CHAPTER I

INTRODUCTION

1.1 Overview and Background

Over the last decade years, the chemistry community has been mobilized to develop new chemistries that are less hazardous to human health and the environment. This new approach has received extensive attention and goes by many names, including Green Chemistry, Environmentally Benign Chemistry, Clean Chemistry, Atom Economy and Benign By Design Chemistry. Under all of these different designations, there are approaches to the synthesis, processing, and use of chemicals that reduce risks to humans and the environment. Many innovative chemistries have been developed over the last several years that are effective, efficient, and more environmentally benign. The Environmental Protection Agency (EPA) developed 12 principles to green chemistry, which go some way in explaining what the definition means in practice. The principles cover such concepts as : (i) the design of processes to maximize the amount of raw material that ends up in the product ; (ii) the use of safe, environment-benign solvents where possible; (iii) the design of energy efficient processes; (iv) the best form of waste disposal, aiming not to create it in the first place.

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Several flow injection analysis (FIA) methods for pharmaceutical assays have been reported in recent reviews [1,2]. Although they are superior to the batch-wise methods in that they provide relatively high sample throughput, low reagents and

sample consumptions, low analysis time and cost effectiveness. Some of which typically requires high reagents consumptions and makes a large amount of waste especially dealing with expensive chemicals, hazardous reagents, or online/remote site applications. Thus, the FIA technique is a relatively expensive method by comparison with the second generation termed sequential injection analysis (SIA).

SIA has been introduced in 1990, as a new concept automatic sample analysis. It is simple, rapid, low sample and reagent consumption. Additionally, it is fully automated system to make it fast and efficient that is important to many routine tasks. In SIA, there is no need for special system reconfiguration of manifold to apply different chemicals into the systems, while all major parameters such as reagents and sample volumes, flow rate, order of mixing and reaction time can be optimized by computer – control. It is compatible with both optical and electrochemical detectors. SIA has been used for pharmaceutical assays that were highlighted in recent reviews [3].

In recent years, interest in the use of micellar mobile phase in reversed phase high – performance liquid chromatography (RHPLC), instead of conventional organic mobile phases, has grown rapidly because of the biodegradability and lower toxicity of surfactants than the conventional organic mobile phase. This technique is termed micellar liquid chromatography (MLC). Most MLC procedure use micelles of the anionic surfactant sodium dodecyl sulfate (SDS). Other useful surfactants include the cationic cetyltrimethylammonium bromide or chloride (CTAB of CTAC) and the nonionic Brij-35. The separations are usually carried out in C_{18} or C_8 columns. The fundamentals of each technique are briefly introduced in following sections.

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1.2 Flow Injection Analysis (FIA)

1.2.1 Introduction

Any measurement in a chemical laboratory involving liquid materials comprises the following operations: solution handling, analyte detection, data collection, and computation of results.

Solution handling requires an arsenal of skills, which a practice chemist has no master, since mixing, decanting, pipetting, and other volumetric operations are still performed manually, even in the advanced laboratories, using tools that were designed more than 200 years ago. It might seem that robots would be suitable tools for automation such manual tasks; but it is likely that their impact will remain limited to repetitive operations like weighing of pulverized materials, mechanization of sample injection into chromatographic columns, handling of radioactive materials, or sample preparation. Because manual handling using robot requires extensive programming and active feedback control, the use of robots is justified only if a large series of repetitive operation is to be handled over prolonged periods. Truly, that seems to be no way of resolving the problem of automated solution handling other than by manual operations, as long as we think in terms of batch operations.

Flow operations are must easier than automate, since they replace the mechanical handling of oddly shaped (and often fragile) containers by sequential movement of liquids in tubes. Flow operations are much easier to miniaturize by using small bore tubing, and the microvolumes are conveniently manipulated and metered by pumping devices, which (unlike pipettes) are not affected by surface tension (or by shaking hands). Flow operations are much easier to control in space and time, since using closed tubing avoids evaporation of liquids, provides exactly repeatable path(s)

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through which measured solutions move, and provides an environment for highly reproducible mixing of components and formation of reaction products. Flow operation are very versatile, since flows can be mixed, stopped, restarted, reversed, split, recombined, and sampled, while contact times with selected sections of reactive or sensing surfaces can be precisely controlled. Finally, flow operations allow most detectors and sensors to be used in a more reproducible manner than when used in batch operations and by hand – as is obvious to anyone who has used both conventional and flowthrough cuvettes.

While many of advantages of flow operations have been exploited in chromatographic method, why has the batch approach not yet been replaced by flow systems in all areas of laboratory practice? The reason must be tradition, and the fact that most chemists are used to thinking in terms of batch operations, where homogenous mixing is thought to be the only reproducible way to bring reactants together and where the homogenously mixed solution is regarded as the only suitable form in which a reproducible measurement can be taken.

The concept of flow injection analysis (FIA) is changing this prevailing attitude. FIA was first described by Ruzika and Hansen in 1975. They modified the technique that air segmentation in continuous flow – analysis by dispersing with the mixing chamber and using flow – induced sample dispersion to provide contact between analyte and reagent. This technique of unsegmented continuous – flow analysis [4].

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1.2.2 Principle and Theory

Flow injection analysis (FI) is a well-established automated technique with numerous and widespread applications in quantitative chemical analysis. It is characteristic that more than 12,000 FI papers have been published in scientific journals since 1975. In the first edition of their well-known monograph [5] the defined FIA as "a method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the absorbance, electrode potential or any other physical parameters, as it continuously changes as a result of the passage of sample material through the flow cell." This technique is based on a combination of reproducible sample injection, controlled dispersion of sample zone, and reproducible timing. The simplest flow injection analyzer (Figure 1.1) consists of a pump, which is used to propel the carrier stream through a thin tube; an injection port or valve, by means of which a well defined volume of a sample solution *S* is injected into the carrier stream in a very reproducible manner; and a reaction coil in which the sample zone disperses and reacts with the components of the carrier stream, forming a species which is sensed by a flow through detector and further recorded. A typical recorder output has the form of a peak (Figure 1.2) the height *H* of which is related to the concentration of the analyte. The time span between the sample injection *S* and the peak maximum, which yields the analytical readout, is the residence time *T* during which the chemical reaction take place. A well-designed FIA system has an extremely rapid response, because *T* is in the range 5 to 20 s, and thus usually at least two samples can be analyzed per minute. The injected sample volumes may be between 1 μl and 200 μl (typically 25 μl), which

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in turn usually requires no more than 0.5 ml of reagent per analysis. This makes FIA an automated microchemical technique capable of having a high sampling rate for sample determinations per hour, with minimum reagent consumption.

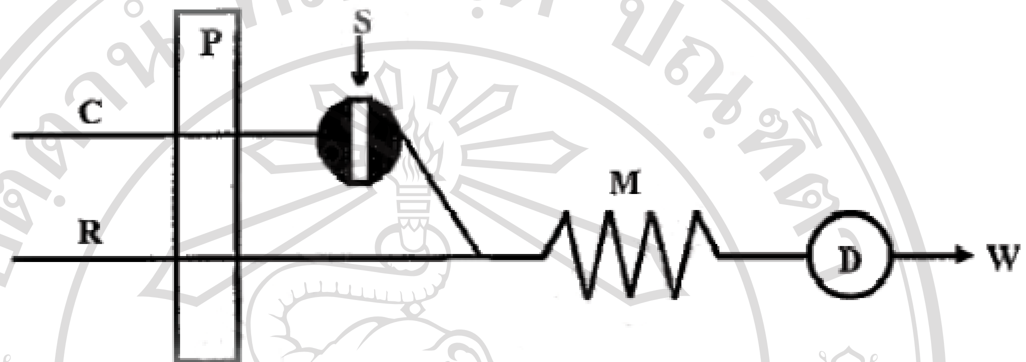

Figure 1.1 The basic components of the FIA system; P = pump, C = carrier stream, R = reagent stream, S = injection port, M = mixing reactor, D = detector, W = waste [6].

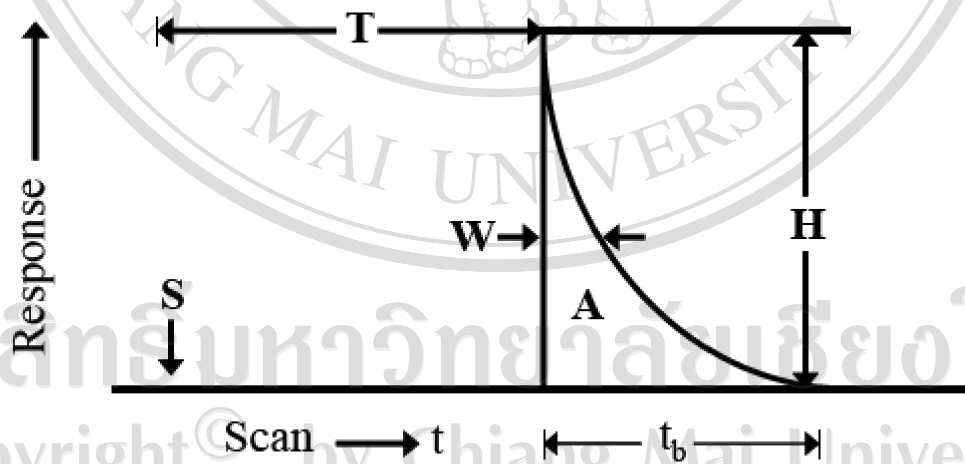

Figure 1.2 The analog output has the form of a FIA peak, the recording starting at S (time of injection), H is the peak height, W is the peak width at a selected level, A is a peak area, T is the residence time corresponding to the peak height measurement and t_b is the peak width at the base line [4].

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1.2.3 Dispersion [4,6-7]

The FIA peak is a result of two kinetic processes, the physical process of zone dispersion and the chemical process resulting from reactions between sample and reagent species, which occur simultaneously. The most common physical phenomenon in manipulation of sample zone in the FIA system is dispersion. The shape of the resulting zone is determined by two main processes: convective transport and diffusion transport. Convective transport occurs from mechanical flow driven by a propelling system. It consists of two processes: turbulent and laminar flows (Figure 1.3a). The turbulent flow occurs in transporting of liquid with air-segmentation. The laminar flow occurs for non-segmented liquids in narrow tubing. In FIA, laminar flow is predominant and causes the sample zone to spread in a parabolic due to higher velocity at the center of tubing (about 2 times the average velocity).

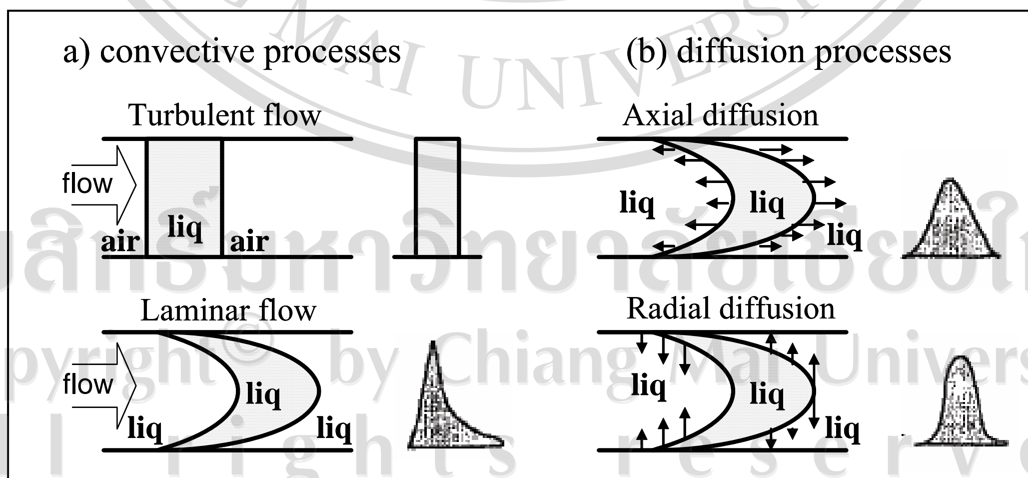

Figure 1.3 General types of transport in closed tubes and the recorded profiles at the detector [4].

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Diffusion transport is caused by concentration gradients. There are two types of diffusion processes: axial and radial, as shown in Figure 1.3b. Axial diffusion is insignificant compared to convective flow, but the radial diffusion contributes more significantly to sample dispersion. This process, termed "secondary flow", results in a washout effect accounting for the low mutual contamination of samples successively injected into the carrier stream and also serves to limit band spreading. At low flow rate it may even be the major mechanism for dispersion. In fact, flow injection analyses usually performed under conditions in which dispersion by both convective process and radical diffusion occurs as shown in Figure 1.4c.

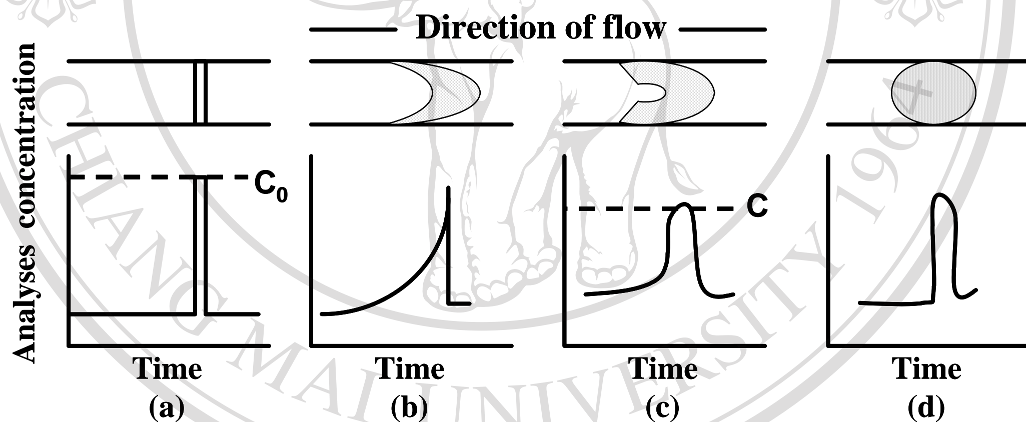

Figure 1.4 Effects of convection and diffusion on concentration profiles of analyses at the detector: (a) no dispersion; (b) dispersion by convective process; (c) dispersion by convective process and radical diffusion; (d) dispersion by diffusion [8].

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A simple dispersion experiment is used to pursuit dispersion by measure dispersion by means of the dispersion coefficient as shown in Figure 1.5. A sample solution is homogeneous and has the original concentration C^0 that would yield a square signal. The height of square signal would be proportional to the sample

concentration (Figure 1.5,left). When the sample zone is injected, it forms a dispersed zone whose form depends on the geometry of the channel and flow velocity. Therefore, the response curve has the shape of a peak reflecting a continuum of concentrations (Figure 1.5, right), which composed of a certain concentration of individual elements of fluid.

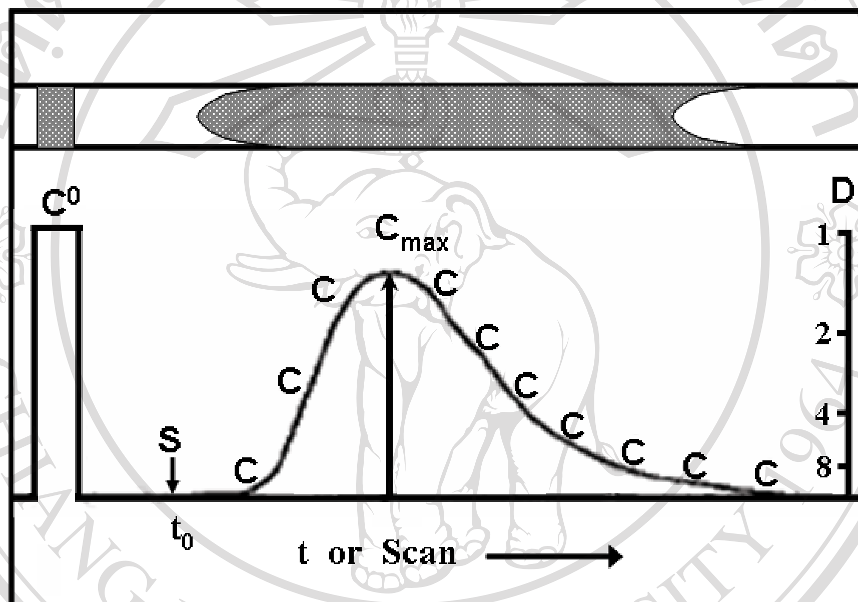

Figure 1.5 Dispersed sample zone in flow system; an original homogeneous sample zone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C^0 to a continuous concentration gradient with maximum concentration C_{max} at the apex of the peak [4].

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The dispersion coefficient (D) is defined as the ratio of the analyte concentration before and after the dispersion takes place:

$$D = C^0/C_{max}$$

Where C^0 is the original concentration of injected sample solution and C_{max} is the concentration of dispersed sample solution.

Dispersion may be considered in terms of the three general categories:

(1) Low dispersion systems ($D < 2$) are used whenever one intends to prevent the original concentration of the analyte in the injected fluid zone being diluted by the carrier.

(2) Medium dispersion systems ($2 < D < 10$) are also used in single channel FI systems, where reagents are used as carrier streams, to attain adequate mixing of sample and reagent.

(3) Large dispersion ($D > 10$) and medium systems are used to achieve sample dilutions, usually to bring the analyte concentration into an appropriate range for readout.

The FI experimental parameters which may influence the dispersion including sample volume, carrier flow rate, flow rate ratio between sample carrier and merging reagent and geometrical dimensions and configurations of manifold components. Varying the values of these parameters confers a significant degree of control over the dispersion characteristics and facilitates optimization of a flow injection system for many diverse applications.

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1.2.4 Reverse Flow Injection Analysis (rFIA)

Johnson and Petty [9] modified the conventional FIA by the variation consists of injecting small volumes of reagent into the flowing sample stream. The modified FIA technique refers as rFIA to emphasize the reversed roles of sample and reagent. In this technique, the sample act as the carrier stream and the reagent is injected into this stream. The reagent and sample mix in exactly the same manner as in a conventional FIA apparatus so that the fundamental principles of FIA (reproducible timing, injection, and dispersion) apply to rFIA as well.

1.2.5 FIA Instrumentations [4,6-8,10]

The basic components of a simple FI manifold typically consist of a propulsion system, an injection or insertion system, a transport and reaction systems and a detection system.

(a) Propulsion System

For FIA system various pump types have been used. The peristaltic pump is the most widely used type of propulsion unit in FIA. It uses a rotating head furnished with several spinning rollers (Figure 1.6). As the head rotates, the rollers are squeezed onto flexible pieces of tubing in order to keep the flowing fluid in motion. Peristaltic pumps deliver a pulsating flow, the rate of which can readily be adjusted via the rotation speed of the pump head and the inner diameter of the tubing. A single pump can simultaneously serve several channels of the same or a different diameter.

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(b) Injection or Insertion System

The injectors employed in FIA are similar in kind to those used in HPLC, but it is necessary for FI valves to withstand extremely high pressures as for HPLC. For a successful analysis, it is vital that the sample solution is injected rapidly as a pulse or plug of liquid; in addition, the injections must not disturb the flow of the carrier stream. The earliest injection system employed in FIA was as simple as a syringe and hypodermic needle. Currently, the injection systems most frequently used are the rotary valve, proportional injector and multi-injection system.

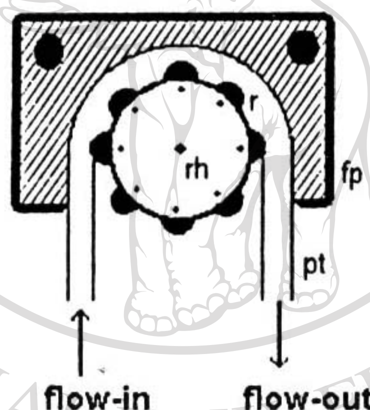

Figure 1.6 Scheme of a peristaltic pump. A flexible tube is placed between the rotating head and a fixed piece. The rollers are squeezed onto the tubing. [11]

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(c) Transport and Reaction System

The transport system is an integral component of any flow analysis system. The function of transport system is to provide connections between the different components of the system. Normally, the transport system consists of small-bore tube of I.D. such as PTFE tubing of 0.35-1.0 mm I.D.

The purpose of using the connectors in FIA system is to join the tubes to one another and to other parts of the system. In FIA, there is a wide range of connectors, but basically there are either dual (linear or V-shaped) or triple (T-, Y- or W-shaped) ways or pieces.

The reactor is a major component of the transport system. The main function of reactor is to promote the reproducible radial mixing of two or more components merged through the system. The reactor is usually made of PTFE tubing. There are many types of the reactor such as straight open tube, coiled tube, mixing chamber, single-bead string reactor (s.b.s.r.) and knitted or 3-D reactor

(d) Detection System

The detection system is the sensing part of the FI manifolds, which allows continuous monitoring of a given property of the sample or its reaction product and provide qualitative and quantitative information of the analyte. In theory, any detection system, which could be adapted for flow through detection may be used as detectors for FIA. These include the spectrophotometer (visible and UV), atomic absorption and inductively coupled plasma spectrometers, nephelometer, fluorimeter, radiometric and various electrochemical detectors.

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1.2.6 Application of FIA for Pharmaceutical Analysis

Pharmaceutical analysis is one of the most important fields in analytical chemistry. The discovery of new drugs and the on-going update of international regulations for the safety and efficacy of pharmaceutical formulations demand the continuous development of new analytical methods. Inevitably, automation plays an

important role, especially when a lot of samples have to be analyzed in the minimum of time. FIA is a well-established automated technique with numerous and widespread applications in quantitative chemical analysis. FIA is generally a simple and inexpensive technique employing common instrumentation such as peristaltic pumps and low-pressure injection valves. Compared to batch methods it offers increased sampling rate, lower reagents consumption, better precision and high versatility.

The above-mentioned advantages of FI have led to a continuously increasing interest in pharmaceutical analysis and quality control applications [12]. The main chemical approach in applying FI to pharmaceutical analysis is through automation of derivatization reactions. UV-vis spectrophotometry seems to be the technique of choice for FI pharmaceutical applications. UV-vis offers the advantages of simple, low cost instruments that are available at all laboratories. Running costs are minimal and no highly-skilled personal is required for their operation. Another significant advantage is that pre-existing batch instruments can be easily converted to flow through by either home- made or commercially available cells.

The present study reviews the applications of flow injection (FI) spectrophotometry to the determination of active pharmaceutical ingredients in their respective formulations. The FIA and rFIA with spectrophotometry were applied to determine of certain drugs in bulk and formulations, and the following tables are the brief reviews of its application in pharmaceutical analysis.

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1.2.6.1 FI Methods Based on Direct UV Detection

The simplest and more straightforward way to determine a compound of pharmaceutical interest is by UV spectrophotometry, based on the measurement of its native absorbance at a pre-defined wavelength. Automation of such a procedure via FI is facile and therefore popular among analytical and pharmaceutical chemists [10–23]. From instrumentation and manifold configuration point of view a single-channelled setup is generally required. Since no reaction takes place, even HPLC instrumentation can be used, by simply replacing the analytical column with suitable tubing connecting the injection valve to the flow-through detector [10]. When developing a FI method based on direct UV measurement, special attention should be paid on the choice of the carrier stream. In order to avoid matrix effects (e.g. pseudo-peaks produced by the Schlieren effect, etc.) or even potential precipitation of the analytes and clogging of the flow channels, the carrier and the solvent of the sample must be as consistent as possible. For this reason, fractions of organic solvents (e.g. MeOH or EtOH) are frequently used as carriers [11,12]. The main disadvantage of these methods is the lack of selectivity of the detector used. It is therefore necessary to apply these assays to samples with a known matrix. This is of course not a problem in pharmaceutical analysis, since the excipients that co-exist with the analyte in the samples are qualitatively and quantitatively known. Through validation of these methods in terms of selectivity is of primary importance. The most appropriate way to do so is to prepare a placebo mixture containing all excipients except for the active ingredient. The developed FI method must tolerate adequate amounts of the placebo, while its accuracy must be checked by analyzing synthetic samples spiked with

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known amounts of the analyte [10,13]. FI procedures based on direct measurement of the absorbance of the analytes in the UV region are summarized in Table 1.1

1.2.6.2 FI Methods Based on Metals–Drugs Interactions

A considerable group of FI methods for the determination of active pharmaceutical ingredients is based on the interactions between metals and drugs. These methods can be categorized into two main groups. The first group includes FI methods based on Red–Ox reactions. A very "popular" protocol among scientists involves oxidation of the analyte by Fe(III) [27–30]. Fe(II) produced by the reaction is determined through a second step using suitable reagents such as 1,10-phenanthroline [27,29,30], 2,2-bipyridyl [29] and 2,2-dipyridyl-2-pyridylhydrazone (DPPH) [28]. This two-step indirect approach has been recently applied to the FI determination of *N*-acetyl-l cysteine using the "merging zones" procedure [27], captopril [28], indapamide [29] and a variety of cephalosporins [30]. Alternatively, Misiuk and Halaburda reported a direct FI method for the determination of perazine based on its on-line oxidation by Ce(IV) in acidic medium. The coloured free radical produced by the reaction was monitored at 510 nm [31]. The second group of published FI procedures is based on the on-line formation of coloured complexes between the pharmaceutical compounds of interest and metal ions. Typical examples appeared recently include tetracycline Al(III) in Tris-buffer (pH 7.0, λmax = 376 nm) [32], ofloxacin—Fe(III) in sulfuric acid medium (λmax = 420 nm) [32], methyldopa—Mo(VI) (λmax = 410 nm) [34], cimetidine—Cu(II) in acetate buffer (pH 5.9, λmax = 330 nm) [35] and epinephrine/isoproterenol—Fe(II) in aminoacetic–

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Table 1.1 FI methods utilizing various colour-forming reactions for pharmaceuticals

Analyte	λ_{max} (nm)	Carrier stream	Detection limit (mg L^{-1})	Linear range (mg L^{-1})	Sampling rate (h^{-1})	R.S.D. (%)	Ref.
Famotidine	265	0.1 M phosphate buffer (pH3)	0.1	20 – 60	30	<1.0	[13]
Cefuroxime	281	H_2O/MeOH (90:10 v/v)	0.06	0.4 – 2.5	-	1.64	[14]
Leflunomide	260	H_2O/EtOH (75:25, v/v)	0.07	0.7 – 29.7	-	1.41	[15]
Gemfibrozil	276	0.1 M NaOH	1.4	20 -100	30	<1.0	[16]
Albendazole	-	MeOH/HCl/H_2O (49:1:50, v/v/v)	0.003	0.07 – 0.8	-	1.87	[17]
Diazepam	360	0.1 M HCl	0.6	2 – 110	100	3.3	[18]
Venlafaxine	274	0.1 M HCl	1.5	30 – 150	30	<1.0	[19]
Ketoprofen	261	Citrate buffer (pH 6.5)	0.3-0.4	7.5 – 75	85	<1.07	[20]
Deflazacort	247	0.2 M phosphate buffer (pH6)/EtOH (80:20, v/v)	0.1	4.4 – 22.1	-	<0.4	[21]
Paroxetine	293	0.1 M acetate buffer (pH 3.1)	0.1	0.35 – 1.76	-	<1.9	[22]
Levofloxacin	288	0.2 M acetate buffer (pH3)/MeOH (90:10, v/v)	0.1	0.3.6 – 1.8	-	0.83	[23]
Doxazocin	365	0.1 M acetate buffer (pH4)/MeOH (90:10, v/v)	0.7	5.9 – 2.9	-	1.2	[24]
Sildenafil	292	0.2 M phosphate buffer (pH8) MeOH (90:10, v/v)	0.14	0.47 – 2.35	-	1.9	[25]
Lansoprazole	292	0.01 M NaOH	0.22	2 - 20	-	1.72	[26]

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carbonate buffer (pH 8.3, λ_{max} = 530 nm) [36]. The advantages of using metal ions as complexing agents in FI include simple manifolds, readily available and cost effective reagents, while the sampling rate is generally very high ranging between 60 h^{-1} [35] and 210 h^{-1} [34]. Alternative FI approaches based on metal–drug interactions include the indirect determination of captopril based on its inhibitory effect on the complex formation between Co(II) and 2,2_-dipyridyl-2-pyridylhydrazone (DPPH) [37]. Fernadez- Gonzalez et al. also reported a FI method for the determination of lactamic antibiotics (amoxicillin and ampicillin) based on the catalytic effect of Cu(II) ions on the hydrolysis of the drugs. The coloured hydrolysis products exhibited enhanced absorbance in micellar medium [38].

1.2.6.3 FI Methods Based on Various Color-Forming Reactions

FI methods based on homogeneous reactions represent the majority of recent spectrophotometric applications to pharmaceutical analysis. The selected chemical systems depend on the structure and properties of the pharmaceutical compounds of interest. A variety of FI procedures are based on the reaction of hexacyanoferrate(III) with pharmaceutical compounds. Olazanpine was determined indirectly based on the measurement of the unreacted oxidant at 425 nm [39]. The well-known reaction of phenolic compounds with 4-aminoantipyrine in the presence of hexacyanoferrate(III) was the basis for a FI method for the determination of salbutamol. Automation of the reaction via FI eliminated the solvent extraction step which is necessary in batch procedures [40]. Based on a similar mechanism, Al-Abachi et al. reported a FI assay for amoxicillin using *N*,*N*-dimethyl*p*-phenylenediamine in alkaline medium [41]. Alternatively, diclofenac and mefenamic

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acid can be oxidized by hexacyanoferrate (III) under flow conditions to form coloured products which are monitored spectrophotometrically [42]. The potentials of FI are pointed out to a great extend when unstable reagents have to be used. These reagents can be produce *in situ* in the FI manifold, offering the advantages of simplicity, rapidity and eliminating the need for frequent standardization and storage under specific conditions [43]. Bromine can be produced on-line by oxidation of bromide by bromate in acidic medium. Acetylcycteine was determined in pharmaceuticals indirectly, based on the decrease of bromine absorbance [44]. Similarly, the decrease of the absorbance of on-line produced iodine by a group of compounds (amoxicillin, cephalexin, ampicillin, cephadrin) was the basis for the development of a FI assay [45]. On-line triiodide ion generation was achieved by mixing iodate and iodide solutions. The decrease of the absorbance of the complex between triodide ion and starch by metamizol was used by Pereira et al. for the determination of the latter [46]. Other reagents recently applied to the FI spectrophotometric analysis of pharmaceutical compounds include 4,6 dinitrobenzofuroxan [47,48], 4-chloro-5,7-dinitrobenzofurazan [49], *N*-bromosuccinimide [50], *p*-toluidine/periodate [51], ammonium molybdate [52], periodate [53], sodium nitrite [54], sodium hypochlorite/salicylate [55], bromocresol green [56], *p*benzoquinone [57] and immobilized polyphenol oxidase [58]. More data on the analytical characteristics of these procedures can be found in Table 1.2.

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1.3 Sequential Injection Analysis (SIA)

1.3.1 Introduction

FIA is well established as a powerful sample handling procedure for laboratory and on-line process analytical chemistry [59], with numerous applications in heavy industrial [60] and environmental [61] situations. However, the latter application is often hampered by the complexity of the required manifolds particularly for multicomponent analyses. In certain circumstances multicomponent process analytical chemistry is only feasible by utilizing several independent FIA systems. In order to compete with other techniques, the newest methodology of flow injection measurements, called sequential injection analysis (SIA), has been developed [62]. It was first reported by Ruzika and Marshall at the University of Washington in 1990 [63].

1.3.2 Principle of SIA

SIA is an automated approach to sample handling that will allow researchers to automate manual wet chemistry procedures in a rapid, precise, and efficient manner. Small solution zones are manipulated under controlled dispersion conditions in narrow bore tubing. Sharing many characteristics with FIA, some would argue that it is simply an extension of FIA. Nevertheless, more than 100 journal articles have been published on SIA since the first paper in 1990. While, like FIA, it is fundamentally dependent on the dispersion of zones in a flowing stream, conceptually, the practice of SIA is different from FIA.

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Table 1.2 FI methods utilizing various colour- forming reaction for some common drugs.

analyte	Method principle	Detection limit (mg L^{-1})	Linear range (mg L^{-1})	Sampling rate (h^{-1})	R.S.D. (%)	Ref.
Sulfanilamides	Derivatization with 4, 6-dinitrobenzofuroxane	0.12 – 0.24	0.25 – 5.5	12- 20	-	[47]
Benzylpenicillin	Derivatization with 4, 6 – dinitrobenzofuroxane	0.14	0.28 – 10.0	30	4 – 5	[48]
Indole derivatives	Derivatization with 4-chloro-5, 7- dinitrobenzofurazane	0.04 – 0.61	0.01	25 – 35	4 – 5	[49]
Levofloxacin	Oxidation with *N*- bromosuccinimide	3.0	10 – 300	90	< 2.7	[50]
Dipyrone	Reaction with ammonia molybdate to produce molybdenum blue	32	166.5 – 2666.4	60	1.7	[52]
Paracetamol	Nitration with sodium nitrite	-	180 – 300	120	<1.0	[54]
Paracetamol	Reaction with sodium hypochlorite followed by reaction with sodium salicylate	0.4	5.0 – 125.0	60	1.5	[55]
Diphenylhydramine	Ion pair formation with bromocresol green	1.0 and 15.0	5 – 21 and 75 – 188	100	1.6	[56]
Diaminopyrimidines	Reaction with *o* – benzoquinone	20 – 70	120 – 300	-	1 – 4	[57]
Isoproterenol	Oxidation by polyphenol oxidase immobilized on controlled pore silica	13.2	26.0 – 156.0	37	< 1.0	[58]

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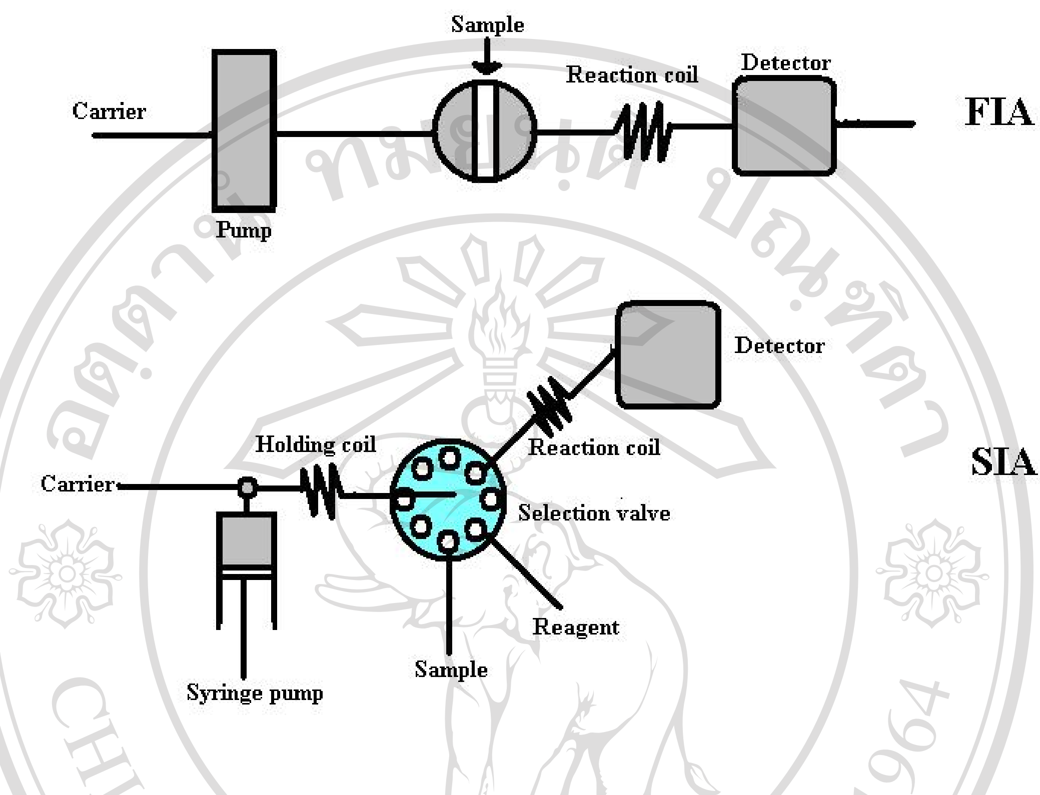

Figure 1.7 The comparison of FIA and SIA

Let's look at a simple FIA experiment and compare it to its SIA equivalent. Consider a single line FIA experiment where we inject a sample into a carrier stream containing a reagent. The FIA manifold is depicted in the above diagram. A sample is pumped into the sample loop of a two-position injection valve and the carrier is flowing constantly through the detector. The length of the sample loop determines the volume of sample injected. When the sample loop is loaded, the valve is switched and the sample is introduced into a flowing carrier stream. The carrier carries the sample through the reactor (usually a reaction coil) to the detector. En route, the sample reacts with the reagent to form a detectable species. The detectable species gives rise to a peak when it passes through the flow cell of the detector. A calibration curve is then

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used with the peak height, area, or width to determine the concentration of the analyte in the sample.

SIA on the other hand does not make use of an injection valve. Rather, a multi-position selection valve replaces the injection valve. Usually, the frequently used FIA peristaltic pump is replaced with a syringe pump and an additional coil called the holding coil is added. Compare the schematic of an SIA manifold to that of the FIA manifold. To achieve the same measurement as described above, the syringe is filled with carrier solution containing the reagent. Then the selection valve is advanced to a port that is connected to the sample line. A small volume of sample is drawn up into the holding coil. The flow program determines the volume of sample; viz. the volume of sample that is drawn up by the pump into the holding coil. The selection valve is then advanced to a port that is connected to the detector, and the carrier transports the sample through the reactor to the flow cell of the detector. Again, a detectable species is formed and is registered as a peak by the detector. The concentration of the analyte in the sample is determined in a similar manner as for FIA.

It is worth noting that at the moment of injection in an FIA experiment, an undispersed plug of sample is introduced into the carrier stream. In SIA, already during aspiration of the sample into the holding coil, dispersion begins to take place and the flow reversal that takes place when the sample is sent off to the detector plays a dramatic role in mixing the sample with the carrier. The next figure depicts graphically what happens at the point of sample injection (1) in an FIA manifold (top) and during the flow reversal inherent to SIA manifold (bottom) and a few seconds after the carrier has started to move (2) the sample towards the detector. This phenomenon may cause an SIA peak to look slightly different to an FIA peak. As

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long as there has been good mixing between sample and reagent, this will not affect quantification because samples and standards are treated alike. In fact, it has been shown that the flow reversal contributes significantly to the mixing of zones.

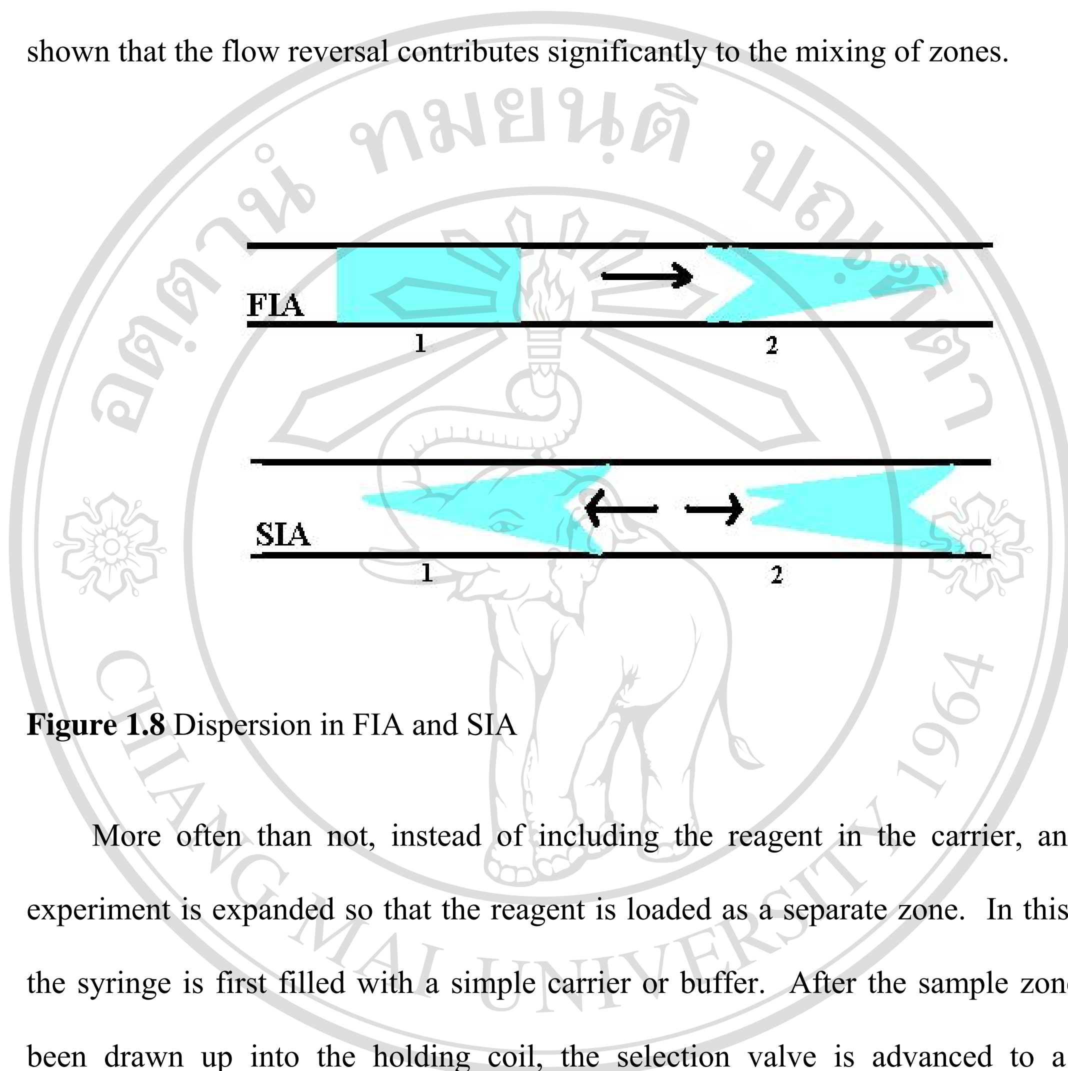

Figure 1.8 Dispersion in FIA and SIA

More often than not, instead of including the reagent in the carrier, an SIA experiment is expanded so that the reagent is loaded as a separate zone. In this case the syringe is first filled with a simple carrier or buffer. After the sample zone has been drawn up into the holding coil, the selection valve is advanced to a port connected to a reagent reservoir and a small reagent zone is drawn up into the holding coil. In this way, it is possible to construct a stack of well defined zones which can be mixed together giving rise to a detectable species. Researchers will appreciate that unlike FIA, which requires re-plumbing when a more complex chemical addition scheme is required, in SIA, all that is required is a change to the flow program. The manifold remains the same. The additional advantages of lowering reagent consumption and minimizing the production of potentially hazardous wastes are both

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important advantages of SIA. The other ports of the selection valve can be used for calibration standards, additional reagents and as locations where more sophisticated operations such as dilution, trace enrichment, and incubation of reactants can take place.

SIA has several advantages over FIA.

- Reagent use is drastically reduced. Typical FIA experiments make use of at least 1mL of reagent per measurement. SIA typically makes use of 50µl. This means that in a 24-hour period assuming one measurement per minute, the FIA analyzer would consume 1440ml of reagent. The SIA analyzer would consume 72ml. It has been noted that the most frequent reason for process analyzer failure is running out of reagents.

- Flow manifolds are simple and robust typically comprising a pump, selection valve, and detector connected by tubing. The same manifold can be used for widely different chemistries simply by changing the flow program rather than the plumbing. Analyzer maintenance is therefore simplified.

- The selection valve replaces the injection valve and provides a means for selecting different sample streams and calibrants. This enables convenient automated calibration.

- Components used in a SIA manifolds are amenable to laboratory, field, and plant operation. In addition to these, SIA enjoys all of the benefits of FIA.

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A typical SIA manifold has been described above and comprises the following main components:

- Pump
- Selection Valve
- Reactor and Holding Coil
- Detector
- Software

Pump

Syringe pumps have been most widely used to aspirate zones and propel the stack of zones through the detector. Some researchers have used peristaltic pumps. The requirements for the pump are that it is precise, reproducible, bi-directional, and able to measure small volumes. Computer control is imperative.

Selection Valve

The selection valve must allow random access of the ports. Small dead volume and zero cross contamination between ports are essential features of a good selection valve.

Reactor and Holding Coil

Although the idea of knotting reactors in FIA has long been advocated; many users of these flow-based techniques make use of reaction coils. They have shown that if excellent mixing without increasing dispersion is required, then a serpentine flow path provides the optimum conditions

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Detectors

The wide ranges of detectors that are employed for FIA are suitable for SIA. The only requirement is that they must be equipped with a flow cell. As for FIA, low dead-volume and immunity to bubbles are key requirements. We have a range of colorimetric and electrochemical detectors which have been developed for FIA and SIA.

Software

The crux of SIA is the flow program. This sequence of events results in the assembly of the stack of zones in the holding coil and subsequent transport to the detector flow-cell. Microprocessor control is imperative. Several packages have been written to achieve this.

1.3.3 Applications of SIA to Pharmaceutical Assays analysis

Since it was first described in the literature in 1990, SIA has been applied to pharmaceuticals analysis. Examples of these applications are summarized in Table 1.3 and are discussed below.

The samples for pharmaceutical assays vary in form, being solids (tablets, capsules), pastes (ointments, creams) or liquids (emulsions, suspensions, solutions). In addition to the active ingredient, auxiliary substances are added to pharmaceutical preparations (e.g., fillers, binders, suspending agents and preservatives). In many cases, the non – active ingredients do not interfere with assay of the active component and can be removed easily. Another advantage is that the approximate composition of the assayed material is almost always known.

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Systematic analysis of pharmaceuticals during their synthesis, extraction and purification is vital to assure their quality at all stages of production, since it affects manufacturing capacity and production efficiency. Speed and high sensitivity are additional requirements for quality control in routine laboratory assays. Spectrophotometry, fluorescence and chemiluminescence have been used most frequently. Since the analytical application of chemiluminescence gained momentum with the growth of flow methods [64], this section presents a short account of its uses. Barnett determined morphine in aqueous and in non-aqueous solutions [65]. Detection was based on chemiluminescence produced by the reaction of morphine with acidic potassium permanganate in the presence of sodium hexametaphosphate. Pasekova. and Pola. s_ek used chemiluminescence detection for the assay of sulphonamides [66] and local anesthetics [67]. They used sulphanilamide as a model substance. Luminescence was emitted during the oxidation of the analyte by potassium permanganate in sulfuric acid medium. A number of enhancers were tested (formic acid, sodiumhexametaphosphate, glycolaldehyde, glutaraldehyde, Rhodamine B). Finally, 0.1 M glutaraldehyde was chosen to enhance the signal. The substances analyzed were sulphanilamide, sulphacetamide, sulphadimidine, sulphafurazole, sulphametoxypyridazine, sulphaguanidine. Assay of local anesthetics used the same principles. Procaine hydrochloride, benzocaine and tetracaine hydrochloride were oxidized by permanganate in aqueous sulfuric acid. Enhancers of cheminiluminescence intensity were 4-hydroxybiphenyl, Rhodamine B, glycolaldehyde, glutaraldehyde and formic acid, of which the latter (0.37M) was found most effective.

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Potentiometric detection with ion-selective electrodes (ISEs) and titrimetry has been used most frequently. For captopril [68,69], the determination proceduren was based on colorimetric measurement of the captopril-palladium complex in acid and was compared with potentiometric titration of captopril with a 10^{-3} mol/l Ag(I) solution. Colorimetry has the advantage of selectivity even in the presence of high levels of matrix components, while potentiometric titration is more repeatable.

1.4 Surfactant and Micelle [92, 93]

1.4.1 Introduction

The word surfactant is a contraction of surface active agent, referring to the main property of this class of molecules: their adsorption at any interface that modifies the surface or interfacial tension. Surfactant molecules are also called detergents and amphiphiles from the greek words " amphi" and "philo," which mean respectively, both and loving. The amphiphile molecules love both polar and nonpolar media. The amphiphilic character of surfactant molecules is due to the association of two parts with very differing polarities inside the same molecule. One part is highly nonpolar, hydrophobic or lipophilic, usually an alkyl chain. Another part of the surfactant of the surfactant molecule is polar or hydrophilic. It can be a nonionic chain with polar groups, such as ether, alcohol or amine groups, or an ionic (anionic or cationic) group. Figure 1.9 shows the schematic representation of a surfactant molecule. Some surfactants have two nonpolar tails or two polar heads, as illustrated in the figure 1.9. The nature of the surfactant polar head is used to classify the molecules.

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Table 1.3 Applications of SIA to pharmaceutical assays analysis

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample throughput (h^{-1})	Ref.
Ascorbic acid (Vitamin C)	Drug Formulations	Oxidation of Vitamin C by Ce(IV) λ_{max} = 410 nm	30 – 200 mg L^{-1}	-	-	[69]
Ascorbic acid (Vitamin C)	Tablets	Redox reaction between ascorbic acid and permanganate in acidic medium , λ_{max} = 525 nm	0 – 1200 mg L^{-1}	-	60	[70]
Boron	Eye lotion	Complexation between D-sorbitol and boric acid, then acid-base reaction with methyl orange, λ_{max} = 520 nm	0.06 – 12 mg L^{-1}	0.06 mg L^{-1}	30	[71]
Bromazepam	Tablets	Complexation reaction of bromazepam with iron(II) in hydrochloric acid, λ_{max} = 585 nm	5 x 10^{-4} – 1.5 x 10^{-4} mol L^{-1}	-	-	[72]
Calcium	Effervescent tablets	Complexation reaction with cresolphthalein complexone , λ_{max} = 578 nm	0 – 20 mg L^{-1}	0.05 mg L^{-1}	43	[73]

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Table 1.3 Applications of SIA to pharmaceutical assays analysis (continued)

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample throughput (h^{-1})	Ref.
Ciprofloxacin	Tablets infusion	Complexation reaction with iron(III), λ_{max} = 447 nm	50 -500 mg L^{-1}	-	-	[74]
Norfloxacin	Tablets	Complexation reaction with iron(III), λ_{max} = 430 nm	50 – 400 mg L^{-1}	-	-	[74]
Iodide	Pharmaceutical preparations	Catalytic effect of iodide on the reaction between Ce(IV) and As(III), λ_{max} = 410 nm	0.002 – 0.5 mg L^{-1}	1.5 μg L^{-1}	15	[75]
Iron (II)	Anti-anaemic pharmaceuticals	Complex of Fe (II) and 2,2-bipyridyl at pH 4.5, λ_{max} = 523 nm	5.0 – 40.0 mg L^{-1}	0.97 mg L^{-1}	100	[76]
Oxprenolol	Tablets	Oxidation of oxprenolol with Ce(IV), λ_{max} = 480 nm	50 – 400 mg L^{-1}	-	-	[77]
Paracetamol	Tablets, Powder, Combined preparations	Oxidation of paracetamol by hexacyanoferrate (III) and reaction with phenol in the presence of ammonia, λ_{max} = 630 nm	0 – 60 mg L^{-1}	0.2 mg L^{-1}	27	[78]
promethazine	Tablets, Syrup, Elexir	Complexation reaction of promethazine hydrochloride with Pd (II), λ_{max} = 540 nm	50 – 400 mg L^{-1}	-	-	[79]

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Table 1.3 Applications of SIA to pharmaceutical assays analysis (continued)

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample throughput (h^{-1})	Ref.
Theophyline	Solution of theophyline and caffeine	Microcolumn packed with anion exchage beads; adsorbed theophyline eluted by HCl, λ_{max} = 274 nm	0.022 – 0.28 mmol L^{-1}	-	-	[80]
Trimeprazine	Pharmaceutical preparations	λ_{max} = 515 nm	50 – 400 mg L^{-1}	-	60	[81]
perphenazine	Pharmaceutical preparations	Complexation reaction with Pd (II) λ_{max} = 560 nm	50 – 500 mg L^{-1}	-	-	[82]
zinc	Pharmaceutical samples	Reaction with xylenol orange, λ_{max} = 678 nm	10 – 60 mg L^{-1}	0.42 mg L^{-1}	30	[82]
sulphafurazole	Tablets	Oxidation of drug by permanganate in acidic medium, chemiluminescence determination	0.009 – 0.88 mmol/l	0.009 mmol/l	120	[83]
Sulphanillamide	Standard solutions	Oxidation of drugs by permanganate in acidic medium, chemiluminescence determination	0.015 – 01.50 mmol/l	0.013 mmol/l	120	[84]

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Table 1.3 Applications of SIA to pharmaceutical assays analysis (continued)

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample throughput (h^{-1})	Ref.
Trimethoprim	Tablets	Oxidation of trimethoprim by $KMnO_4$, chemiluminescence determination	20 – 100 mg/l	0.1 mg/l	30	[85]
Procaine hydrochloride	Injections	Oxidation of the analytes by permanganate, chemiluminescence determination	0.5 – 50 mg/l	0.3 mg/l	120	[86]
Morphine	Morphine in non – aqueous solution	Chemiluminescence reaction of morphine with acidic potassium permanganate in the presence of sodium polyphosphate	0.01 – 0.1 % m/v	0.0003% m/v	120	[87]
Morphine	Standard solutions	Chemiluminescence reaction of morphine with acidic potassium permanganate in the presence of sodium hexametaphosphate	2.5×10^{-6} – 3.0×10^{-5} mol/l	10^{-8} mol/l	-	[88]
Captopril	Tablets	Petentiometric titration with Ag(I) solution	Linear to 1.4×10^{-3} mol/l	-	-	[89]
S- captopril	tablets	Amperometric biosensor, based on L-amino acid oxidase	0.05 – 1.50 μmol/l	16 nmol/l	80	[90]

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Table 1.3 Applications of SIA to pharmaceutical assays analysis (continued)

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample throughput (h^{-1})	Ref.
S-captopril	Synthesis process monitoring	Enantioselective membrane electrode based on maltodextrin, potentiometric determination	$10^{-6} – 10^{-3}$ mol/l	4.4 x 10^{-7} mol/l	38	[91]

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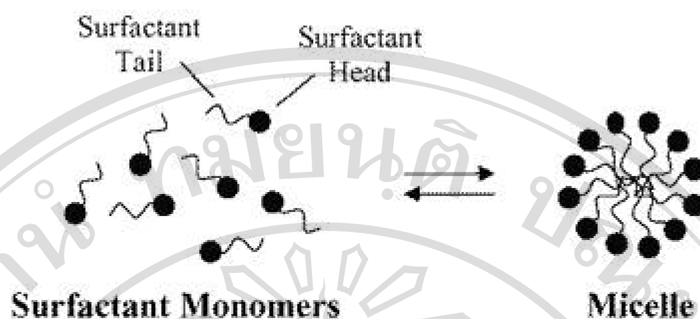

Figure 1.9 The schematic representation of a surfactant molecule

1.4.2 Classification

According to the electrical charge of their polar head, the three main classes of surfactants are classified by their hydrophilic group. Anionic, cationic, nonionic and zwitterionic surfactants contain anion, cation, noion and zwitterion respectively, as their hydrophilic group.

1.4.2.1 Anionic Surfactant

Anionic surfactants are surface active agents in which the surface active ion is an anion when the surfactants dissociate in water. Consequently, anionic surfactants are now being used most widely and extensively in detergent, shampoo, body cleansers and so on. All of the soaps (the fatty acid salts) are anionic surfactants. Commonly used anionic surfactants are the alkyl benzenesulfonates, alkyl sulfonates and alkyl phosphates as shown below.

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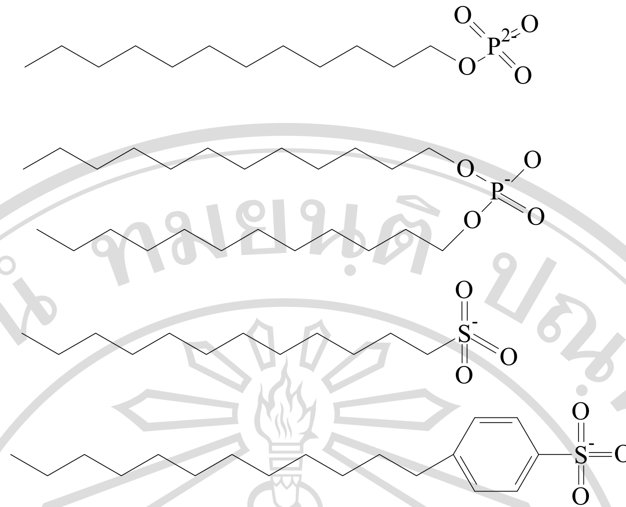

Figure 1.10 The molecular structure of alkyl phosphate, alkyl sulfonate, and alkyl benzene sulfonate.

1.4.2.2 Cationic Surfactant

Cationic surfactants are also surface active agents in which the surface active ion is a cation when the surfactants dissociate in water. It is fairly easy to recognize the cationic surfactants, as they exhibit a positive charge. Fatty amine salts (or ammonium salts) were developed as the first cationic surfactants as shown in Figure 1.11.

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Figure 1.11 The molecular structure of fatty amine salts which were the first style of cationic surfactant synthesized

Cationic surfactants are typically used in things like hair-conditioner and fabric softeners. The fatty amine salts proved quite useful in blends with nonionic surfactants, giving good stability over a range of pH levels. The principal uses of the fatty amine salts are in ore-flotation, corrosion inhibition and wetting. They are rarely marketed on their own, rather in proprietary formulations. The development of the pH insensitive cationic surfactants allowed much wider use of this technology. The quaternary amine is a strong hydrophile, making these good surfactants. They are primarily used in domestic fabric softeners, as the positively charged hydrophilic head tends to interact strongly with negatively charged fabric fibers.

1.4.2.3 Nonionic Surfactants

Nonionic surfactants differ from both cationic and anionic surfactants in that the molecules are actually uncharged. The hydrophilic group is made up of some other very water soluble moiety, (e.g. a short, water- soluble polymer chain) rather than a charged species. Traditionally, nonionic surfactants have used poly(ethylene oxide) chains as the hydrophilic group. Poly (ethylene oxide) is a water soluble polymer; the polymers used in nonionic surfactants are typically 10 to 100 units long.

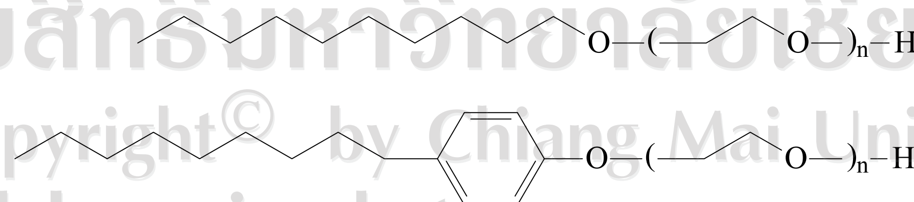

Figure 1.12 The molecular structure of an alcohol ethoxylate and an alkylphenol ethoxylate

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1.4.2.4 Zwitterionic Surfactants

A zwitterionic surfactant is a zwitterions that has surface- active properties. A zwitterions is also known as an internal salt, having an anion and a cation in the one molecule chemically joined together. Zwitterionic surfactants are also called amphoteric surfactants as they often comprise a base coupled to an acid.

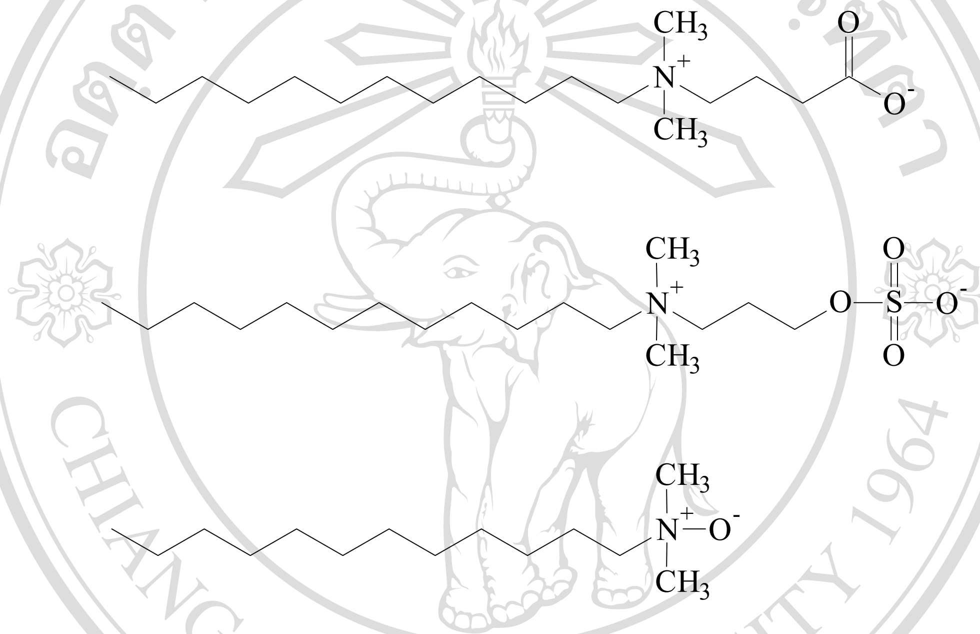

Figure 1.13 Three zwitterionic surfactants : an ammonium carboxylate, an ammonium sulfate and an amine oxide

1.4.3 Micelles

When the concentration of the surfactants in aqueous solution is increased above a characteristic value known as the critical micelle concentration (CMC), they associate to form relatively well-defined aggregates known as micelles. As shown in Figure1.14, these aggregates of amphiphiles assemble such that the tails of the molecules are packed together in the core of the micelle while the polar head groups form a boundary zone between the nonpolar core of the micelle and the polar aqueous

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solution beyond. The charged interfacial zone is referred to as the Stern layer of ionic micelles. Nonionic micelles do not have charged head group, but rather polar structure such as polyoxyethylene groups are presented to the bulk solution. The microscopic order provided to the solution by micelles gives rise to perhaps the most important characteristic of micellar solutions, which is the ability to solubilize otherwise water insoluble molecules in what is essentially an aqueous matrix.

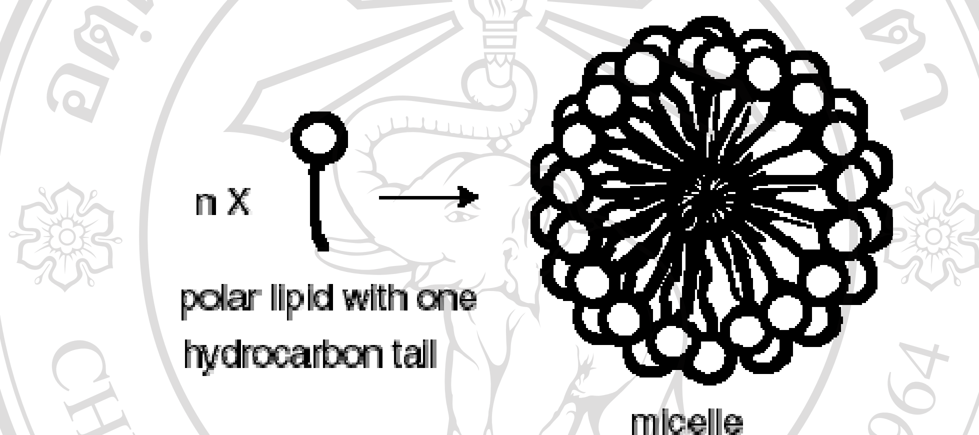

Figure 1.14 The aggregation of N monomers to form a normal, aqueous micelle. The open circles represent polar head groups and may be anionic, cationic, nonionic or zwitterionic [94].

At concentration above the CMC, the phenomenon can be observed by changing in any of several physical properties (i.e., absorbance maxima, surface tension and conductance) of the solution with increasing concentration of amphiphile. The concentration of free amphiphile remains fairly constant with added surfactant. It is important to realize that the concentration of free amphiphile does slowly increases above the CMC although the change is often small enough so as to be unconstructive to the analytical technique at hand.

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1.5 Micellar Liquid Chromatography

1.5.1 Introduction

The first report on the analytical use of an aqueous solution of a surfactant, above its critical micellar concentration (CMC), as a mobile phase in reversed – phase liquid chromatography (RPLC) was published in 1980 [95]. The technique, named micellar liquid chromatography (MLC), is an interesting example of the modification of the chromatographic behavior taking advantage of secondary equilibria to vary both retention and selectivity.

Most MLC procedures use micelles of the anionic surfactant sodium dodecyl sulfate (SDS). Other useful surfactants include the cationic cetyltrimethylammonium bromide or chloride (CTAB or CTAC) and the nonionic Briji-35 [96]. The separations are usually carried out in C_{18} or C_8 columns.

Inside the column, solutes are affected by the presence of micelles in the mobile phase and by the nature of the alkyl-bonded stationary phase, which is coated with monomers of surfactant (Figure 1.15). As a consequence, at least two partition equilibria can affect the retention behavior. In the mobile phase, solute can remain in the bulk water, be associated to the free surfactant monomers of micelle surface, be inserted into the micelle palisade layer, or penetrate into the micelle core. The surface of the surfactant – modified stationary phase is micelle-like and can give rise to similar interactions with the solutes, which are mainly hydrophobic in nature. With ionic surfactant, the charged heads of the surfactant in micelles and monomers adsorbed on the stationary phase are in contact with the polar solution, producing additional electrostatic interactions with charged solutes. Finally, the association of

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solutes with the no modified bonded stationary phase and free silinol groups still exists.

The most serious limitations of pure micellar solutions are their weak elution strength and poor efficiencies. As early as 1983 [97], the addition of a small percentage of 1- propanol was found to enhance the efficiencies and decrease the asymmetries of chromatographic peaks. Later, the term "hybrid micellar mobile phases" was given to the ternary eluents of water/organic solvent/micelles. Although 1-propanol is still the most frequently used additive, other alcohols (methanol, ethanol, 1-butanol, and 1-pentanol) and organic solvents common in conventional RPLC (acetronitrile and tetrahydrofuran) have also been used. It should be noted that micellar solutions increase the solubility of butanol and pentanol in water to reach concentration levels considered useful in chromatography.

The concentration of organic solvent should be low enough to make the existence of micelles possible. Such maximal amount depends on the type of surfactant and organic solvent, and is usually unknown. For SDS, the maximal volume fractions of acetonitrile, propanol, butanol, and pentanol that seem to gurantee the presence of micelles are 20%, 15%, 10%, and 7% (v/v), respectively. However, analytical reports where authors claim the use of hybrid micellar mobile phases and these maximal values are exceeded – micelles do not exist- are not unusual. In such conditions, the system bears closer resemblance to an aqueous – organic system, although the surfactant monomers still affect the retention and efficiencies.

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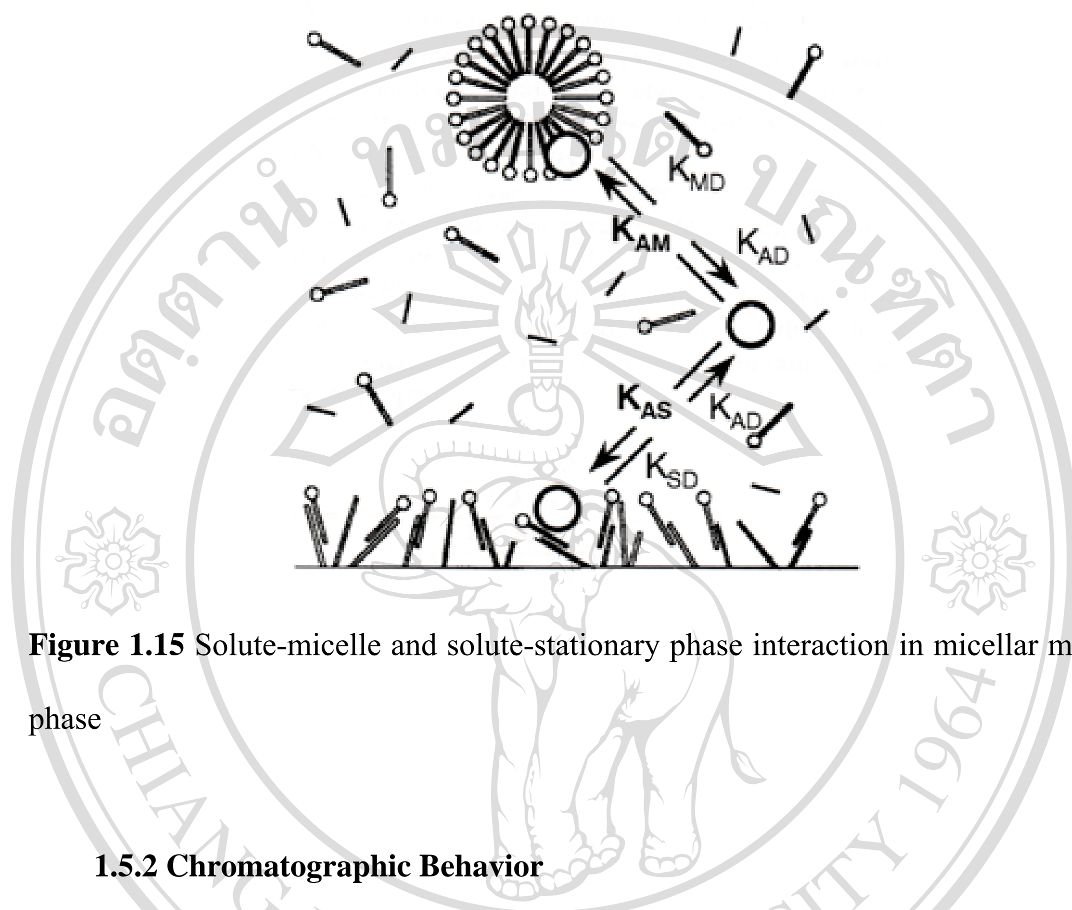

Figure 1.15 Solute-micelle and solute-stationary phase interaction in micellar mobile phase

1.5.2 Chromatographic Behavior

Micellar systems share the basic components of a RPLC system, that is, a nonpolar stationary phase and a polar aqueous mobile phases are homogeneous, whereas micellar solutions are microscopically heterogeneous, being composed of two distinct media: the amphiphilic micellar aggregates (micellar psudophase) and the surrounding bulk aqueous or aqueous – organic solvent that contains surfactant monomers in a concentration approximately equal to the CMC. In micellar solutions, the solute is preferentially solubilized into or onto the micellarassembly, a process which is dynamic. The solutes localized in micelles experience a microenvironment that is dramatically different from that of bulk solvent in terms of polarity and fluidity. This is reflected by micelle-induced perturbations of physico-chemical

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properties of the solutes, including changes in solubility, acidity, photophysical properties and reaction rates.

The complexity of MLC is much greater than that conventional RPLC with aqueous-organic solvents, because of the number of possible interactions with both mobile and stationary phase (Figure 1.16). The solutes in the mobile phase can interact electrostatically with the charged outer-layer of ionic micells, and hydrophobically with their lipophilic interior. The steric factor can also be important. The modification of the stationary phase by adsorption of surfactant monomers, which creates a “micelle-like” surface, gives rise to similar interactions with the solutes. The combination of these interactions cannot be duplicated by any traditional pure or mixed solvent system. While micellar solutions will never totally replace traditional aqueous-organic eluents, they offer several interesting alternatives to separation work.

The basic mechanism of separation in MLC is fairly well understood and there is a reasonable theoretical foundation on which to build. MLC is a fascinating example of the use of a secondary chemical equilibrium in liquid chromatography. The primary equilibrium is the partitioning of the solute between bulk mobile phase and stationary phase, and the secondary equilibrium is the partition to micelles. This secondary equilibrium is affected by a great variety of parameters: type and concentrations of surfactant and additives such as salts or organic modifiers (for instance, alcohols), and pH.

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1.5.3 The Three-Phase Model

Armstrong and Nome [98] proposed a three-phase (stationary phase, bulk aqueous solvent and micellar pseudo-phase) model, to explain the chromatographic behavior in a RPLC system of a solute eluted with a mobile phase containing a surfactant above the CMC. Such a treatment has allowed a theoretical description of MLC, and greater understanding and utilization of this chromatographic technique.

According to the three-phase model, the reaction of a solute is controlled by three competing reversible equilibria, namely partitioning from bulk aqueous solvent to micelles, partitioning from bulk aqueous solvent to the alkyl-bonded stationary phase, and direct transfer from micelles to the stationary phase. The first equilibrium takes place inside the mobile phase, and the latter equilibrium can be neglected in most situations, but it is significant for highly nonpolar (water-insoluble) solutes, which have a great affinity for both stationary phase and micelles. The partitioning equilibria are described by three coefficients: P_{wm} (between water and micelles), P_{ws} (between water and stationary phase), and P_{ms} (between micelles and stationary phase). It is the first partition coefficient, P_{wm}, that imparts uniqueness to MLC. The coefficients P_{ws} and P_{wm} have opposing effects on the retention of solutes: as P_{ws} increases the retention increases, whereas P_{wm} increases the retention is reduced due to increased partitioning into micelles.

The retention behavior of solutes will depend on the type of interactions with the micelles and with the surfactant-modified stationary phase. Nonpolar solutes should only be affected by hydrophobic intractions (Fig.1.16a). For these solutes, different proportions of nonpolar, dipole-dipole and proton donor-acceptor interactions

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between solutes and surfactants are expected. But for solutes that are charged, two distinct situations can be considered:

(i) the sign of the charges on the solute and surfactant are the same (Figure1.16b), or

(ii) the sign of the charges on the solute and surfactant are opposite (Figure16c)

The first situation is encountered when an anionic solute is eluted with an anionic surfactant, or a cationic solute is eluted with a cationic surfactant [*e.g.,* dissociated phenol with the anionic surfactant sodium dodecyl sulfate (SDS), and protonated benzylamine with the cationic surfactant dodecyl trimethylammonium bromide (DTAB), on a C_{18} column] [99]. Electrostatic repulsion from the micelle should not affect the retention as the solute will still reside in the bulk solvent phase, and therefore, it will move down the column. In contrast, repulsion from the surfactant-modified stationary phase should cause a decreased retention, and the solute may elute in the dead volume. However, it may be retained by hydrophobic interaction with the stationary phase, although this effect will be reduced by the electrostatic repulsion. Due to different hydrophobic interactions, dissociated phenol and 2-naphthol are well separated with SDS.

The second situation appears when a solute is chromatographed with an oppositely charged surfactant, where electrostatic attraction occurs between both species. Electrostatic attraction between solute and micelle will complement any hydrophobic interaction, and thus, it can be expected that the solute will remain in the mobile phase for a longer period of time, decreasing the retention. However, electrostatic and hydrophobic interactions with the stationary phase are often

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sufficiently large to offset micellar attraction and thus the retention will increase. That is why dissociated phenol and 2-naphthol are retained to a greater extent with DTAB than with SDS on a C18 column [99].

Nonionizable solutes should only experience hydrophobic interactions. It has been shown, however that any molecule with a dipole moment can profoundly be affected by electrostatic effects [97].

1.5.4 Micellar Liquid Chromatography in Pharmaceutical Analysis

MLC was born in the late 1970s, together with the development of other HPLC techniques, based on the works of Armstrong, Berthod, Cline-Love, Dorsey, Fendler, Hinze, Mullins, Pramauro, Pelizzetti and Sybsilska [98,100]. After the development of the theory and its first applications, MLC was more or less forgotten, but, in the 1990s, the work of other (mainly Spanish) groups demonstrated its usefulness in modern pharmaceutical analysis. MLC is a mode of reversed-phase liquid chromatography (RPLC), in which the mobile phases are aqueous solutions of a surfactant at a concentration above the critical micellar concentration (cmc). It is successfully used in separation analysis, especially in liquid chromatography. In most cases, long alkyl chain surfactants such as sodium dodecyl sulfate (SDS) are added to the mobile phase [101-103]. The enhancement of selectivity and the adjustment of retention of ionic solutes can be easily achieved by MLC compared with the typical ion-pair liquid chromatography. Therefore MLC is applicable for the simultaneous separation of a wide variety of active ingredients in pharmaceutical formulations such as cold medicines or ointments. Other than the selectivity manipulation, through the protein solubilization capability of the miceiles, direct plasma/serum injection can be

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performed by MLC [104,105]. The determination of drugs, whose blood concentration level is relatively high, is clinically performed by using MLC mode. Separation of small ions also can be successful by employing gel filtration columns and micelles [106]. The micellar solutions have been used in thin-layer chromatography (TLC) [107-109] to improve the selectivity as in HPLC. The briefly reviews on pharmaceutical applications of MLC is shown in Table 1.4.

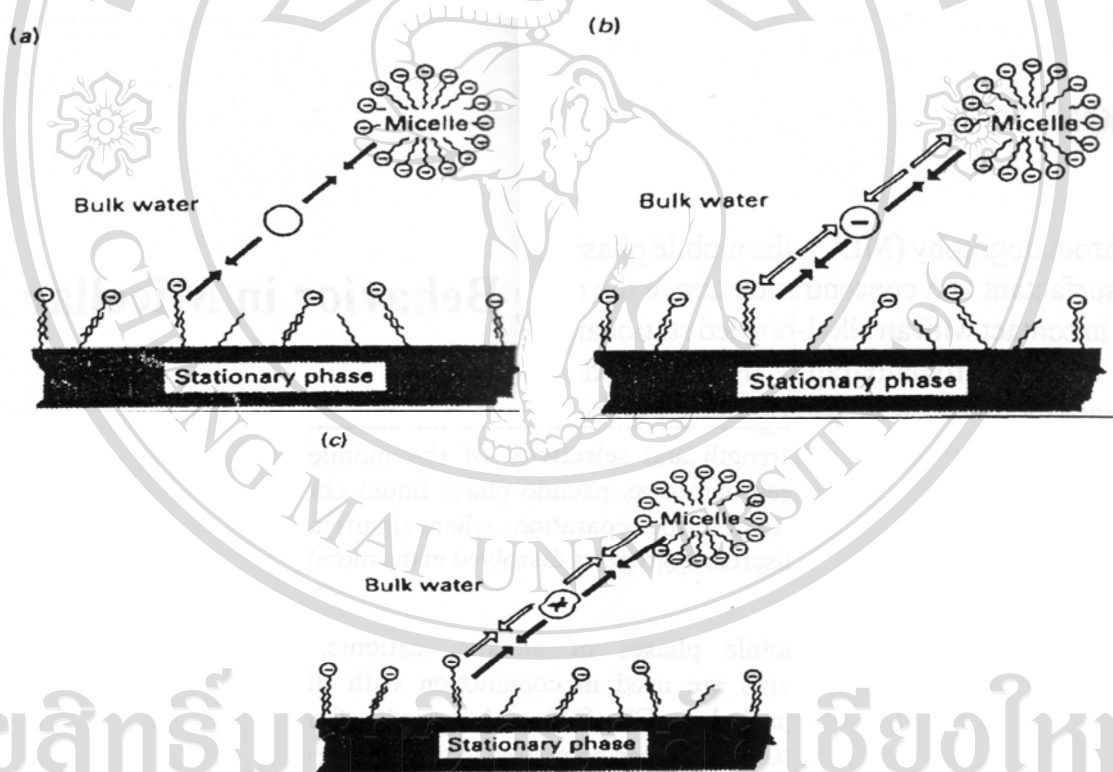

Figure 1.16 Solute – micelle and solute – stationary phase hydrophobic (🡹) and electrostatic interaction (⇧) with an anionic surfactant : (a) non polar solute, (b) anionic solute and (c) cationic solute

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1.6 Zinc

Zinc is commonly found in the Earth's crust. It is found as a mineral (most commonly sphalerite, zinc sulphide), often associated with the ores of other metals (e.g. copper, lead and cadmium). The mineral zinc is present in all organs, tissues, fluids and secretions. About 90% of total body zinc is found in skeletal muscle and bone, with much smaller amounts in the liver, gastrointestinal tract, skin, kidney, brain, lung, prostate and other organs.

1.6.1 Uses

As a component of every living cell in the body, zinc has a multitude of diverse regulatory functions. It is best known for its involvement in enzyme functions and structures such as for DNA synthesis, RNA transcription, mitosis, and cell activation [122]. Zinc-dependent enzymes are involved in metabolism of proteins, lipids and carbohydrates. It plays as essential role in cell membrane integrity; helps manage insulin action and blood glucose concentration, essential roles in development and maintenance of the body's immune system. Zinc is required for bone and teeth mineralization. Moreover is involved with normal taste and wound healing. Zinc is required for the synthesis of various biological markers of nutrition and of collagen [123]. Zinc is essential in regulating gene expression. Zinc has long been considered to have anti-inflammatory properties [122]. In enzymatic system such as carbonic anhydrase, lactate dehydrogenase in intermediary metabolism during exercise are involving of zinc [124]. Zinc is particularly important for cells that are rapidly turning over such as those in the immune system; as well as in the maintenance of the central nervous system [125].

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Table 1.4 Application of MLC in pharmaceutical analysis

Drugs	Stationary phase	Mobile phase	Detection (nm)	Ref.
antihistamine drugs(10)	Spherisorb ODS C18 250 mm x 4.6 mm i.d., 5µm	CTAB 0.04 M 3% 1-butanol, pH 3	225-260 nm	[110]
Vitamin A and E	Spherisorb ODS 100mm×3.9 mm, 5 µm	76.9 mM SDS 11.7% 1-butanol, pH of 6.73	285 nm	[111]
B6 vitamin	Kromasil C-18 (Scharlab, 120 x 4.6 mm i.d.5 µm	150 mM SDS 2% pentanol, pH 3.	290 nm	[112]
lidocaine and tolperisone	Zorbax C18 12.5 mm x 4.6 mm i.d ,5 µm	0.075 M SDS 7.5% isopropanol pH 3	210 nm	[113]
sulphonamides	Sperisorb ODS-2 C18 125 x 4.6 mm, 5 µm	0.05 M SDS 2.4 % Pentanol pH 7	550/490 nm	[114]
Barbiturates(6)	Sperisorb ODS-2 C18 120 x 4 m.m., 5 µm	0.07 M SDS 0.4 % 1 – PrOH pH 7.4	240 nm	[115]
Diuretics (19)	Hypersil C18 150 x 3 mm, 5 µm 50 ^{0}C	0.04 M SDS 4 % tetrahydrofuran pH 3.2	190 – 360 nm	[116]
Cough and Colds (5)	ODS – 2 C18 120 x 4.6 mm, 5 µm	0.15 M SDS 6% pentanol	260	[117]
Benzodiazepines	C18	0.06 M SDS 5 % Butanol pH 7	230	[118]
Anticonsultants (3)	C18	0.05 M SDS 7 % butanol, pH 7	UV	[119]
Barbiturates (4)	C18 120 mm x 4.6 mm i.d.	0.10 M SDS 4% butanol, pH 7	230	[120]
Vitamin B (3)	C18	0.10 M SDS 4 % pentanol pH 3	270, 290 and 325	[121]

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1.6.2 Deficiency

Deficiency in humans can result in Zinc can be deficient even if plasma levels appear to be "normal." A practical criterion is clinical response to zinc supplementation [126]. Traditional indicators of zinc status such as plasma levels and measuring activity of zinc metalloenzymes in blood are relatively resistant to changes in dietary zinc. A good way to measure zinc levels is by looking at granulocytes and lymphocytes because they reflect the body's zinc status fairly accurately. A quantitative assay of alkaline phosphotase activity in the granulocytes is also very useful [127]. Numerous factors play a role in zinc deficiency. These include poor dietary zinc intake, excessive dietary phytate intake, chronic illness, malabsorption, or over-supplementation with iron or copper [128]. Incidence of zinc deficiency in well-nourished humans is unknown due to difficulties in sufficiently diagnosing zinc deficiency and the diversity of its metabolic roles. Among the most sensitive enzymes to dietary zinc intake are deoxythymidine kinase (involved in skin collagen formation) and alkaline phosphotase (involved in the function of granulocytes) [122]. Symptoms of zinc deficiency include poor growth and development, appetite loss, dermatitis, hypogonadism, alopecia, reduced taste acuity, delayed wound healing, impaired reproduction and poor immune function. Growth retardation and delayed sexual maturation have been some of the most characteristic features of zinc deficiency [122]. Clinical zinc deficiency in breastfed infants is accompanied by rashes, dermatitis, failure to thrive, decreased zinc levels in the serum and irritability. Severe zinc deficiency is rare and caused by genetic or acquired conditions. Acrodermatitis enteropathica is a deficiency. Symptoms include eczematoid skin lesions, alopecia, diarrhea, bacterial and yeast infections, and eventually death if left

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untreated [129]. Moderate deficiency leads to rough skin, poor appetite, mental lethargy, abnormal neurosensory change. Mild deficiency symptoms include low testosterone levels, oligospermia, decreased natural killer cell activity, decreased interleukin-2 activity and decreased activity of T helper cells. In addition it may lead to decreased thymulin activity, hyperammonemia, hypogeusia, decreased adaptation to darkness, and a decrease in lean body mass. It's also been suggested that immature B cells accumulate in the spleen in zinc deficiency and as a result lead to enlargement of this organ.

1.6.3 Toxicity

It is generally assumed that zinc is non-toxic because of the strong homeostatic regulation of processes controlling the absorption and secretion of this mineral. However, if zinc is ingested in very high doses it may lead to gastrointestinal distress and vomiting [130].

Zinc requirements are greatest during times of rapid growth such as infancy, adolescence, pregnancy, and lactation. Zinc deficiency affects epidermal, gastrointestinal, central nervous, skeletal, and reproductive systems. Adult women are more likely to consume inadequate amounts of zinc. Results from the Total Diet Study in 1991 indicated that the amounts of zinc provided by the typical diet are below the required daily allowance for children, adolescent females, and women during their reproductive years. The side effects commonly exhibited may include amenorrhea, weight and appetite loss, and skin abnormalities. During pregnancy, there are increasing zinc requirements to meet the needs of the mother as well as the developing fetus. Since nutrient stores are deposited during the last three months of

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pregnancy, a premature fetus is greatly compromised. Zinc is usually the first mineral to be immediately supplemented when it comes to the premature infant. Since zinc is deposited into the body's tissues, low birth weight or intra uterine growth retardation could limit zinc reserves and lead to quick zinc deficiency once the child is born. Both men and women are at risk for zinc deficiency especially between puberty and the age of 25 due to low dietary intake of zinc as well as increased urinary zinc loss secondary to estrogen and/or stress. Zinc deficiency is commonly found in people with eating disorders, malabsorptive syndrome, alcoholic liver disease, chronic renal disease, sickle cell anemia, chronically debilitated individuals, and in those cases when patients present with geophagia [123]. Genetic disturbances in zinc metabolism occur in acrodermatitis enteropathica which result in severe zinc deficiency. It's been noted that supplementation with zinc results in increased hedonic tone, motivation, alertness, responsivity, and a decrease in nervousness and restlessness in these patients [131]. Patients on TPN may also suffer from zinc deficiency if not adequately replaced since the body loses 6-12 mg of zinc per day. Zinc deficiency can be fatal if left untreated. Common signs of the zinc deficient TPN patient include pustular dermatitis, alopecia, diarrhea, immune dysfunction, weight loss, intercurrent infections due to cell mediated immune dysfunctions and hypogonadism in males [121, 123]. Because of Zinc's role in cellular growth it is of particular importance in early childhood as well as for patients needing tissue repair [132]. There is also an increased risk of pneumonia in children with zinc deficiency [128]. Reduced rates of anorexia, cough, diarrhea, fever, and vomiting among zinc supplemented children with stunted growth have also been observed. Studies suggest that zinc may be a more important limiting factor to growth among children in the first two years of life when

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zinc requirements are higher than compared to older children. Special attention also needs to be placed on children with potential zinc deficiency because, unlike adults, they tend to fail to adapt to the increased absorption of zinc that happens naturally in adult when there are decreased levels of zinc in the body. Older adults often have marginal zinc status. Individuals totally dependent in IV feedings without added zinc can experience severe zinc deficiency.

Therapeutic dosages usually between 5-10 mg of zinc have been used for the treatment of acute diarrhea in infants and children [133]. Therapeutic doses of zinc for the common cold usually range between 10-50 mg of zinc given every 6-8 hours throughout the day. Zinc is usually the preferred therapy to treat Wilson's disease and sickle cell anemia since it has proven to be an effective treatment and is relatively non toxic. The increasing number of reports that daily supplementation with zinc affects the activities of selective metalloenzymes along with specific cellular and organ processes, further point out the need to differentiate between meeting the requirements for this nutrient and optimal nutrition. In addition, zinc(II) is present in vitamins and minerals preparations [134]. For this reason, the determination of zinc in pharmaceutical preparations is an important analytical field.

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1.6.4 Determination of Zinc in Pharmaceutical Preparations

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Various analytical techniques are available for the determination of zinc in pharmaceutical preparations. Various complexing agent have been used to react with zinc (II) ion, and a complexation product can be detected by spectrometric instruments(colorimeter , spectrophometer and spectrofluorimeter).

1.7 Tetracycline and Derivatives

Tetracycline (TC) and its derivatives (chlortetracycline(CTC), oxytetracycline (OTC), etc.) are employed extensively as bacteriostatic and antibiotic drugs. There is, therefore, a continuing requirement for reliable, sensitive and selective means for their determination. Procedures that have been proven to be useful including electrochemical methods [147,148], uv-visible spectrophotometry [149-154], liquid chromatography [155] and an immunoaffinity-based procedure [156]. The analytical performance of these methods is summarized in Table 1.6.

Table 1.5 Comparison of reagents for the spectrophotometric determination of zinc

Reagent and conditions	Linear range	Detection limit	RSD (%)	Ref.
Reagent = dithizone pH = 5.0	3.0 x 10^{-6} – 1.8 x 10^{-5} mol l^{-1}	0.05 μg ml^{-1}	1.2	[135]
Reagent = BPH[1] pH = 10 λ_{max} = 440	0.04 – 1.2 ppm	-	-	[136]
Reagent = azo-dye derivatives pH = 9.3 λ_{max} = 530 nm	0.06 – 2.70 μg ml^{-1}	-	0.9990	[137]
Reagent = PAN[2] λ_{max} = 550 nm	0.2 – 0.4 μg ml^{-1}	0.05 μg ml^{-1}	2.1 – 2.3	[138]
Reagent = TAN[3] pH = 5.5 λ_{max} = 582 nm	0.04 – 4.0 mg l^{-1}	10 μg l^{-1}	3.3	[139]
Reagent = xylenol orange pH = 5.0 -6.0 λ_{max} = 580 nm	1 – 20 μg , 25 ml^{-1}	-	< 2	[140]

Table 1.5 Comparison of reagents for the spectrophotometric determination of zinc

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(continue)

Reagent and conditions	Linear range	Detection limit	RSD (%)	Ref.
Reagent = PAR[4] pH = 9.11 λ_{max} = 494 nm	0.025 – 13 ppm	-	<3	[141]
Reagent = PPST[5] pH = 6.5 λ_{max} = 424 nm	-	0.010 $\mu g\ ml^{-1}$	-	[142]
Reagent = PKSH[6] pH = 4.5 λ_{max} = 376 nm	-	62.1 nM	-	[143]
Reagent = 2,4 – DHBINH[7] pH = λ_{max} = 568 nm	10 -60 $\mu g\ ml^{-1}$	0.42 $\mu g\ ml^{-1}$	< 1	[146]

[1] BPH = biacetyl mono(2-pyridyl)hydrazone

[2] PAN = 1-(2-pyridylazo)-2-naphtol

[3] TAN = 1-(2-tiazolylazo)-2-naphtol

[4] PAR = 4-(pyridyl-2-azo)-resorcinol

[5] PPST1 = (phenyl-2-pyridyl)carbylidene-5-salicylidene

[6] PKSH = di-2-pyridyl ketone salicyloylhydrazone

[7] 2, 4 – DHBINH = 2,4-dihydroxybenzaldehyde isonicotinoyl hydrazone

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Table 1.6 Analytical characteristics of some methods for the determination of tetracycline, chlortetracycline and oxytetracycline

Drugs	Method	Linear Range	Limit of Detection	Ref.
TC OTC	Voltammetry	$5x10^{-8}$ – $1.2x10^{-7}$ mg l^{-1}	5 X 10^{-8} M (DPSV) 1.2 X 10^{-7} M (DCSV)	[147]
TC	FI with biamperometric	25 – 400 μg ml^{-1}	-	[148]
TC	Derivative fluorimetry	-	0.05 μg ml^{-1} (serum) 0.125 μg ml^{-1} (kidney)	[149]
OTC	Derivative spectrophotometry	-	0.87 – 2.10 μg ml^{-1} (depended on wavelength)	[150]
OTC	Fluorimetry	0.05 – 0.6 μg ml^{-1}	0.002 μg ml^{-1}	[151]
TC CTC OTC	FI-Spectrophoto-metry	20 – 200 μg ml^{-1} 40 – 300 μg ml^{-1} 40 – 260 μg ml^{-1}	-	[152]
TC CTC OTC	Spectrofluorimetry	10-200 ng ml^{-1}	2 ng 4 ng 1 ng	[153]
TC CTC OTC	Fluorosensor	9 – 800 nM 25 – 1000 nM 20 – 1000 nM	4.0 nM 9.0 nM 7.5 nM	[154]
TC OTC	Time resolved fluorescence	10 –1000 ng ml^{-1} 5 – 1500 ng ml^{-1}	5.0 ng 2.4 ng	[155]
TC CTC OTC	Liquid chromatography	0.01 – 10 μg ml^{-1} - -	2 ng 4 ng 1 ng	[156]
TC	Fluorescence and immunoaffinity	0 – 400 ng ml^{-1}	19 ng ml^{-1}	[157]

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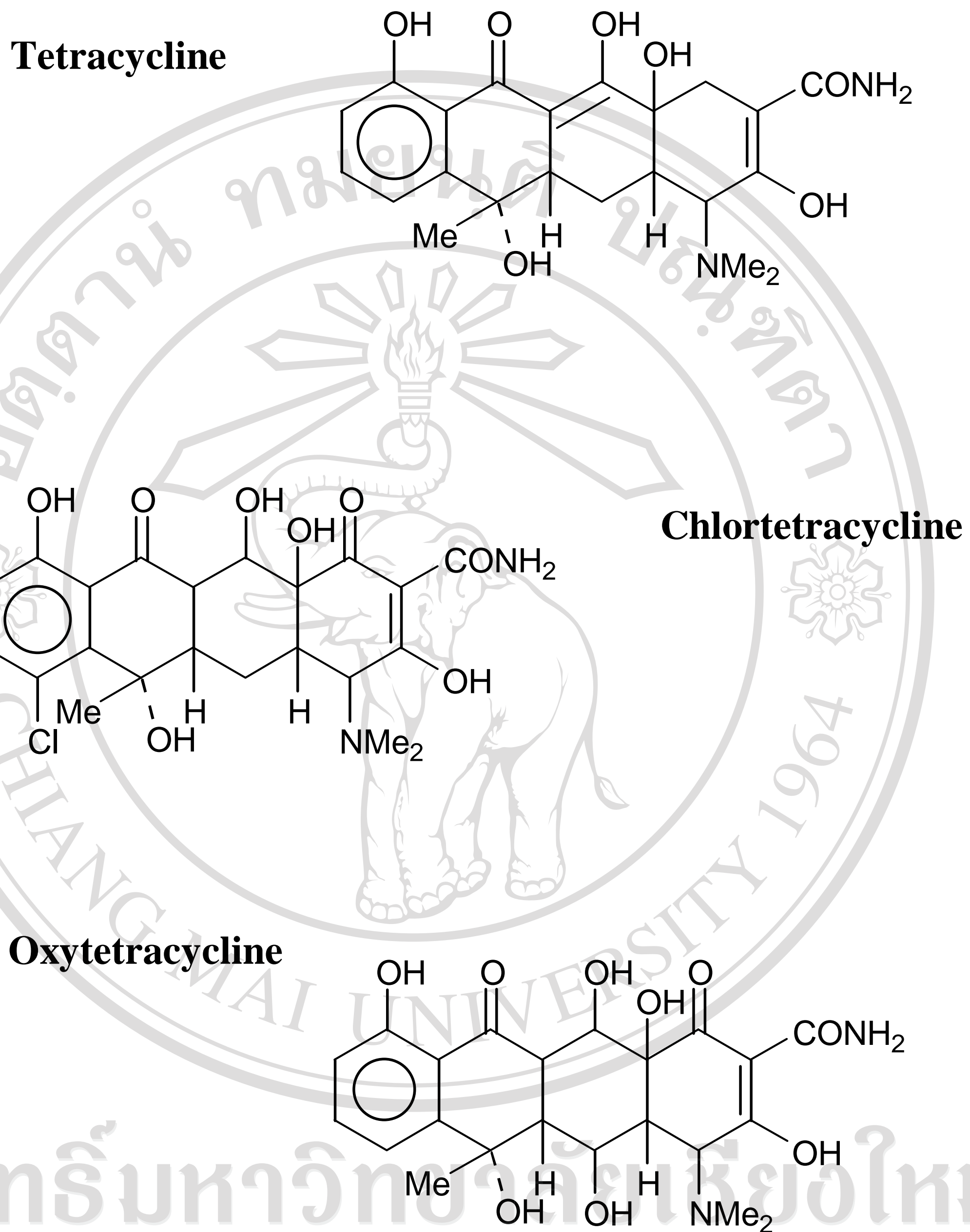

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Figure 1.17 Structures of tetracycline and its derivatives

1.7.1 Chortetracycline

Chlortetracycline is the first member of the group of the tetracycline antibiotics that be discovered. It was discovered in a soil sample yielding an actinomycete, *Streptomyces aureofaciens* [157]. It has been widely employed as a bacteriostatic and antibiotic in human, animal nutrition as feed additives and veterinary medicine. In human, it widely used to control bacterial infections. CTC is commonly used in livestock production because of its remains the most versatile antibiotic, high potency and low cost. Moreover, it is licensed for the treatment of respiratory and systemic infections in pigs, poultry and other farm animals [158]. For this reason, it is necessary to develop the high sensitivity and accuracy method for determination of the chlortetracycline in pharmaceutical preparations. Various methods were proposed for determination of this drug in a variety of samples such as HPLC with UV detector [159-162], fluorescence [163-165], MS [166-169], electrochemical detector [170-171] also micellar liquid chromatography (MLC) [172]. These analytical methods are included on the chromatographic analyses that appear in comprehensive review [173]. However, some of these methods are still some problem for improvement from the point of recovery and reproducibility that have also been reported by other authors. It is therefore recommended to use SPE cartridges and LC analytical columns containing a high purity silica or polymer materials [174].

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Several flow injection analysis (FIA) methods for pharmaceutical assays have been reported in recent reviews [175,176] because the requirements demanded by pharmaceutical industries concerning automation and higher sampling rate therefore the flow system with various detections have been developed. Amperometric flow systems were described [177-179]. They provide high analytical sensitivity but

accomplish low sampling throughput. Chemiluminescence (CL) flow systems were a better alternative, lucigenin or hexacyanoferrate [180], bromine [181], $[Cu(HIO_6)_2]^{5-}$ [182] and silver(II) ion [183]were used as oxidizing agents. In these CL systems, some of the oxidants are unstable, they are so strong that they can oxidize a large number of inorganic and organic substances, expensive and the oxidizing reagents used are highly toxic.

1.8 Cough and Cold Drugs [184,185]

Cough and cold combinations are used mainly to relieve the cough due to colds, influenza, or hay fever. They are not to be used for the chronic cough that occurs with smoking, asthma, or emphysema or when there is an unusually large amount of mucus or phlegm with the cough. Cough and cold combination products contain more than one ingredient. For example, some products may contain an antihistamine, a decongestant, and an analgesic, in addition to a medicine for coughing. Since different products contain ingredients that will have different precautions and side effects, it is important that the patients should know the ingredients of the medicine they are taking. The different kinds of ingredients that may be found in cough/cold combinations include:

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1.8.1 Antihistamines

Antihistamines are used to relieve or prevent the symptoms of hay fever and other types of allergy. They also help relieve some symptoms of the common cold, such as sneezing and runny nose. They work by preventing the effects of a substance called histamine, which is produced by the body. Some examples of antihistamines

contained in these combinations are: bromodiphenhydramine, brompheniramine, carbinoxamine, chlorpheniramine, dexchlorpheniramine, diphenhydramine, doxylamine, phenindamine, pheniramine, phenyltoloxamine, pyrilamine, promethazine, and triprolidine .

1.8.2Decongestants

Decongestants, such as ephedrine, phenylephrine, and pseudoephedrine, produce a narrowing of blood vessels. This leads to clearing of nasal congestion. However, this effect may also increase blood pressure in patients who have high blood pressure.

1.8.3Antitussives

To help relieve coughing these combinations contain either a narcotic [codeine, dihydrocodeine, hydrocodone or hydromorphone] or a nonnarcotic [carbetapentane, caramiphen, or dextromethorphan] antitussive. These antitussives act directly on the cough center in the brain. Narcotics may become habit-forming, causing mental or physical dependence, if used for a long time. Physical dependence may lead to withdrawal side effects when stop taking the medicine.

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1.8.4Expectorants

Guaifenesin works by loosening the mucus or phlegm in the lungs. Other ingredients added as expectorants (for example, ammonium chloride, calcium iodide, iodinated glycerol, ipecac, potassium guaiacolsulfonate, potassium iodide, and sodium citrate) have not been proven to be effective.

1.8.5 Analgesics

Analgesics, such as acetaminophen, aspirin, and other salicylates [such as salicylamide and sodium salicylate] are used in these combination medicines to help relieve the aches and pain that may occur with the common cold. The use of too much acetaminophen and salicylates at the same time may cause kidney damage or cancer of the kidney or urinary bladder. This may occur if large amounts of both medicines are taken together for a long time. However, taking the recommended amounts of combination medicines that contain both acetaminophen and a salicylate for short periods of time has not been shown to cause these unwanted effects.

1.8.6 Anticholinergics

Anticholinergics such as homatropine may help produce a drying effect in the nose and chest. Some of these combinations are available only with the doctor's prescription. Others are available without a prescription; however, a health care professional may have special instructions on the proper dose of the medicine for patient medical condition.

1.8.7 Determination of Some Cold-Cough Drugs

In this work, acetaminophen, guaifenesin, phenylephrine , pseudoehedrine hydrochloride, and phenylpropanolamine hydrochloride are discussed. Their structure are presented below.

Acetaminophen (ACT) is an analgesic and antipyretic (lowers fever). It works by lowering a chemical in the brain that stimulates pain nerves and the heat-regulating center in the brain. It is used for treatment of minor aches and pains due to headache,

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muscle aches, backache, arthritis, the common cold, flu, toothache, menstrual cramps, and immunizations, and for the temporary reduction of fever.

Guaifenesin (GUA) is an expectorant. It is loosens phlegm and increases the lubrication of your lungs allowing for a productive cough and decreased chest congestion. It is also used to reduce chest congestion caused by the common cold infections, or allergies.

Phenylephrine (PE) or Neo-Synephrine is an α-adrenergic receptor agonist used primarily as a decongestant, available as an oral medicine or as a nasal spray., as an agent to dilate the pupil and to increase blood pressure. Phenylephrine has recently been marketed as a substitute for pseudoephedrine, (e.g. Pfizer's *Sudafed (Original Formulation)*) but there are recent claims that oral phenylephrine may be no more effective as a decongestant than placebo.

Pseudoehedrine hydrochloride (PSE) is a decongestant. It works by constricting (shrinking) blood vessels (veins and arteries) in the nose, lungs, and other mucus membranes. By decreasing blood flow to the nose, lungs, and other areas, pseudoephedrine decreases congestion, and airways are opened up. It is also used to treat congestion associated with allergies, hay fever, sinus irritation, and the common cold.

Phenylpropanolamine hydrochloride (PPA) is the same group of PSE. PPA is an ingredient found in many OTC and prescription cold and cough medicines, nasal decongestants, appetite suppressant and weight loss products. However, the U.S. Food and Drug Administration (FDA) took the step to remove PPA from all drug products since 2000, due to the report by scientist at Yale University School of Medicine [186], in " Phenylpropanolamine and Risk of Hemorrhagic Stroke Project". This study

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reported that taking PPA increases the risk of hemorrhagic stroke. Nowadays, in Thailand, PPA has also been banned in drug product since 2001 by FDA.

There are a number of works describing in the determination of active ingredients in cough and cold drugs, multicomponent drugs, in which the common techniques are HPLC and derivative spectrophotometry as reviewed in Table 1.7.

Table 1.7 Determination of active ingredients in cold preparations

Drugs	Sample	Method	Linear range μg ml^{-1}	LOD μg ml^{-1}	Tr min	Ref.
ACM PSE DMP CPM	Tablet	CE with electrochemical detection Electrolyte : tris-H_3BO_3 (+27 kv)	0.5 – 200 8 – 300 2.5 – 350 0.5 - 330	0.1 0.55 1 0.2	<12	[187]
PNE PSE PPA CPM DCPM	Tablet, capsule Powder, injection Syrup (single and binary mixture)	MLC C18, 120 x 4.6 mm 0.15 M SDS – 6% pentanol (pH 7)	05 – 30 0.5 – 50 0.5 – 50 0.5 – 30 0.5 – 30	0.02 0.2 0.2 0.01 0.01	3.8 4.6 5.7 6.3 6.3	[188]
ACM PSE CPM	Tablet	HPLC C18, 150 x 4 mm (205 nm) 25% CH_3OH in 0.05 M NaH_2PO_4	-	0.15 1.82 1.16	-	[189]
PPA GPN DPP	Syrup	HPLC C8, 250 x 4.6 mm CH_3CN:triethylamine 35:65, pH 3.5	10 – 30 50 – 150 1.5 – 4.5	0.010 0.025 0.025	2.17 3.56 7.37	[190]
DBPM PSE LRD	Binary mixture Tablet	HPLC C18, 200 x 4.6 mm 0.01 M $(NH_4)_2PO_4$ in CH_3OH : H_2O pH 8.3	10 – 175 225 – 3500 5 - 40	-	4.2 1.0 -	[191]
DMP ACB CAF ACM	Sachet	HPLC Tetrabutylammonium, hydrogen sulfate, pentane sulfonic acid	-	-	-	[192]

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1.9 Research Aims

This research is focused on the development of analytical methods, including flow injection analysis, sequential injection analysis and micellar liquid chromatography for the determination of some metal ions and some pharmaceuticals. The objectives of this research work are as follows:

1. To design and fabricate a more environmental friendly SI system to reduce chemical/sample consumption and waste release

2. To design the MLC procedure for the simultaneous determination of some pharmaceutical preparations

3. To apply the home-made SI analyzer and MLC for determining some metal ions and some certain drugs in pharmaceutical samples

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