

# FLOW INJECTION IMMUNOANALYSIS (FIIA) FOR THE DETERMINATION OF PESTICIDES IN WATER

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## SUMMARY

The principle of a heterogeneous competitive enzyme immunoassay was applied to a flow injection system. This flow injection immunoassay (FIIA) can be run automatically. Antibodies to the triazine herbicide atrazin were immobilized on membranes. Antibody containing membranes were mounted in a cross-flow reactor and changed after each assay. In the assay atrazin competed with an atrazin-peroxidase conjugate for the antibody binding sites. The product of the enzyme reaction was measured fluorimetrically. One assay is completed within 15 minutes. Sensitivities of the FIIA proved similar to the ELISA and depended mainly on the properties of the antibodies and conjugation strategies. The potential and limitations of the FIIA system will be discussed.

## INTRODUCTION

Immunoassays for the control of pesticides in water find increasing interest, especially since the appearance of pesticides in drinking water has alarmed the public [1]. Stronger regulations for water control require highly sensitive assays and the ability to test large numbers of samples. As the established chromatographic methods are time consuming and costly, immunoassays should find their place in water control, at least as a screening tool.

Immunoassays for pesticides have been recently reviewed [2,3].

Flow injection analysis has been described as a tool for the detection of the product of the immunochemical reaction [4]. Recently, a FIIA system for the detection of theophylline and insulin using an immobilized secondary antibody and a preincubation step was reported [5].

Here we present a method with the potential for automatic immunoassays, combining the solid phase techniques with flow injection analysis. The concept is demonstrated for the assay of atrazine, a triazine herbicide.

#### THE FIIA SYSTEM

The assay follows the principle of a heterogeneous, competitive enzyme immunoassay. Antibodies were immobilized on activated membranes (Immunodyne, 3  $\mu\text{m}$  pore diameter, PALL Corp., Glen Cove, USA). For saturation the membranes were incubated with bovine albumin. The treated membranes were fixed in a cross-flow reactor and replaced after each assay. The immunochemical reactions are shown in Fig. 1 and the FIIA system is illustrated in Fig. 2. A process timer (Alphotronic, Karlsruhe, FRG) controls the pumps and valves. The protocol is listed in Table 1.

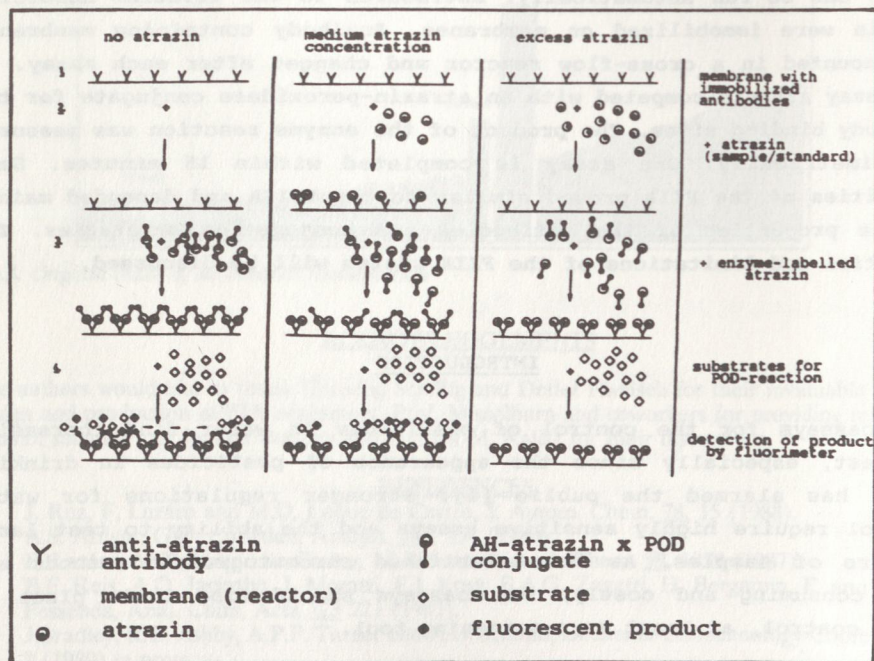


Fig. 1: Principles of the immunochemical reactions in FIIA

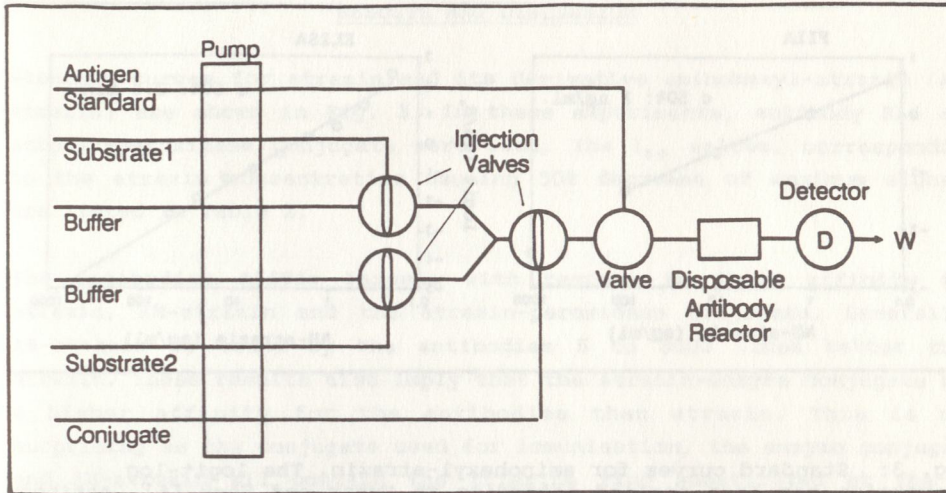


Fig.2: Illustration of the FIIA (flow-injection-immunoanalysis) system

Table 1: FIIA standard protocol. The flow rate was 0.69 ml/min.

step	solution	vol. (ml)	time (min)
1	buffer	0,69	1
2	hapten (atrazin)	1,26	2
3	conjugate (injection vol.)	0,24	0,5
4	buffer	0,14	0,2
5	buffer (cycles) <sup>1</sup>	0,35	2,5
6	buffer	2,76	4
7	substrate 1 substrate 2 (injection vol.)	40 µl 40 µl	0,5
8	buffer	0,46	0,7
9	-- (incubation)	0	2
10	buffer	1.38	2
total time per assay: (without membrane change)			16 min.

<sup>1</sup> 30 cycles with 1 sec. pumping and 4 sec. incubation

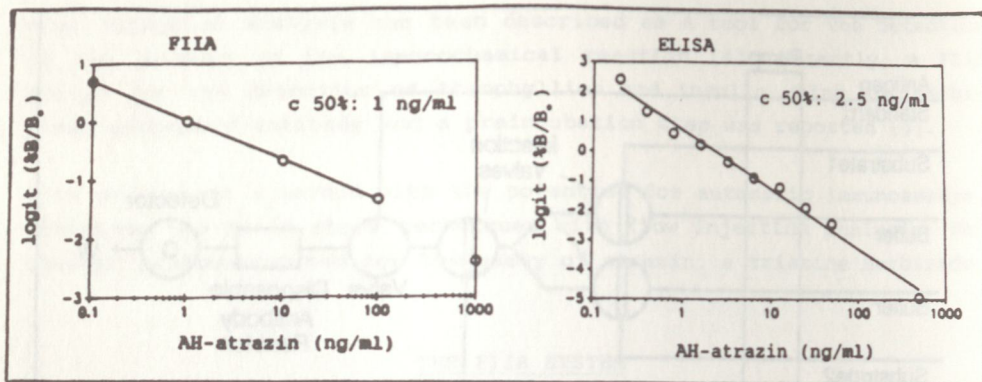


Fig. 3: Standard curves for aminohexyl-atrazin. The logit-log conversion has been applied according to Huber and Hock [6]. Antibody R14 was used. The AH-atrazin-peroxidase conjugate was diluted 1/200 (FIA) and 1/6000 (ELISA)

Table 2: Determination of the 50% values for atrazin and aminohexyl-atrazin by FIIA and ELISA. - "c 50%" is the concentration of the hapten causing 50% reduction of conjugate binding. The anti-atrazin antibodies are polyclonal (rabbit sera).

antibody	hapten	c 50% (ng/ml)	
		FIIA	ELISA
R14	atrazin	700	--
	AH-atrazin	4	4
R15	atrazin	130	30
	AH-atrazin	3	4.5
R19	atrazin	--	200
	AH-atrazin	2	2.5

RESULTS AND DISCUSSION

Standard curves for atrazine and its derivative aminohexyl-atrazin (AH-atrazin) are shown in Fig. 3. In these experiments, antibody R14 and atrazine-peroxidase conjugate were used. The  $I_{50}$  values, corresponding to the atrazine concentration causing 50% decrease of maximum signal, are listed in Table 2.

The antibodies differ largely with respect to their affinity for atrazine, AH-atrazin and the atrazine-peroxidase conjugate. Generally, AH-atrazin is bound by the antibodies 5 to 3000 times better than atrazine. These results also imply that the atrazine-enzyme conjugate has a higher affinity for the antibodies than atrazine. This is not surprising as the conjugate used for immunisation, the enzyme conjugate and AH-atrazin all contain the triazine ring substituted at the C2 position. Conjugates synthesized by other methods may have lower affinities for the antibodies and could thus be replaced more easily by atrazine. In fact, desethyl-aminocapryl-atrazin-peroxidase and ametryn-sulfoxide-atrazin-peroxidase conjugates (Prof. Hock, TU München-Weihenstephan) have been observed to be replaced by lower amounts of atrazine. The weaker binding of such conjugates might be overcome by a longer incubation time for the enzyme reaction, if ELISA techniques are used. In the FIIA system, however, longer incubation times are not feasible. This problem can be solved either by using higher amounts of bound antibody or, preferably, by applying antibodies with higher affinity. It also proves important to have access to different conjugates for immunisation.

The FIIA system offers several advantages over the ELISA and conventional chromatography methods, especially with respect to the relatively short time needed for analysis and its potential for automation. Sample pretreatment is not necessary. FIIA might be the best method for the continuous measurement of pesticides in waste water of production processes. There are also limitations to the FIIA method in that only one analyte (and some cross-reacting substances) can be detected and samples cannot be analyzed in parallel. However, large numbers of samples, e.g. from water works, can be analyzed sequentially and automatically once the automatic membrane exchange mechanism is established. Different haptens (e.g. pesticides) might be analysed by alternating the flow between several parallel reactors.

Automation of the FIIA including computer control, autosampler and a membrane exchange mechanism is presently in progress. In addition, various new antibodies are being tested.

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