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Short Communication

Comparison of FT-IR and AAS for metalloimmunoassay of a tricyclic antidepressant drug (desipramine)*

F. MARIET,† P. BROSSIER,†§ F. DICAIRE‡ and A.A. ISMAIL‡

† *Unité d'Immunoanalyse, Faculté de Pharmacie, 7 Bd Jeanne d'Arc 21033, Dijon Cedex, France*
‡ *Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montréal, Québec H3A 2K6, Canada*

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Introduction

The use of metal complexes as labels in immunoassays was first proposed by Cais *et al.* nearly a decade ago for the assay of oestrogens [1]. They termed this method *metalloimmunoassay* (MIA) and used AAS for the detection of the *metal*-contained in the label. Such an approach, however, was limited by the high background signal due to free metal contamination in the sample. This problem was resolved by the use of an organic solvent extraction method which reduces the interference from free metal ions in the sample [2].

In order to avoid metal contamination, another approach consists of measuring *carbonyl* ligands of metallotracers instead of the metals themselves and a new immunoassay was developed in this laboratory in which infrared spectroscopy was used to detect metal carbonyl complexes [3]. This technique owes its feasibility to the availability of Fourier transform infrared (FT-IR) spectrometers with ultrasensitive liquid-nitrogen-cooled detectors and to the fact that the ν(CO) absorptions of metal carbonyl complexes exhibit very high absorptivities in the infrared spectrum where no contamination by natural carbonyls appears. This new immunoassay method was termed *infrared-immunoassay* (IRIA).

This paper compares the results obtained with FT-IR and AAS for the titration of polyclonal antisera produced against desipramine.

Experimental

The synthesis of desipramine labelled with the organometallic marker (called Cy-desipramine) was recently described [4]. The structure of the metallotracer is shown in Fig. 1.

Infrared spectra were recorded on a Bomem Michelson FT-IR spectrometer equipped with an InSb detector and operating at a 4 cm^{-1} resolution. Routinely, 16 scans were co-added. Peak heights were measured using a subroutine included in the FT-IR software.

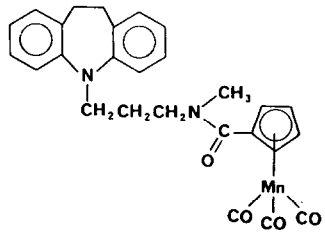

Figure 1
Structure of metallotracer (Cy-desipramine).

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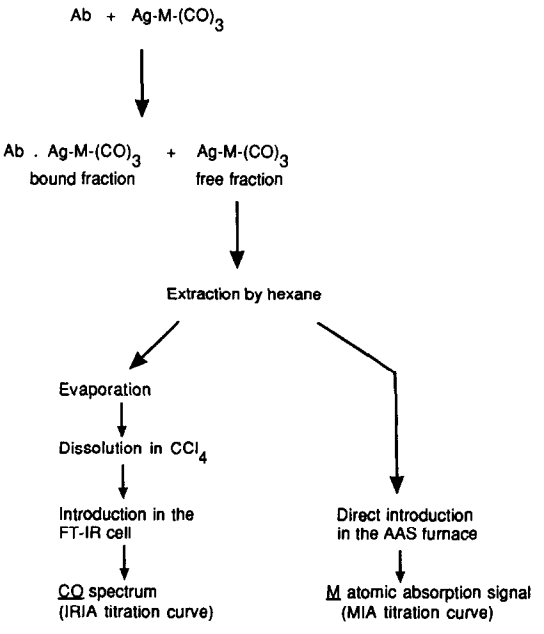
§Author to whom correspondence should be addressed.

Atomic absorption measurements were carried out on a Zeeman Hitachi model Z.7000 as previously described [5].

Antisera against desipramine were obtained by inoculating "fauves de bourgogne" rabbits with a BSA-desipramine conjugate [5].

The titration curves were constructed from successive dilutions of antisera in phosphate buffer pH 7.2, incubating with a fixed concentration of Cy-desipramine. Subsequent to incubation at 4°C for 4 h, the "free" Cy-desipramine fraction was extracted from phosphate buffer into 1000 µl of hexane. For the AAS detection, 25 µl of the organic solvent was introduced into the furnace of the Hitachi spectrophotometer and the signal recorded. For the FT-IR measurements, a portion (500 µl) of the hexane solution was transferred to a glass test tube and the solvent was evaporated under centrifugal vacuum. The Cy-desipramine "free" fraction was redissolved in 15 µl of carbon tetrachloride and transferred to a sodium chloride microcavity cell (1 mm pathlength). Appropriate controls were included to determine total amounts of the tracer.

The IRIA titration curves were plotted by measuring the change in intensity of the v(CO) signal at 2030 cm^{-1} obtained from a fixed and known amount of Cy-desipramine incubated with increasing amounts of antibodies. The MIA titration curves were obtained by measuring the absorbance of the signal at 279.6 nm.



Scheme 1
Protocol for the generation of IRIA and MIA titration curves. Ab, antibody; Ag-M-$(CO)_3$, metallotracer.

Results and Discussion

The characterization of the antibody titre is an essential step in the development of an immunoassay. Traditional methods for the evaluation of antibody titre generally involve the use of radioisotopes. However, because of the licensing restrictions on handling and disposal of radioactive materials, many laboratories are developing non-radioisotopic methods.

A new approach has been employed using organometallic complexes and the protocol followed to generate the titration curve of the antisera is described in Scheme 1. Several preliminary studies were carried out to test the stability of the organometallic-labelled hapten under the immunoassay conditions. It was found that the organometallic tracer is stable in ethanol solution in the refrigerator for a period of many months, and in buffer for at least 24 h when incubated under the assay conditions. More than 70% of the free labelled hapten is extractable into hexane (paper submitted for publication).

Representative FT-IR spectra corresponding to successive antiserum dilutions are shown in Fig. 2. The signal-to-noise ratio is sufficient for accurate measurement of the variation in the signal as a function of the antiserum dilution. The v(CO) absorption at 2030 cm^{-1} for each spectrum was used for the construction of the antiserum titration curve shown in Fig. 2.

For the same antiserum dilutions, Fig. 3 shows both AAS absorption signals and resultant titration curve.

The curve obtained from the FT-IR experiments was very similar to that obtained by using the atomic absorption technique. The good correlation between the two curves proved that the use of the metal-carbonyl labels is equally well performed by IRIA and by MIA.

Conclusion

The feasibility of using metal-carbonyl complexes as infrared labels in antibody titration has been demonstrated. The principal advantage of the FT-IR method is the absence of trace metal contamination.

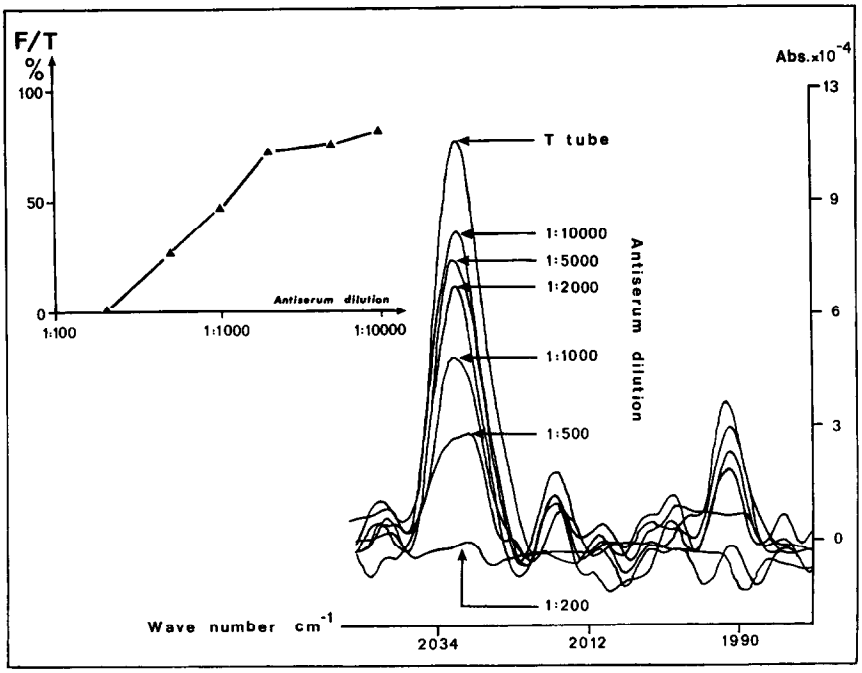


Figure 2
FT-IR spectra for the free fraction of metallotracer after incubation with antiserum, and consequent titration curve.

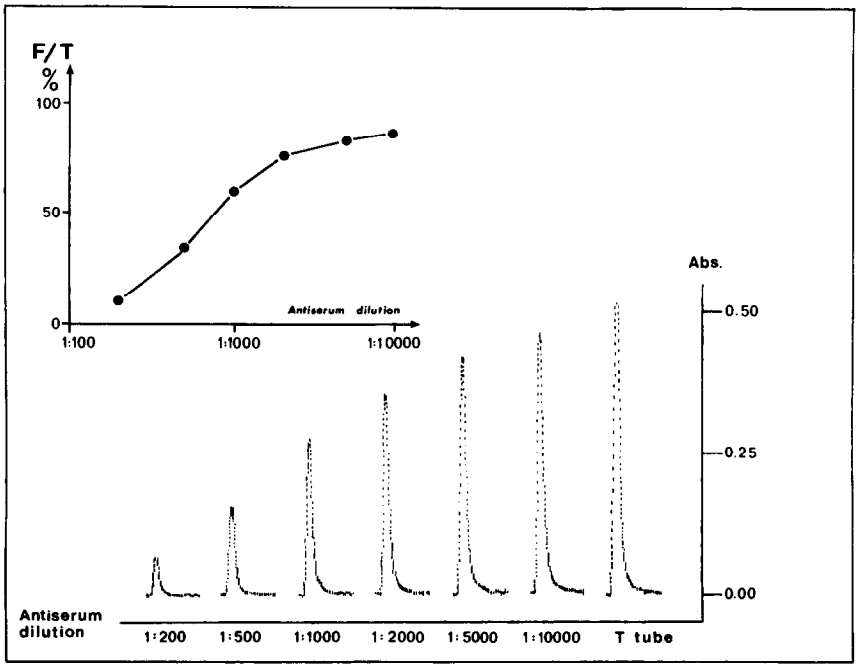


Figure 3
AAS absorption signals for the free fraction of metallotracer after incubation with antiserum, and consequent titration curve.

A large number of metal-carbonyl derivatives will be synthesized and tested for future use as suitable markers in the development of an IRIA screening method for antisera raised against drugs of abuse and against environmental pollutants.

Further refinements of the IRIA are in hand with a view to producing standard calibration curves for different drugs.

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