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FLOW INJECTION SPECTROPHOTOMETRIC AND AMPEROMETRIC DETERMINATIONS OF AMMONIA WITH GLUTAMATE DEHYDROGENASE REACTOR

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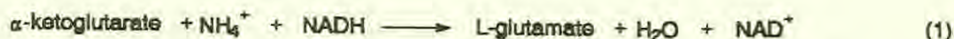
ABSTRACT. A flow injection method for indirect spectrophotometric and amperometric determinations of ammonia and ammonium, based on a 100-µL-glutamate dehydrogenase (GlDH) reactor, is described. GlDH was immobilized on controlled pore glass after silanization and glutaraldehyde activation. Injections of 50-µl standard ammonium chloride solutions to the carrier (water) produced peaks proportional to 3-1400 µM ammonia with a detection limit of 3 µM. The reagent stream delivered 0.2-0.6 mM nicotinamide adenine dinucleotide (NADH), 3.0 mM α-ketoglutarate and 4.0 mM adenosine-5'-diphosphate (ADP, activator) in 0.1 M Tris-acetate or phosphate buffer. Monitoring the decreasing NADH concentration after the enzymatic reaction produced peaks which formed the basis for measuring ammonia. Detection was made with a flow-through spectrophotometer (at 340 nm) or amperometric detector with a wall-jet phenoxazine-modified graphite electrode, held at 50 mV *vs* saturated calomel electrode (SCE). pH optima were 7.7-8.5 for the optical detector and 7.5-7.9 for the electrochemical detector. The system was applied to the determination of ammonium salt in commercial crude tryptophanase. The reactor was stable only for five days when used with Tris (pH 8.0-8.5) and stored in 0.1 M phosphate buffer (pH 7.0) at 4 °C between uses but stability improved when potassium phosphate buffer (pH 7.8) was the reagent carrier and the storage buffer contained 10 mM ammonium chloride.

INTRODUCTION

Ammonia is one of the enzymatic products resulting from several redox, hydrolysis and deamination reactions in biological processes [1,2]. L- and D-amino acid oxidases as well as dehydrogenases, for example, catalyze oxidations [1] while deaminases catalyze deamination of amino acids [2], all resulting in the formation of ammonia.

Nitrogen-containing biomolecules, such as urea and creatinine, also yield ammonia upon enzymatic reactions [3-7]. Direct and indirect detection methods of the ammonia produced from enzyme-catalyzed reactions have, therefore, been the basis of assaying various substrates [3, 5-16]. The indirect approach includes the use of GlDH for indicating ammonia formation [3, 10, 14-16] and, in bioanalytical systems, due consideration has been given to this reaction because of the highly favoured equilibrium towards L-glutamate formation [17].

The enzyme requires adenosine-5'-phosphate (ADP) as activator.



$$\alpha\text{-ketoglutarate} + NH_4^+ + NADH \longrightarrow \text{L-glutamate} + H_2O + NAD^+ \quad (1)$$

Based on soluble or immobilized GlDH, applications of this reaction for the determination of ammonia in biological fluids [18-20], lake and waste waters [21, 22] as well as in foods [23, 24] have been reported. An indirect detection system, based on this reaction includes a creatinine biosensor in a flow injection system, with membrane-entrapped creatinine iminohydrolase and GlDH on phenoxazine-modified graphite electrode [15]. The reaction consumes NADH and the flow injection peak of creatinine is, therefore, negative. The current decrease is due to the electrocatalytic oxidation of the remaining NADH at the modified electrode.

Tryptophanase also catalyzes the hydrolysis of a number of L-amino acids by α,ß-elimination reaction, yielding ammonia as one of the products [25]. The reactions represented in Equation 2, take place in presence of pyridoxal-5'-phosphate (cofactor) and potassium (or ammonium) ion as activator.

$$\text{L-}RCH_2CHNH_2COOH + H_2O \longrightarrow \text{pyruvate} + NH_3 + RH \quad (2)$$

The substituent group, R, in the amino acid may be indoyl (in the case of tryptophan), NH_2 (in the case of L-α,ß-diaminopropionic acid, DAP), SH, SH_2CH_3, OH etc. There are a few examples of coupling tryptophanase with GlDH for assaying substrates. A recent case is the use of an automatic analyzer for serum potassium based on soluble tryptophanase/GlDH and consumption of NADH (Equation 1) [26]. At least one paper indicates that, ß-N-oxalyl-L-α,ß-N-diaminopropionic acid (ODAP) also undergoes hydrolysis by this enzyme, possibly giving α-ß elimination reaction products as detected from the pyruvate formed [27]. ODAP is a neuro-toxic amino acid that causes human neurolathyrism and is found in the highly protein-rich seeds of *Lathyrus sativus [28].*

Our interest in tryptophanase arises from the need to develop flow methods incorporating immobilized enzymes to detect ODAP, either directly or through DAP after its hydrolysis. There has been an urgent call by agronomists and the biomedical community working on the eradication of neurolathyrism, for a fast and selective analytical method for the neurotoxin. The most widely used method is based on a chemical hydrolysis of the toxin to DAP and oxalate, followed by a colorimetric reaction of DAP for indirect determination [29]. The method is not fast nor selective as its α-isomer (α-ODAP) also gives the same hydrolysis products.

One possible approach towards this call is to study co-immobilized tryptophanase/GlDH for the determination of ODAP via DAP formation or directly. Optimization of the reaction conditions (Equation 1) for a flow injection determination of ammonia (the product in Equation 2) with an immobilized GlDH reactor should thus be the first step before studying the double-enzyme system.

This report presents a study of a flow injection system with immobilized GlDH reactor for the determination of ammonia and ammonium salts, based on the consumption of NADH using either spectrophotometric or amperometric detection. An application is also included on the measurement of ammonium salt in a commercial crude tryptophanase. The assay of the ammonium salt in the enzyme preparation is also important since removal of the ammonium ions is required prior to immobilization and this can be easily checked by the present ammonia-detecting system.

EXPERIMENTAL

Reagents. The following chemicals from Sigma were used in this work: α-ketoglutarate (K1750), NADH (N8129), ADP (A5771), tris(hydroxymethyl)aminomethane (Tris, T1503), 3-aminopropyl(triethoxy)silane (A3648), glutaraldehyde (G6257), partially soluble crude tryptophanase (T0754, from *E. coli*, 1 mg preparation releases 15-40 μg indole 10 min^{-1} from L-tryptophan) and the other biochemicals. Ammonium chloride (Analar) and dipotassium hydrogen phosphate (BDH) were also used. All the chemicals were used as received except glutaraldehyde, which was pretreated with activated carbon to remove possible polymeric products.

Buffer and reagent solutions were prepared in double-distilled and degassed water. The reagent solution, which was prepared every day, contained 0.2-0.6 mM NADH, 3.0 mM α-ketoglutarate and 4.0 mM ADP in 0.10 M Tris-acetate or in 0.1 M phosphate buffer (pH 7.8). pH (with Tris-acetate) and reagent effects were studied by varying each factor while maintaining the others constant as described in this section. Standard solutions were prepared from stock solutions of 10 mM ammonium chloride. Ammonia and ammonium are interchangeably used in this report.

Immobilization of enzyme. Controlled pore glass, CPG (CPG-10, particle size 0.075-0.125 mm, pore size 500 Å, Serva) was initially silanized with 3-aminopropyl(triethoxy)silane in toluene [30]. About 3 ml of 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.0) was added to 190 mg of the silanized CPG and the reaction allowed to take place under reduced pressure at room temperature for 30 min and at 4 °C for additional 30 min. 15 mg (480 U) of GlDH (from bovine liver, Sigma G7882), in 3.0 ml of the same buffer, was then added to the activated product for immobilization under reduced pressure (30 min) and at 4 °C (overnight). The immobilization yield was 85% as calculated from the absorbance readings of the enzyme solution before and after the immobilization. A packed-bed GlDH reactor was finally prepared in a-100 μl Plexiglas tube (i.d. 2 mm) with polypropylene filters at the two ends.

Detection systems. Spectrophotometric and amperometric flow-through cells were used to indirectly detect ammonia through the decreasing NADH concentration which resulted from the enzymatic reaction. LKB 2151 UV-Vis monitor, set at 340 nm, was used for the optical detection.

An amperometric detector, Zäta LC4 (Höör, Sweden) was connected to a three-electrode electrochemical cell, of the type used earlier [31] but reduced in size (10 x 1.25 mm) and equipped with a Radiometer K701 SCE. One end of a 6-cm long graphite rod (Ringsdorf-Werke GmbH, diameter 3.1 mm) was chemically modified by adsorption of 5,5'-[1,4-phenylene bis(carbonylamino)]bis-9-diethylaminobenzo[a]phenoxazin-7-ium (CA reg. no. 135656-95-0) (Mirak's Blue) using a few drops of the solution in acetone [32]. The graphite electrode was modified with the mediator every day. Prior to modification, the surface of the graphite rod was cleaned with wet fine emery paper, preheated at 700 °C for 90 s and cooled in a desiccator [33].

The flow injection system. The flow injection set-up for ammonia determination is represented in Fig. 1. It consisted of a two-channel Gilson Minipuls 2 peristaltic pump to deliver the sample carrier (water) and the reagent solution. Sample injection was made from a 50 μl loop using a pneumatically operated injection valve (Cheminert, type SVA). The two streams were allowed to mix in a knot of Teflon tubing (about 1 m long, i.d 0.5 mm) before reaching the reactor. All connections in the system were made with Teflon tubes (i.d. 0.5 mm).

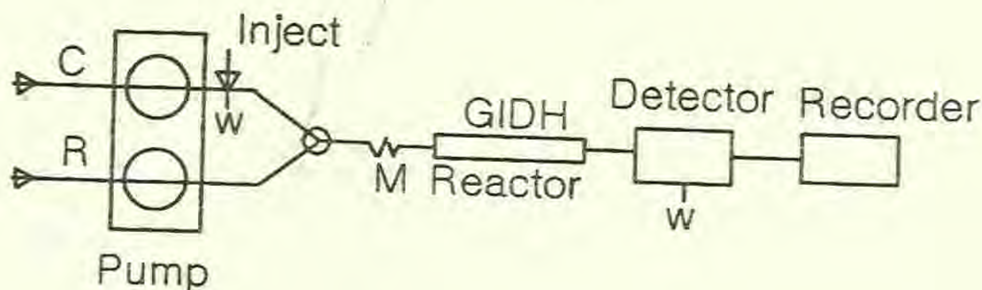
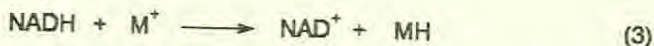


Fig. 1. Manifold for determination of ammonia. C (carrier, water), R (reagents: 3.0 mM α-ketoglutarate, 4.0 mM ADP and 0.2-0.6 mM NADH in 0.1 M Tris-acetate or phosphate buffer, pH 7.8), M (mixing coil) and W (waste). The flow rate from the outlet was 0.50 $ml.min^{-1}$)

RESULTS AND DISCUSSION

The basis of measuring ammonia concentration with the flow system using both detectors is the peak-height resulting from the decrease in NADH concentration. Similar responses to consumption of NADH based on other enzymatic reactions were earlier applied in flow injection determinations of ADP [34], creatine [35], creatinine [15, 35], and glucose [36].

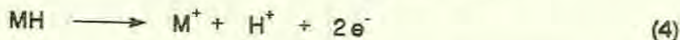
The mediator used in modifying the graphite electrode was first synthesized by coupling two Nile Blue molecules with terephtaloyl chloride and applied to construct a wall-jet glucose biosensor, based on the electrocatalytic oxidation of NADH [32]. The mechanism of its electrocatalytic action is believed to be the same as that of other phenoxazines, e.g. Meldola's Blue [37]. The chemical step at phenoxazine-modified graphite surfaces is the oxidation of NADH,



$$NADH + M^+ \longrightarrow NAD^+ + MH \quad (3)$$

where M^+ is the oxidized mediator adsorbed at the electrode surface.

The mediator is then regenerated to its oxidized form by fast electrochemical reaction at the oxidizing potential applied to the working electrode.



$$MH \longrightarrow M^+ + H^+ + 2e^- \quad (4)$$

In the present flow injection system the steady state response to 0.4 mM NADH was about 1.05 absorbance units (AU) for the optical detector and 1500-1600 nanoamperes (nA) for the amperometric detector at a flow rate of 0.5 ml/min. The steady state response to NADH was thus the baseline and the decreasing peak heights to ammonia injections were taken as positive.

Detector responses with pH. The effect of pH, with Tris-acetate buffer, on the responses of both detectors was examined by injection of standard ammonium chloride solutions. The curve for the spectrophotometric flow injection peaks with pH was an inverted U, with an optimum pH range of 7.7-8.5 (curve not shown). This range is

wider than the reported optically measured optimum for the native bovine liver enzyme in solution which was pH 8.0-8.5 [38]. It was also reported earlier that the pH-activity profile of native and immobilized GIDH were indistinguishable [39]. The immobilization method was, however, different from the method reported in this paper.

The influence of pH on the amperometric peaks was distinctly different from that of the optical method as shown in Fig. 2. The pH-dependence is very flat within the range of pH 7.5-7.9. Responses due to the electrocatalytic oxidation of NADH by the mediator decreases with increasing pH [32]. The combined effect of pH on the kinetics in the reactor, and the pH dependence of the electrocatalytic oxidation of NADH should,

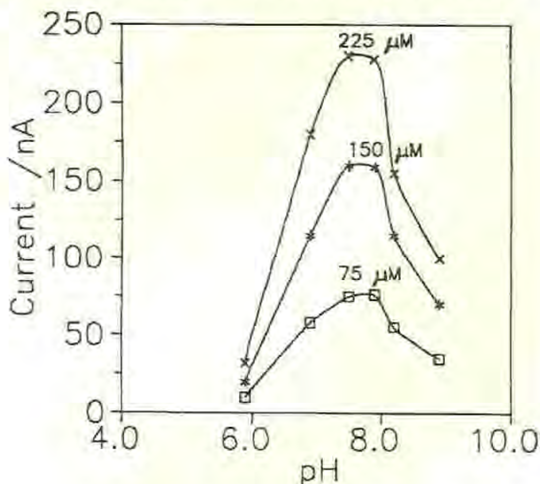


Fig. 2. The effect of pH (Tris-acetate) on the amperometric flow injection peaks of standard ammonium chloride. Other conditions are as in Fig. 1.

One advantage behind the use of this mediator is its alkaline and operational stability [32], unlike other phenoxazines such as Meldola's Blue [40].

The enzyme was inactivated within days in the Tris buffer, particularly at pH > 8. Replacing the reagent buffer with potassium phosphate and lowering the pH seemed to improve the stability of the reactor as will be discussed in a later section.

Reagent effects. The concentration of NADH in the reagent was adjusted to 0.2-0.6 mM depending on the injected concentration of the analyte. Because of dispersion of the injected sample in the flowing stream, the minimum NADH concentration is about 40% of the injected analyte concentration. The dispersion coefficient of the system was about 4.1. Linear responses were obtained for 3-1400 µM ammonia when the reagent stream delivered 0.2-0.6 mM NADH.

At high concentrations, NADH is an inhibitor to both soluble [41, 42] and covalently immobilized GIDH [42]. No inhibition of the enzymatic reaction was, however, noted when the reagent stream delivered as much as 0.75 mM NADH.

Varying concentrations of α-ketoglutarate were also used to examine its effect on responses to 400 µM ammonia. 2.0-3.5 mM solution of the substrate was sufficient to saturate the enzyme. The response to injection of 400 µM ammonium chloride decreased by about 10 percent when 4.0 mM of α-ketoglutarate was used in the reagent stream.

ADP activates both native [43] and immobilized GlDH [42]. The effect of ADP on activity was examined by varying its concentration from 0.0 to 7.0 mM, as shown in Fig. 3. The result reveals about 50-56 percent increase in activity when the enzyme was saturated with ADP. In early reported works, saturating concentrations of ADP (with NADPH as the co-enzyme) caused activity increases by about four-fold for the soluble enzyme [43] and by nearly 2-fold for the immobilized form [42].

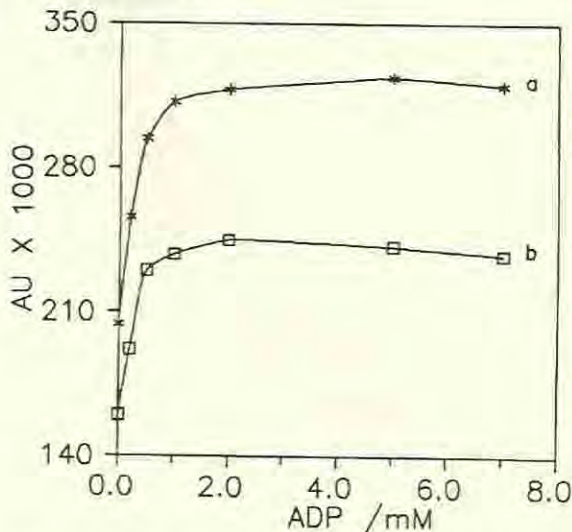


Fig.3. The effect of ADP concentration on the optical flow injection peaks of (a) 225 μM and (b) 300 μM ammonia.

Flow rate variations. The conversion efficiency of an enzyme reactor in FIA depends on the kinetics in the reactor and the flow rate which is inversely related to the residence time [44]. In an amperometric method, a change in flow rate influences mass transport of the electroactive species to the wall-jet electrode. The steady state or flow injection current of a given NADH concentration will, therefore, increase with increasing flow rate.

Figure 4 illustrates the effect of flow rate (varied between 0.1-1.1 ml/min) on the amperometric steady state response to 300 μM NADH as well as on the flow injection peaks of 300 μM NADH and ammonium chloride. The steady state and flow injection peak currents of NADH increased with flow rate (Fig. 4a and 4b). It should be noted that the plot of peak current for NADH injection *vs* flow rate is inverted with respect to that of the steady state for comparison with ammonia injections (Fig. 4c).

With increasing flow rate, the peaks of the ammonium standard injected to the carrier reached a maximum at 0.45-0.54 ml/min and then gradually decreased (Fig. 4c), despite the positive effect of flow rate on mass transport to the modified electrode surface. This decrease in the peak current of ammonium chloride injection at flow rates beyond 0.54 ml/min should be ascribed to an incomplete reaction in the reactor due to decrease in the mean residence time.

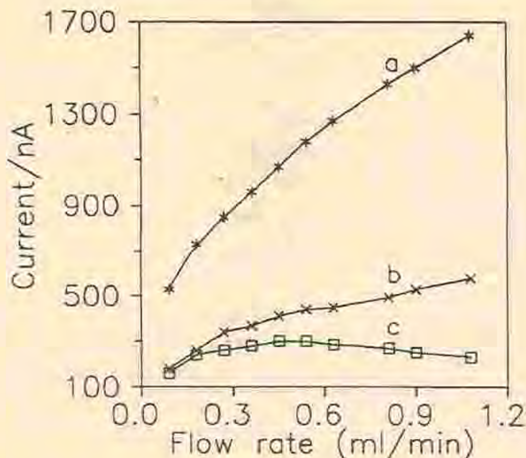


Fig. 4. The influence of flow rate on the base line current of 300 μM NADH in the flow stream (a); amperometric flow injection peaks of 300 μM NADH (b); and 300 μM ammonia (c).

The flow injection peaks of 300 μM NADH and 300 μM ammonium chloride at different flow rates were examined using spectrophotometric detection (data not shown). Since the mass transport factor in the spectrophotometric flow-through cell is non-existent, dispersion in the flowing stream is the only factor that caused a slight decrease in the peaks of injected NADH. With increasing flow rate, the ammonium peaks decreased faster than those of NADH (data not shown) because of lower reaction rate in the reactor and, to some extent, increased dispersion. Similar observations were made in one of our earlier works when NADH peaks were compared with substrate peaks in relation to flow rate variations [35].

Routine measurements with both of the detection systems were made at a flow rate of 0.5 ml/min.

Stability of immobilized enzyme. Responses to injections of standard ammonium chloride were consistent for 5 days using the same bovine liver GlDH reactor, giving a sensitivity of 1.00-1.06 mAU/μM in Tris-acetate buffer at pH 8.5. The response, however, decreased gradually followed by a sharp decline until the ninth day (see Fig. 5). Lowering the pH to 7.7-7.9 with the same buffer system did not significantly improve the stability of the immobilized enzyme. Between uses, storage of the reactor was made at 4 °C in phosphate buffer (pH 7.0). Because of its instability, three batches of reactors, from the same product, were used to study the flow system. During the study period (seven weeks) there was no loss of activity of the stock of immobilized GlDH under the same storage conditions.

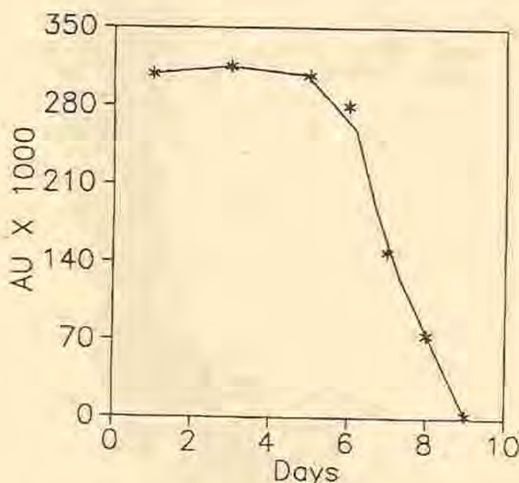


Fig. 5. Optical flow injection responses to 300 μM ammonia with the same batch of a 100 μl GlDH reactor and using Tris-acetate buffers (pH 8.3) throughout the reactor life time.

Some earlier reports on the stability of GlDH reactors are rather conflicting (Table 1). A flow injection system with beef liver GlDH reactor (immobilized on glutaraldehyde-activated CPG) had been useful only for a few days [14]. In contrast to this report another flow system coupled with a CPG-based GlDH reactor is reported to have 80% residual activity after 3 months [24] (Table 1, row 5). Nylon tube and Eupergit (epoxyacryl resin) GlDH reactors, enzymes from *Proteus species* and beef liver, respectively, were also very stable [10, 45]. The performance of the nylon tube reactor was very good for six months with a slight loss of activity after about 40 days [10], while the Eupergit-based reactor lost 50% activity in 73 days [45]. No remarks were, however, given in these papers if the stability factor was associated with the extent of binding of the enzyme with the supports or with the type of storage buffer.

A much earlier work had shown that native bovine liver GlDH is inactivated within an hour in Tris-HCl, loosing 75% and 97% of its activity at pH 8 and 9, respectively [46]. From a long list of inorganic and buffer salts, the study showed that alkaline phosphates and ammonium salts were the best protective agents to preserve the activity of the enzyme. This suggests that a systematic study of selecting storage and working buffer for GlDH reactors could possibly help alleviate the stability problem.

Packing of immobilized GlDH for the third reactor was made after the stability results were made available and, as a working buffer, potassium phosphate (pH 7.8) replaced Tris-acetate for the quantitation of ammonium salt in tryptophanase samples. Between intermittent applications of the reactor for four weeks (8 h per week), the flow injection responses decreased to 50% of the first days when the storage buffer contained 10 mM ammonium chloride. No detailed and systematic investigation was made, however, to observe the effect of this or other storage buffers. The use of buffers above pH 8 is not generally recommended to prevent the hydrolysis of the glass support and a decrease in reactor life-time. The improved stability of the reactor should, therefore, be partly ascribed to the use of a low-pH working buffer. Further

improvement of reactor stability may be attained by critical examination of the supports, as well as the working and storage buffers.

Table 1. Stability of GIDH reactors

Enzyme support	Working buffer (pH)	Storage buffer (pH)	Stability (time*, % remaining activity)	Reference
Eupergit	phosphate (8.0)	phosphate	73 d, 50%	45
Nylon tube	Tris (8.5)	-	> 6 m**	10
CPG	phosphate, EDTA, ADP (7)	phosphate (7)	few d	14
CPG	phosphate (8.0)	phosphate (7.0)	1 d	14
CPG	phosphate (8.0), EDTA	phosphate (7.0)	3 m, 80%	24
CPG	Tris-acetate (8.0-8.5)	phosphate (7.0)	5 d, 100%	this paper
CPG	K-phosphate (7.8)	K-phosphate (7.0), 10 mM NH_4Cl	28 d, 50%	this paper

*days (d), months (m);
**destroys >98% of 100-600 μM ammonia interference.

Calibrations. Standard ammonia (as ammonium chloride) solutions were injected from the 50 μl sample loop to calibrate the responses of both detectors. The sensitivity of calibration curve with the spectrophotometric detection (not shown) was about 1 AU/mM. Sample through-puts of the system, at a flow rate of 0.5 ml min^{-1}, was 35-40 h^{-1}. With both detectors the linear range for ammonia was 3-1400 μM using the recommended pH and reagents. The buffer type did not practically affect the detection sensitivity. The baseline noise of the amperometric system, however, increased to 5-10 nA when NADH was beyond 0.5 mM. Blank (water) injections produced peaks equivalent to 1 μM ammonia. The relative standard deviation (n = 5) and the detection limit were 1.5% and 3.0 μM for the optical detector and the corresponding values for the amperometric detector were 2.1% and 3.0 μM. Calibrations with the reactor using phosphate as working buffer (Table 1, last row) are shown in Fig. 6 for the amperometric detector. Curve 6b shows linearity up to 1000 μM ammonia after 3 weeks.

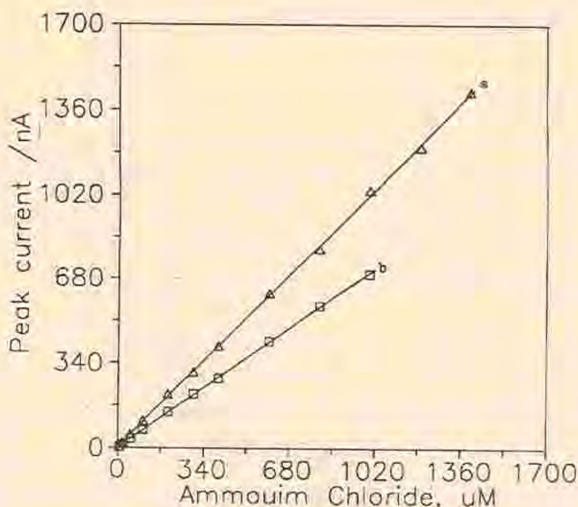


Fig. 6. Typical calibration curves with the amperometric detection system with phosphate buffer as reagent carrier (pH 7.8); Calibration within the first days of reactor (a); and after three weeks (b).

Determination of ammonium salt in tryptophanase. We noticed the presence of an ammonium salt in crude partially soluble preparations of tryptophanase when an attempt was made to monitor a time-dependent ammonia formation from tryptophanase-catalyzed hydrolysis of DAP (see Equation 2), using the GlDH reactor in the flow injection system. Within 30 min, the same peak was obtained from injections of a reaction mixture and a blank. 1 ml of the reaction mixture contained 500 μM DAP, 1.0 mM pyridoxal-5'-phosphate, 1.0 mM glutathione and 0.33 mg/ml of tryptophanase in 0.1 M potassium phosphate buffer (pH 7.8), while the blank contained all except DAP. Both were maintained at 37 °C in a thermostated water bath with a shaker before injection. Plots of injection peaks with time for the reaction mixture and the blank solution are shown in Fig. 7.

Further screening of the reagents by injecting their solutions at room temperature confirmed that the peaks arose from the tryptophanase sample. It is established that other amines (such as methylamine) are not substrates to GlDH and the enzyme is specific to ammonium ions [47]. No specification from the manufacturer was made as to the presence and content of any ammonium salt in the enzyme preparation. An earlier paper reported, however, soluble apotryptophanase from Sigma contained ammonium salt which was removed by dialysis [48].

The reaction did not start until after 30 min as shown in Fig 7a. Such a delay was also observed when the same reaction was monitored with detection of the pyruvate formed using a lactate dehydrogenase reactor [49]. From the part of the plot with the highest slope and calibration data, the estimated rate of formation of ammonia is 10^{-3} μmol.min^{-1} and the calculated activity of 1 mg of crude enzyme with respect to DAP is 1.5×10^{-4} μmol.min^{-1}. The result also indicates that homogeneous kinetic assay for

DAP could be designed with purified tryptophanase and GlDH. The reaction with soluble apotryptophanase is currently under study.

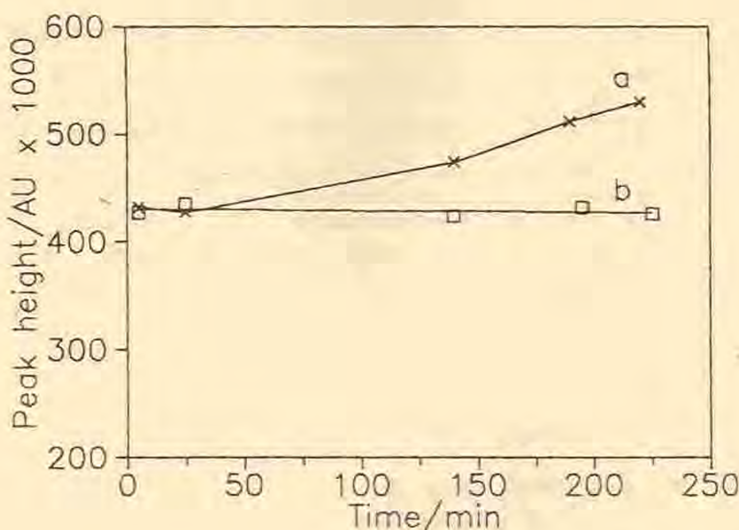


Fig. 7. Responses of the optical detection system with time to injection of tryptophanase (0.33 mg/ml) (a) in presence of its substrate, DAP, and reagents described in the text (at 37 °C), and (b) the same as (a) but with no DAP.

If immobilization of tryptophanase (via glutaraldehyde activation of the glass) is desired, removal of the ammonium salt (and the insoluble part) should, evidently, be made from the crude preparation and the flow system with the enzyme reactor could be used to check its removal after a separation step.

The content of ammonium (as ammonium sulphate) in the crude enzyme was determined using both detectors from the supernatant or filtrate of the enzyme solution in phosphate buffer. The results of 5 injections for sample sizes ranging between 0.15-0.75 mg/ml of the same crude preparation are shown in Table 2. Measurements with the two detectors were made on different days.

Although the mean values (n = 5) of the two detection systems are in good agreement, the precision of the amperometric method is lower than that of the spectrophotometric one. Upon injections of the supernatant solutions of tryptophanase, the amperometric base-line of NADH in the reagent stream as well as the flow injection peaks gradually decreased with time. 85% of the initial steady state value of NADH in the reagent solution was recorded in 30 min with 10 injections of tryptophanase solution containing 0.75 mg/ml. The response was, however, recovered when the modified electrode in the cell was changed. From this observations it should be concluded that electrode fouling, due to adsorbed protein and impurities, should be responsible for the decreasing amperometric responses and the low precision observed (Table 2) when tryptophanase samples were injected. No significant improvement was noted in the consistency of the base-line even if the enzyme solution was filtered.

Table 2. Ammonium salt determination in crude partially soluble tryptophanase

Tryptophanase added mg/ml	$[NH_3]$ /μM	NH_4^+ as sulphate % (w/w)	C.V (n = 5)	Detector*
0.15	195	17.1	2.3	spec.
0.20	250	16.4	7.0	amper.
0.25	319	16.7	2.0	spec.
0.25	324	17.0	5.1	amper.
0.75	959	16.7	1.8	spec.

*Spectrophotometric or amperometric

CONCLUSION

The immobilized GIDH reactor, incorporated into a flow injection system, has been shown to be a useful means of indirectly quantitating ammonia and ammonium salts, using both amperometric and spectrophotometric detectors. The presence of ammonium salts interfere with the immobilization of enzymes on glutaraldehyde-activated glass, and the ammonium salt in tryptophanase samples should thus be removed before co-immobilizing it with GIDH for a reactor preparation that could be used to study the reactions of L-amino acids including DAP and ODAP. The removal of ammonium salt in tryptophanase (or in other enzymes) could be simply checked with the proposed flow injection system. To prolong the stability of the GIDH reactor, systematic selection of immobilization method and buffer is highly desirable.

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