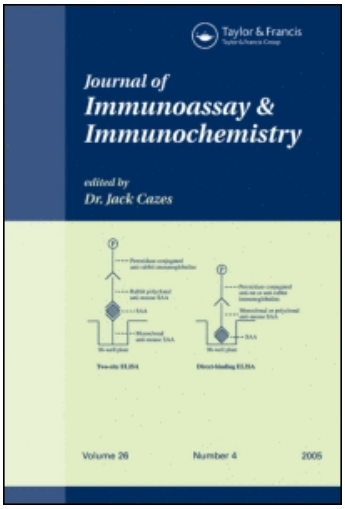


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# Determination of (+)-3,3′,5-Triiodo-L-thyronine (L-$T_3$) from Serum Using a Sequential Injection Analysis/Immunosensor System

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## ABSTRACT

A sequential injection analysis/immunosensor system is proposed for the analysis of $T_3$ in serum with a rate of 75 samples/hr. The immunosensor design is based on the physical immobilization of anti-$T_3$ in carbon paste. The working concentration range of the immunosensor in a sequential injection analysis system is between 3.4 and 340 ng/mL with a limit of

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detection of 2.19 ng/mL. The system is very reliable and very easy to design and operate.

*Key Words:* Amperometric immunosensor; Thyroid hormone; Sequential injection analysis; L-$T_3$.

## INTRODUCTION

(+)-3,3′,5-Triiodo-L-thyronine (L-$T_3$) is the major active thyroid hormone with L-$T_4$ functioning as prohormone. Its concentration in blood reflects the hormonal states of hyperthyroidism and hypothyroidism. The determination of L-$T_3$ in blood requires high sensitivity and also high selectivity over the other thyroid hormone L-$T_4$ that has a similar molecular structure. Most of the methods proposed up to now for the assay of L-$T_3$ are based on HPLC,[1] radioimmunoassay,[2,3] and direct amperometry.[4,5]

Due to the need of determining L-$T_3$ from a high number of patients' blood, it is necessary to decrease the cost of analysis and to have a high rate of determinations per hour. This can be done by utilization of the immunosensor, designed previously[4] as a detector in a sequential injection analysis (SIA) system.

The emphasis of this paper is on the SIA/immunosensor system utilized for the determination of L-$T_3$ in serum. No sample preparation is necessary for the serum samples. The consumption of the serum and buffer is very low (270 μL of each).

## EXPERIMENTAL

### Amperometric Immunosensor Design

The antiserum was diluted to a working dilution of 1 : 30 in 0.01 mol/L phosphate buffer saline, pH 7.4, containing 0.1% sodium azide. The graphite powder was heated at 700°C for 15 sec in a Muffle furnace and cooled to ambient temperature in a dessicator. The paraffin oil and graphite powder were mixed in a ratio of 1 : 4 (w/w) and then added to the diluted anti-L-$T_3$ to obtain a final composition of 0.9% (w/w) in anti-L-$T_3$. The carbon paste (graphite powder and paraffin oil) was filled into a plastic pipette tip leaving about 3–4 mm empty in the top to be filled with the chemical modified carbon paste that contains anti-L-$T_3$. The diameter of the immunosensor was 3 mm. Electric contact was made by inserting a silver wire in the carbon paste.

Before each use, the surface of the electrode was wetted with double distilled water and then polished with an alumina paper (polishing strips

30144-001, Orion). When not in use, the amperometric immunosensor was stored in a dry state at 5°C.

## Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 20 and a software version 4.9 were used for all amperometric measurements. A platinum electrode and a Ag/AgCl (0.1 mol/L KCl) electrode served as counter and reference electrodes in the cell.

## Reagents and Materials

The immunological system composed from L-$T_3$ and monoclonal anti-L-$T_3$ was supplied by Sigma (St. Louis, MO). Graphite powder with a particle size of 50 μm was supplied by Merck (Darmstadt, Germany). Paraffin oil was supplied by Fluka (Buchs, Switzerland). All other reagents were of the highest analytical grade. All the solutions were prepared using de-ionized water.



## Sequential Injection System

The immunosensor was incorporated into the conduits of the SIA system (Fig. 1) constructed from a Gilson Minipuls peristaltic pump and a 10-port electrically actuated selection valve (Model ECSD10P, Valco Instruments, Houston, TX). Tygon tubing (0.76 mm i.d. for the holding coil and 0.89 mm i.d for the mixing coil) was used to construct the manifold; coils were wound round suitable lengths of glass tubing (15 mm o.d.); 0.1 mol/L NaCl was used as carrier. The capacity of the system is about 75 samples per hour. The device operating sequence is shown in Table 1. The device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa). The FlowTEK[6] software package (obtained from MINTEK) for computer-aided flow analysis was used thoughout for device control.

# RESULTS AND DISCUSSION

An optimum flow rate of 3.61 mL/min was used to propel the solutions. In the SIA system, the sample and buffer consumption is only 270 μL, each,



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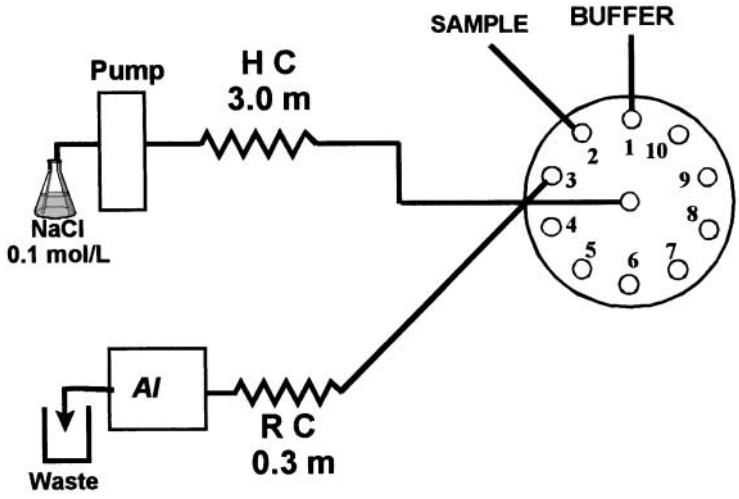


***Figure 1.*** Schematic flow diagram of SIA/immunosensor system used for the determination of L-$T_3$ in serum samples. HC, holding coil; RC, reaction coil; AI, amperometric immunosensor cell. (*View this art in color at www.dekker.com.*)

***Table 1.*** Device sequence for one cycle of the SIA system.

| Time (sec) | Pump | Valve | Description |
|---|---|---|---|
| 0 | Off | Buffer | Pump stops, select buffer stream (valve position 1) |
| 5 | Reverse | Buffer | Draw up buffer solution |
| 9.5 | Off | | Pump stops |
| 10.5 | | Sample | Select sample stream (valve position 2) |
| 11.5 | Reverse | Sample | Draw up sample solution |
| 16 | Off | | Pump stops |
| 17 | | Amperometric immunosensor cell | Select amperometric immunosensor cell line (valve position 3) |
| 18 | Forward | | Pump stack of zones to amperometric immunosensor cell |
| 48 | Off | | Pump stops, return valve to starting position (valve position 1) |





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per measurement of the concentration of L-$T_3$, which is very economical. All measurements were performed at 650 mV vs. Ag/AgCl.

## The Response of the Amperometric Immunosensor in SIA System

The equation of calibration for L-$T_3$ is:

$$H = 0.22 + 4.15C$$

where $H$ (nA) is the peak height and $C$ is the concentration (ng/mL) of L-$T_3$. The correlation coefficient, $r$, is 0.9997. The linear concentration range for the immunosensor is between 3.4 and 340 ng/mL, with a limit of detection of 2.19 ng/mL.

## The Selectivity of the Amperometric Immunosensor

The selectivity of the amperometric immunosensor was checked using both: separate and mixed solutions methods, vs. L-$T_4$, D-$T_4$ and polyvinylpyrolidine (PVP). The selection of L-$T_4$ for the selectivity test is to prove the suitability of the method to be used for the assay of L-$T_3$, in the presence of L-$T_4$ in blood samples. It is also essential to determine L-$T_3$ in the presence of D-$T_4$ and PVP if one has to perform tests of purity and enantiopurity of the pharmaceutical compounds containing L-$T_3$.

Amperometric selectivity coefficients were determined following the method proposed by Wang.[7] The amperometric selectivity coefficients values obtained for L-$T_4$ ($pk_{amp} = 8.56$), D-$T_4$ ($pk_{amp} = 7.05$), and PVP ($pk_{amp} = 7.77$) demonstrate the specificity of the immunosensor for the assay of L-$T_3$ using the SIA/immunosensor system. The inorganic cations like $Na^+$, $K^+$, $Ca^{2+}$ do not interfere in the assay of L-$T_3$.

## Analytical Applications

The response characteristics, as well as the specificity of the immunosensor, makes possible the utilization of the proposed analysis system for clinical and drug analysis.

The results obtained (Table 2) for the determination of L-$T_3$ in serum demonstrated the suitability of the proposed immunosensors/sequential injection analysis system for on-line determination of the thyroid hormone in blood





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***Table 2.*** Determination of L-$T_3$ in serum using sequential injection analysis/amperometric immunosensor system and the method proposed by Hay et al.[8]

| L-$T_3$ (pmol/L) | |
|---|---|
| Immunosensors/SIA | Comparison method[8] |
| 12.49 | 12.40 |
| 11.56 | 11.62 |
| 10.42 | 10.50 |
| 4.73 | 4.74 |
| 5.72 | 5.63 |
| 2.36 | 2.40 |
| 6.27 | 6.25 |
| 8.15 | 8.20 |
| 9.14 | 9.08 |

samples. Furthermore, they correlate very well with those obtained using the method proposed by Hay et al.[8]

## CONCLUSIONS

The SIA/amperometric immunosensor system provides excellent features for the immunoassay of L-$T_3$ in blood samples, as well as in pharmaceutical products. The design of the SIA system is simple, fast, and reproducible. The reliability of the analytical information is assured by the low RSD values obtained in the recovery tests, by the high rate of analysis, and by its large working concentration range.

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