The Versatility of Sequential Injection and its Adaptability to Stoichiometry, Kinetics and Titrimetry

Salah M. Sultan

Chemistry Department, King Fahd University of Petroleum and Minerals
KFUPM Box 2026, Dhahran 31261, Saudi Arabia, E-mail smsultan@kfupm.edu.sa

Abstract

This paper highlights the versatility, the potential and new trends of the sequential injection analysis technique in three areas of research: First, Stoichiometry and in this connection the contineous variation method, the mole ratio method, were all discussed. Second, Kinetics and reaction rate measurements and and hence postulating the mechanism and adopting new kinetic methods of analysis. Third, newly introduced titrimetric methods of analysis. Finally the advantages of SIA over traditional methods were mentioned.

Keywords Sequential Injection, Stoichiometry, Kinetics and Titrimetry

1. Introduction

1.1 Objectives

The versatility and new trends of Sequential Injection Analysis (SIA) technique would be exploited as a tool:

a. to be applied to some fundamental studies, such as to determine the stoichiometry of the reaction, equilibrium constants.
b. to monitor chemical kinetics and to determine reaction rate constants whenever possible and to use the kinetic data to elucidate a plausible reaction mechanism and adopt validated kinetic methods for quantitative determinations.
c. to automated micro titration methods.

1.2 The SIA technique

Sequential injection analysis (SIA) technique earlier introduced by Ruziüka and Marshall[1] is considered a state-of-the-art technology and a striking revolution to the analytical chemistry world and a potential introduction in literature. It is proved to be successfully replacing the traditional old cumbersome techniques such as titrimetry as well tools such as burettes, pipettes and measuring cylinders.

SIA is based on the sequential aspiration of sample and reagent zones through a selector (rather than injection valve) in a channel or a holding coil (Fig. 1). In this manner a stack of well-defined zones is obtained which is then allowed to penetrate each other by flow reversal. The flow reversal creates a composite zone a section of which can be trapped inside the observation zone of the detector, by stopping the flow, for reaction rate measurements. Advantages of SIA[2-4] include mechanical simplicity, since only a single valve and a single pump is needed, and reliability, because once configured the components and associated flow channel do not need physical restructuring. Tuning the length can influence the degree of zone dispersion and the number of flow reversals, and the reaction time can be adjusted through the duration of a stopped flow period.

The measuring cycle of the SI technique involves forwarding the carrier solution until the holding coil, reactor and detector havebeen washed out. Aspiration of sample solution

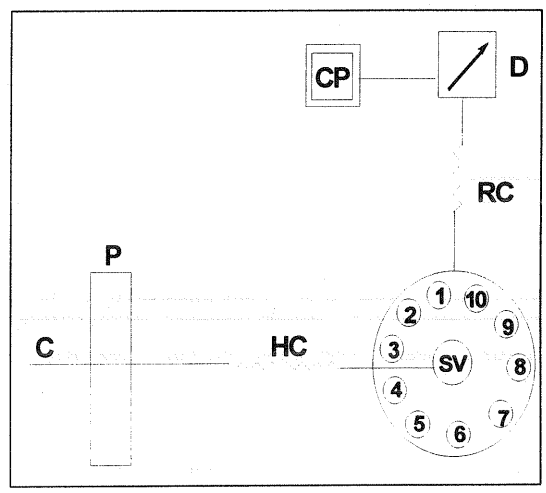

Fig. 1 Typical SI-manifold; C, carrier; P; pump; HC, Holding coil; SV, selector valve; RC, reaction coil; D, detector; CP, computer.

and/or reagent solution with the selector valve in one position by flow reversal into the holding coil. Forwarding the carrier solution to propel the composite sample reagent zone through the reactor into the detector. Stop of the flow for reaction rate monitoring.

2. Stoichiometry

2.1 The traditional continuous variation method

This method was first introduced by Ostromisslensky[5] and later developed by Job[6] to determine stoichiometries and formation constants of complexes. In the method a series of solutions is prepared by mixing different volumes of equimolar solutions of the ligand and the metal ion and diluting to a constant volume to give solutions of the same total molar concentration but different mole fractions. If a single, stable complex is formed, a plot of absorbance versus mole fraction of one of the reacting species gives a characteristic triangular plot.

The mole fraction of the maximum of this plot gives the stoichiometry of the complex. Likussar[7] has developed a theoretical approach to the continuous variation method in which no approximations are necessary.

2.2 The continuous variation method by SIA

The SIA technique was successfully used for the investigation of reaction stoichiometry of both trimeprazine and perphenazine, of the phenothiazine family, when complexed with palladium (II) utilizing the Job's plot method[7-9]. Both compounds were found to form stable complexes with palladium(II) in sulphuric acid media and spectrophotometrically monitored at 515 nm for the trimeprazine and at 560 nm for perphenazine. With SIA, the palladium(II)-to-drug mole ratio was varied, for the Job's plot analysis, by changing the aspiration timing (volume) of equimolar solutions of palladium(II) and the drug into the holding coil. The total volume of the two reagents was held at a constant value of 352.8 μL in each run. The ability of the SI apparatus to vary the volume of a reagent sequentially with millisecond precision is considered a great advantage and a revolution in flow injection methodology. Fig. 2(a) and Fig. 2(b) demonstrate typical Job's plots for absorbance versus molar fraction of palladium(II) using equimolar solutions of 8.24×10^{-4} M at pH 4.70 for trimeprazine system and of 5.70×10^{-4} M at pH 4.85 for the perphenazine system thus resulted in a stoichiometry of 1:1 for both systems.

Again SIA was used for the investigation of reaction stoichiometry of complexation of ciprofloxacin and/or norfloxacin, of the fluoroquinolone antibiotic, with iron(III) in sulfuric acid media utilizing the Job's plot method[10-12]. In the method different aliquots of equimolar solutions of iron(III) and of the drug were mixed to give a total volume of aspiration of 162.5 μL maintained constant by adjusting the aspiration times or the aspiration volume. The volume of each was varied between 14.8 μL (0.5 s) and 147.5 μL (5.5 s). Fig. 3(a) is a typical Job's plot generated by using equimolar solutions of iron(III) and ciprofloxacin of 1.0×10^{-3} M. Fig. 3(b) is a typical Job's plot generated by using equimolar solutions of iron(III) and norfloxacin of 2.0×10^{-3} M. Both plots were used using 5.0×10^{-3} M sulfuric acid and an ionic strength of 0.2 M. It is clear that the curves $A = f(X_{drug})$ exhibit a maximum for the mole fractions close to 0.66, indicating that the ratio of iron(III) : drug in the complex is 1:2. Similar plots made at sulfuric acid concentrations greater than 0.025 M, gave different results and the 1:1 iron(III): fluoroquinolone complex was found to be the dominant species. These findings are in agreement with the results published by two independent groups, where 1:1 complex was obtained by one group[13] when iron(III) was complexed with ciprofloxacin at acidities higher than 0.025 M, and 1:2 complexes were obtained for the complexation of norfloxacin by the other group[11] when 5×10^{-3} M was used. It can also be observed that only ten minutes are needed to generate the Job's plot using the SIA and less than 10 mL are enough to repeat the procedure for, at least, five times. This topic has been comprehensively covered by Vanstaden[13] by writing theory on determination of reaction stoichiometries by SIA.

2.3 The traditional molar ratio method

This method was introduced by Yoe and Jones[14] and developed by Momoki et.al[8]. It is based on graphical

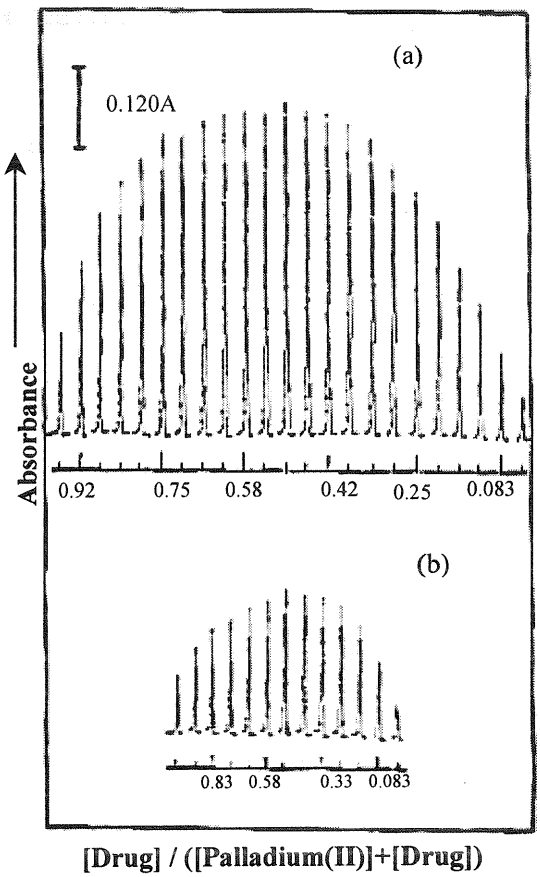

Fig. 2 Job's plot: (a) [palladium(II)] = [trimeprazine] = 8.24×10^{-4} M; total volume is euivalent to 358.2 μL and aspiration times were varied in 0.50 s steps; (b) [palladium(II)] = [perphenazine] = 5.70×10^{-4} M; total volume is equivalent to 358.2 μL and aspiration times were varied in 1.0 s steps

representation of the observed absorbances versus the molar ratios of the two components of the complex when the concentration of one component is held constant while that of the other component is varied.

2.4 The molar ratio method utilizing the SIA technique

The SIA technique was successfully used for the investigation of reaction stoichiometry of both ciprofloxacin and norfloxacin by their complexation with iron(III) utilizing the molar ratio method[10]. In the molar ratio method ideally two straight lines are obtained when the absorbance is plotted versus the iron(III) to drug ratio, and the point of intersection of these two lines correspond to the stoichiometric ratio upon interpolation to the mole ratio axis. Therefore the total volume of the ligand was maintained constant by aspiration of 147.5 μL into the holding coil by flow reversal, whereas iron(III) was varied between 14.8 μL (0.5 s) and 192.0 μL (6.5 s). The ionic strength of all solutions was adjusted to 0.20 M with ammonium sulphate and the sulfuric acid concentration was 5.0×10^{-3} M. Fig. 4(a) shows a typical molar ratio plot for the complexation of iron(III) with ciprofloxacin with peaks 1 to 11 produced by varying the mole ratio of iron(III) to drug from 0.1 to 1.1 in 0.1 steps.

Fig.3a

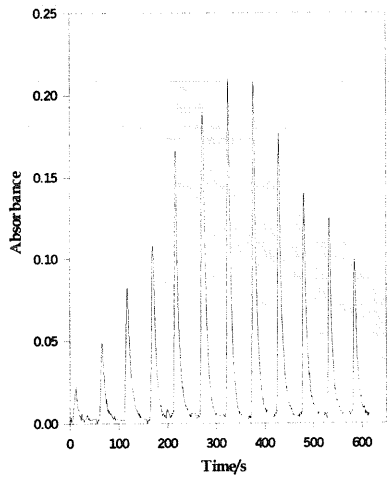

Fig. 3b

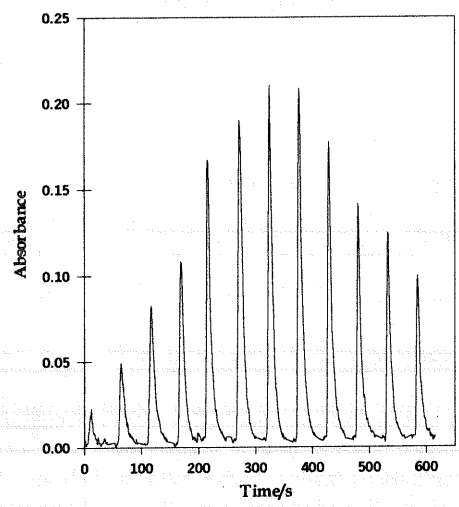

Fig.3 SIAgram representing Job's plot: (a) [iron(III)] = [ciprofloxacin] = 1.00 x10^{-3} M; (b) [iron(III)] = [norfloxacin] = 2.00 x10^{-3} M; ionic strength = 0.20 M; total volume is equivalent to 162.0 mL and aspiration volumes were varied between 14.5 µL and 147.5 µL. Mole fractions of the drug were: (1) 0.0; (2) 0.1; (3) 0.20; (4) 0.30; (5) 0.40; (6) 0.50; (7) 0.60; (8) 0.70; (9) 0.80; (10) 0.90; (11) 0.95; (12) 0.97

Fig. 4a

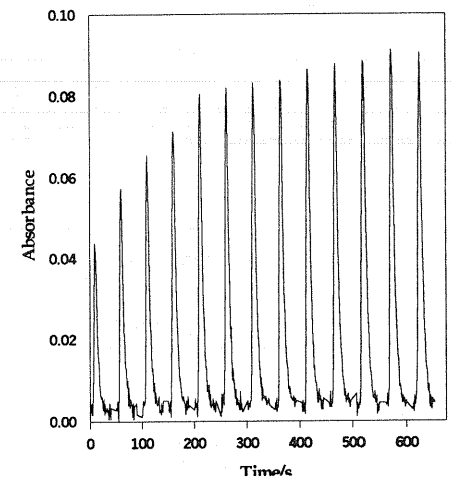

Fig. 4b

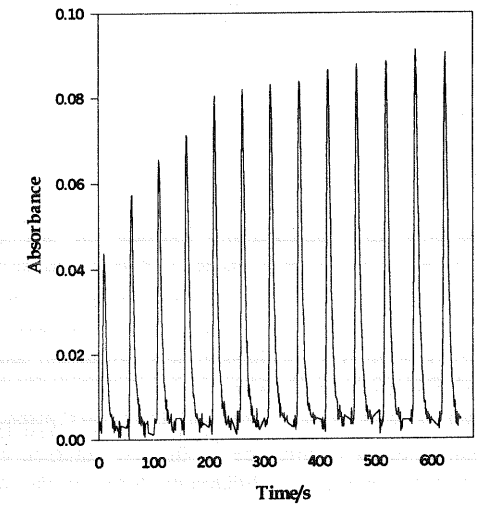

Fig. 4 SIAgram representing molar ratio plots: (a) [iron(III)] = [ciprofloxacin] = 1.00 x10^{-3} M; (b) [iron(III)] = [norfloxacin] = 2.00 x10^{-3} M; [sulfuriacid] = 5.00 M; ionic strength = 0.20 M; total volume of the drug was 147.5 µL. and aspiration volumes of iron(III) solutions were varied between 14.5 µL (peak 1) and 192 µL (peak 13).

Most of the methods available for determining formation constants are mainly based on preparing a series of solutions containing known proportions of the complex-forming species, in which the concentration of one of the reactants or products is followed directly or indirectly by a suitable analytical technique[15]. The method was further developed by Schwarzenbach[16] and Ringbom[17] and recently by introducing computer programs[18-20].

The mean values of log K_f obtained by these numerical methods for ciprofloxacin and norfloxacin complexes with iron(III) in 5.0x10^{-3} M sulfuric acid and 0.20 M ionic strength were 7.756 and 7.839 respectively, which is close to what was reported earlier[21].

3. Kinetics utilizing the SIA technique

3.1 Reaction rate measurements and flow/sequential injection

Slowness of some of the reactions investigated deemed necessary a study of these reactions using kinetic methods of analysis[22-23]. Most of the criticism about kinetic methods is due to the lack of accurate reproduction of the reaction conditions in each experimental determination. Reproducibility in the experimental conditions is therefore a major concern in kinetic methods compared to equilibrium methods especially regarding the time, which is more critical in the former[22]. From the foregoing it can be concluded that the major requirements of a successful kinetic method of analysis are,

accurate timing, careful adjustment of experimental conditions, precise sample and reagent measurements and accurate measurement of the response signal.

SIA is strikingly successful for kinetic determinations because injection volumes, reaction times and zone dispersion can all be changed readily and precisely by varying sequenced volumes, flow rate, stopped-flow times and reversals via computer control of the pump[24].

In stopped-flow injection the sample is injected into a carrier stream, reagent is added, and the mixture transported into the flow cell where the flow is stopped. Advantages of this method over the continuous flow method are[24,4] sensitivity is increased, accuracy is improved, kinetic discriminations could be carried out, consumption of reagent solutions is greatly reduced.

Gradient stopped-flow FIA[25-30] is ideally suited for measurement of reaction rates and rate laws of chemical reactions because the FIA peak profile provides as infinite number of reactant ratios, so that stopping the flow at suitable positions on the peak allows the adjustment of conditions to first order, second order, etc. Sultan proposed a fixed-time method for the determination of bromazepam in pharmaceutical preparations[30]. The method was based on stopping the flow for a given time just after sample injection using single line flow injection manifold.

Fig. 5

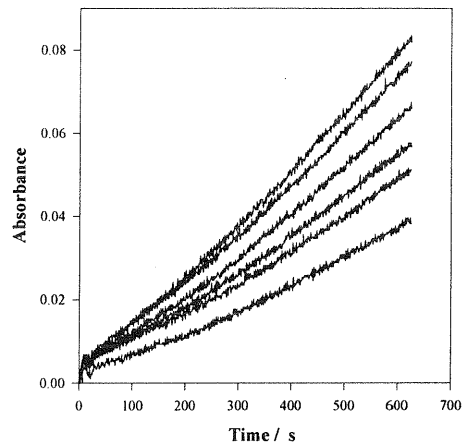

Fig. 5 Absorbance-time curve sfor the determination of the order of the reaction with respect to hydrogen ions, [iron(II)] = [BRZ] = 4.5800 x10^{-3} M; [HCl] = 0.0400 (1); 0.00200 (3); 0.0150 (4); 0.0115 (5) M; delay time (t_d) = 12.0 s; stopped flow (t_s) = 200 s.

3.2. Kinetics and mechanism by sequential injection:

SIA technique was successfully utilized to the full investigation of the complexation reaction of bromazepam (7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one), with iron(II) in hydrochloric acid media[28,29,31]. The reaction was kinetically studied, the mechanism was postulated and a kinetic method of analysis was adopted.

The partial reaction orders with respect to the different variables assumed to have influence on the rate equation was carried out by considering the differential form of the rate equation involving the pseudo zero-order reactions; when the rate of formation of the products are virtually negligible.

Rates and reaction orders were calculated from the plot of absorbance versus concentration by applying the fixed-time method[33,34].

Reaction orders with respect to [H^+], iron(II), and bromazepam for this reaction was found to be inverse one(-1), one(1), and one(1) respectively.

It was observed that the reaction rate decreases as the acid concentration is increased as represented by Fig. 5.

Arrhenius Activation energy was calculated to be 41.7 KJ mol^{-1}.

3.3. Formation constant

V-Reaction mechanism :

The precise SIA kinetic results resulted in easy and acceptable postulation of the mechanism of bromazepam-iron(II) reaction. The kinetics of the reaction indicates that reaction rate accelerates as the acid concentration decreases and slows down as the acid concentration increases suggesting that the deprotonated form of the bromazepam is the active complexing species and that the reaction starts once a deprotonation equilibrium step takes place before the rate-determining step as follows:

$BRZ\text{-}N_2H_2^{2+} <==> BRZ\text{-}N_2H^{1+} + H^+$ ..... (1)

The the deprotonated species reacts with iron(II) as follows:

$BRZ\text{-}N_2H^{1+} + Fe^{2+} = (BRZ\text{-}N_2)Fe^{2+} + H^+$ .....(2)

$BRZ\text{-}N_2H^{1+} + (BRZ\text{-}N_2)Fe^{2+} = (BRZ\text{-}N_2)_2Fe^{2+} + H^+$.... (3)

$BRZ\text{-}N_2H^{1+} + (BRZ\text{-}N_2)_2Fe^{2+} = (BRZ\text{-}N_2)_3Fe^{2+} + H^+$ ...(4)

The mechanism clearly explains that higher acid concentration favours the existence of lower drug to iron(II) mole ratio while lower acid concentrations favours higher ratios as indicated by reactions in equations (4) and (5) following the rate determining step.

3.4 The kinetic method

The SIA fixed-time method[22,30] was found to be suitable for the determination of bromazepam. From the kinetic data, 0.0100 M iron(II) in 0.0115 M hydrochloric acid concentrations were taken fixed for the determination of the bromazepam at different fixed times of 50.0, 75.0, 100.0 and 150.0 seconds. Based on the correlation coefficient (r^2) and the intercept (b_o), of the regression of the bromazepam concentration versus absorbance of the complex, the fixed time of 100.0 second was found to be suitable for taking measurements. Therefore, the analysis volume (V_A) was aspirated for exactly 100 seconds where it reaches the detector for absorbance measurements. Regression plots were found to be linear for bromazepam concentration in the range 5.0 x 10^{-4} to 1.5 x 10^{-3} M.

3.5 Kinetic application

The sequential injection fixed-time method was applied to the determination of bromazepam in the proprietary drug lexotanil tablets without suffering interferences from excipients already found.

The work thus presented proved to be a typical demonstration for the capacity and versatility of the sequential injection (SI) as a powerful technique, which goes beyond reaction rate measurements and superficial kinetic studies undertaken by the inventors. For the first time, the (SI) technique is employed for a full kinetic investigation leading to a direct postulation of a reaction mechanism thus validating a comprehensible method of analysis. This opens a way to mechanistic scientists to use an easy computer-controlled instrument they have been lacking for decades to fill the gap and replace the time-consuming manual dilution procedures being carried out to date.

Tc-99 in nuclear waste was determined by stopped flow SIA system employing radiometric detection[34]. The principles of stopped-flow by SIA and its application to the kinetic determination of traces of a proteolytic-enzyme has been well documented by Ruzicka and Gubeli[2]. Recently a stopped-flow SIA spectrophotometric method for simultaneous determination of phosphate and silicate has been reported by Mastorres eta[35].

4. Titrimetry utilizing the SIA technique

4.1 Traditional titrimetry compared to FIA and SIA titrations

If a sample zone (S) of an acid (for example) is injected into a carrier stream of a base (R), the dispersed zone will become gradually neutralized by the base penetrating through the interfaces with the carrier stream at the leading and trailing boundaries.

4.2 Typical SIA titrations

SIA technique was employed in a titration procedure thus indicating new versatility, utmost capability and considered a breakthrough in the field of analytical chemistry. It was successfully utilized for fully automated titrations and well performed to titrimetric analysis of vitamin C [36]. The method is based on the oxidation of vitamin C to the dehydro-ascorbic acid with cerium(IV) in sulfuric acid media. In the method no mixing chamber was introduced and no external indicator was used, instead a holding coil was utilised and the decrease in the absorbance of cerium(IV) was spectrophotometrically monitored at 410 nm. Water was used as the carrier blank to adjust the absorbance to zero before commencing the titration. Cerium(IV) and vitamin C were sequentially aspirated in the holding coil with the former used at a higher concentration with at least ten folds larger than that of the latter. The difference in the absorbance of cerium(IV) before and after adding the analyte was measured to calculate the vitamin C initial concentration.

The holding coil is considered the heart of the titration process by SIA and substitutes the mixing chamber in the conventional FIA titration assembly. The volume of the holding coil is variable and could be adjusted by changing the length as required and this allows the introduction of a large volume of a reagent compared to the analyte which, could be aspirated to penetrate into the reagent with the efficient axial and radial dispersion. The situation thus described exactly resembles the conventional titration methodology but with the merits that the sequential titration method is fast, automated, and precise with the consumption of reagents in micro-quantities.

Similar SIA titrations methods have recently been reported; sulfuric acid is determined in-process effluents[37] and titrations of strong and weak acids[38]. SIA titrimetry has also been applied in some titrations without mixing or dilution using chemical sensing membranes[39].

4.3 Optimization of SIA titration

Chemometrics and optimization of the parameters involved in SIA titration was found to be easily conducted for vitamin C - cerium(IV) reaction[36]. The super modified simplex computer program was employed for the optimisation of sulfuric concentration, the cerium(IV) concentration and the flow rate of dispensing the reactants to the spectrophotometer.

The SI titrimetry proved far beyond comparison with the conventional titration method with respect to precision, accuracy, time consumption, reagent economy and speed. The SI titrimetry is much more advanced and novel compared to the FIA titrimetry by the fact that the holding coil replaces the gradient mixing-chamber and peak broadening is eliminated by the high pressure exerted by the action of the syringe pump and a multi-position valve with a standard pressure of 600 psi (liquid); the peristaltic pump in FIA exerts a pressure not more than 60 psi. In this connection peak height of the narrow baseline were used to calculate for concentration, and not peak width at half peak height, thus increasing precision in measurements. With a suitable SIA instrument, precise volumes flow rate and the computer board resulting in high accuracy of the determination controls time.

5. Conclusion

In the light of the above applications and new trends employed by the SIA one could deduce the remarkable development and the potentials of the technique as due to the following advantages:

- The SIA allows flexible and easy selection of the injected volume via a computer board.
- With SIA all operations are easily computerised and automated for precise volume uptake and accurate determinations
- The technique successfully replaces traditional hands-on operations and tools thus minimizing human errors, economizing manipulations and reducing man power and cost for micro titration methods.
- A valve could be assigned for a mixing chamber where reactants could be mixed stored to generate a set of diluted standard solutions or arrested for kinetic measurements or automated titrimetry.
- Reaction rate measurements can easily be adjusted and monitored through the duration of a stopped-flow period.
- Mixing is thorough and complete over a small time interval and reaction products could be monitored with sufficient reproducibility.
- The SIA technique affords the study of reactions with half-lives of a few seconds without the need for a fast detection system.

Acknowledgement

Thanks is due to KFUPM, Dhahran, Saudi Arabia for the facilities provided. Thanks is also due to the Japan Society for Analytical Chemistry (JSAC) for their invitation as a keynote speaker to present this work to the International Congress on Analytical Sciences (IUPAC) held in Tokyo during 6-10 August, 2001.

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(Received October 22, 2002)