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Optimization of Wave Forms for Pulsed Amperometric Detection of Cyanide and Sulfide with Silver-Working Electrode

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A continuous potential pulse is applied to a silver-working electrode on a pulsed amperometric detector (PAD) for detection of free cyanide and sulfide. The moving phase is 0.1 M sodium hydroxide, 0.5 M sodium acetate and 5% (v/v) ethylenediamine mixture, and the flow rate is 0.7 mL/min. Optimized pulse conditions include a −200 mV (*vs.* Ag/AgCl reference electrode) detection potential (E_d) for 60 msec and 50 mV cleaning potential (E_c) for 120 msec. The silver working electrode surface is not poisoned by cyanide or sulfide, and the PAD maintains long-term stability without loss of sensitivity and reproducibility at these pulse conditions. The detection limit of cyanide and sulfide separated by ion chromatography using an anion exchange column is 0.1 ppm and 0.05 ppm, respectively.

Introduction

The analysis of cyanide and sulfide which are fatal to humans at low concentration levels is very important for forensic or environmental chemistry. These ions can be easily separated from common anions by ion chromatography using an anion exchange column. However, unlike common mineral acid anions, they are barely detected by a suppressed conductivity detector because very weak acidic species, HCN and H_2S, that exhibit low conductivity are formed in an anion suppressor column.

Argentometric or iodometric titration, potentiometry,[1,2] spectrophotometry,[3,4] gas chromatography[5,6] and voltammetry[7] have been proposed for determining cyanide and sulfide. However titration method is interfered by co-existing ions, and gas chromatography method is time consuming.

The detection method employing single potential amperometry for a flowing liquid system is easy to handle and shows excellent sensitivity. It has been widely used for the determination of various electroactive species at very low concentrations.[8~10] However, certain samples having contact with the electrode surface (especially, carbohydrates, proteins or alcohols) can deposit on the electrode surface and alter the surface characteristics of the electrode materials.[11] These poisoned electrode surfaces should be cleaned periodically with a mechanical or electrochemical method for a stable and reproducible analysis.

Whereas single potential amperometry applies single potential and measures the constant current, pulsed amperometry applies multiple potentials (detection, cleaning and conditioning pulses) with a repeating sequence and measures the current only during a short sampling interval.[12,13] Successive appropriately positive or negative potential pulses remove reaction products that would cause the electrode fouling. The long-term stability and outstanding reproducibility of pulsed amperometric detector (PAD) makes it one of the most widely interfaced detectors for liquid chromatography systems.[14,15]

The first amperometric analysis of free cyanide that forms a soluble complex with a silver electrode[16] was accomplished by Phillar *et al.*[17,18] and followed by Koch.[19] Phillar *et al.* applied a continuous −0.5 V (*vs.* Ag/AgCl) detection potential to the cylinder type silver working electrode located in the carrier flow path. The main conclusion of their work was that the silver working electrode surface was not poisoned by cyanide because cyanide forms a soluble complex with the silver electrode. However, an excessive amount of sulfide or strong oxidants existing with cyanide may poison the silver electrode.

Rocklin and Johnson also analyzed cyanide and sulfide ions with a silver working electrode[20] appling a continuous 0.0 mV detection potential *vs.* the Ag/AgCl reference electrode. They concluded that cyanide and sulfide do not poison the silver electrode in spite of the fact that sulfide deposits onto the silver electrode as Ag_2S.

The Dionex Corporation (USA) which is the manufacturer of the PAD used by the author recommended an optimum potential wave form for cyanide and sulfide analysis, that is, 0.0 mV of detection potential (E_d) *vs.* the Ag/AgCl reference electrode for 60 msec without any cleaning pulse. However, the authors found that this pulse form was satisfactory only for a short-term analysis of cyanide or sulfide. After approximately a half a day of continuous analysis of cyanide or sulfide, if a mechanical or electrochemical regeneration processes were not employed, the electrode surface was darkened, and the electrode characteristics were altered. Effects include increased noise, reduced response and reduced reproducibility. At this point, the black film on the electrode surface should be removed by polishing with alumina for regenerating the sensitivity of the electrode.

In the present paper a new amperometric pulse form is reported that has been shown to allow stable and reproducible continuous flow injection analysis, and to be free from electrode fouling without the need for mechanical polishing.

Experimental

Apparatus. All current measurements were performed with a Dionex flow through-cell. The working electrode was 1.7 μm silver disc and all potentials were reported with respect to the Ag/AgCl reference electrode. Pt disc was used as a counter electrode. The silver electrode surface was polished with 0.3 μm alumina on microcloth with water as a

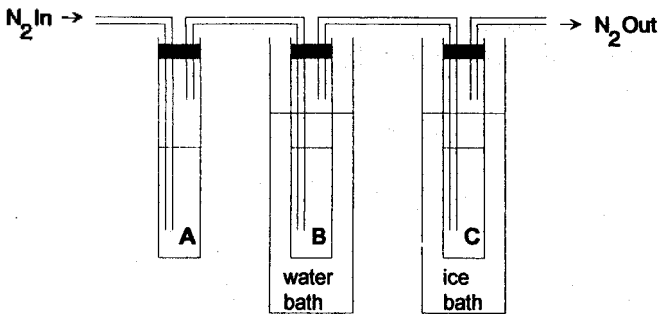

Figure 1. The schematic diagram of a sample aeration apparatus. A: NaOH solution for CO_2 elimination. B: 10%(v/v) sulfuric acid solution. C: 0.1 N NaOH solution.

lubricant prior to use. Potential pulses were generated with a programmable Dionex Ionchrome/Amperometric detector.

The mobile phase was pumped into the flow-through cell with a Dionex 2000I ion chromatography pump system. All measurements were performed at a flow rate of 0.7 mL/min. The samples were introduced into the carrier stream of the IC by means of a 200 μl sample loop injector. Separation of cyanide and sulfide by IC was performed using a Dionex HPIC-AS6 10 μm particle size anion exchange column having a hydrophobic functional group, and 5% cross linkage. Tap water was treated in a serial fashion by passage through a Millipore Milli-RO 60 water purification system.

The blood samples for recovery tests were filtered with Satorius SM 13200 dialysis membrane.

Reagents. All solutions were prepared from reagent grade chemicals that were used as received. Standard solutions containing cyanide or sulfide ion were prepared in 0.1 M sodium hydroxide. Solutions of lower concentration were prepared by serial dilutions with 0.1 M sodium hydroxide, 0.5 M sodium acetate and 5% (v/v) ethylenediamine mixture. Mobile phase was filtered through a 0.3 μm Millipore membrane filter prior to use. Dissolved oxygen was removed by dispersion with N_2 gas and a N_2 atmosphere was maintained over the carrier solutions during the operation. All experiments were performed at room temperature.

Whole blood treatments. For Conway microdiffusion method, a whole blood sample containing cyanide or sulfide is added onto 3 M sulfuric acid solution to release free cyanide and sulfide. Two ml of 0.1 N sodium hydroxide solution is used as an absorbing solution. The diffusion is performed at 50 ℃ for 2 hrs. For dialysis membrane filtration, 5 mL of a whole blood sample is filtered by a Satorius SM 13200 dialysis membrane with a reduced pressure. A simple apparatus, as shown in Figure 1, is used for blood aeration. Five ml of a whole blood sample is placed in 10% (v/v) sulfuric acid solution(B). The temperature is maintained at 60 ℃ by water bath. The solution is purged with N_2 gas passed the CO_2 trap(a) for 40 min with a 1.2 l/min flow rate. Released free cyanide or sulfide is absorbed in a cold 0.1 M NaOH solution(C).

Results and Discussion

PAD wave form optimization. The PAD wave form used in the present experiments is described in Figure 2.

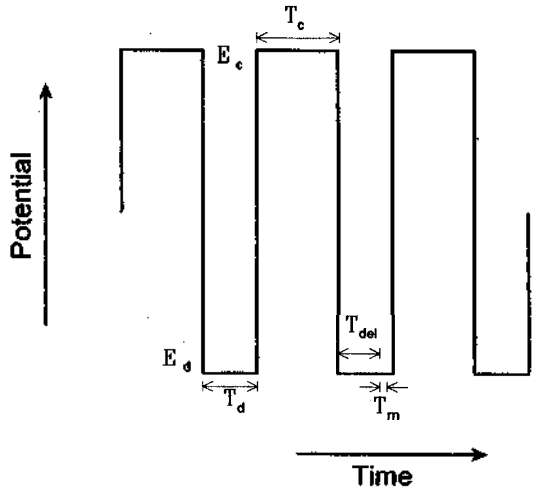

Figure 2. Schematic diagram of wave form used for the pulsed amperometry. E_d denotes a detection potential held for T_d, and E_c denotes a cleaning potential held for T_c. T_m denotes current sampling time fixed to 16.67 msec which begins 20 msec before the end of E_d.

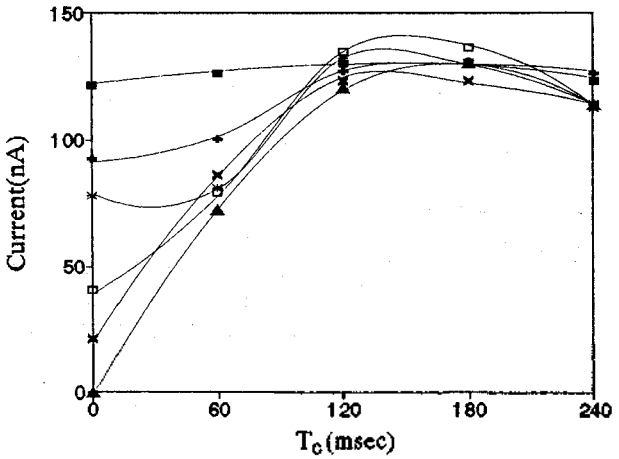

Figure 3. The effect of E_d and T_c on 1 ppm cyanide responses. T_d=60 msec, E_c=50 mV.
−■−: E_d=0 mV, −+−: E_d=−100 mV, −✳−: E_d=−200 mV, −□−: E_d=−300 mV, −×−: E_d=−400 mV, −▲−: E_d=−500 mV.

E_d is the detection potential applied for the time period T_d. A positive cleaning potential (E_c) that removes the oxidizable contaminant on the electrode surface is applied for the time period T_c following E_d. T_m is the current sampling time that begins from 20 msec before the end of T_d and continues for 16.67 msec. T_{del} is time delay before the initiation of T_m. The time delay allows the capacitive charging current to diminish to near zero.

Figure 3 and Figure 4 represent the response variations for 1 ppm cyanide and 1 ppm sulfide according to E_d and T_c variation. T_d is held at 60 msec and E_c is controled at 50 mV. Each marked line represents the current variation as E_d varies from 0 mV to −500 mV. When the cleaning potential is not applied (T_c is 0 msec), the current decreases as E_d increases toward the negative direction. The cyanide

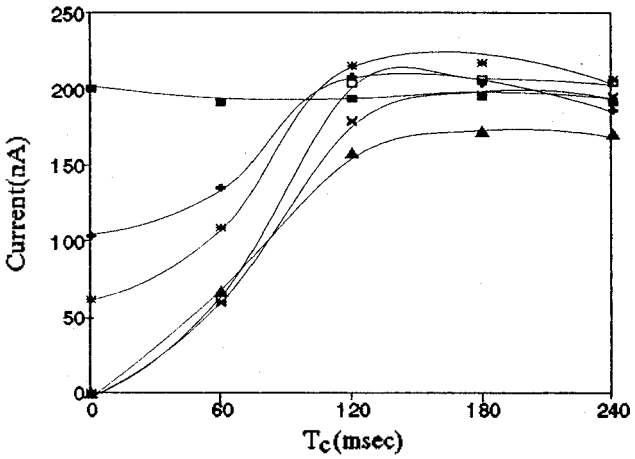

Figure 4. The effect of E_d and T_c on 1 ppm sulfide responses. T_d=60 msec, E_c=50 mV.
−■−: E_d=0 mV, −+−: E_d=−100 mV, −✳−: E_d=−200 mV, −□−: E_d=−300 mV, −×−: E_d=−400 mV, −▲−: E_d=−500 mV.

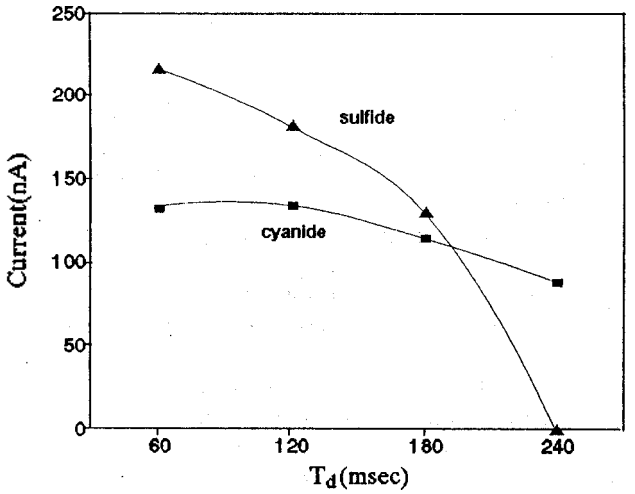

Figure 5. The effect of T_d on cyanide and sulfide responses.

response decreases faster than that of sulfide, and neither ion responds at the silver electrode beyond −500 mV. With 50 mV of cleaning potential, the response of cyanide and sulfide increases as T_c increases until it reaches plateau at 120 msec. However, without the mechanical polishing with alumina, the electrode can be reconditioned only with a 50 mV cleaning pulse of 120 msec duration. This means that cyanide or sulfide poisons the silver electrode surface. This is especially true for sulfide which forms a Ag_2S film.[21]

Figure 5 shows the dependence of the PAD response for cyanide and sulfide on T_d at −200 mV of E_d. E_c is 50 mV and T_c is 120 msec. It shows that the current decreases rapidly with each 60 msec T_d value increment from 60 msec to 240 msec. The response for sulfide diminishes more rapidly than that of cyanide. This fact is attributable to more rapid fouling of the silver electrode surface because the silver sulfide film produced is insoluble, whereas cyanide forms a soluble complex with the silver electrode.

Figure 6 is the response variations of 1 ppm cyanide and 1 ppm sulfide with E_c variation. Negative 200 mV of E_d is

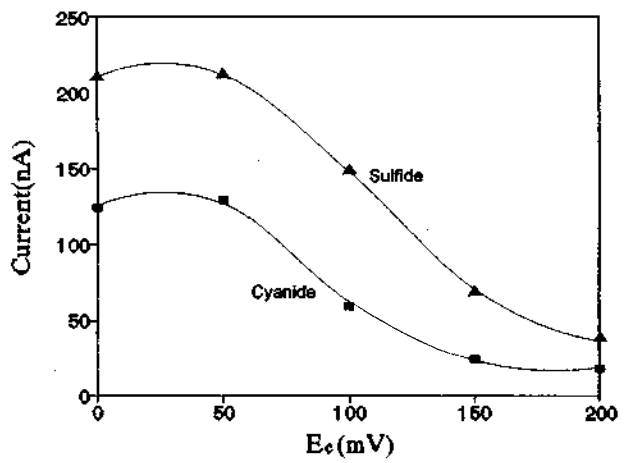

Figure 6. The effect of E_c on cyanide and sulfide response.

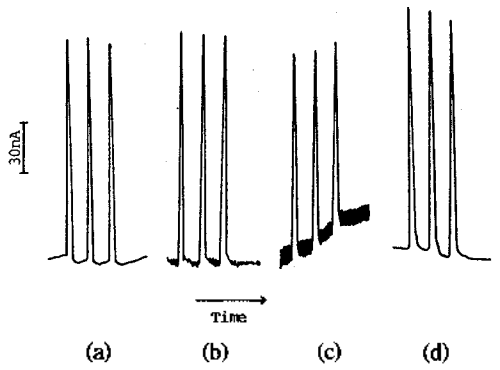

Figure 7. PAD responses for 1 ppm cyanide with two different pulse forms. (a), (b) and (c): E_d=0 mV, T_d=60 msec and T_c=0 msec. (d): E_d=−200 mV, T_d=60 msec, E_c=50 mV and T_c=0 msec.

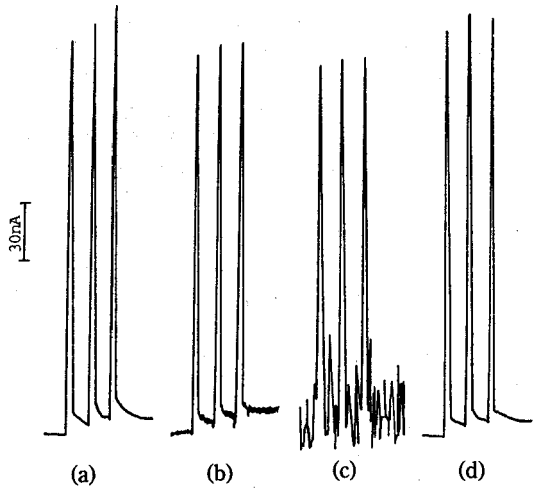

Figure 8. PAD responses for 1 ppm sulfide with two different pulse forms. (a), (b) and (c): E_d=0 mV, T_d=60 msec and T_c=0 msec. (d): E_d=−200 mV, T_d=60 msec, E_c=50 mV and T_c=0 msec.

applied for 60 msec duration, and T_c is fixed to 120 msec throughout these experiments. As shown in Figure 6, the response of either cyanide or sulfide at the silver electrode

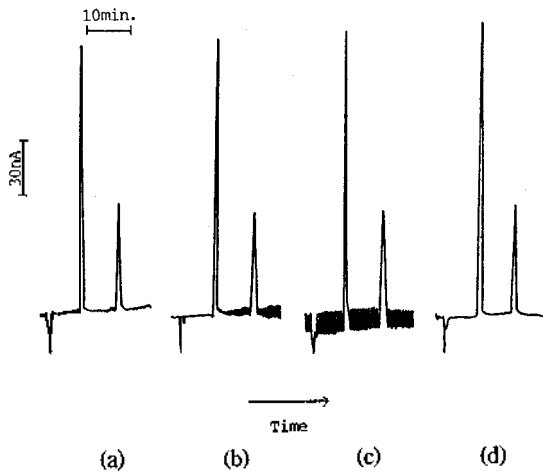

Figure 9. Ion chromatograms of cyanide and sulfide, separated with HPIC AS6 column and detected with PAD. The pulse form of (a), (b) and (c) is the same as that with Figure 7 (a) and (d) is the same as that with Figure 7 (b). (a): the first chromatogram after polishing, (b): the 15th chromatogram after polishing, (c): the 16th chromatogram after polishing, (d): the 17th chromatogram after polishing, cleaning pulse is added to the original pulse.

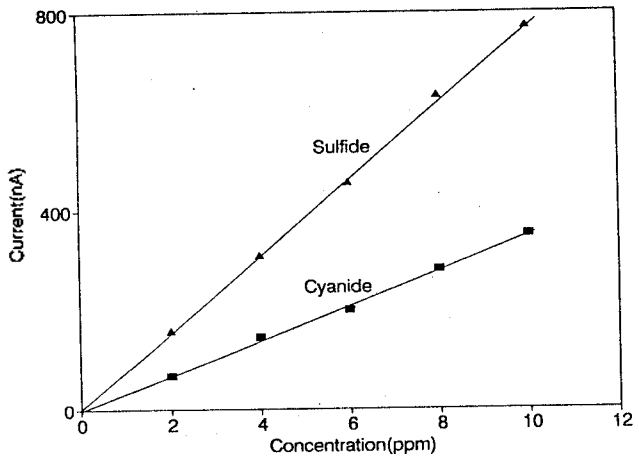

Figure 10. Calibration curves for cyanide and sulfide. Detector pulse conditions are the same as those with Figure 7(d).

is almost constant from 0 mV to 50 mV of cleaning potential, and the response decays beyond 50 mV. The response decrease with increase of the potential E_c is probably due to the interferences of oxide or hydroxide formed at the surface of the silver electrode. The response for sulfide decays more rapidly than that of cyanide.

Flow Injection Analysis. Figure 7 and Figure 8 depict the responses of three consecutive 1 ppm cyanide or sulfide injections, respectively, with a 1 minute interval between injections over a period of continual use employing two different pulse forms. The Dionex recommended pulse form (0 mV of E_d for 60 msec without cleaning pulses) is employed for (a), (b) and (c), and the authors' modified pulse form (-200 mV of E_d for 60 msec, and 50 mV of E_c for 120 msec) is employed for (d). The silver electrode surface was polished with alumina before the (a) injections.

The (a)s having smooth baseline represent the first three injection results. However, after continuous analysis of cyanide or sulfide for approximately 3 hrs, the baseline becomes noisy as shown in (b)s, and this noise becomes larger 4 hrs after an initial injection as shown in (c)s.

The (d)s are cyanide or sulfide responses measured with the author's modified PAD pulse form followed after the (c) analysis without mechanical polishing of the silver electrode surface. As shown in (d)s, the silver electrode surface is regenerated with the modified pulse form with a cleaning pulse added. The sensitivity increases slightly and the baseline noise disappears.

IC analysis. Figure 9 shows the ion chromatograms of 1 ppm cyanide and 1 ppm sulfide obtained with two different pulse forms. These ions are separated by Dionex HPIC-AS6 column with an eluent of 0.1 M sodium hydroxide, 0.5 M sodium acetate and 5% (v/v) ethylenediamine mixture at a flow rate of 0.7 mL/min. The first peak appears at about 10 min, following the negative dip, corresponding to a sulfide peak which exists as SH^- because of high pH, and the second peak that appears at about 17 minutes is a cyanide peak which exists as CN^-.

The (a) is an ion chromatogram obtained with the Dionex recommended pulse form, at a newly polished electrode. The figure shows that both ionic species can be determined with stable baselines, as indicated in Figures 7 and Figure 8. The (b) is the 15th ion chromatogram for the same sample solution. All the chromatographic conditions including the pulse form are the same as in (a). As shown in the figures, the peak intensities are the same with that of the first injection but the baseline noise increases after the sulfide elution. The (c) is the 16th determination of both ions without changing any of the experimental conditions. It shows that the silver electrode surface was not regenerated with the elapse of time. The number of injections at which the baseline noise appears would varies depending on the concentration of analyte, injection interval, solvent and reagents purity, etc.

The (d) is the 17th determination of both ions after changing pulse conditions suggested by the authors and without alumina polishing for regeneration of the silver electrode. When the new cleaning step was added to the pulse form, the baseline of ion chromatogram became smooth, while the response remains constant. According to these results, it can be seen that the silver electrode surface is poisoned by long-term cyanide or sulfide analysis without a loss of signal response to 1 ppm concentrations. However, introduction of a 50 mV of cleaning potential for 120 msec can regenerate the electrode surface resulting a stable baseline.

Calibration curves of cyanide and sulfide obtained with the modified pulse form are shown in Figure 10. The current increases linearly with concentration increments. The detection limit of each ion is 0.1 ppm and 0.05 ppm, respectively.

Cyanide and sulfide analysis from whole blood. Free cyanide and sulfide contained in a whole blood sample is separated by three different ways before IC injection; Conway microdiffusion, dialysis membrane filtration and aeration with N_2.

Free cyanide and sulfide released from a whole blood sample, with the above methods, are analyzed with IC-PAD, and

Table 1. Recoveries of cyanide and sulfide after three different sample treatments

added amount	Conway Microdiffusion		dialysis membrane filtration		aeration	
(ppm)	cyanide	sulfide	cyanide	sulfide	cyanide	sulfide
0.20	0.19	0.18	0.10	0.13	0.18	0.18
0.40	0.36	0.36	0.25	0.25	0.34	0.37
0.60	0.58	0.54	0.39	0.53	0.53	0.56
0.80	0.73	0.73	0.54	0.57	0.72	0.73
ave. recovery	93.7± 2.6	91.5± 0.7	66.8± 2.6	72.6± 5.4	88.8± 1.5	92.5± 0.5

their recoveries are shown in Table 1. The filtration method is simple and fast compared with the other two methods. However, the recoveries of cyanide are lower than the others because the cyanide binds with heme, hemoglobin or methemoglobin that could not pass through the pores of the dialysis membrane. The recoveries of sulfide are also decreased because sulfide could adsorb on the large protein molecule.

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Quantitative Structure-Activity Relationships (QSAR) Study on C-7 Substituted Quinolone

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To see the quantitative relationship between the structures of the C-7 substituted quinolones and their antibacterial activities, theoretical parameters such as the molecular van der Waals volume, surface area and some electrostatic parameters based on the molecular electrostatic potential, which represent lipophilicity , and some quantum mechanical parameters are introduced as descriptors. The sixteen substituted quinolone derivatives and twenty bacteria are used for the study. It is found that the QSARs of C-7 substituted quinolones are obtained for eleven bacteria and our descriptors are more useful for Gram positive organisms than negative ones. It is also shown that molecular surface area (or molecular Waals volume) of the C-7 substituent and net charge of C-7 atom of the quinolones are the descriptors of utmost importance.

Introduction

The basic assumption of Quantitative Structure-Activity Relationships (QSAR) is that there are some quantitative relationships between the microscopic (molecular structure) and the macroscopic (empirical) properties (particularly biological activity) of a molecule.[1] The term structure does not necessarily mean the spatial arrangement of atoms in a mo-