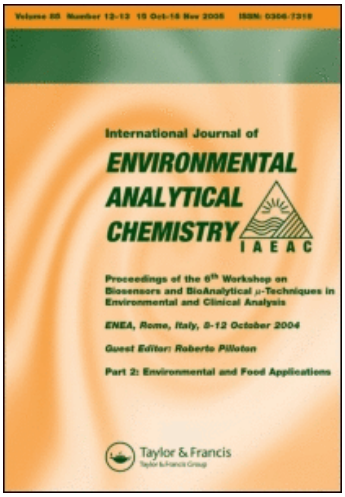

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Immunoassays in the Analysis of Water†

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Radioimmunoassays were first described some 25 years ago and have since been widely applied. In general, immunoassays depend on the principle of competitive binding or saturation analysis. More recently, non isotopic techniques have been utilised in attempts to overcome some of the disadvantages associated with the use of radioactivity.

Immunoassays are simple to perform, cost effective and universally applicable to molecules greater than 150 daltons mol. wt. Undoubtedly, their main advantage lies in the exquisite sensitivity that can be achieved ($10^{-9}-10^{-10}$ molar). Immunoassays are ideally suited to the quantitation of analytes in water, especially as sensitivity can be further increased by a simple concentration step.

The specificity of immunoassays depends on the particular antiserum used. Group specificity, useful for screening procedures, is readily achieved. With a careful choice of immunogen antisera with absolute specificity is also possible.

The potential for immunoassays in water analysis has not yet been realised although few problems should arise in adapting existing assays or in developing new assays of direct relevance to the water industry.

KEY WORDS: Immunoassay, microcontamination, antisera, water.

INTRODUCTION

Immunoassay techniques have now been applied to analytical problems in many fields of study. Radioimmunoassay (RIA) was first

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described a quarter of a century ago,[1] and until now has remained the most widely used type of immunoassay. Lately though, other forms of immunoassay, especially those utilising enzymes have become more common. Most innovations have been in clinical chemistry but the advantages of the technique have been recognised in pharmacology, therapeutic drug monitoring, forensic medicine, veterinary practice, epidemiology and food science. Immunoassays have not yet been widely applied to environmental analyses in spite of their advantages of simplicity and specificity coupled to high sensitivity. The principles and techniques of immunoassay and their use for the measurement of organic microcontamination in water samples will be outlined.

IMMUNOASSAY PRINCIPLES

Many publications have dealt with the principles of immunoassay[2,3] and only a simple review of the subject can be given here. Immunoassays are based on the principle of competitive binding or saturation analysis and require two key reagents: an antiserum with the desired specificity towards the analyte and an immunoreactive form of the analyte which has been labelled e.g. with a radioisotope or an enzyme. A fixed amount of the labelled analyte competes with the analyte (present as either standard or sample) for a fixed and limited number of antibody binding sites. At the end of a period of incubation, the antibody bound fraction is separated from the unbound or "free" fraction using a suitable "phase separation" procedure.

The amount of label associated with one or both fractions is measured and the distribution of label between the fractions can then be related to the amount of unlabelled analyte present. An increasingly popular and often extremely sensitive form of immunoassay, the immunometric assay utilises purified labelled antibodies.[4]

Phase separation techniques have been reviewed[5] and rely on physical, chemical or immunological differences between the phases. For small molecules, dextran-coated charcoal is often used to adsorb the free fraction whereas the antibody bound fraction can be precipitated using reagents such as ammonium sulphate, poly-

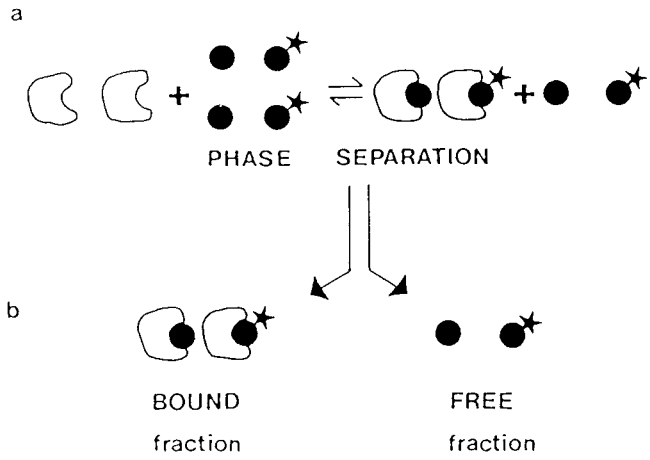

FIGURE 1 *The basic principle of immunoassay.* A known and limited amount of antibody, a known amount of labelled antigen (●★) and an unknown amount of unlabelled antigen(●) (sample or standard) are incubated. (b) Following equilibrium, the bound complex is separated from the unbound antigens (labelled and unlabelled antigen). (Reprinted with permission from *Trends on Analytical Chemistry* **3**, 215 (1984).

ethylene glycol or a second (double) antibody. The latter reagent is an antiserum raised against the immunoglobulins of the species in which the first antibody was produced. Centrifugation of the assay tubes results in a pellet containing either the free or bound fraction. The distribution of label between the phases can then be estimated either in an aliquot of the supernatant or (following aspiration of the supernatant) in the pellet. Solid phase methodology simplifies phase separation. Either the antigen or antibody is adsorbed to some solid support e.g. cellulose or glass beads or even the walls of the tubes. Following incubation, aspiration with or without centrifugation is all that is required to achieve the separation of the free and bound fractions. Phase separation is a critical stage in an immunoassay and the cost and time involved, precision and extent of "misclassification" should be carefully examined.

PRODUCTION OF ANTISERA

An antiserum contains antibodies to a specific antigen which are

produced by an animal in response to an injection of an immunogen which is "foreign" to that animal. Antibodies are divided into a number of different classes, the principal one used in an immunoassay being immunoglobulin G (Ig G). Antibody molecules consist of two heavy chains (the amino acid structure of which determines the class of the immunoglobulin) and two light chains. Each chain possesses a variable region which together form the specific binding sites (of which there are two) for a particular antigen. Polyclonal antibodies are derived from several clones of lymphocytes which are stimulated in response to an immunogen, and an antiserum will thus contain populations of antibodies with similar but not identical specificity and avidity for the antigen.

Antisera have now been produced to a huge range of substances and many of these are available commercially. Small molecules (<5000 daltons) are known as haptens and are not normally immunogenic. They can be made so by covalently linking them to a carrier protein which is foreign to the animal e.g. bovine serum albumin, ovalbumin, thyroglobulin, synthetic polypeptides. Antisera produced in response to a conjugate will contain antibodies not only to the hapten but also to the carrier protein.

Antisera for use in immunoassays should be of high avidity, preferably of high titre and exhibit the desired specificity. It is vitally important that the specificity of an antiserum is well documented and to determine which substances of similar structure to the antigen cross react. Cross reactants may be precursors, metabolites or degradation products of the analyte or endogenous substances that have a similar structure to the analyte.

For haptens, the site of conjugation has a profound but predictable effect on the specificity of the antiserum produced. In general, the part of the molecule which is furthest from the site of conjugation will be the most antigenic.[6] For example, in this laboratory antisera have been produced in response to a 6-succinyl morphine conjugate which cross reacts with the congeners of morphine (diamorphine and codeine) as well as with its main metabolite, morphine-3-glucuronide. These antisera are group specific and are ideally suited to drug screening programmes and forensic work. In contrast, an antiserum raised to a N-succinyl morphine[7] conjugate is specific (Table 1) and has a minimal cross reaction with morphine-3-glucuronide and is suitable for studies on the disposition of morphine.

TABLE I

The effect of site of conjugation on the specificity of morphine antisera

Compound	6-Succinyl morphine-BSA Antiserum G/R/6	Antiserum G/G/1	Succinyl normorphine-BSA Antiserum G/S/7
Morphine	100	100	100
Codeine	100	100	5
Diamorphine	100	100	2.5
Normorphine	<1.0	<1.0	16
Morphine-3-glucuronide	31	2.3	0.26

As has already been mentioned, antisera which are produced in an active immunisation schedule are polyclonal in nature. Since 1976[8] it has been possible to produce monoclonal preparations of antibodies using hybridoma technology. Lymphocytes from the spleen of an immunised animal (usually a mouse) are fused with a mouse myeloma cell line. The resulting hybrids each produce one type of antibody derived from a single lymphocyte. Monoclonal antibodies are of enormous benefit in the analysis of complex biological systems and as diagnostic tools. Their advantages over polyclonal antisera in immunoassays are still being evaluated.

LABELLED ANTIGENS

The most widely known type of immunoassay has until recently been RIA, where the analyte is labelled with a radioisotope. The sensitivity of an assay depends to a great extent on the mass of label added to the assay tube. Thus high specific activity labels are a prerequisite for high sensitivity. Low specific activity labels can however be immensely useful for antiserum screening.

Tritium and ^{14}carbon are in some ways ideal labels for small molecules since their incorporation causes minimal structural and immunoreactive change. For some haptens though, it has been impossible or too expensive to obtain high specific activity β-emitting labels and radioiodinated ligands have been used. Although counting techniques are quicker and cheaper than liquid scintillation

counting, there are other problems associated with the use of ^{125}I. Because of the short half life of ^{125}I (60 days), antigens have to be labelled at regular intervals. The immunoreactivity of the antigen can be grossly altered by the direct incorporation of a radioiodine atom. For haptens, where direct radioiodination is usually difficult, a moiety which contains a tyrosine or histidine-like moiety—a tag—is used, often reducing or abolishing immunoreactivity.

Increasingly stringent regulations on the use of radioactivity as well as the potential health hazard of the frequent use of millicurie amounts of radioiodine, have led to the use of alternative, nonisotopic labels (Table II). One important aspect of the use of nonisotopic labels is that homogenous (non-separation) assays can be developed and opportunities for the development of simple fast, portable kits for "yes-no" answers become possible.

Presently, enzyme immunoassays (EIA) and especially ELISA

TABLE II

Non-isotopic labels used in immunoassay

Label type	Examples
Enzymes	Peroxidase
	Alkaline Phosphatase
	Urease
	β-D-Galactosidase
Fluorescence	Fluoroscein
	Rhodamine
	Umbelliferones
	Rare earth metal chelates
Luminescence	Luminol/Isoluminol
	Acridinium esters
	Peroxidase
	Luciferin/Luciferase
Particles	Red blood cells
	Latex
Others	Proteins
	Viruses
	Free radicals
	Co-enzymes
	Substrates

(*E*nzyme *L*inked *I*mmuno *S*orbent *A*ssay) techniques[9] are becoming increasingly used. ELISA assays, the sensitivity of which can now rival that of RIAs, are currently being applied to quantitative, semiquantitative and qualitative determinations in a variety of applications, some of which can be carried out without laboratory services. Chemiluminescent[10] and fluorescent[11] labels are also becoming more widely used.

IMMUNOASSAYS AND WATER ANALYSIS

In spite of their advantages, immunoassays have not yet been widely applied to the analysis of water. Immunoassays are universally applicable to analytes of <150 daltons and are not subject to limitations that often apply to more conventional techniques e.g. the need for volatility in gas chromatography. The most important aspects of immunoassays is their exquisite sensitivity, which can in most cases be achieved without prior extraction. No other *readily* available analytical technique can rival immunoassay for sensitivity, especially when high specific activity radio- or chemiluminescent-labels are employed. For many small molecules detection limits can be as low as 1–50 pg (10^{-10} mol/l). In clinical chemistry, matrix effects (e.g. from plasma) due to the presence of proteins, enzyme modifiers, endogenous fluorescence often reduce sensitivity, but such problems are unlikely to occur with aqueous samples. Problems often arise in clinical practice when sample volumes are limited. Such limitations are not usual in the analysis of water and since robust immunoassays are relatively tolerant of changes in ionic strength, a simple sample concentration step achieved by lyophilisation or evaporation can increase aasay sensitivity even further.

The specificity of immunoassays has often been criticised. Such criticisms are more often than not unfounded. Results obtained with an immunoassay like those obtained with any analytical technique should be interpreted with caution. Specificity and cross reactivity studies should be as thorough as possible and results should always be viewed in the context of each particular application. Class specificity, ideal for screening purposes is relatively simple to achieve, and more expensive and time consuming techniques can be used for confirming the presence of a particular analyte in immunoreactive

samples. Specificity for an analyte itself can often be achieved by a careful choice of the hapten-protein conjugate. If necessary a simple extraction step designed to improve specificity can be included in the assay procedure. Antisera especially cross reacting ones have also been used as sensitive detectors for HPLC.

Immunoassays are simple, quick and reproducible techniques which can handle high sample numbers. Automation or semiautomation of the assays are possible and the present and future innovations in immunoassay technology will be of interest to the water analyst in such areas as Catchment Quality Control and Intake Monitoring. Already, visually readable ELISA techniques which can be carried out under "field" conditions are available.

The main disadvantage of immunoassays is their high developmental costs, especially if reagents are not readily available. For RIA especially, the cost of capital equipment is high. It may take up to two years to develop a properly validated immunoassay, the main factor in this period being the unpredictable time for antisera with the requisite properties to be produced.

Most of the antisera available commercially (or from research laboratories) are understandably derived from clinical chemistry and related subjects. Antisera to compounds of more relevance to the water analyst are not so readily available. Antisera and immunoassays have however been described for paraquat,[12] parathion,[13] dieldrin,[14] S-bioalletrin,[15] and benomyl,[16] amongst others. Several laboratories, in this country and abroad, including that of the author can provide the facilities and expertise for antiserum production. Immunoassays are potentially important for the analysis of an immense number of pesticides[17] and herbicides, organic contaminants and plant toxins e.g. Microcystis Aeroginosa, and have an important role to play in determining the fate of pharmaceutical chemicals including antibiotics and steroids, in the aquatic environment.[18]

Many antisera are already available for drugs including steroids and an initial study has shown the feasibility of adapting clinical assays to the analysis of water.[19] Assays for methotrexate, progesterone, norethisterone and ethinylestradiol were used, and after sample concentration by lyophilisation, detection limits of between 5-10 ng/litre were achieved. Oral contraceptives were apparently absent in the samples of potable water tested. The significance of

very low concentrations of steroids in potable water is not known, but had they been present at concentrations close to the assay detection limit, only a minute fraction of the prescribed daily dose would be ingested by an individual during the course of a day. It was also deduced that there was no risk of exposure to the general population from methoetrexate, a potent anticancer drug often used in large doses. The drug was detected in water from a sewer downstream from a large oncology clinic but not in river or water samples derived therefrom.

FUTURE PROSPECTS

Immunoassays have many attributes which should be attractive to water analysts. They can be viewed as valuable additional and complementary techniques to GC, GC-MS and HPLC, and can also offer cost effective screening procedures. Future developments in devising simple quick methods which can be carried out *in situ* should make this type of assay even more useful. The introduction of immunoassays to water analysis is likely to make as big an impact as their introduction made to clinical practice 25 years ago.

References

1. R. S. Yalow and S. A. Berson, *J. Clin. Invest.* **39,** 1157 (1960).
2. R. P. Ekins, *Br. Med. Bull.* **30,** 3 (1974).
3. A. Voller, A. Bartlett and D. E. Bidwell (Editors), *Immunoassays in the 80's* (MTP Press Ltd.), (1981).
4. J. S. Woodhead, G. M. Addison and C. N. Hales, *Br. Med. Bull.* **30,** 44 (1974).
5. J. G. Ratcliffe, *Br. Med. Bull.* **30,** 32 (1974).
6. K. Landsteiner, *Specificity of Serological Reaction* (Thomas, Springfield III, 1945).
7. B. A. Morris, J. D. Robinson, E. Piall, G. W. Aherne and V. Marks, *J. Endocrinol.* **64,** 6 (1974).
8. G. Köhler and C. Milstein, *Eur. J. Immunol.* **6,** 511 (1976).
9. A. Voller, D. E. Bidwell and A. Bartlett, *The Enzyme Linked Immuno Sorbent Assay* (ELISA), (Dynatech Europe, Guernsey) (1979).
10. W. P. Collins, G. J. Barnard, J. B. Kim, D. A. Weerasekera, F. Kohen, Z. Eshbar and H. R. Lindner, *Immunoassays for Clinical Chemistry* (Eds. Hunter and Corrie, Churchill Livingstone, Edinburgh, 1983) Chap. 3, pp. 373–397.
11. E. Soini and I. Hemmilä, *Clin. Chem.* **25,** 353 (1979).
12. Y. Levitt, *Lancet* **II,** 358 (1977).

13. R. P. Vallejo, E. R. Bogus and R. O. Mumma, *J. Agric. Food Chem.* **30,** 572 (1978).
14. J. J. Langone and H. Van Vunakis, *Res. Commun. Chem. Pathol. Pharmacol.* **10,** 163 (1975).
15. K. D. Wing, B. D. Hammock and D. A. Wustner, *J. Agric. Food Chem.* **26,** 1238 (1978).
16. W. H. Newsome and J. B. Shields, *J. Agric. Food Chem.* **29,** 220 (1981).
17. K. D. Wing and B. D. Hammock, *California Agriculture* **34,** March 1980.
18. M. L. Richardson and J. M. Bowron, *J. Pharm. Pharmacl.* **37,** 1 (1985).
19. G. W. Aherne, J. English and V. Marks, *Ecotoxicol. Environ. Safety* **9,** 79 (1985).