J. Flow Injection Anal., Vol. 24, No. 2 (2007) 119–124

Development of Flow Injection Triiodide System as an Automated Iodometric Assay in Pharmaceutical Analysis

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Abstract

An automated iodometric assay, a potential analytical tool for general applications in various quality control and analytical laboratories, was extended for the determination of iodine reactive components in pharmaceutical formulations such as benzylpenicillin. The method was developed based on flow injection analysis (FIA) that utilizes a triiodide (I_3^-)-selective membrane detector. The principle of the system for penicillin determination was based on the standard iodometric assay, where the penicilloic acid that was produced from the enzymatic hydrolysis reaction was merged and reacted with I_3^- solutions in the flow system. The excess I_3^- was then detected by a flow-through potentiometric I_3^- detector. Under the adopted conditions, a sampling frequency of 75 samples per hour was obtained. The response was linear from 0.05 to 1.0 mmol L^{-1} and the limit of detection was 0.0025 mmol L^{-1}. Good reproducibility (RSD ±3.80 (n = 4)) was obtained for the determination of 0.5 mmol L^{-1} benzylpenicillin. Good agreement was obtained between the proposed method and the manufacturer's claim values when applied to the determination of benzylpenicillin in pharmaceutical formulations.

Keywords: Benzylpenicillin, flow injection analysis, triiodide detector, iodometric assay.

Introduction

Iodometric assay has been a very important method in analytical applications dealing with multiple reactions concerning iodine and analytes. Iodine is either consumed or liberated upon reaction with analytes and it was quantified by titration method using sodium thiosulphate. The related reactions include oxidation/reduction process involving oxidizing agents such as halogen (chlorine, bromine), Fe(III), and Cu(II) and addition reaction involving double bond in fatty acids (determination of iodine value). The samples types have been a wide variety ranging from aqueous samples such as disinfectants and waste water to organic samples such as edible oils. In pharmaceutical analysis, iodometric assay plays an important role for drugs compounds that complex iodine such as ascorbic acids and penicilloic acids. It was a standard analytical method [1] for β-lactam containing compounds as in penicillin groups.

An automated iodometric assay based on flow injection triiodide (FI - I_3^-) potentiometric system utilizing a 2-nitro octhyl ether blank membrane without any ionophore, was thus developed as a low cost, simplified and rapid method for iodometric analysis in general analytical applications. Generally, I_2 would exist as I_3^- form in iodide excessive solutions. The I_3^- responsive blank membrane was first reported by Ortuno et al., 2005 [2] for the determination of chlorine containing disinfectant. FI system utilizing this membrane was further explored for its capability to determine chlorine species [3] and

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oxidation value [4, 5] (peroxide value and anisidine value) in bleach solutions and edible oils respectively. The speciation of chlorine (total available chlorine, free available chlorine, and combined available chlorine) can be achieved based on kinetic discrimination between free and combined available chlorine after a temporary masking by Fe^{2+}, before the active chlorine is converted to I_3^- by iodide and detected by I_3^- responsive membrane. KI solution was used in the determination of peroxide value based on redox reaction between hydroperoxide (primary oxidation product) and iodide to produce I_3^-.

Chloramines-T reagents was used in the determination of anisidine value based on nucleophilic reaction between chloramines-T and alkenals (secondary oxidation product), where excessive chloramines-T was oxidized to I_3^- in the flow system. All the reactions carried out between oil and reagents have been accelerated by vortex action using a reacting chamber [4, 5]. The applications and analytical characteristics of some of the reported FI – I_3^- were summarized in Table 1.

In this paper, we intend to extend the application of FI – I_3^- system to pharmaceutical formulations. There are numerous iodine reactive pharmaceutical compounds such as polyol [6], captopril [7], selenium sulphide [8], dimethyl titanocene [9] and cephalosporin [10] which are quantified using iodometric assay. Benzylpenicillin (penicillin G), an important derivative of penicillin was one of the frequently analyzed compound using this method. Despite the conventional iodometric titration where penicillin is hydrolyzed in NaOH to produce penicilloic acids which consume iodine, Opstal et al., 1990 reported an analytical method for the determination of penicillin in pharmaceutical formulations using immobilized penicillase reactor (penicillase enzyme act as catalyst for the hydrolysis of β-lactam group in penicillin) and spectrophotometric detection [11]. However, the sensitivity was rather lower using this method. Other than that, there were other reported method using HPLC [12] which continue to be the most widely applied technique for the identification and quantification of penicillin, and it has to a large extent replaced the microbiological techniques. However, HPLC techniques require relatively long analysis time. Biosensor approaches, by virtue of their low cost and high specificity, have attracted a lot of attention. The underlying principle of most of these techniques was based on the detection of pH changes that resulted from the penicilloic acid formation.

Table 1 Several applications of FI – I_3^- system

	Aqueous sample		Organic sample	
	TAC [1]	FAC, CAC, chlorite [2]	Peroxide value [3]	Anisidine value [4]
Sample matrix	Disinfectants	Bleach and tap water	Edible oil	Edible oil
Reactive component		Hypochlorite ion (OCl^-), chloramine (NH_2Cl), chlorite ion (ClO_2^-)	Hydroperoxide (ROOH)	2-alkenals (ROH)
Reagent	KI	KI	KI	Chloramine-T, KI
Reaction involved	I_3^- sample was pre-prepared from oxidation of I^- by chlorine	I^- oxidized by chlorine species at different rate and pH to produce I_3^-	Aqueous I^- oxidized by oil hydroperoxide by vortex action to produce I_3^- in aqueous phase	Oil alkenals consume aqueous chloramines-T by vortex action, excess chloramines-T is reduced by I^- to produce I_3^-
Analytical range	$5x10^{-6}$ – $1x10^{-4}$ M	$2.8x10^{-6}$ – $2.8x10^{-4}$ M	0.35 – 28.0	1.0 – 23.0
Detection limit	-	$1.4x10^{-6}$ M	0.32	0.9
Sampling frequency, h^{-1}	80	75	80	40

TAC- Total available chlorine; FAC- Free available chlorine; CAC- Combined available chlorine

Numerous biosensing systems that incorporate surface plasmon resonance (SPR) [13,14,15], pH glass electrode [16], ion-selective electrodes [17, 18,,19], field effect transistors (FET) [20] and amperometric [21] have been reported. Lapierre et al.,reported on a biosensor approach that required stopped flow and rotating sequences to obtain complete hydrolysis and thus better sensitivity [22]. Other methods such as capillary electrophoresis [23] and microbiological diffusion analysis [1] have also been described.

Here, our interest is more on extending the multi-analytical capability of the FI – I_3^- system to pharmaceutical products, primarily benzylpenicillin, based on the reduction of I_3^- level upon production of penicilloic acids by enzymatic hydrolysis. With appropriate modifications, the system can be utilized in most of the quality control and analytical laboratories as an automated approach for iodometric assay, not only for benzylpenicillin but for any kind of samples that deal with iodine or iodine reactive components. Finally, the proposed method withstands numerous advantages of low cost, easy of membrane fabrication and multiple analytical applications, without sacrificing its natural characters of high sampling frequency, accuracy and sensitivity.

Experimental

2.1 Apparatus

The FI system consisted of a peristaltic pump (Cavro), a four way injection valve (Cheminert) that was controlled by the ASA (Advanced System Automation) software. Potentiometric measurements were performed at 25.0 ± 0.2 °C using an Orion Digital Ion Analyzer (Model 701) that was connected to a flow-through detector. The flow-through cell was of wall jet design with an in-built reference electrode (Model FIP-3) that was supplied by Chemflow Devices (Thornbury). All the FIA tubings (0.35, 0.56 and 0.80 mm i.d.) used were made from PTFE. A glass calomel electrode that was connected to an Orion Research Expandable Ion Analyzer (model EA 940) was used for measurement of pH of aqueous solutions.

2.2 Chemicals and reagent

All reagents used were of analytical grade. Immobilised penicillin G amidase (180 U/g) in white powder form was purchased from Fluka. 2- nitrophenylocthyl ether (NPOE), benzylpenicillin (potassium salt) were obtained from Sigma and polyvinyl chloride (PVC) was purchased from Aldrich. I_3^- solution was prepared by diluting appropriate amount of 0.1 N Wij's iodine solution (BDH) in 250 mL solution that contained 1 x 10^{-3} $mmol^{-1}$ and 1 x 10^{-2} mol L^{-1} of acetic acid and potassium iodide, respectively. It was diluted to the mark to obtain 2.4 x 10^{-4} mol L^{-1} I_3^- solution. Acetate buffer solution was prepared by pipetting 0.3 mL of acetic acid (BDH) in 100 mL volumetric flask that contained deionised water. Sodium acetate (0.01 M) (Sigma) was added to adjust the solution to the required pH (2.5-7.0).

2.3 Preparation of membranes

I_3^- selective cocktail was prepared by dissolving 0.200 g of NPOE and 0.100 g of PVC powder in 3 mL tetrahydrofuran (Merck) [2]. The mixture was stirred for 30 minutes to ensure complete dissolution of all components. Four drops of the mixture were applied drop-wise to the tip of a coated- wire platinum substrate of the electrode body and was left to dry. The electrode was conditioned in I_3^- solution (0.100 M) for 1 hour before use.

2.4 FIA set up

The FIA set-up used is shown in Figure 1. The benzylpenicillin sample was passed through the packed reactor (12.5 cm x 0.5 cm i.d) that contained immobilized penicillin G amidase at a flow rate of 0.1 mL min^{-1}. The penicilloic acid that was formed was merged with the I_3^- stream, resulting in the consumption of I_3^-. The unreacted I_3^- is then injected into the acetate buffer carrier and is finally detected by the I_3^- detector.

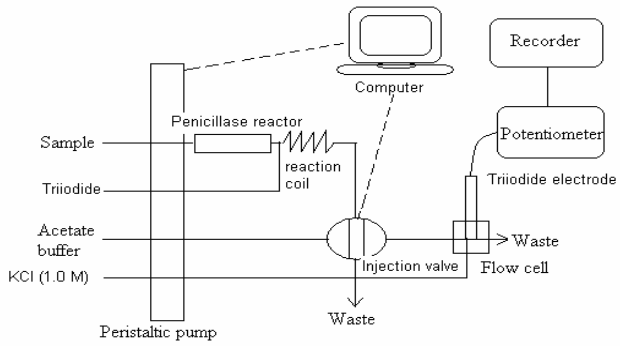

Fig. 1 FIA set-up used in the studies.

2.5 Analysis of Samples

Four Safcillin tablets (contain 125 mg benzylpenicillin) and two ampicillin capsules were obtained from a local drug store. The samples were grinded, dissolved in different volumes of water to obtain different concentrations of the drug, and were analyzed using the proposed method.

Results and Discussion

3.1 Response of triiodide sensor

When the potential signal (y, mV) was plotted versus log concentration I_3^- (x, log mol L^{-1}), the response was found to be linear from 2.8 x 10^{-4} to 2.8 x 10^{-6} mol L^{-1} (regression coefficient, 0.996). The straight line is described by the equation y = 37.8x + 215 (Figure 2). This information is important as it provides the information on the approximate I_3^- concentration that is needed to be prepared to achieve the desired linear range. 2.8 x 10^{-4} mol L^{-1} I_3^- was used as the carrier stream. The detection limit (S/N = 3) of the sensor was 1.6 x 10^{-6} mol L^{-1} I_3^-.

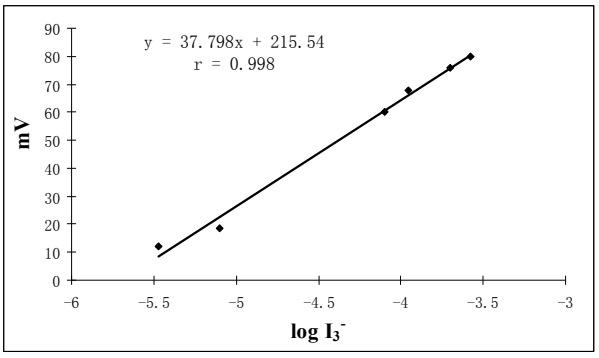

Figure 2 Calibration curve determined as potential versus log I_3^-

3.2 Optimization of FIA parameters

3.2.1 Effect of pH of carrier stream

The effect of pH of the acetate buffer on the signal was studied by injecting 2.8 x 10^{-5} mol L^{-1} I_3^-standard. The result shows that the system is most sensitive over the pH range 2.5 to 4.7 (Figure 3).

3.2.2 Effect of sample flow rate, sample injection volume and carrier flow rate

The effect of volume of I_3^- solution injected (20 - 150 µL), carrier buffer and I_3^- flow rate (0.6- 2.5 mL min^{-1}) were studied. Different flow rates were achieved by manipulating the diameter of PTFE tubings. The benzylpenicillin solution flow rate was adjusted to the lowest rate to flow through the reactor but at the same time resulting in minimum back pressure.

Using a larger volume of sample and increasing the carrier flow rate resulted in higher sensitivity of the system. 50 µL of sample and carrier flow rate of 2.0 mL min^{-1} were chosen to obtain higher sampling frequency without sacrificing the sensitivity.

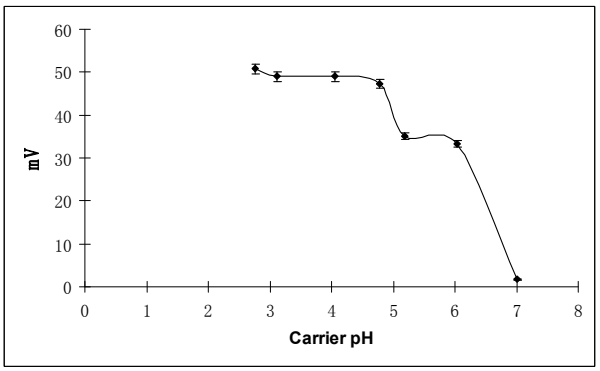

Figure 3 Effect of pH of acetate buffer carrier solution on the response of the sensor (I_3^- concentration 2.8 x 10^{-5} mol L^{-1}). Other conditions are shown in Table 3.

3.2.3 Effect of mass of penicillase beads and incubation time

Four penicillase reactors (A - 6 cm x 0.2 cm i.d.; B – 10 cm x 0.2 cm i.d.; C – 8 cm x 0.5 cm i.d.; D – 12.5 cm x 0.5 cm i.d.) that contained different mass of penicillase G amidase (Table 2) were prepared and were tested by first injecting with distilled water followed by 0.25 mmol L^{-1} benzylpenicillin standards. The potential difference that resulted from the injection of the two solutions was measured. The results show that the sensitivity was better when bigger mass of beads were used (Table 2). However, beads' mass larger than 1.3 g was not recommended due to the higher back pressure.

For the study of effect of incubation time (using 1.3 g penicillin G amidase), the pump was stopped for 5 minutes when the benzylpenicillin was present in the reactor, before it was reactivated. The effect of this incubation procedure was compared to when the pump was continuously operated by

injecting 0.25 mmol L^{-1} benzylpenicillin. Straight lines with regression equations y = -37.8x + 19.0 (correlation coefficient, 0.993) and y = -36.8x + 28.5 (correlation coefficient, 0.999) when incubated for 5 minutes and continuous pumping, respectively, was found. It was found that the incubation time has resulted in a slight increase in the sensitivity of the system as more penicilloic acid was produced when the pump was stopped. However, continuous pumping was preferred as the sampling frequency is greater.

Table 2 Effect of mass of benzylpenicillase beads on sensitivity

Reactor (length x i.d., cm)	Mass of penicillinase beads (g)	Enzyme units	Difference in potential* (mV)
A (6 x 0.2)	0.35	64.4	0.0
B (10 x 0.2)	0.64	117.8	0.0
C (8 x 0.5)	1.30	239.2	6.0
D (12.5 x 0.5)	2.50	460.0	17.6

*Difference in potential when injected with 0.25 mmol L^{-1} benzylpenicillin and distilled water.

3.3 Adopted parameters and its analytical characteristics

The adopted parameters and the analytical characteristics of the FIA system are shown in Tables 3 and 4, respectively. The proposed method has better sensitivity (linear range, 0.05 - 1.0 mmol L^{-1}) compared to the earlier report that used a pH responsive tridodecylamine membrane (linear range, 2.0 – 17.0 mmol L^{-1}) [18,19]. The earlier method also has a lower sampling frequency (30 h^{-1}) and the chemical immobilization procedure required 24 hours of incubation time. On the other hand, the preparation of the I_3^- membrane is much simpler and it was found to remain operational without significant decrease in sensitivity when continuously pumped for 25 hour with the carrier solution [3]. As a whole, the proposed method gives relative higher sampling frequency and lower sample volume compared to standard iodometric titration.

Table 3 Adopted FIA parameters from optimization results

Parameters	
Sample flow rate, mL min^{-1}	0.1
I_3^- concentration, mol L^{-1}	2.4 x 10^{-4}
I_3^- pH	4
I_3^- flow rate, mL min^{-1}	1.0
Injection volume, µ L	60
Acetate buffer carrier pH	3.5 - 4.5
Acetate buffer carrier flow rate, mL min^{-1}	2.0
Mass of penicillin G amidase, g	1.3

Table 4 Analytical characteristics of benzylpenicillin using the proposed FIA system.

Linear range, mmol L^{-1}	0.05 – 1.0
Regression equation (x, log mmol L^{-1} , y, mV)	y = -23.4x + 22.1
Correlation coefficient	0.991
Detection limit, mmol L^{-1} (S/N = 3)	0.0025
Sampling frequency, h^{-1}	75
Reproducibility, %	± 3.8

3.4 Analysis of Samples

The optimized method was applied for the determination of benzylpenicillin in pharmaceutical formulations. The results show good agreement between the values obtained when compared to the manufacturer's claimed values (Table 5). Typical calibration signals are shown in Figure 4.

Table 5 Results for the determination of benzylpenicillin using the proposed FIA method

Sample #	Manufacturer's claim, mmol L^{-1}	Obtained, mmol L^{-1}	Relative error, %
1	0.25	0.262	4.8
2	0.10	0.096	-3.8
3	0.50	0.475	-5.0
4	0.05	0.050	0.0
5	0.25	0.240	-4.0
6	0.50	0.52	4.0

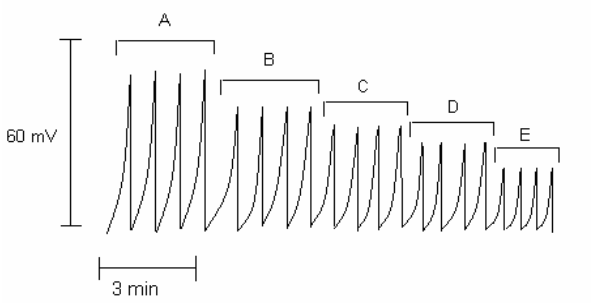

Figure 4 Typical FIA peaks for obtained when injected with different concentrations of benzylpenicillin (A- 0.05 mmol L^{-1}, B- 0.1 mmol L^{-1}, C- 0.25 mmol L^{-1}, D- 0.5 mmol L^{-1}, E- 1.0 mmol L^{-1})

Conclusions

An automated iodometric assay for the determination of benzylpenicillin in a flow injection configuration by incorporating an enzyme reactor and I_3^- membrane sensor was described. The method offers adequate selectivity and sensitivity, enabling it to be applied successfully for the determination of benzylpenicillin in pharmaceutical formulations. While the ability of potentiometric sensors to function in turbid, coloured samples is notable advantages, the system is potentially applicable for iodometric analysis of a wide range of pharmaceutical products, as well as other chemical substances, which might play an important role in future automation approaches in quality control and analytical laboratories

Acknowledgement

The authors greatly acknowledge the financial support of the Ministry of Science and Innovation Malaysia through a Science Fund scheme (305/PKIMIA/613307) and a UiTM internal grant (600-IRDC/ST 5/3/1066) for funding this project.

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(Received September 28, 2007)
(Accepted December 10, 2007)