ABSTRACT

Flow Injection Analysis and its Application to the Quantification of Selected Biochemicals

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The work presented herein has as its focus the application of the recent advances in flow injection analysis (FIA) to the development of novel/improved analytical methods for the quantification of biochemicals, such as human serum albumin, immunoglobulin G, creatinine, urea, glucose and proteolytic enzymes, illustrating in the process the versatility of FIA as an analytical technique.

The albumin methodology combines low pressure liquid affinity chromatography with FIA. The pseudo-affinity matrix used is Cibacron Blue covalently linked to a 2-fluoro-1-methyl-pyridinium salt-activated Fractogel support. The albumin is readily eluted at pH 7.5 using a Tris saline buffer. Linear albumin calibration curves $r = 0.999$, $n = 7$, up to at least 250 mg dL^{-1} and recovery yields in the range 95% to 103% are obtained. Within day and day to day CV's are < 4.3%.

The FIA / chromatographic method for the immunoglobulin (IgG) quantification utilizes a novel

synthetic IgG receptor affinity gel. This system, under optimized conditions, generates calibration curves linear up to at least 2.5 mg dL^{-1} of human IgG. Excellent recovery yields (99 - 101%) and within day CV's (< 4.2%) are achieved.

A FIA method is also developed for creatinine quantification in serum. It is based on the rapid colour that develops (λ_{max}, 546 nm) when creatinine reacts with alkaline 3,5-dinitrobenzoate. Calibration curves are found to be linear up to 15 mg dL^{-1}. Up to 60 samples can be manually analysed per hour. Recovery yields varied between 95 - 103% and a within day precision of < 3% was achieved. Interferences are observed with acetone, ascorbate, bilirubin and haemoglobin.

The urea / FIA methodology is based on the colour that develops (λ_{max}, 517 nm) when urea reacts with o-phthalaldehyde in the presence of naphthylethylenediamine under acidic conditions. Calibration curves are found to be linear up to 51 mg urea N dL^{-1} when the FIA manifold is operated under optimized conditions. Up to 40 samples can be analysed per hour. Recovery yields varied between 95 - 99% and within day CV between 2.1 - 3.9%.

The development and evaluation of an immobilized enzymatic FIA method for the quantitation of glucose in serum is described in Chapter VI. In this method Fractogel co-immobilized glucose oxidase / peroxidase

bioreactors are utilized employing 3-methyl-2-benzothiazolinone hydrazone and 3-(dimethylamino) benzoic acid as the colorimetric agents. Optimum activity was achieved when a total of 0.4 mg protein per mL of support in the ratio of 3:1 (glucose oxidase : peroxidase, in terms of their activities) was offered for coupling. The immobilized enzyme retained 98% of its initial activity after 40 days. Linear calibration up to 9 mg dL^{-1} with detection limit of 0.07 mg dL^{-1} was observed using D-glucose standards. Recovery yields varied between 98 - 101% and within day CV's between 1.8 - 5.0%. Up to 35 serum samples per hour can be analysed manually.

Lastly, a rapid and sensitive FIA method is described for the fluorometric determination of the proteolytic activity of bromelain, chymotrypsin, ficin, papain and trypsin. The solid substrate utilized was fluorescein-labelled bovine serum albumin coupled to a 2-fluoro-1-methyl pyridinium salt– activated Fractogel support. The amount of fluorescein released from enzymatic cleavage of the substrate is found to be directly proportional to the activity of the proteolytic enzyme.

In each FIA method developed statistical comparisons are made with established independent methods.