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Reviews

Immunoassays and Environmental Studies

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Abstract

Immunoassays (immunochemical methods - IMA_S) are now being seen as useful analytical tools, and supplements to conventional analytical methods: gas chromatography - GC and high performance liquid chromatography - HPLC. Immunochemical methods provide rapid, sensitive, and cost-effective analyses for a variety of environmental contaminants. Development of these methods is multidisciplinary. IMA_S combine principles of immunology and chemistry into tests that are used by scientists in practically every discipline, including fields as diverse as molecular biology and environmental science. All immunoassays rely on interaction between an antibody as analytical reagents and target analytes (antigen). Environmental immunoassays have been developed and evaluated for analytes including major classes of pesticides, organic compounds as polychlorinated biphenyls (PCB_S), polyaromatic hydrocarbons (PAH_S), pentachlorophenols (PCP_S), BTEX (benzene, toluene, ethylbenzene, and xylene), dioxins and furans, and some inorganics, for example cadmium, lead, mercury, and microbial toxins.

This paper provides an overview of the possibilites of immunoassays as a detection method for environmental contaminants. The principles and the history of immunoassay methodology are reviewed.

Keywords: antibodies, antigen, environmental contaminants, immunoassays

Introduction

Environmental contamination (inorganic and organic) is recognized as a worldwide problem.

Many contaminants can be analyzed using sometimes highly sophisticated analytical techniques. Much effort has been put into research concerning the development of new and improved existing methods for contaminant analysis. The methods generally used to measure contaminants are HPLC and GC. These methods require extensive purification, experienced technicians and expensive equipment and reagents. As a consequence, attention has been directed to new methods. The immunoassay seems to be a good alternative, at least for screening purposes. The immunoassay is not new, because it has been used for many years in clinical chemistry as a reliable, sensitive, and selective method to determine low concentrations of organic compounds in, for example, blood, urine, tissue extracts, etc. [8, 29, 37].

Immunoassay technology originated in the late 1950s when Yalow and Berson [38] (Nobel Prize awarded) published of immunological assay which could detect human insulin at the picogram level in small samples of blood. In the following years this technology found wide application in biochemistry, endocrinology, and clinical chemistry [29].

The possibility to adopt immunoassays for environmental studies was recognized more than a decade ago. Ercegovich introduced IMA techniques for ecosystem contaminants in the early 70's [6]. IMA_S have been developed for a broad range of pesticides and contaminants of industrial origin [1, 10, 12, 20]. Several articles have reviewed recent progress in the development of IMA for environmental contaminants, and their use in field studies and human biomonitoring [9, 13, 22, 33-36].

Table 1 presents properties of traditional and IMA techniques. IMA_S can help provide timely data that are often needed when dealing with industrial spills and

environmental hot-spots. The driving force in the development of immunochemical methods is the need for rapid and simple tests that can be performed on site without requiring sample transfers to an analytical laboratory. Field-portable immunoassays enable rapid determination of compounds needed to effectively direct hazardous waste site remediation and clean-up. They can be used by personnel unfamiliar with analytical chemistry methodologies [35].

These methods provide rapid, sensitive and cost-effective analyses for a variety of environmental contaminants. They can be adopted to the laboratory and field study. Probably the next few years will be critical in the development of immunochemical technology for use in environmental analysis. The increasing popularity of field immunoassay analysis is due, in large part, to the highly portable equipment and minimal setup requirements [36].

The Immunoassay

Immunoassays are generally termed as assays that employ antibodies to detect and quantify antigens. In environmental analysis the potential of immunoassays for quick and large-scale screening of contaminants has been recognized and an increasing number of modification of assays have been developed [9, 17]. The main component of immunoassays is the antibody that specifically binds a target molecule.The antibodies used in IMA_S belong to the immunoglobulin's gamma (IgG) [18, 31].

The antibodies can be derived from antiserum - polyclonal antibodies (PAb) or from hybridomas - monoclonal antibodies (MAb) [5, 16, 18, 27, 37]. PAbs are easy to obtain while MAbs are expensive to screen and maintain. During recent years, the fast development in the field of cloning and recombination DNA technology and the progressive knowledge of the molecular structure of Abs and their genes have allowed these problems to be solved by the *in vitro* synthesis of Ab. The RAbs (recombinant antibodies) have some advantages over the PAb and MAb. They are: stability and affinity, have the smaller size of the Ab and low production cost. On the other hand, the procedure for their production is very complex and needs new bioengineering techniques [18, 21].

Nowadays, the potential of this technique allows the possibility of designing a receptor for each particular antigen and modulating the characteristics of a given Ab.

The second step in developing a successful immunoassay involves a suitable marker. The marker serves to facilitate the rapid detection of antibody-antigen binding. The different types of markers used over time are: isotopes, enzymes, coenzymes, and fluorigenic substrates.

The main principle of IMA can be illustrated by the following reaction [20]:

$$Ab + Ag + Ag^* <-> AbAg + AbAg^*$$

where:
Ab - antibody,
Ag - antigen, and
Ag* - labelled antigen

GLOSSARY

Antibodies	– a class of proteins known as immunoglobulines which are produced in response to a foreign substance
Antigen (or immunogen)	– a foreign compound which can be bound by antibodies and stimulate their formation; it can be biological and synthetic
Hapten	– small foreign molecules do not induce an immune response but is recognize by some antibodies
Monoclonal antibodies (MAb)	– a homogenous antibody population produces by a hybridoma cell line
Polyclonal antibodies (PAb)	– a serum sample that contains heterogenous population of antibodies, varying in specifity and affinity
Recombinal antibodies (RAb)	– antibodies produced by *in vitro* synthesis (cloning and recombination of DNA)
Hybridoma	– the product of the fusion of two different parental cells that contains genomic material from both cells. This cell combination (hybridoma) can produce a single type of antibodies
Epitope	– a specific chemical domain on an antigen that is recognized by an antibody; also called antigen determinant

The AbAg interaction is week and involves bonds, for example Van der Waals interaction and electrostatic bonds - usually predominate, another hydrogen and hydrophobic bonds.

Generally, there are two immunoassays:

- first system is termed radioimmunoassay (RIA) and uses radioactive labels as markers,
- second system uses enzymes, and is termed enzyme immunoassay (EIA).

Radioimmunoassay (RIA)

The successful introduction of RIA has revolutionized many areas of clinical and biological sciences. In this method different radioisotopes were used, for example ^{125}I, ^{3}H, and ^{14}C [28, 37]. There are disadvantages associated with the use of radioactive elements in this system, for example: the need of radiological protection, and separated areas to perform the assay, the generation of radioactive waste, the short half-life of some radioactive isotope used, and the necessity of expensive analytical equipment. Such disadvantages have led to the development of immunoassay systems using alternative labels. Some of these systems use fluorescent or chemiluminescent, enzymes.

Table 1. Comparison of traditional and immunoassay techniques.

PROPERTIES	TRADITIONAL TESTING	IMMUNOASSAYS
– TURNAROUND TIME	1 – 4 weeks	less than 2 hours
– TEST COST	$ 65 – $ 250 per test	$ 10 – $ 25 per test
– PORTABILITY	laboratory bound	in-lab or on-site
– ACCURACY	high	high
– RELIABILITY	high	high
– EASE OF USE	dificult	moderate to simple

Enzyme immunoassay (EIA)

Enzyme immunoassays were first introduced to environmental analysis in the early 1970's [6, 28, 33]. The assays use enzymes as a marker substance. In most such systems the antibody was immobilized on a solid surface, such as on the internal walls of the wells in the microtiter plate. The level of antibody present is limiting, labelled and unlabelled antigen compete with each other for binding. The labelled antigen (antigen-enzyme conjugate) is retained by the immobilized antibody. After reaching equilibrium (antibody-antigen binding) unbounded antigen is removed by a washing step. The amount of enzyme-labelled antigens retained is detected by enzymatic activity. Figure 1 presents the principles of EIA [37].

Different modification of the EIA are involved. One of the most popular is ELISA (enzyme-linked immunosorbent assay). Sometimes this assay is termed "double antibody sandwich techniques" [37]. The principle of the system is illustrated in Figure 2. ELISA is one of the most common immunoassay schemes. On the base of the immunoassay originated different modern modifications, like immunoaffinity chromatography (IAC), flow injection immunoanalysis (FIIA), immunosensor probes, and multianalyte microspot IA_S [28].

Antigen present is bound to the immobilized antibodies. In the mixture a second antibody labelled with an enzyme is present. This antibody also recognizes the antigen, and binds to the retained antigen and the enzyme label is conjugated to this second antibody. Monoclonal antibodies must recognize an epitope on the antigen surface which differs from the epitope recognized by the primary or immobilized monoclonal antibody. After removing an unbounded antibody-enzyme conjugate, the activity of the enzyme is detected. The activity is proportional to the quantity of the antigen present in the sample [22, 37].

The first commercially available immunoassays kit was developed by biochemist Bruce Ferguson [20]. This kit was used for the detection of pesticides.

A wide variety of enzymes have been used as markers in various EIA systems. Many of the enzymes used produce a coloured product which should be easily monitored by colorimetric or other methods. Enzymes most often used as labels include: alkaline phosphatase, horseradish peroxidase, β-galactosidase, glucose oxidase,

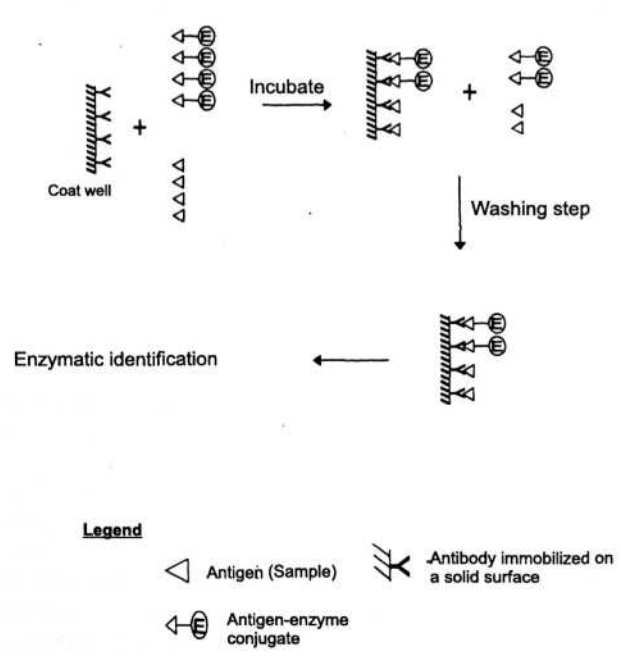

Fig. 1. Principles of competetive solid-phase EIA (Ref. [37]).

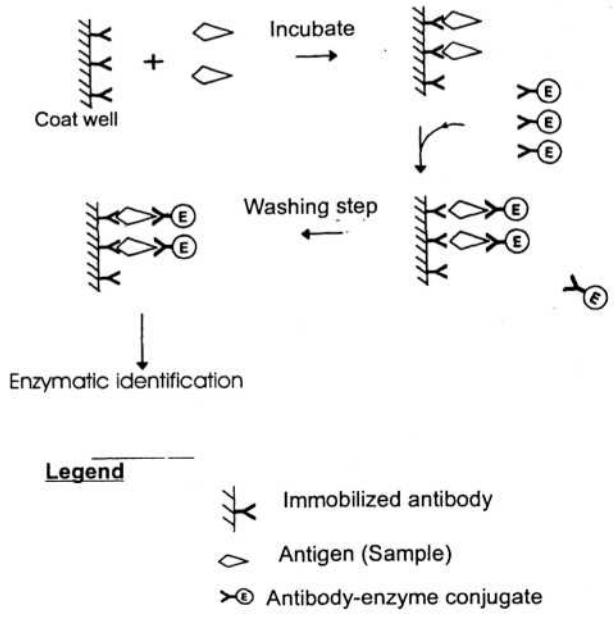

Fig. 2. Principle of non-competitive ELISA (Ref. [37)].

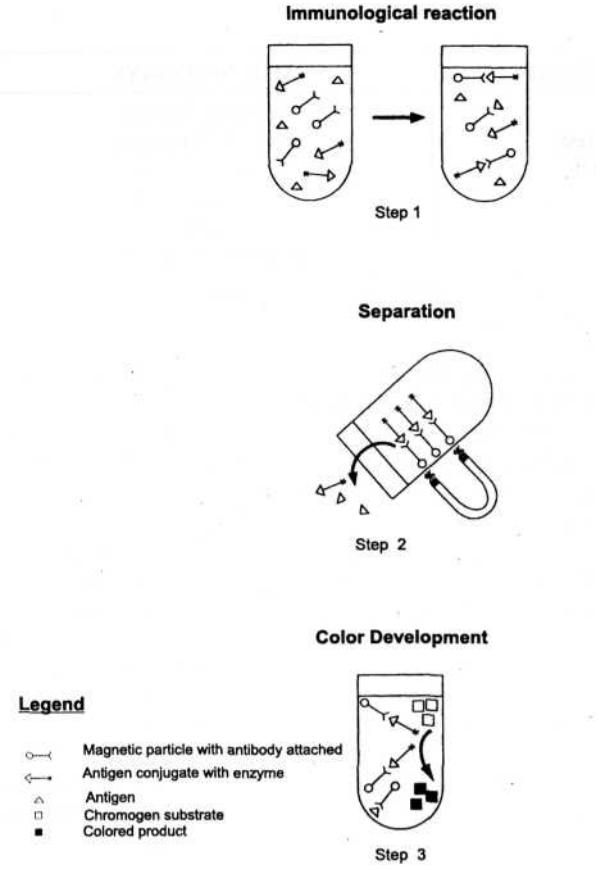

Fig. 3. Principle of Ohmicron's RaPID Assay (Ref. [25]).

alkaline phosphatase and urease. The enzymes are mainly isolated from microorganisms such as *E. coli, Rhizopus niveus, Aspergillus niger, Bacillus pasteurii.* The attached enzyme catalyzes the reaction to form an easily detectable coloured product. The enzyme can catalyze a reaction in which a colorless substrate is converted into a coloured product. The catalytic properties and the activity of enzymes chosen should be easily monitored [22].

The development of the immunoassay systems is connected with using monoclonal antibodies. Since the introduction of monoclonal antibody techniques by Kohler and Milstein [15], these precise immunochemical reagents have been applied in a wide variety of fields [5,15, 29]. For environmental monitoring MAbs have some advantages over PAbs. The MAbs is a uniform, invariant reagent that may be widely distributed, easily standarized and incorporated into regulated methods. The MAbs immunoassay show greater specificity, specially for insecticides where the differences in chemical structure is very small [16].

Another example, Chiu *et al.* [4], used a monoclonal immunoassay for the detection of coplanar polychlorinated biphenyls. Monoclonal antibodies have also been isolated for dioxins and furans [3, 30]. These antibodies recognize tetrachloro- and pentachloro-dibenzodioxins and -dibenzofurans.

The use of monoclonal antibody technology is suitable for the development of rapid, inexpensive screening assays for monitoring the presence of different compounds in biological, soil and other environmental samples.

Immunoassays for Environment Monitoring

A few immunoassays are now beginning to be developed for environmental monitoring, mainly of pesticides and toxic waste chemicals [19, 20, 29, 33-36]. Environmental monitoring by immunoassays is a new technique. It offers great potential for the screening of compounds like dioxins where conventional methods are costly and difficult to implement. Some environmental compounds (for which IMA_S have been developed) are presented in Tab. 2.

Table 2. Some environmental compounds for which IMA_S have been developed (Ref. [35, 36])

PESTICIDES	INORGANICS
Alachlor	Cadmium
Aldicarb	Indium
Aldrin/dieldrin	Lead
Atrazine	Mercury
Benomyl	
Bentazon	**ORGANICS**
Captan	
Carbofuran	Benzene, toluene, ethylbenzene
2,4 – D	xylene compounds (BTEX)
DDT	Diesel
Endosulfan	Fuel
Malathion	Gasoline
Metalaxyl	Polyaromatic hydrocarbons (PAH_s)
Metolachlor	Pentachlorophenol (PCP)
Parathion	Polychlorinated biphenyl (PCB)
Paraquat	Trinitrotolune (TNT)
Triazines	Microbial toxins: aflatoxins, ochratoxins

Ercegovich discussed using immunoassays for the detection of some pesticides: DDT, malathion, and aminotriazole [6]. It was the first strong suggestion that immunoassays can be used as alternative analytical methods. In 1980, Hammoch and Mumma started to publish their experience in synthesizing hapten-protein conjugates and producing polyclonal antibodies [10]. Their work showed that immunoassays are useful tools for analytical analysis.

In the next years, IMA_S were used to detect different types of contaminants in water, soil, and sediment samples [2, 7, 14, 23, 26].

There are two main screening strategies: one is to detect biological and biochemical effects of contaminants, the second is to detect selective target contaminants. IMA screening techniques are beneficial in the case of trace contaminants that require complex clean-up procedures and sophisticated instrumental quantification. They lower average analytical costs and eliminate very expensive sample clean-up. Approximately

Table 3. Some advantages and disadvantages of IMA_S for environmental analysis (Ref. [28, 29]).

ADVANTAGES	DISADVANTAGES
– Wide applicability – Sensitive and specific – Rapid and easy to use – Reduction preparation – Rapid with high sample throughput – Ideal for large sample loads; easily automated – Suited to lab and field use – Cost-effective analysis of small-volume samples	– Development costs – Hapten synthesis can be difficult – Can be vulnerable to cross reacting compounds and non-specific interferences – Require independent confirmation – Not suited to small sample loads or multi-residue determinations – Lack acceptance, conservative attitudes

$1 billion a year is spent in the United States to monitor environmental contaminants [29]. A considerable savings could be made with regards to environmental monitoring by the use of techniques such as IMA.

Dioxins (PCDD,) and furans ($PCDF_S$) are very expensive to determine and time consuming; range from $1000 to $2000 per sample, depending on sample complexity. Much of the cost is associated with sample preparation. Dioxins and furans are normally extracted from soil or sediment with their extract subjected to a very tedious clean-up procedure. Both RIA and EIA have been developed for the detection of these compounds [29, 30, 32, 34]. In these kits polyclonal and monoclonal antibodies were used. The MAb and DNA structures were licensed, leading to the commercial development of IMA screening tools for assessing exposures to dioxins in human populations.

IMA have been also used to detect PCB_S [4, 29] Most interest in IMA for PCB_S has focused on the various Aroclors as an antigen. The Aroclors are used as coolants in electrical transformers and capacitors. Aroclors differ in their chlorine content and in the number of chlorine atoms per PCB molecule. Another compound for which IMA_S have been developed are polynuclear aromatic hydrocarbons such as benzo-α-pyrene and their DNA or protein adducts [26, 29, 32].

Presently, numerous companies market IMA kits for the detection of contaminants in food and in environmental matrices. ImmunoSystems Inc. of Scarborough, ME (USA), was one of the first companies to offer IMA kits for environmental contaminants. One popular IMA kit is Ohmicron Environmental Diagnostics which develops a broad spectrum of RaPID Assay test kits to detect contaminants, such as PAH, BTEX, TNT, PCB, PCD, and many pesticides [11, 24-25]. The kits use magnetic particle as the solid support for the detection of environmental contaminants. Ohmicron holds an exclusive license for this technology. Antibodies are attached to microscopically small magnetic particles instead of test tubes, thus speeding up the chemical reaction between antibody and contaminants. This modification provides more precise results than earlier immunoassay technology [Fig. 3]. More precise results mean fewer costly false positives, and thus more savings for remediation customers. Mainly, the kits are used during site remediation to detect contaminants and monitor the clean-up process. They can also be used to locate and map sites, screen laboratory samples and monitor industrial processes.

Traditional kits are composed of the following parts: soil collection and sample extraction kits, reagents kit, magnetic rack and analyzer. Based upon the collective experiences of the authors, it is recommended that once chosen, the user stick with the same product from a manufacturer during the monitoring process as results between manufacturers do not correlate well [11, 24]. In the end of the review some advantages and disadvantages of IMA_S are presented in Tab. 3.

Summary

Reasons for the use of the immunoassay techniques include the selectivity and sensitivity exhibited by antibodies and the simplicity of performing the immunoassays. IMA screening techniques are used for the detection of a broad variety of pollutants, including pesticides and industrial contaminants and related compounds. IMA techniques can transform many difficult and costly routine environmental analysis, reducing sample overloads and lowering overall analytical costs. IMA_S can help provide real time data are often needed when dealing with industrial spills and environmental hot-spots. But IMA methods are not a *panacea* for everything and should be used as a qualitative rather than a quantitative tool for assessing environmental contamination.

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Mention of trade names and commercial products does not endorse or recommend their use.

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