will bring an innovation to the ultra high-sensitive immunoassay on polymer lab-on-a-chips. In this work, the obtained LOD was 0.4 µIU/mL with a dynamic range of 0.4 - 55 µIU/mL.

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REFERENCES:

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