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A portable on-chip assay system for absorbance and plasmonic detection of recombinant bovine growth hormone in milk

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ABSTRACT

This paper reports a portable device and method to extract and detect recombinant growth hormone from milk samples for point-of-care and point-of-need applications. Growth hormone from milk samples was extracted by solid-phase extraction, and detection was carried out using the plasmonic property of gold nanoislands deposited on a glass substrate. Trace levels of growth hormone in milk were analyzed by their optical absorbance property using a microfluidic chip. To perform detection at the point of need, we built a portable assay system using disposable lab-on-chip devices. The proposed method is able to detect recombinant growth hormone in milk at concentrations as low as 5 ng/mL.
Key words: bovine growth hormone, lab-on-chip, plasmonic detection

INTRODUCTION

Bovine growth hormone, also known as somatotropin (bST), consists of a polypeptide chain of 191 AA; it is naturally produced in mammals and is known to influence the growth and reproductive system of cows (Rochut et al., 2000). Somatotropin has frequently been used as a growth promoter in dairy farming to increase milk production (Saha et al., 1994); it increases milk production by partitioning the nutrients absorbed by the cows. The commercialization of bST became possible with the discovery of recombinant DNA technology that allowed artificial hormone to be produced in large amounts (Ahmed and Khosa, 2010). The use of recombinant (**r**)bST to increase milk production is allowed in some countries, including the United States. However, many countries, including Canada and the European Union, have banned the use of rbST. The use of rbST is controversial mainly because of its potential effects on the health of animals and consumers (Buttel, 2000; Gohary et al., 2014). To regulate and monitor the administration of growth hormone in production of milk and its derivatives, a simple, low-cost, and portable device with a reliable detection procedure is required.

Typically, a 100-g sample of cow milk contains water (85.4 g), proteins (3.1 g), fat (3.5 g), sugar (4.4 g), cholesterol (10 mg), calcium (100 mg), SFA (2.3 g), MUFA (0.8 g), and PUFA (0.1 g) (Hadjipanayiotou, 1995). Milk contains a large variety of proteins; the majority are caseins and the remainder are arrays of enzymes, proteins responsible for transporting nutrients, proteins responsible for resisting diseases (antibodies), and proteins that control growth. The concentration of natural growth hormone in milk is 1 to 10 ng/mL (McGrath et al., 2008); however, the milk of animals treated with rbST may contain rbST up to 100 ng/mL, depending upon its administration in the animal (Le Breton et al., 2010).

Various methods to analyze proteins have been used to detect growth hormone. The traditional approaches of detection of growth hormone are ELISA (Castigliego et al., 2007), RIA (Jindal and Ludri, 1990), bioassay methods, and surface plasmon resonance-based immunoassays (Heutmekers et al., 2007). Recently, Le Breton et al. (2010) proposed a detection method of rbST in milk, using liquid chromatography, combined with mass spectroscopy (Rochut et al., 2000). Most of the reported methods are expensive and involve complex analytical processes. They are laboratory based, not portable, very expensive, labor intensive, and time consuming. Hence, bioassays using lab-on-a-chip (**LOC**) devices are attractive because of several inherent advantages; they have high sensitivity, are low cost, and require only minute amounts of samples and reagents. We have recently reported on the labeled and label-free detection of bST (Ozhikandathil et al., 2012b; Ozhikandathil and Packirisamy, 2012b) and rbST (Ozhikandathil et al., 2012a; Ozhikandathil and Packirisamy, 2013) using optical LOC devices. However, due to the complexity of the experimental setup, the LOC were not portable and the detection experiments could not be done on a point-of-need basis. In addition, in our previous work, the detection experiments were not carried out

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in milk samples. The present work proposes a method of extraction of rbST from milk and detection using a portable device.

The separation of proteins is an essential process in the detection of growth hormone in milk. A large variety of separation processes can be used; however, solid-phase extraction (**SPE**) is the most powerful method because it is faster and more accurate than other methods. Traditionally, SPE systems were used with chromatographic systems for the quantitative and qualitative analysis of bio-molecules. In this work, we report an SPE protocol for the separation of proteins from milk, combined with specific detection of rbST using gold nanoislands in a portable device. In our previous work, a simple and low-cost approach was reported for the deposition of gold nanoislands on glass substrate for plasmonic detection. However, that method was not investigated for the detection of growth hormone directly in milk samples.

To regulate the use of growth hormone in dairy farming, it is necessary to develop LOC devices and a portable testing apparatus. The main drawback of the most of the LOC reported in the literature is that they are not portable because of complexities in test setup. This work reports a low-cost and simple procedure to detect growth hormone in milk samples using a portable device and a microfluidics chip. The main motivation of the present work was to take microfluidics-based assays to the field where rapid testing is required. The proposed portable optical device is capable of measuring the absorbance of biological species and nanostructures for screening antigen–antibody interactions using microfluidics chips.

The schematic of the portable setup for the measurement of absorbance of the localized surface plasmon resonance (**LSPR**) band is shown in Figure 1(a). The setup consists of a UV-visible source coupled to a spectrometer through 2 lens collimators attached on 2 metal slots. The metal slots are designed such that the microfluidic chips can be inserted and self-aligned in the optical path. Glass substrates with gold nanoislands are used for the plasmonic detection experiments. As shown in Figure 1(b), the substrate with gold nanoislands is inserted into the metal slot and optical measurements are taken. The microfluidic chip is designed and fabricated for absorbance measurements. Figure 1(c) shows the schematic of the microfluidic chip used in the device. The size of the microfluidic chip was 3×1.5 cm.

Gold nanoparticles are extensively used for label-free detection of proteins due to their strong optical absorbance properties in the UV and visible regions of the electromagnetic spectrum (Heutmekers et al., 2007; Hoa et al., 2007; Willets and Van Duyne, 2007; Huang et al., 2009). The plasmon band, also called LSPR, is due to the collective oscillation of electrons at certain frequencies. The LSPR is sensitive to changes in the refractive index of the surrounding environment; hence, they are useful in immunoassays. The gold nanoislands were formed on the glass substrates by convective assembly

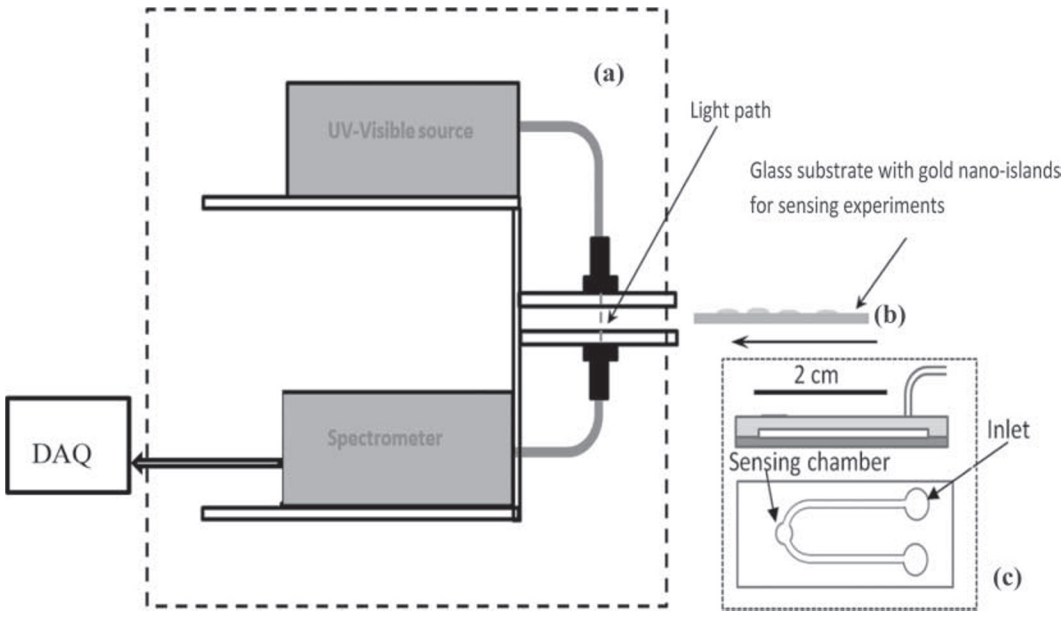

Figure 1. (a) Schematic of the portable device (DAQ = data acquisition laptop), (b) glass substrate with gold nanoislands used for the plasmonic sensing of recombinant bST (rbST) in milk, and (c) schematic of the microfluidic chips used for the measurements of absorbance of rbST. Color version available online.