Flow Injection Analysis of Naphthalene and Substituted Naphthalene Dialdehyde Derivatives of Amino Acids with Fluorescent Detection

M. AMINUDDIN[1] AND J.N. MILLER[2]

[1]*Chemistry Department, Islamia University, Bahawalpur, Pakistan*

[2]*Chemistry Department, Loughborough University of Technology, Leicestershire, LE11 3TG, England*

(Received 26th September, 1992, revised 26th October, 1993)

Summary: A flow injection manifold incorporating fluorigenic reagent has been developed for the determination of glycine. The experimental approach is little different than from the usual procedure. A reverse flow injection analysis system is adopted in which the fluorigenic reagent has been injected into the non-segmented continuous carrier stream containing the buffer. Various factors which affect the fluorescence signal have been examined in this study in order to benefit from these findings.

Introduction

The determination of species in flowing solution is becoming increasingly popular by virtue of its important area in the automation of analysis and monitoring. The technique of flow injection analysis (FIA) originally developed by Ruzicka and Hansen [1] in 1975 is a rapid and precise method for screening large number of samples. The use of FIA also provides an efficient sample introduction technique, making it even more attractive because of the simple instrumentation required and improved responses under dynamic conditions. This has found application as a suitable tool for the determination of glycine and albumin [2] and of primary amines in sea-water [2]. The combination of flow injection analysis with fluorescence detection has been of much interest. It is sensitive and allows detection even at the $pgml^{-1}$ level. It has been preferred to other detection system especially in automatic analyses [3]. FIA system also finds its use for increasing the sensitivity of a technique [4]. Determination of amines and amino acids, using FIA, previously made use of *o*-phthalaldehyde (OPA) as a fluorigenic

reagent for the on-line derivatisation reaction [2]. However, the reaction product is unstable and thus complicating the use of the reagent [5].

In this work we investigated the use of newly synthesised fluorescent labels e.g. naphthalene-2,3-dicarboxaldehyde, NDA [6] and 1-phenylnaphthalene-2,3-dicarboxaldehyde, θNDA [7] with an FIA system to enhance the sensitivity, and reproduci bility for the determination of amino acid (glycine). The results have been compared with OPA as a labelling reagent for amino acids. Further, in order to economise in the use of expensive fluorigenic reagents, NDA and θNDA, a "reverse flow injection analysis" system has been employed (Fig. 1). In this method the sample (glycine) is pre-mixed with thiol, borate buffer, and β-cyclodextrin and is continuously pumped through the connector T piece to the detector. The fluorigenic reagent is injected into the non-segmented continuous carrier stream containing the buffer. The labelling reagent meets the sample in a connector T and the reaction product then passes on to the detector and the fluorescene signal is recorded.

Parameters investigated in this study include:

(a) Pumping rate (b) tube length (c) relationship between the thiol and amino acid contents. (d) reaction time and (e) sample size.

Results and Discussion

Fluorescence signal intensity as a function of reaction time

The effect of gradually increasing the time lapse between injection and measurement of fluorescence signal was investigated. For this the fluorescent derivative of glycine formed separately with NDA, θNDA or OPA using FIA were suspended temporarily in the reaction coil by halting the carrier flow and then allowed to pass on to the detector. Thus, by switching the pump 'off' and 'on' the fluorescence signal corresponding to the time lapse was obtained on a recorder. In order to minimise dispersion, the length of a 0.58 mm i.d. Teflon tubing between the connector 'T' and the detector was 10 cm. Figure 2 shows that as the time lapse between the pump switching 'off' and 'on' increase the fluorescence of the derivative also seems to increase to a plateau. For OPA-glycine the plateau was reached in 3 minutes, whereas both NDA and θNDA

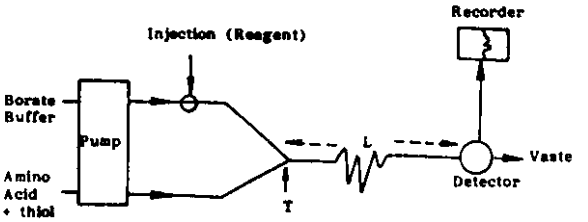

Fig.1: Schematic diagram for reverse flow injection analysis.

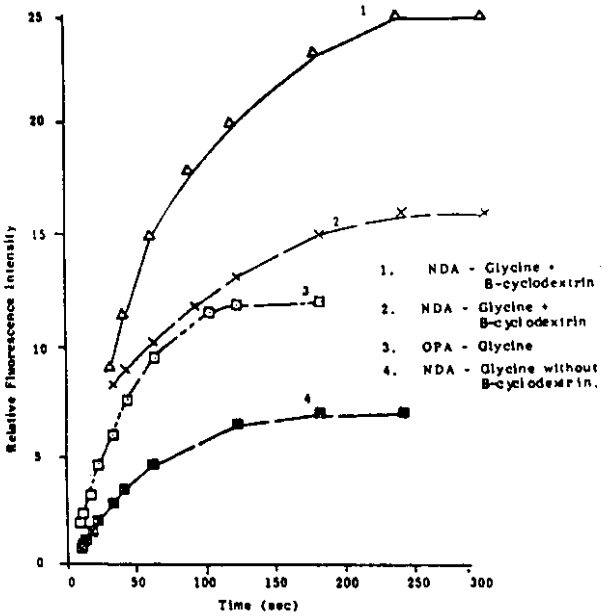

Fig.2: Reaction time Derivatisation of glycine using NDA, θNDA and OPA as fluorigenic reagents.

- glycine took 5 minutes for the fluorescence to become constant. The plateau in each case seems to suggest that the reaction was complete within this time.

By applying the same stopped - flow mode in FIA, the fluorigenic reaction of NDA with glycine in the absence of β- cyclodextrin showed the fluorescent derivatisation reaction to be complete in about the same time (Figure 2) as that with β-cyclodextrin. This shows that the reaction time remains unaffected by β-cyclodextrin. The use of β-cyclodextrin in the fluorigenic reaction is for enhancement of the fluorescence intensity of the derivative.

The NDA and θNDA seem to be equally efficient labelling reagents for glycine as OPA using FIA.

Dependence of the fluorescence signal on the amount of thiol

For the fluorigenic reaction of OPA, NDA and θNDA with glycine, a thiol is required, without which the fluorecent derivative, an isoindole, would not be formed. However the quantity of thiol used for a static system may not be the same an that required in FIA. The study showed that fluorescence intensity was found to level off as the ratio of thiol:glycine was around 10 mmol for both OPA and NDA - derivatives. It was assumed from this result that the same would be true for θNDA-derivatives as well.

The addition of thiol:glycine in this ratio may be necessary to obtain the maximum fluorescence signal for a fluorescent product.

Relationship between flow rate and fluorescence intensity

Fluorescent labelling in flowing solution is a novel approach to determine species in trace quantities. In order to achieve enhanced fluorescence signal there is need to maintain proper flow rate. The present study indicated that the flow rates had a considerable effect on the fluorescence intensity. The peak height of the OPA and NDA-glycine derivative decreased with the increase of flow rate. Figure 3 shows that the fluorescence intensity for both the derivatives was almost halved when the flow rate was altered from 1.0 to 1.5 ml/min. The fluorescence signal was further adversely affected as the flow rate was changed to 2.0 ml/min., reducing the signal to virtually zero. Such results may seem to arise from the shorter time available for the reaction and may possibly be a combined effect of high dispersion [8] with increasing flow rate.

Effect of tube length on fluorescence peak height

Useful information can be had about the fluorigenic reaction in flowing solution by changing the tube length 'L' in between the merging point 'T' and the detector in flow injection analysis. Figure 4 shows that change in tube length greatly affects the fluorescence signal monitored. The peak height, corresponding to the signals for all of these

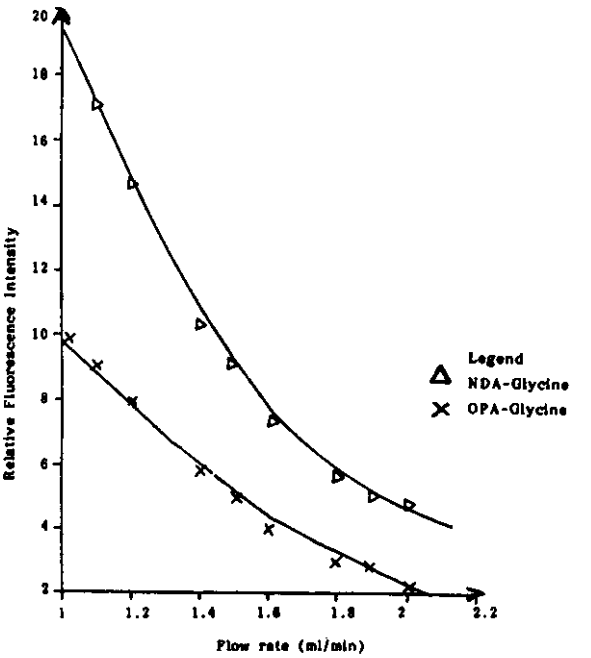

Fig.3: Variation of peak height with flow rate.

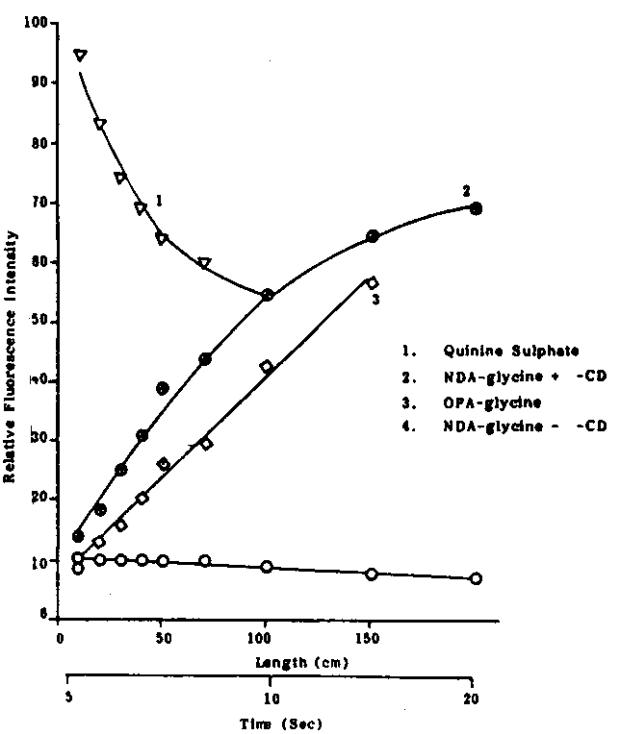

Fig.4: Effect of tube length on peak height of the fluorescing molecules.

derivatives, OPA and NDA-glycine, were at their minimum value when the shorter length of tube 10 cm, was used. The peak heights kept on increasing as the length of 0.58 mm i.d. Teflon tubing was increased from 10 cm to 20, 30 and upto 200 cm. By observing the trend of this change in terms of peak height, the progress of the fluorimetric reaction between glycine and the labelling reagents has been studied. The yield of the fluorescent product is very very low when the reactants/products travel through only 10 cm of tubing. In contrast the yield of the fluorescent derivatives constantly rises as the residence time increases by increasing the tube length. Increasing manifold length helps mixing and promotes chemical reaction but leads to increased dispersion and loss of sensitivity. The increasing peak height with increasing tube length seems to indicate that more fluorescent product is being formed overriding the effect of the dispersion process. Since the reaction is not complete and may take longer than 20 seconds, it would be difficult to accommodate such a long reaction time in an FIA system.

However, in the standard quinine sulphate, there is a decrease in peak height with increasing tube length. This is due to the increased dispersion as expected.

Calibration curve

Glycine solutions at different concentrations (0.945×10^{-1} mol/ml to 3.77×10^{-8} mol/ml) were found to form fluorescent derivatives using reverse flow injection analysis. NDA-glycine derivatisation, maintaining a flow rate of 1 ml/min. offered peak height versus concentration a linear relationship. A very dilute solution of glycine as low as 0.9425×10^{-8} mol/ml was successfully derivatised to obtain fluorescence signal of significance. This may be equally applicable for reaction with any other amino acid, using NDA or θNDA as fluorigenic reagents.

Experimental

Apparatus

The flow injection analysis (FIA) manifold shown in Figure 1 was used. 'L' is the length of 0.58 mm i.d. Teflon tubing (R.S. components Ltd.) which was 10 cm for the investigation of reaction time. The FIA manifold was directly attached to a Perkin-Elmer model 2000 fluorimeter, fitted with a flow cell. Injections were made using a Rheodyne 5020 valve fitted with a 50 µl loop. The selection of a 50 µl loop offered a dispersion value of 2.54. Reagents and buffers were pumped using a Gilson Minipuls 2,4-channel peristatic pump. A model 28000 recorder (Bryans Ltd.) was used to record the FIA fluorescence data.

Detection

Fluorescence detection was carried out at the appropriate wavelengths, 520 nm for NDA and θNDA-amino acid derivatives and 450 nm for OPA-derivatives with excitation at 460 nm and 350 nm respectively.

Solutions

(i) Glycine sample solution together with other reagents

10^{-4} M glycine solution (20 ml) was mixed with 10.9 mM β-cyclodextrin, BCD (4.8 ml) and 0.1% v/v 2-methyl-2-propanethiol (1.7 ml) and borate buffer, pH 10.0 (1 ml).

(ii) Fluorigenic reagent solution

A suitable quantity of the fluorigenic reagent NDA, θNDA or OPA, was dissolved in a minimum volume of ethanol (5-10 ml) and the final volume made upto 25 ml with either distilled water or 0.025 M sodium borate buffer, pH 10.0 to give a solution of the required concentration.

Experimental procedure

The glycine sample solution, pre-mixed with B-CD, thiol and borate buffer, was continuously pumped (1.0 ml/min) through one of the two lines of the manifold (Figure 1) for reaction time investigation. A carrier stream containing the borate buffer was flowing through the other line (1.0 ml/min. for reaction time study) into which the fluorigenic reagents, NDA, θNDA or OPA was introduced. The fluorigenic reagent and glycine entered the merging point T *via* a connector before passing on to the detector. The pump was stopped five seconds after the fluorigenic reagent had been injected into the carrier stream. For reaction time studies the reactants were kept in the reaction coil for 5, 10, 15, 30 and

upto 300 seconds, after which the pump was re-started.

For all other experiments the conditions remained the name unless stated otherwise.

References

1. J. Ruzicka and E. H. Hansen, *Anal. Chim. Acta,* **78**, 145 (1975).
2. J. I. Braithwaite and J. N. Miller, *Anal. Chim. Acta,* **106**, 395 (1979).
3. Froehlich, in Modern Fluorescence spectroscopy, E. L. Wehry (Ed.), Vol. 2, Heyden, London, 1976.
4. M. R. Pereiro Garcia, M. E. Diaz Garcia and Alfredo Sanz Model, *J. Anal. Spectrom.,* 4, 699 (1987).
5. J. F. Stobaugh, Ph.D. Thesis, The University of Kansas, 1982.
6. J. W. Cook, L. Hunter and R. J. Schoental, *J. Chem. Soc. Suppl., 228 (1949).*
7. M. Aminuddin, Ph.D. Thesis, Loughborough University of Technology (U.K.), 1987.
8. J. Ruzicka and E. Hansen, Flow injection Analysis, John Wiley and Sons, New York, pp. 37 (1981).