Diffusion of Choline Chloride in Aqueous Solutions of Chondroitin Sulfate

Ok Sun Jung and Si-Joong Kim

Department of Chemistry, Korea University, Seoul 135, Korea

Hyoungman Kim†

Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology, P.O. Box 150 Chongyangni, Seoul 131, Korea *(Received July 28, 1984)*

Mutual diffusion coefficients of choline chloride were determined by using the diaphragm cell method in aqueous solutions of chondroitin sulfate A at 25°C. The diffusion coefficients of choline chloride in 0.1g/100m*l*, 0.5g/100ml and 1g/100m*l* respectively of chondroitin sulfate solutions were compared with those of binary systems of water–choline chloride. At low concentrations, the diffusion coefficients of the choline chloride in the presence of chondroitin sulfate were significantly smaller than the values obtained in the absence of chondroitin sulfate, indicating a strong interaction between these solutes. The effect of this interaction on the diffusion of choline ion is largest at higher chondroitin sulfate concentrations and at lower choline chloride concentrations. The influence of chondroitin sulfate is overcome at higher choline chloride concentrations. Self-diffusion coefficients of choline ion in the presence of chondroitin sulfate are also obtained. Excellent agreements were obtained between the experimental data and the calculated values obtained by using the Manning's equations. These observations suggest that the interaction between choline chloride and chondroitin sulfate involves primarily a long range electrostatic effect and there is no appreciable "condensation" or binding of choline ion to the chondroitin sulfate.

Introduction

Diffusion studies of choline chloride in the presence of other components in aqueous solutions is of interest because of the importance of this electrolyte in certain physiological functions. Detailed studies were made for the systems H_2O–choline chloride–KCl[1] and H_2O–choline chloride–HCl[2]. The work by Jordan and Kim[2] was an extension of the earlier studies on ternary diffusion of electrolytes including acids[3,4], and one of its objectives was to see whether the negative value of the cross-term diffusion coefficient of choline chloride in the presence of hydrogen chloride concentration gradient is large enough to suggest any influence of acetic acid on the retardation of the diffusional outflow of choline ion from the synaptic gap after the acetyl choline is hydrolysed. It was concluded that the size of the cross-term diffusion coefficient is too small to have this effect. Since, however, there is a high concentration of polyanionic glycosaminoglycans, such as chondroitin sulfate, within the synaptic gap[5] it is of interest to study the effect of these polyanions on the diffusion of choline ions. In the present study, the diffusion coefficients of choline chloride in chondroitin sulfate solutions were determined by using the diaphragm cell method. Although this is really a 4-component system, it is assumed that the diffusion of chondroitin sulfate in the absence of its own initial concentration gradient is negligible. Thus the chondroitin sulfate solution itself is assumed to be the solvent and the diffusing systems are regarded as binary systems.

Experimental

Diffusion Experiments. Stokes type diaphragm cells were fabricated by joining a Corning 40F sintered glass (40mm dia., 4 mm thick) as the diaphragm with a glass tubing (40 mm inner dia.) for each of the cells. The volumes of the upper and lower compartments were about 40 m*l*. The stirrers are made of soft iron wires sealed into glass envelopes. The solutions in the upper and lower compartments were stirred with these bars by rotaing a magnetic assembly similar to the one described by Mills and Woolf[7]. The diffusion experiments were performed in a water bath which was maintained at 25 ± 0.01°C. The magnetic assembly was rotated at a speed of 50–60 rpm. The general experimental procedure was already given by Stokes and Dunlop[8].

Chemicals. The KCl (Merck, extra pure) for calibrating the cell was recrystallized twice from distilled water and fused in a platimum dish. Choline chloride (Wako Chemical, extra pure) was purified according to the procedure described by Fleming and Gosting[1]. The four-times recrystallized choline chloride was dried in a vacuum oven and then in a desiccator over P_2O_5. This sample was weighed in a glove box.

Sodium chondroitin sulfate A (Sigma) from whale cartilage was used without further purification. The analysis by the Lowry method gave the protein content of less than 0.1 %.

The tritium labeled choline chloride was purchased from the New England Nuclear. Dioxane (Burdick and Jackson, spectroscopic grade), and all other reagent grade chemicals were used without further purification.

Concentration Determinations. KCl solution was titrated potentiometrically by using a silver electrode and this method determines the Cl^- concentration with a precision of 0.5 %.

The concentration of choline chloride was also determined this way.

Determination of the choline chloride during the diffusion process was made by using tritium labeled choline chloride. A Beckman ST3133 liquid scintilation counter was used for obtaining the cpm. The cocktail solution was prepared by

mixing 8g PPO, 0.2g POPOP, 80g naphthalene, 900 m*l* dioxane, 10 m*l* ethylene glycol and 90 m*l* methanol to make up one liter solution. 12 m*l* of cocktail solution was mixed with each 0.1 m*l* of sample solution for scintillation counting. In order to minimize the effect of quenching, the solutions in the lower compartment was diluted so that the cpm values of the diluted lower solution became about the same as those of the upper solutions. The results of triplicate measurements were averaged.

Calibration of the Cells. The cell constants were determined by using KCl solutions since the integral diffusion coefficient of this solution is well known. The necessary equation is

$$\bar{D}=\frac{1}{\beta_e t}\ln\left(\frac{C_B^0-C_T^0}{C_B-C_T}\right)$$

where $\bar{D}$ is the integral diffusion coefficient, C^0 is the initial solute concentration, and C is the concentration after the diffusion period t is elapsed. The β_e is the cell constant defined as

$$\beta_e=\frac{A}{l}\left(\frac{1}{V_B}+\frac{1}{V_T}\right)$$

where A and l are the effective area and length of the diaphragm, respectively and V are the volumes of the respective comparments. The initial concentration of the upper compartment, C_T^0, is usually zero and that of the lower compartment can be calculated by

$$C_B^0=C_B+C_T\left(\frac{V_T+{}^1/_2V_D}{V_B+{}^1/_2V_D}\right)$$

In the above equations, the subscripts, B, T and D represent the lower compatrment, upper compartment and the diaphragm, respectively.

Calculation of Diffusion Coefficients. The calculation of the differential diffusion coefficients were obtained from experimentally obtained integral diffusion coefficients for the case of non-linear concentration dependency of D. The procedure of the necessary calculation was given in detail by Mills and Woolf[7].

Results and Discussion

Table 1 gives the integral diffusion coefficients obtained for H_2O-choline chloride system at 25°C. The differential diffusion coefficients calculated from these data are plotted against the square root of the concentration in Figure 1. The mutual diffusion coefficients obtained by Fleming and Gosting[1] are also represented for comparison in this diagram. The curves are extrapolated to the calculated value at infinite dilution using the limiting ionic mobilities. The deviation of the present values and those obtained by Flemind and Gosting[1] from a Gouy optical diffusiometer is within about 0.1 % at low concentrations. At higher concentrations, however, the deviation increases with increasing concentration reaching to about 2 % at the miximum concentration studied.

Three sets of diffusion coefficients of choline chloride in chondroitin sulfate solutions were obtained at three different concentrations of the macromolecular component. Table 2, 3 and 4 give the experimental integral diffusion coefficients of choline chloride in 0.1g/100 m*l*, 0.5g/100 m*l* and 1 g/100 m*l* chondroitin sulfate solutions, respestively. These values are plotted in Figure 2 and compared with the integral diffusion coefficients of choline chloride in water. It can be seen that the diffusion coefficients of choline chloride is primarily dependent on the choline chloride to chondroitin sulfate concentration ratio; the smaller the ratio, the smaller the diffusion coefficient of the choline chloride. Although an increase in the viscosity may cause a decrease in the diffusion coefficient to some degree, it can be explained qualitatively in terms only of the primary electrostatic effect.

The diffusional movement of choline in this complicated system is determined not only by its own concentration but

TABLE 1: Integral Diffusion Coefficients of Choline Chloride in Water at 25 °C

C_B^0 (mole/*l*)*	$\bar{D}_{obs}\times10^5$ (cm²/sec)
0.0331	1.296
0.0454	1.276
0.0780	1.253
0.1169	1.240
0.2201	1.181
0.3982	1.154

*C_T^0 is zero in all cases.

TABLE 2: Integral Diffusion Coefficients of Choline Chloride in 0.1 g/100 m*l* Chondroitin Sulfate Solution

C_B^0 (mole/*l*)	$\bar{D}_{obs}\times10^5$ (cm²/sec)
0.0291	1.18_2
0.0359	1.18_5
0.0852	1.17_8
0.1470	1.17_3

TABLE 3: Integral Diffusion Coefficients of Choline Chloride in 0.5 g/100 m*l* Chondroitin Sulfate Solution

C_B^0 (mole/*l*)	$\bar{D}_{obs}\times10^5$ (cm²/sec)
0.0158	0.896
0.0255	0.921
0.0285	0.879
0.0717	1.02
0.1195	1.16
0.1478	1.17
0.1946	1.19
0.3664	1.13

TABLE 4: Integral Diffusion Coefficients of Choline Chloride in 1.0 g/100 m*l* Chondroitin Sufate Solution

C_B^0 (mole/*l*)	$\bar{D}_{obs}\times10^5$ (cm²/sec)
0.0169	0.722
0.0169	0.635
0.0361	0.814
0.0512	0.787
0.0511	0.751
0.1099	0.926
0.1804	0.967
0.3140	1.12

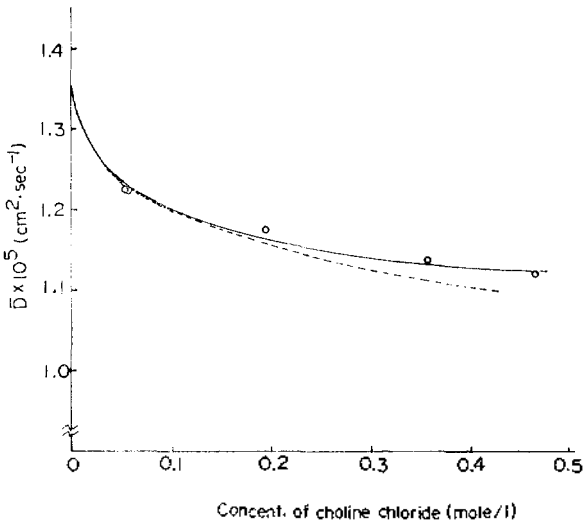

Figure 1. The mutual diffusion coeffcients of choline chloride in water at 25 °C. differential ∘, from Fleming and Gosting. ·····, diffusion coefficients obtained here.

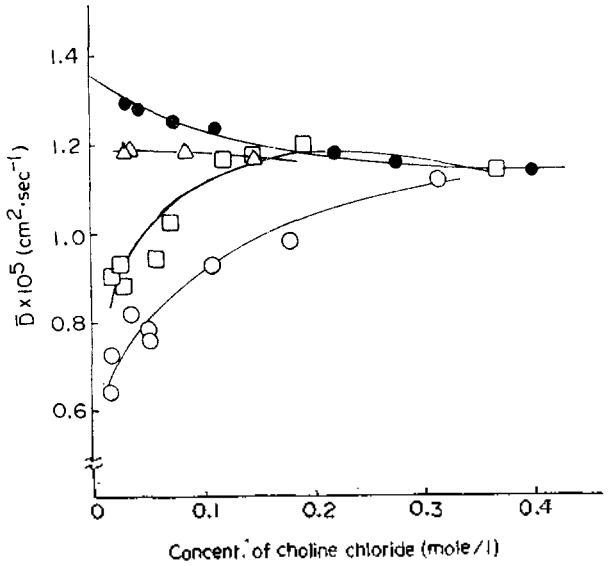

Figure 2. Integral diffusion coefficients of choline chloride in chondroitin sulfate solutions. ●, 0.0 g/100 m*l* chondroitin sulfate; △, 0.1 g/100 m*l*; □, 0.5 g/100 m*l*; 0, 1.0 g/100 m*l*.

also by those of other ions present. When choline chloride alone is diffusing, the overall diffusion coefficient is decided by the average mobility of choline ion and chlorde ion, That is, choline ion is "pulled" by the fast moving chloride ion and, in doing so, chloride ion is slowed down. When sodium chondroitin sulfate is present uniformely in this solution, choline and chloride ions no longer are required to be transported together to satisfy the macroscopic electroneutrality. The Na^+ and choline ion can simply "ion exchange" and a part of choline ion can stay with chondroitin sulfate as the counter ion. When the diffusion of choline ion is determined, the measured value represents an average of the counter ion and the species traveling together with the cholride, As the

TABLE 5: Comparison of Self-Diffusion Coefficients of Choline Chloride in 0.5 g/100 m*l* Chondroitin Sulfate Solutions with Those of Calculated Values Using Manning's Equations

Conc. (mole/*l*)	$D_{obs} \times 10^5$ (cm^2/sec)	$D_{cal} \times 10^5$ (cm^2/sec)	
0.0881	0.937	0.932[a]	0.932[b]
0.0921	0.951	0.938	0.934
0.1109	0.945	0.953	0.943
0.1376	0.952	0.962	0.952
0.1548	0.960	0.969	0.955

[a] Equs. (4) and (5); [b] Equs. (2) and (3).

concentration of the chondroitin sulfate increases, more choline ion will stay with the polyanion and the diffusion coefficient of the choline ion will approach zero as the chondroitin sulfate is expected to be stationary under the experimental conditions. Since the diffusion coefficient cannot be obtained at extreme dilution with the present method, this is not apparent in Figure 2.

As the ratio of concenrtation of choline chloride to that of chondroitin sulfate increases, however, the effect of the polyelectrolyte diminishes and the diffusion coefficient of choline chloride approaches the value without the polyelectrolyte present. That the choline chloride concentration which is needed to bring up the D value in the presence of the chondroitin sulfate to the values of the binary diffusion coefficient is roughly proportional to the latter's concentration can readily be seen in Figure 2.

Table 5 presents the self-diffusion coefficient of the choline chloride in 0.5 g/100 m*l* chondroitin sulfate solutions. Figure 3 compares these self-diffusion coefficients wtih the integral diffusion coefficients of choline chloride in 0.5 g/100 m*l* chondroitin sulfate solution. Two features stand out in this figure. First, the mutual diffusion coefficient increases with increasing concentration faster than the self-diffusion coefficient. This may be simply the results of "pulling" effect of chloride ion for the integral diffusion coefficient while this effect is absent from the self-diffusion coefficients, Second, both diffusion coefficients converge into a single low value at infinite dilution.

Manning developed a theory of self-diffusion of cations in polyelectrolyte solutions[9,10] and it will be of interest to compare the experimental values with those predicted by Manning's equations. According to this theory, the self-diffusion coefficient of a monovalent cation in the presence of an anionic linear polyelectrolyte is expessed by

$$D_i = (1+X^{-1})^{-1}[D_i^{(c)}(1-\xi^{-1}) + D_i^{(p)}(\xi^{-1}+X^{-1})]$$

where

$$X = n_e/n_s$$
$$\xi = e^2/\epsilon kTb$$

and

$$b = L/P$$

In these equations, $D_i^{(c)}$ is the self-diffusion coefficient of "condensed" cation which corresponds to the diffusion coefficient of the polyelectrolyte, $D_i^{(p)}$ is the self-diffusion coefficient of uncondensed cation, n_e and n_s represent the equivalent polyanion concentration and the concentration

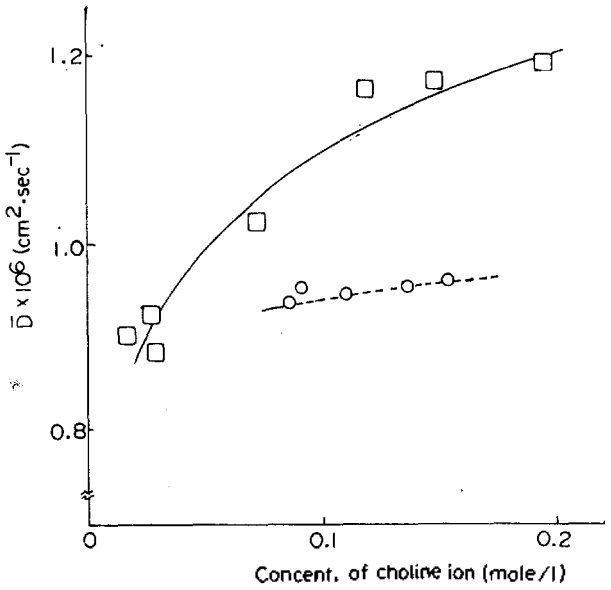

Figure 3. Mutual and self-diffusion coefficients of choline in 0.5 g/100 ml chondroitin sulfate solution. □, mutual diffusion coefficients; ○, self diffusion coefficients.

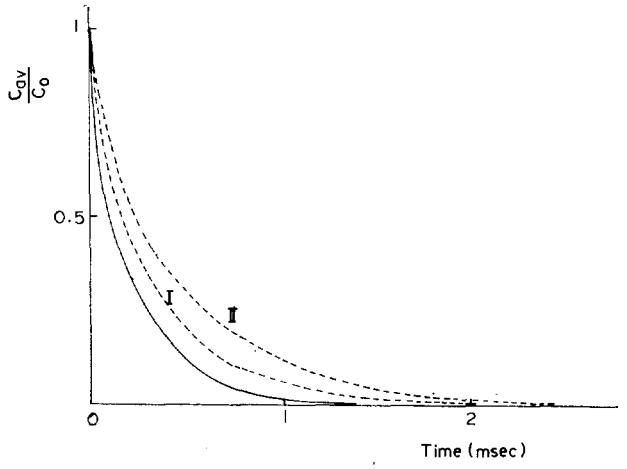

Figure 4. The ratio of the average concentration, C_{av}, to the initial concentration, C_0, of choline chloride in the synaptic gap plitted against time. C_0=0.01 *M*, solid line, 0.0 g/100 ml chondroinit sulfate; I, 0.5 g/100 ml; II, 1.0 g/100 ml.

of the added univalent electrolyte, respectively, e is the elementary charge, ε is the dielectric constant of the solvent, kT is the Boltzman factor, L is the total length of the polyelectrolyte, and P is the total unmber of charged groups in the polyelectrolyte.

It is seen that the self-diffusion coefficient of a cation in the presence of a polyanoin is the average of the diffusion coefficient of condensed and uncondensed species. Since the diffusion coefficient of polyanion is very small compared to that of uncondensed cation, one can approximate

$$D_i^{(c)}=0$$

while

$$D_i^{(u)}=D_i^0\left[1-\frac{1}{3}A(1,\xi^{-1}X)\right] \quad (1)$$

where D_i^0 is the self-diffusion coefficient of the cation at infinite dilution and

$$A=(\xi, X)=\sum_{m_1=-\infty}^{\infty}\sum_{m_2=-\infty}^{\infty}[\pi\xi^{-1}(m_1^2+m_2^2)+1+2X^{-1}]^{-2} \quad (2)$$
$$(m_1, m_2\neq 0, 0)$$

From these equations, manning obtained[10]

$$D_i/D_i^0=(\xi^{-1}X+1)(X+1)^{-1}\times\left[1-\frac{1}{3}A(1;\xi^{-1}X)\right],\ \xi>1 \quad (3)$$

For the case of small X values, eq. (2) may be simplified to

$$A(\xi, X)=\frac{1}{2}\xi X+0(X^2) \quad (4)$$

which can be introduced into eq. (1) to obtain

$$D_i/D_i^0=1-[1-(5/6)\xi^{-1}]X+0(X^2) \quad (5)$$

The calculated self-diffusion coefficients obtained by using eq. (5) are given in the 3rd column of Table 5 and the values calculated by using eqs. (2) and (3) are given in the lats column of the same Table. In these calculations, the value of the charge density parameter, ξ, is taken as 1.28, which was empirically obtained from self-diffusion experiments of Na^+ in the presence of chondroitin sulfate[11]. The agreement between the experimental and calculated values using either the general expession or the case of small X values are equally good within the experimental errors. Similar results were obteained for self-diffusion of Na^+ ion in the presence of chondroitin sulfate[11]. These suggest that the monovalent cations, unlike the cases of divalent cations, do not "condense" to chondroitin sulfate and the long range eletrostatic effect dictates the diffusion behvaior of monovalent cations.

An important yet unresolved question is what portion of choline ions, produced within the cholinergic synapse and neuromuscular junction by the hydrolysis of acetylcholine during the synaptic transmission, is re-absorbed by the high affinity uptake system in the presynaptic membrane[12-14]. Eccles and Jaeger[15] calculated the rate of acetylcholine depletion from a synaptic gap by assuming that the gap is a cylinder with 2μ diameter and that the acetylcholine diffuses radially into a infinite sink surrounding the gap and found that the diffusional outflow to be very fast. According to their calculations, the depletion was complete within a millisecond. By using their calculation procedure, a similar result was obtained for the case of choline ion depletion from the synaptic gap. Figure 4 gives the concentration decay profile of choline chloride with and without the presence of chondroitin sulfate. It can be seen that the presence of chondroitin sulfate slows down the depletion rate somewhat but its effect on the overall economy of choline cycle is not clear.

The most quantitative choline uptake rate by the presynaptic membrane to date was obtained by Rothlein and Parson[14]. This uptake experiment with *T*-sac from *Torpedo californica* gave a value of V_{max} of 1.5×10^{-13} mole/sec/mg protein at 25°C. Assuming a 50 % protein content, the presynaptic membrane will have 2.46×10^{-11} mg of protein which means the maximum uptake rate per synaps is 3.69

$\times 10^{-24}$ mole/sec. If 0.01 mole/l concentration of choline is initially formed in the synaptic gap during one cycle of nerve impulse transmission, the number of moles of choline wtihin the gap is 1.57×10^{-17}, if the dimension of the gap is assumed to be 2μ in diameter and 500A in width. Even with within the maximum rate of 3.69×10^{24} mole/sec., it will take 4.25×10^{6} sec. to take up all the molecules of choline ion. This means that the rate of diffusional outflow of choline is much larger than the uptake rate and there will be practically no choline uptake. This situation is, of course, untenable and it is obvious that a more accurate choline uptake rate should be obtained experimentally in order to be able to assess the role of chondroitin sulfate within the synaptic gap in affecting the uptake efficiency of choline.

Acknowledgement. The financial support of the Korea Science and Engineering Foundation is gratefully appreciated.

References

(1) R. Fleming and L. J. Gosting, *J. Phys. Chem.*, **77**, 2371 (1973).
(2) M. Jordan and H. Kim, *J. Solution Chem.*, **11**, 347 (1982).
(3) H. Kim, G. Reinfelds and L. J. Gosting, *J. Phys. Chem.*, **77**, 934 (1973).
(4) H. Kim, R. C. Deonier and G. Reinfelds, *J. Solution Chem.* **3**, 934 (1974).
(5) See for example, S. Bon, J. Cartand and J. Mussoulie, *Eur. J. Bicohem.*, **85**, 1 (1978).
(6) R. H. Stokes, *J. Amer. Chem. Soc.*, **72**, 763 (1950).
(7) R. Mills and L. A. Woolf, "Diaphragm Cells", The Australian National University, 1968.
(8) R. H. Stokes and P. J. Dunlop, *J. Amer. Chem. Soc.*, **73**, 5456 (1951).
(9) G. S. Manning, *J. Chem. Phys.*, **51**, 924 (1969).
(10) G. S. Manning, *J. Chem. Phys.*, **51**, 934 (1969).
(11) J. Madgelenat, P. Turqu and M. Chemla, *Biopolymers*, **13**, 1533 (1974).
(12) H. I. Yamamura and S. H. Snyder, *J. Neurochem.*, **21**, 1355 (1973).
(13) M. J. Kuhar and L. C. Murrin, *J. Neurochem.*, **30**, 15 (1978).
(14) J. E. Rothlein and S. M. Parsons, *J. Neurochem.*, **33**, 1189 (1979).
(15) J. C. Eccels and J. C. Jaeger, *Proc. Royal Soc.* **B148**, 38 (1958).

Microprocessor Based Laser Induced Fluorometry
I. Development of System and Application to Liquid Chromatography

Hasuck Kim,† Chanseung Park, Euijin Hwang and O. Won Choi

Depatment of Chemistry, Seoul National University, Seoul 151, Korea *(Received July 31, 1984)*

An analytical applicability of the fluorescence detection with an optical multichannel analyzer to organic dyes was studied in this work. Continuous acquisition of spectra was possible with the use of a microcomputer. The maximum acquisition rate of a spectrum with 70-point average was about 3 seconds. Floppy discs were used to store data for later use in processing. Laser induced fluorescence detector in High Performance Liquid Chromatography was chosen for an application. Fluorescein below 10^{-15}g was detected satisfactorily using this system. With the help of microcomputer, three dimensional chromatograms of time-wavelength-intensity were obtained.

Introduction

Optical Multichannel Analyzer (OMA) which is consisted of a parallel optoelectronic image detector array and a digital signal accumulation system has merits of both spectrograph and spectrometer. So, its use in spectroscopy has been increased over the past several years[1-6].

In fluorescence spectroscopy, although a large variety of spectrofluorometers are commercially available now, the detection system used is entirely based on the use of photomultiplier tube (PMT) .The use of OMA as fluorescence detector is superior to the conventional detector because of its ability of recording entire spectrum simultaneously even though it has lower light detectability[3]. Therefore, it has unique applicabilities to transient spectrofluorometry, such as real time peak detection in liquid chromatography or other time resolved spectroscopic studies in kinetic application.

In chromatographic application, it gives the combination of coventional separation and molecular fingerprinting technique, and it offers the most powerful means of analyzing the complex material[7]. In the case where the components to be analyzed lack sufficient volatility for gas chromatography, liquid chromatography combined with fingerprint technique such as mass spectrometry or rapid scanning UV-Vis absorption spectroscopy offers a path to analysis[8]. But difficulties arise in the corresponding interface to a mass spectrometer and poor sensitivity of UV-Vis absorption spectroscopy, OMA detection of fluorescence spectra in