5
Using Nanoparticles in Agricultural and Food Diagnostics

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5.1
Introduction

There is growing interest in the safety of agricultural raw materials and of food and feed products. During growth, the production process, and the storage of food, sophisticated, low-cost, and rapid tests are increasingly being used. Safety items include the presence of pathogenic micro-organisms [1–4] or the toxins they produce during storage of the raw ingredients [5–14]. The presence of pesticides [15–20], anabolic steroids [21], antibiotics [22–25], or adulterating substances [26–28] is also a matter of concern. New regulation on food labeling requires notification with respect to the (possible) presence of allergenic substances to inform the allergic consumer of potential hazards [29–31]. The presence of the phrase "may contain" on the label is no longer sufficient.

Here we will present biosensors that use nanoparticles as detection labels. Some of these sensors can be applied on-site, needing a minimum amount of resources and training. Biosensor formats that will be discussed include lateral flow (immuno)assays, nucleic acid lateral flow (immuno)assays, flow-through (immuno) assays, antibody microarrays, and the surface plasmon resonance biosensor. The use of nanoparticles during sample pre-treatment will be mentioned and future prospects will be discussed.

5.2
Biosensors

According to the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is "a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with a transduction element" [32]. Biosensors are being developed that recognize the micro-organism or analyte with high specificity, sensitivity, and efficiency [33].

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A special kind of biosensor is an immunosensor that uses the highly specific interaction of antibody and antigen, often with high sensitivity and speed. Immunosensors are among the most often used sensors for toxin and microbial detection in agriculture, food, and feed [34]. The biosensor is constructed on a carrier material onto which the capturing element is immobilized, a transduction element is positioned, and often a connection is made to a device for reading the response and archiving purposes. A set-up for sample pre-treatment and automated delivery can be included. The workhorse of immunosensors is the antibody, which has to be highly specific, sensitive, and efficient. Although very important, this aspect is not the focus of this chapter. We will only mention here that a specific, sensitive, and efficient antibody has to be available. Especially when running rapid tests, the affinity between analyte and capture agent should be high, with short interaction kinetics to allow a relevant number of complexes to be formed within the timeframe of the test.

5.3 Transduction Principles

The transduction technology ultimately gives the result of the test. Although a wide variety of transduction principles are available, the most popular are of an electrochemical [35] or optical nature, although magnetic [36] and piezoelectric transduction [37] principles are gaining more attention. For rapid assays, it is preferable to develop a test where the results can be interpreted by visual examination. To this end, specific antibodies or secondary antibodies are labeled with colored nanoparticles. Today, the most used nanoparticles are based on colloidal gold with a diameter of 40 nm [5, 7, 10–14, 17–19, 21, 25, 38–53], giving a red color. Nanoparticles that are used less often include colloidal carbon [2, 54–57] (black), colored latex [15] (several colors), fluorescent silica particles [58], or dye-encapsulated liposomes [6, 31] (several colors or fluorescent).

Nanoparticles with magnetic properties [12, 36, 59–62], quantum dots having fluorescent properties [63] (several colors), and nanoparticles with up-converting phosphors have been developed as well [64–66]. However, no applications in the agricultural disciplines are known. Magnetic nanoparticles are, apart from signal-generating labels, also used in sample pre-treatment and washing [36, 59]. Although with colored nanoparticles the result is visible, it may be necessary to digitize the results for later evaluation. To that end, specialized readers are available, but a flatbed scanner and image analysis software are often sufficient [2, 4, 54, 56, 57, 67]. For fluorescent and magnetic particles, a dedicated reader is obligatory.

Coupling of the requested biological compound (for example, antibody or (strept)avidin to the nanoparticles) basically can be done using one of two strategies: coupling by adsorption [54] or by covalent interaction by means of a chemical reaction [61, 68].

5.4
Examples of Biosensors in Which Nanoparticles Are Being Used

5.4.1
Lateral Flow (Immuno)assay

One of the most popular immunochemical methods is the lateral flow (immuno) assay, well known from the pregnancy test. The test does not require trained personnel or expensive equipment, and its result is often a visual "yes" or "no" with a certain cut-off value. Usually the results are obtained within 30 minutes. Depending on the analyte, several formats may be developed. For high-molecular-weight analytes, the sandwich format is applicable. On a nitrocellulose strip with dimensions of, for example, 5 cm × 0.5 cm, a transverse stripe of a solution of the specific antibody at an appropriate concentration (100–1000 μg ml^{-1}) in a, preferably, low-salt buffer is sprayed at an appropriate distance, for example, 1.5 cm, from the origin, called the test line. A second line can be included at some distance from the test line, called the control line. This line may contain an antibody against the species of the labeled antibody to provide a test control.

A sample application pad and a conjugate release pad are mounted on one end of the strip and an adsorbance pad is on the opposite end. The conjugate release pad contains the nanoparticles labeled with antibody and used for the evaluation (see Section 5.3). This antibody can be the same as or different from the sprayed antibody. Often, one antibody is a polyclonal and the other is a monoclonal, recognizing different epitopes of the analyte. The strip can be mounted in a device for easier handling. The strip is dried and can be stored in a sealed aluminum pouch with desiccant for later use. Such a strip can be stored for a prolonged time without refrigeration. Running the test is possible by simple addition of a fixed volume, for example, 10–100 μl of (an extract of) the food or feed sample on the sample pad. An appropriate running buffer, for example, 100 mM borate buffer (pH 8.8) with 1–2% bovine serum albumin (BSA) or any other blocking compound and 0.05% Tween 20 can be added to make the volume up to100 μl to run the test when necessary. Using this format, a positive result is obtained when the analyte is present. The response at the control line has to be positive in all cases, to ensure a proper performance of the test. The test format is called lateral flow (immuno) assay (LFIA) or immunochromatography (ICG) in sandwich format [69]; a scheme is presented in Figure 5.1.

When the analyte is of low molecular weight, such as pesticides or antibiotics, the test has to be formatted in another way (inhibition format). A conjugate of an analog of the analyte to a carrier protein has to be sprayed at the test line. It is advisable to use a carrier protein other than the protein that has been used to produce the antibody. For example, if the antibody-inducing antigen used a conjugate to keyhole limpet hemocyanin (KLH), then the protein of the conjugate to be sprayed may be BSA instead.

Again, the conjugate release pad contains the label and a specific antibody that recognizes the analyte. The strips in this format can be stored and the test can be

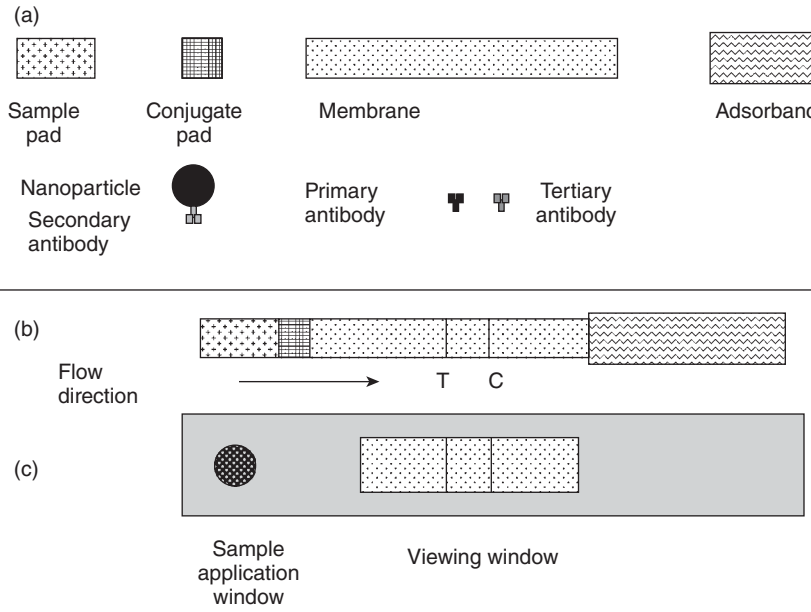

Figure 5.1 Scheme of a lateral flow immunoassay test strip and device in sandwich format: (a) parts of the test, (b) ready-made test strip (test line T; control line C), (c) test strip in device (not to scale).

processed in the same way as outlined above. However, the presence of a colored response at the test line now indicates the absence of the analyte, and the absence of a response indicates the presence of the analyte above a certain threshold. One can say that in this case the technician who performs the test needs more knowledge. The test format is called LFIA or ICG in inhibition format [69]. Several attempts are being made to reverse the response in this layout (presence of analyte yields a positive, colored response) using anti-idiotype antibodies (antibodies against antibodies) [70], or anti-complex antibody fragments produced in an expression system [71]. A scheme of this principle and layout is presented in Figure 5.2.

5.4.2 Nucleic Acid Lateral Flow (Immuno)assay

Another quite different format has to be developed when a specific and sensitive antibody cannot be generated. This is especially true when the absence of pathogenic micro-organisms has to be proven. To design such a test, a species-specific deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequence has to be amplified using one of the currently available amplification procedures such as the polymerase chain reaction (PCR) [2, 38, 54, 72–77], loop-mediated isothermal amplification (LAMP) [43, 78–81], and the nucleic acid sequence-based amplification (NASBA) [68, 82].

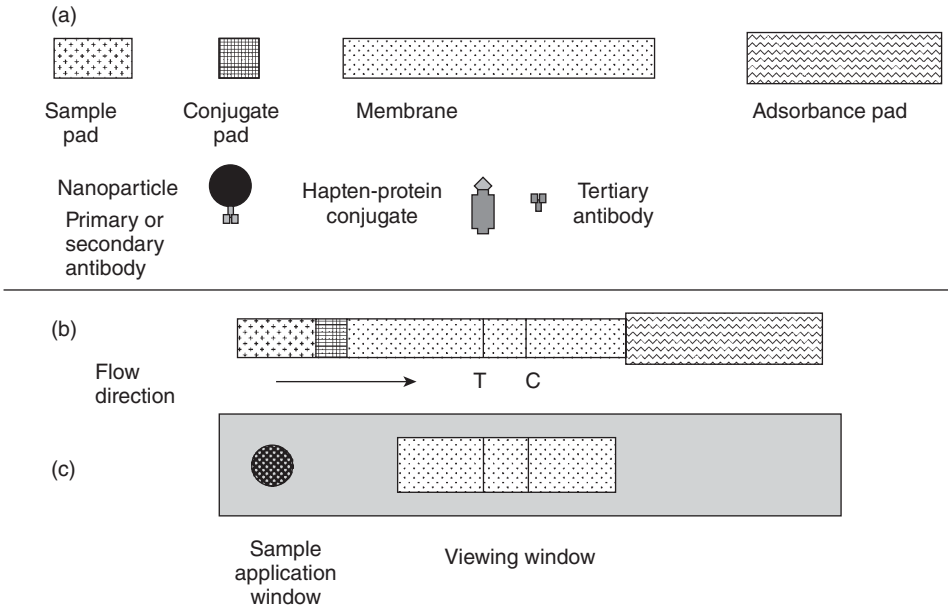

Figure 5.2 Scheme of a lateral flow immunoassay test strip and device in inhibition format: (a) parts of the test, (b) ready-made test strip, (c) test strip in device (not to scale).

As an example, a strategy of PCR-amplified species-specific templates is illustrated in Figure 5.3. A set of two primers is used, of which the forward primer usually contains a discriminating tag and the reverse primer has a biotin. Such primers can be ordered from primer suppliers according to the sequence required. Antibody against the discriminating tag is sprayed at the test line and a conjugate of the nanoparticles to (strept)avidin is used for visualization of the signal. Discriminating tags used include Texas Red (TxR), fluorescein isothiocyanate (FITC), cyanine 5 (Cy5), digoxigenin (DIG), and dinitrophenyl phosphate (DNP). Antibodies to these tags are commercially available and are sprayed at the test line. The control line contains biotin-labeled immunoglobulin G (IgG), also widely available. The response at the control line has to be positive in all cases, to ensure a proper performance of the test. This test format is called nucleic acid lateral flow (immuno) assay (NALFIA) [2, 4, 57, 59], and the response is positively correlated to the amount of amplicon.

5.4.3 Flow-Through (Immuno)assays

Apart from the above-mentioned format where the sample flows laterally through the membrane, it is also possible to design a format with a vertical sample flow, that is, through the membrane. In this case, several spots may be applied onto the membrane, of which one spot is the test control and the other(s) contain(s) the

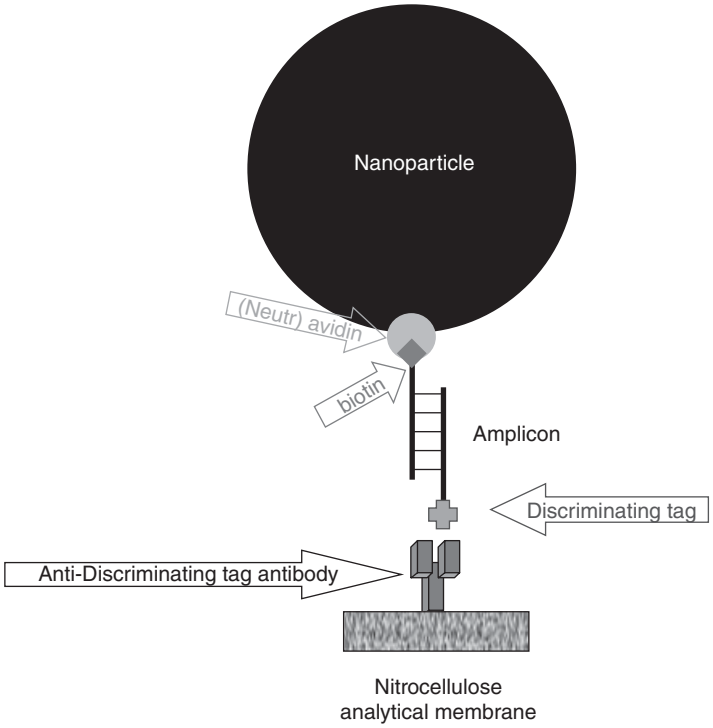

Figure 5.3 Scheme of a nucleic acid lateral flow test principle.

capturing agent(s) [18, 19]. A scheme of this format and test principle is shown in Figure 5.4.

5.4.4 Antibody Microarrays

Protein chips using antibodies as recognition elements are evolving very rapidly [83]. They are not yet as popular as the above-mentioned techniques and formats; this is mainly due to the paucity of suitable antibodies, lack of affinity, cross-reactivity, and loss of functionality upon binding. Antibody microarrays are used for multi-analyte testing [84–86]. Briefly, antibodies raised against the analytes of interest are spotted in an ordered way on a carrier chip, often in a microscope slide format [87]. It is also possible to spot a microarray in the wells of a multi-well plate. Often gold nanoparticles are used for detection upon binding of analyte to antibody. However, we recently showed that carbon nanoparticles are well suited to this task; see Figure 5.5 for a typical layout and principle.

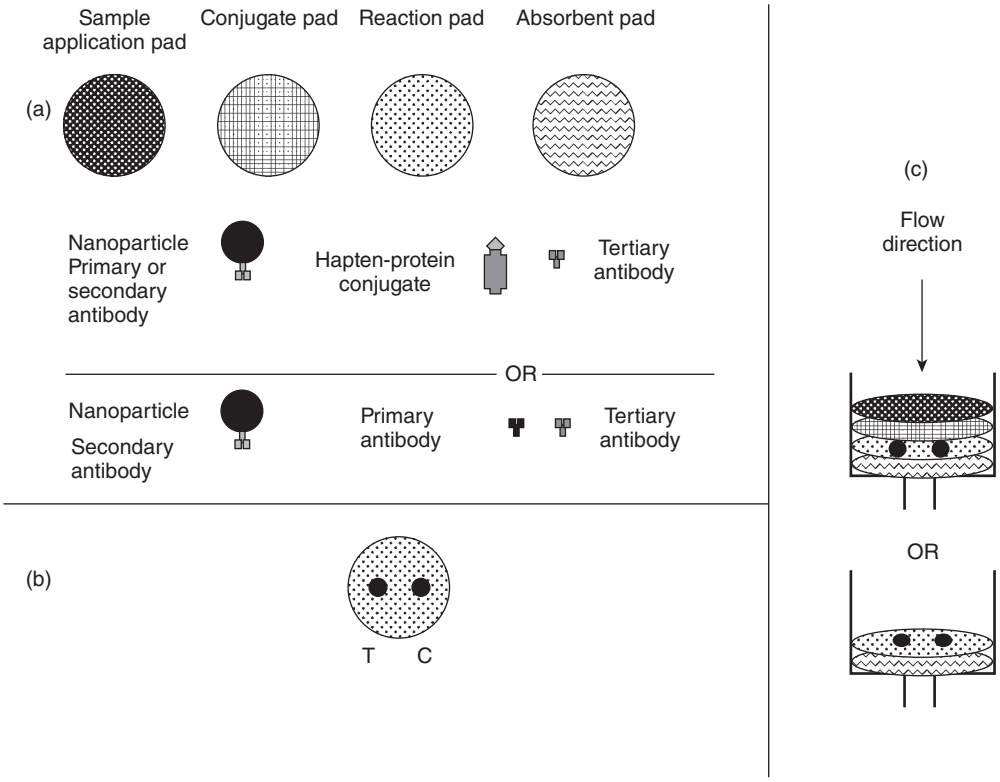

Figure 5.4 Scheme of a flow-through immunoassay, in sandwich or inhibition format: (a) parts of the test, (b) ready-made test pad, (c) test pad in device (not to scale).

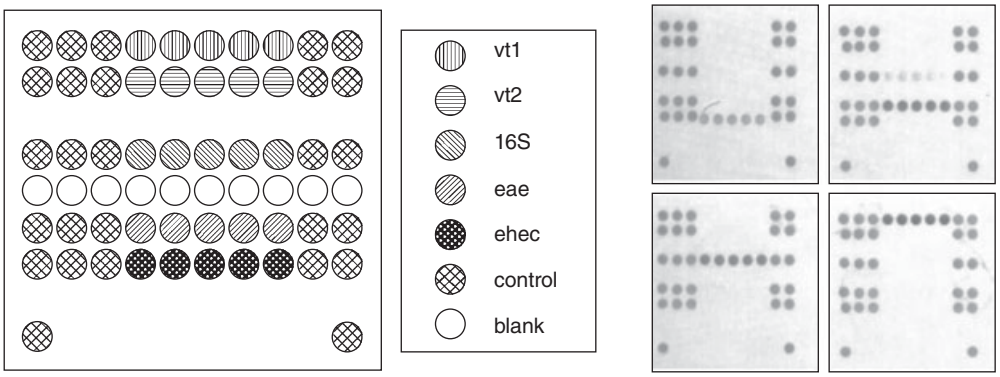

Figure 5.5 Layout of an antibody microarray and a typical set-up of a test.

5.4.5
Surface Plasmon Resonance Spectroscopy

Surface plasmon resonance spectroscopy is a technique where antibody–antigen interaction is visualized using the change of the refractive index upon binding. The technology relies on the generation of plasmons, quasi-particles resulting from the quantization of plasma oscillations that can be compared to photons for light waves. Optical evanescent waves are commonly found during total internal reflection. When a plasmon interacts with a molecule, characteristics depending on the molecular mass are changed and can be measured. Mainly the angle of reflection changes due to the interaction with coated molecules. A biosensor based on surface plasmon resonance is advertised as a label-free technique. However, sometimes nanoparticles are used to increase its sensitivity [88, 89].

5.5
Future Prospects

Preferably, the whole assay covers automated sample-taking until the read-out of the results without any additional handling by the user. To that end, an integrated, so-called "lab-on-a-chip" layout may be used in which all necessary items, including sample pre-treatment, are combined in one, often disposable, housing. However, no applications have been presented for food or feed components, but the principles of those presented in the medical profession [90] use nanoparticles doped with up-converting phosphors. This format will be transferable very well to food and feed safety issues.

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