3.5 An Improved Injection System for On-line High Sensitivity Phenylthiohydantoin Amino Acid Analysis

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The first commercial protein sequencers introduced in 1970 and the more recent "gas-phase" sequencer (Hewick et al., 1981) failed to appreciate the extent of heat destruction of certain derivitized amino acids while sitting in the fraction collector awaiting manual analysis. This problem was largely overcome by on-line PTH-amino acid analysis (Machleidt et al., 1980). In spite of the advantages of on-line PTH-amino acid analysis several shortcomings still exist in the commercial sequencers (Applied Biosystems Inc., models 470A and 477A equipped with model 120A PTH-amino acid analyser). The percentage of sample injected onto the HPLC column is <50% while the remainder is transferred to a non-refrigerated fraction collector where labile PTH-amino acids decrease due to destruction. A second difficulty is maintaining parameters (flow rate, time) which control the transfer of liquid from the conversion flask of the sequencer into the sample loop of the HPLC injection valve.

This report describes our efforts to improve upon the existing commercially available on-line injection system which relies on the large difference in viscosity of the sample solvent compared to that of argon gas (Fig 1c). Relatively large-bore tubing (F) leads from the conversion flask (G) of the sequencer to the 50 μl sample-loop of the Rheodyne injection valve (B) with the exit port of the injection valve connected to capillary tubing (H) leading to a waste container. The flow of sample from the conversion flask into the injection valve and sample-loop is relatively fast since only low-viscosity argon gas is passing through the capillary tubing (H). Once the sample liquid has filled the loop of the injection valve and starts to flow through the capillary tubing, the rate of flow markedly decreases. The
sequencer must be adjusted in order to minimise the further flow of liquid which has entered the capillary tubing since this material is directed to the waste container and lost.

In our injection device (Fig. 1a) a length of large-bore PTFE tubing (1.5 mm o.d., 0.8 mm i.d., Beckman Instruments Inc.) (A) is connected between the outlet port of the Rheodyne injection valve (B) of the HPLC and a solenoid valve (C) which is connected to waste. The percentage volume of sample transferred to the sample-loop can be readily adjusted by introducing a 500 μl gas-tight syringe (D) (Fig. 1b). The stainless-steel plunger is replaced with a hollow PTFE plunger (E) (a suitable length of PTFE tubing (3.0 mm o.d., 1.5 mm i.d.) with a smaller dimension PTFE tubing (1.5 mm o.d., 0.8 mm i.d.) inserted into its full length). Since the liquid comes to a complete stop, critical timing is unnecessary.

The transfer time of the sequencer programs (for both models 470A and 477A) is adjusted to 100 s (twice the time necessary). Control of solenoid valve (C) is by a simple timer (based on the 555 integrated circuit) started at the correct time by a function of the sequencer (Fig. 2). For the 470A sequencer the "LC Start" function is used to start the timer. With the model 477A sequencer, the function "Block Flush" is used as it is unique in that it is the only function where valves 14 and 20 operate at the same time. Connection to both valves 14 and 20 via plug J1 in the 477A sequencer is used to start the timer controlling the injector solenoid valve (C). The function "Block Flush" is used just prior to "Clear Inj to Waste" function so that operation of the injection solenoid valve occurs at the correct time. A set time of 300 s allows for any minor drift in the timer.

Modifications to the existing operational programs for Applied Biosystems sequencers required for installation of this injection device are listed in Table 1.

With a gas pressure of 3.5 psi the volume of the PTFE tubing connecting the injection valve to the solenoid valve (A in Fig. 1a) should be four times the volume of the tubing connecting the conversion flask to the injection valve (F) plus the volume of the sample loop. Variations in any individual sequencers can be easily compensated for by adjustment of the length of PTFE tubing (A in Fig. 1a). Typical length of PTFE tubing (A) (0.8 mm i.d.) required for 100% sample injection was 1650 mm. For 100% sample