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OPTIMIZATION IN PHOTOCHEMICAL REACTION DETECTION

APPLICATION TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–PHOTOLYSIS–ELECTROCHEMICAL DETECTION

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SUMMARY

Chemometric techniques have been applied to optimization of response in photochemical reaction detection. Multivariate optimization using a factorial experimental design was employed to assess the effects of mobile phase pH and UV irradiation time on the amperometric response obtained by high-performance liquid chromatography–photolysis–electrochemical detection for four cardiovascular drugs. The procedure is more representative of the dynamic analytical conditions encountered in photochemical reaction detection than previous methods employing univariate optimization or off-line static methods. Stepwise multiple linear regression with backward elimination was used to fit the experimental data to empirical reduced third-order polynomial models. Plots of response were used to determine optimum conditions and visualize the interactions between pH and irradiation time. The design and construction of a low-cost on-line photochemical reactor for high-performance liquid chromatography is also discussed.

INTRODUCTION

Photochemical reaction detection is a variation of on-line post-column derivatization which has found particular usefulness in pharmaceutical and forensic analysis. It is employed to extend the sensitivity and selectivity of detection in high-performance liquid chromatography (HPLC) and to minimize sample preparation. Reviews of photochemical reaction detection in HPLC have been published[1–4]. Combining photoderivatization with electrochemical detection techniques allows rapid and unique photochemical reactions to be advantageously coupled with the high selectivity and sensitivity of electrochemical detection[5]. Krull and co-workers[6–14] have extensively studied HPLC–photolysis ($h\nu$)–electrochemical detection (ED) using conventional thin-layer amperometric cell detectors.

In the previously reported $h\nu$–ED methods, optimization of electrochemical response with respect to solution pH has been performed off-line using photolysis–cyclic voltammetry[12–14], on-line, but independently of irradiation time or flow-

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rate[7,8], or the pH dependence was either not reported or not optimized[9–11]. Although static off-line methods such as cyclic voltammetry (CV) are useful for scouting purposes and indicating trends, the results may not accurately represent the actual dynamic conditions of the on-line photochemical reactor (PCR) employing amperometric detection. We have observed situations in which a compound may show no activity at a glassy carbon electrode by CV, yet produce an electrochemical response by HPLC–amperometric detection under similar conditions. In both experiments, the same solvent was used for CV and as the HPLC mobile phase. Slow electron transfer under the CV conditions may be responsible for this phenomenon. Alternatively, the enhanced mass transfer of electroactive species to the electrode under hydrodynamic conditions of HPLC–amperometry may result in the greater sensitivity observed[15].

With respect to both the off-line and on-line optimization procedures, these methods have systematically varied one experimental variable of the analytical system at a time while holding all others constant (univariate optimization). Conditions were optimized for pH then optimized for irradiation time. Because the influences of these parameters on the formation of electroactive photoderivatives are not mutually independent, both parameters should be varied simultaneously (multivariate optimization).

In the previous hν–ED methods, irradiation time was evaluated by altering the flow-rate incrementally to produce a range of residence times within an irradiation coil of fixed length. The peak areas or heights representing the electrochemical responses as a function of flow-rate (residence time) were used as a measure of optimum irradiation time. A new coil was then constructed corresponding to the length required to give an equivalent residence time at the flow-rate desired for the chromatographic separation. However, for concentration-dependent detectors such as amperometric detectors, variation in flow-rate has a pronounced effect on the observed response[16]. The area response for a constant amount of electroactive compound is inversely proportional to the flow-rate. The peak height is inversely proportional to the standard deviation of the peak, which in turn is influenced by flow-rate. When a post-column reactor is added to an HPLC system, additional factors resulting from dispersion in the reactor further compound the effect of flow-rate on response. In light of these influences, it is apparent that variation of flow-rate to optimize irradiation time incorporates response factors related to flow-rate that are unresolved from the effect of irradiation on electrochemical response. A better approach to optimization of irradiation time requires that the flow-rate required by the chromatographic separation be maintained and the reactor length and pH be varied simultaneously. Alternatively, a system might be employed in which the flow-rate and reactor length remain constant and the photon flux and pH are varied.

The application of hν–ED to the analysis of antihypertensive drugs in dosage forms and biological fluids is currently being investigated in our laboratory. One of the objectives of the study has been to demonstrate the utility of chemometric techniques in the optimization of electrochemical response obtained by photochemical reaction detection. This paper describes a multivariate optimization procedure in which the mobile phase pH and irradiation time parameters are simultaneously varied under the actual dynamic conditions of analysis. A rigorous statistically based factorial experimental design was employed. No previous papers employing multivariate chemometric techniques to optimize response in photochemical reaction detection have been reported.

EXPERIMENTAL

Standards, reagents and test solutions

Spironolactone, hydrochlorothiazide, clonidine, and chlorthalidone reference standards were all obtained from the United States Pharmacopeial Convention (Rockville, MD, U.S.A.). Reagent-grade sodium dihydrogen phosphate, sodium hydroxide, and phosphoric acid were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). HPLC-grade methanol was purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.).

Stock solutions (0.25 mg/ml) of spironolactone, hydrochlorothiazide, clonidine and chlorthalidone were prepared in methanol. Working standard solutions (12.5 μg per ml) were prepared by diluting a 5-ml aliquot of the stock solution to 100 ml with 42% aqueous methanol.

Mobile phase solutions were prepared from a bulk solution containing methanol–0.1 *M* NaH_2PO_4 (45:55). A set of eight mobile phases within the pH range 2.0–8.0 was prepared from portions of the bulk solution by adjusting the pH with concentrated phosphoric acid or 50% aqueous sodium hydroxide to the required pH. All mobile phases were degassed before use in an ultrasonic bath.

Instrumentation

Fig. 1 illustrates the arrangement of the HPLC–hν–ED system components. The system included a Beckman Model 110B solvent delivery module (Fullerton, CA, U.S.A.) delivering a flow-rate of 1.0 ml/min and a Rheodyne Model 7125 loop injector with a 20-μl loop. An Uptight guard column (Upchurch Scientific, Oak Harbor, WA, U.S.A.) packed with Vydac reversed-phase packing, 30–40-μm pellicular particles (Universal Scientific, Atlanta, GA, U.S.A.) was used. The analytical column was a Zorbax CN Reliance cartridge column, 5-μm spherical particles, 8 cm × 4 mm I.D. (DuPont, Wilmington, DE, U.S.A.). The photochemical reactor was constructed using a Model SC3-9 low-pressure mercury discharge lamp with a Model SCT-4 power supply (UVP, San Gabriel, CA, U.S.A.). A set of five irradiation coils of different length was knitted from PTFE tubing 0.3 mm I.D. × 1.59 mm O.D. (Anspec, Ann Arbor, MI, U.S.A.) corresponding to irradiation times of 4.17, 8.35, 16.70, 25.05 and 33.40 s at a flow-rate of 1.0 ml/min. A 7.62-cm diameter fan (Rotron, Woodstock, NY, U.S.A.) provided air cooling for the reactor. Reactor components were contained in a 31.5 × 16.5 × 15.5 cm box constructed from galvanized sheet steel and perforated masonite. The bottom and back sides were perforated to improve

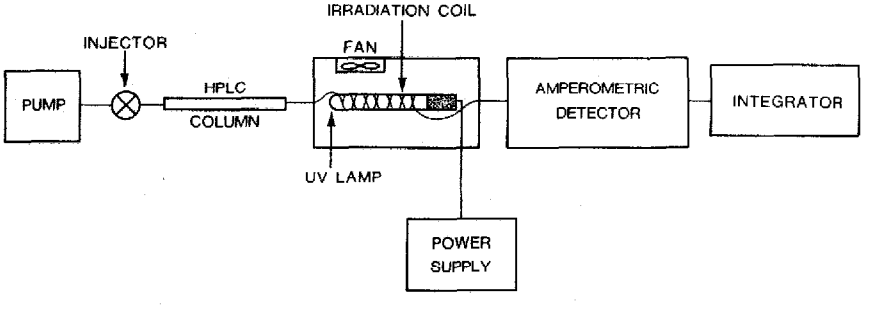

Fig. 1. Diagram of HPLC–photolysis–electrochemical detection system.

air flow for cooling purposes. The detector used was a BAS LC-4B amperometric controller and LC-17 thin-layer transducer with glassy carbon working electrode and Ag/AgCl reference electrode (Bioanalytical Systems, West Lafayette, IN, U.S.A.). The operating potential was +1.0 V and a range of 20 nA was used. Connections between the analytical column, irradiation coil and the thin-layer transducer were all made with Upchurch Fingertight fittings (Upchurch Scientific). Area responses were recorded using an HP 3392A Integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

Optimization procedure

Electroactive photoderivative formation was determined simultaneously as a function of irradiation time and mobile pH for spironolactone, hydrochlorothiazide, chlorthalidone and clonidine. Irradiation time was varied by changing the coil length in the photoreactor using one of the five coils in the set. Working standard solutions of the model compounds were injected in duplicate into the HPLC–hν–ED system. Amperometric responses were recorded for each coil and also with the UV lamp of the reactor off to provide response values without irradiation. This time series was repeated in each of the eight mobile phases.

RESULTS AND DISCUSSION

Experimental design, data analysis and mathematical modeling

Chemometric techniques similar to those used in the optimization of chromatographic resolution[17] may be used to optimize analyte response. In fact, many of the same variables affecting resolution also influence photoderivatization: mobile phase pH, per cent and type of organic modifier, presence of inorganic salts, etc. The additional parameter which must be considered in hν–ED is the irradiation time or photon flux. In general, optimization includes the selection of optimization criteria and the parameters to optimize, selection of an experimental design, and evaluation and interpretation of the results. In hν–ED, of course, the criterion to optimize is the photoinduced electrochemical response (peak area or height). On a practical level, the basic reaction conditions are constrained by the general chromatographic limitations (pH range 2–8 for silica-based columns, choice of solvents, etc.), the specific requirements of a given separation (*e.g.* conditions for reversed-phase or ion-pair chromatography as dictated by the analyte), and the need for a suitable electrolyte for hν–ED. Frequently, existing HPLC methods have been adapted to use hν–ED. Considering these restrictions, the parameters to optimize are usually limited to the mobile phase pH and irradiation time.

A factorial experimental design was implemented in which the photoinduced amperometric response was acquired at eight levels of mobile phase pH and six levels of irradiation time. This 8 × 6 factorial design parameter space encompasses the region of pH 2–8 recommended for silica-based HPLC columns and the irradiation times (coil lengths) possible with the UV lamp used in the study (0–33.4 s). A factorial type design was chosen rather than a simplex design because it was desired to obtain an accurate representation of the response surfaces encountered in HPLC–hν–ED over the entire parameter space and because no information of this type has been reported. The possibility also existed that the surfaces contained local optima, a situation in which simplex or univariate techniques may fail to find the best response

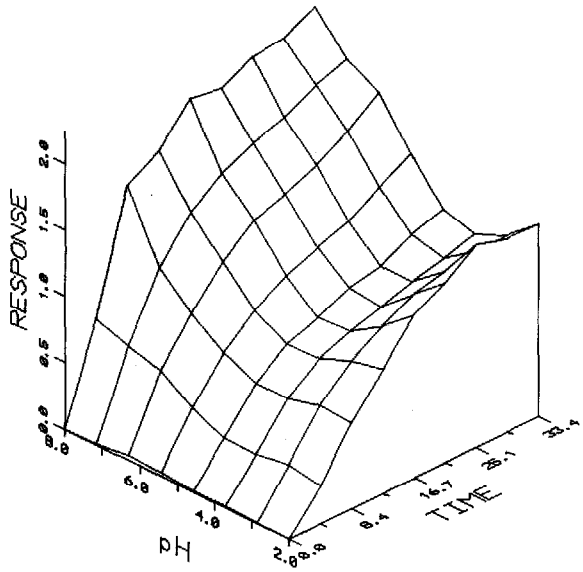

Fig. 2. Experimental amperometric response surface as a function of irradiation time and mobile phase pH for spironolactone. Time in s.

conditions or global optimum. It was also desired to establish the extent of interaction between pH and irradiation time.

Fig. 2 is a plot of the response surface constructed from the experimental data for spironolactone. Spironolactone is electro-inactive in the absence of UV irradiation as indicated by zero response along the pH axis at zero time. Photoinduced response generally increased with increasing pH and irradiation time. The global optimum is found at a pH of 8.0 and irradiation time of 33.4 s. A depression is evident in the surface along values collected at pH 4.0. Response at pH 2.0 and 33.4 s represents a local optimum within the parameter space.

The hydrochlorothiazide response surface is given in Fig. 3. Hydrochlorothiazide is electroactive without irradiation. The left forward edge of the surface (time zero) represents the effect of the observed native amperometric response of hydrochlorothiazide. As is well known, a significant enhancement in response for electroactive compounds is possible by optimizing the mobile phase pH. At a pH of 8.0, the response is constant regardless of irradiation time (relative standard deviation of $\pm 3.5\%$). Equivalent responses are attainable at any irradiation time at this pH. At low pH values where hydrochlorothiazide has no native electroactivity, the response can be increased by UV irradiation.

Chlorthalidone, like spironolactone, is inactive without irradiation. The response surface (Fig. 4) is similar to that of spironolactone with the exception that a ridge occurs rather than a depression. Optimum response is found along this ridge at pH 6.0 and 33.4 s. The clonidine data produced a response surface (Fig. 5) which indicated low-level response only at high pH and no significant response at low or intermediate pH. However, a large enhancement in response with increasing irradiation time is observed at high pH.

Empirical polynomial models of photoinduced electrochemical response as a

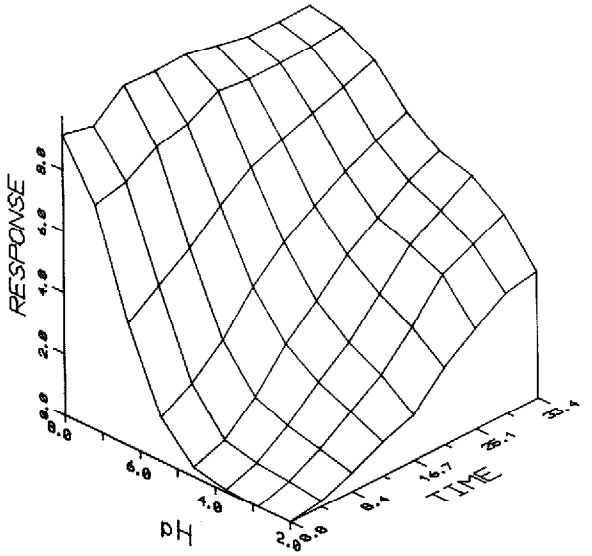

Fig. 3. Experimental amperometric response surface as a function of irradiation time and mobile phase pH for hydrochlorothiazide. Time in s.

function of pH and irradiation time were fit to the experimental data using matrix least squares regression analysis. Polynomial models allow interaction effect terms to be included in the model. A full second-order polynomial was first employed because they are frequently useful in describing response surfaces of phenomena over limited factor domains[18]. The resulting regression equation is:

$$R = a + bt + c\text{pH} + dt^2 + e\text{pH}^2 + ft\text{pH}$$

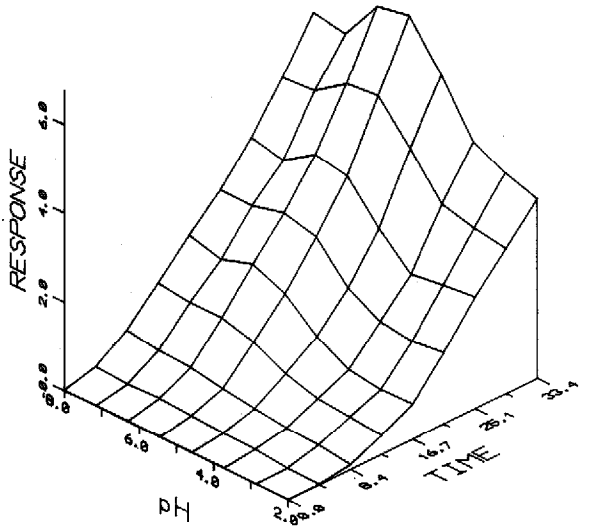

Fig. 4. Experimental amperometric response surface as a function of irradiation time and mobile phase pH for chlorthalidone. Time in s.

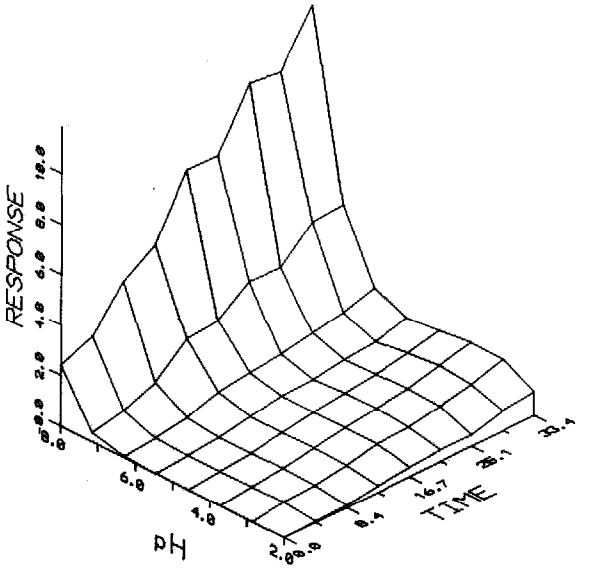

Fig. 5. Experimental amperometric response surface as a function of irradiation time and mobile phase pH for clonidine. Time in s.

where R is the amperometric response, t is the irradiation time in seconds, pH is the mobile phase pH and a–f are the estimated numerical coefficients of the equation. Values obtained for the numerical coefficients and the coefficients of determination (R^2) for the second-order model for each compound are given in Table I. Adequacy of the models was assessed using the coefficients of determination and the F-test for the significance of regression[18]. In three of the four sets of experimental data, an R^2 of 0.941 or greater was obtained which indicates that the second-order model accounts for 94.1% (or more) of the variation in the data. In the fourth case, the data for clonidine, the model explained only 80% of the variance of the data. For cloni-

TABLE I

ESTIMATED REGRESSION COEFFICIENTS, COEFFICIENTS OF DETERMINATION (R^2) AND STANDARD ERROR OF ESTIMATE (S.E.E.) FOR SECOND-ORDER POLYNOMIAL MODEL

Coefficient	*Spirono-lactone*	*Hydrochloro-thiazide*	*Chlorthal-idone*	*Clonidine*
a	0.721	−1.721	−0.850	6.150
b	0.085	0.298	−0.036	−0.093
c	−0.399	−0.311	0.369	−3.086
d	−0.002	−0.001	0.004	0.000
e	0.045	0.206	−0.035	0.327
f	0.004	−0.026	0.010	0.035
R^2	0.941	0.944	0.968	0.800
S.E.E.	0.162	0.815	0.374	1.176

dine, the change in photoinduced amperometric response *versus* pH and irradiation time was less gradual than the three other compounds. Response increased sharply at high pH and with increasing irradiation time. The rapid changes in response within a small area of the parameter space resulted in a poorer fit using the second-order equation for clonidine.

In order to develop a model that encompassed all the compounds studied, higher-order polynomials were investigated. Although it is always possible to make R^2 large by adding more terms to the model, the new model is not necessarily a better description of the experimental data unless the error sum of squares in the new model is reduced by an amount equal to the original error mean square[19]. If it is not, the new model will have a larger error mean square than the old model due to the loss of degrees of freedom for error. It is also recommended that the order of the model be kept low to eliminate higher order oscillations. In order to obtain a model that explains the data with minimal error yet does not contain a large number of non-significant terms, an alternative technique, stepwise multiple regression using backward elimination was employed[19]. The initial regression equation was a full third-order model:

$$R = a + bt + c\text{pH} + dt^2 + e\text{pH}^2 + ft\text{pH} + gt^3 + h\text{pH}^3 + it^2\text{pH} + jt\text{pH}^2$$

where R, t and pH are as previously defined and a–j are the estimated regression coefficients. Backward elimination attempts to find a good model with a small set of significant variables by starting with a model containing all the terms to be considered and eliminating them one at a time. In each step, the partial F-statistic is calculated for each term in the model as if it were the last variable to enter the model. The smallest of the partial F-statistics is compared with a user-selected F-to-remove. The term is removed from the model if the F-statistic is smaller than the F-to-remove. Using this technique, third-order models reduced in the number of terms from the full model were obtained. In all cases, R^2 values (adjusted for degrees of freedom) were greater than 0.952. Because of the variety of response surfaces that are possible, the number of terms in the model and the particular terms retained in the model as well as the signs and values are different for each compound. The coefficients of determination corrected for degrees of freedom were greater using this reduced third-order model than the full second-order model and were not statistically different from R^2 values for a full third-order regression. Coefficients of determination and the estimated regression coefficients for the reduced third-order model are found in Table II.

Model response surfaces generated from the regression coefficients for the reduced third-order model more accurately approximated the shapes of the experimental response surfaces than using the full second-order coefficients. Response surfaces generated from the model equations for spironolactone and hydrochlorothiazide are shown in Figs. 6 and 7,. respectively.

Chromatographic results

Mixtures containing spironolactone and hydrochlorothiazide or chlorthalidone and clonidine were analyzed by HPLC–hν–ED using the optimal conditions determined by visual inspection of the experimental response surface plots. For the four compounds studied, mathematical analysis of the model equations could not be used

TABLE II

ESTIMATED REGRESSION COEFFICIENTS, COEFFICIENTS OF DETERMINATION (R^2) AND STANDARD ERROR OF ESTIMATE (S.E.E.) FOR REDUCED THIRD-ORDER MODEL

E designates term eliminated from regression model.

Coefficient	*Spirono-lactone*	*Hydrochloro-thiazide*	*Chlorothal-idone*	*Clonidine*
a	0.458	2.261	2.370	−11.890
b	0.102	E	−0.125	0.220
c	−0.248	−2.200	−1.693	9.259
d	$-3.43 \cdot 10^{-3}$	E	$4.46 \cdot 10^{-3}$	E
e	$2.62 \cdot 10^{-2}$	0.391	0.345	−2.143
f	E	0.109	$5.19 \cdot 10^{-2}$	−0.109
g	$6.20 \cdot 10^{-5}$	E	E	E
h	E	E	$-2.13 \cdot 10^{-2}$	0.151
i	$-2.75 \cdot 10^{-4}$	$-2.66 \cdot 10^{-4}$	E	E
j	$1.28 \cdot 10^{-3}$	$-1.26 \cdot 10^{-2}$	$-4.16 \cdot 10^{-3}$	0.014
R^2	0.966	0.977	0.977	0.952
S.E.E.	0.124	0.525	0.316	0.577

to predict optimal responses because the functions did not maximize within the experimental parameter space. These pairs of drugs were analyzed because they occur in commercially available diuretic–antihypertensive combination dosage forms.

Fig. 8 shows the chromatograms obtained for spironolactone and hydrochlorothiazide using a pH 8.0 mobile phase without the PCR and with the PCR containing a 33.40-s irradiation coil. As indicated by the response surfaces, spironolactone

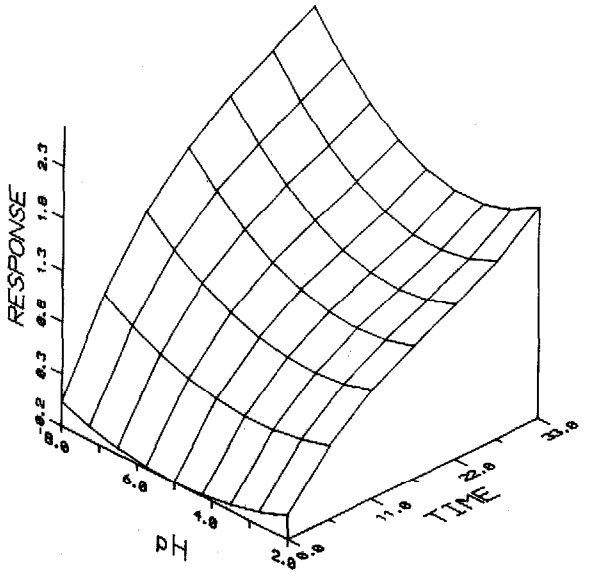

Fig. 6. Reduced third-order regression model response surface as a function of irradiation time and mobile phase pH for spironolactone. Time in s.

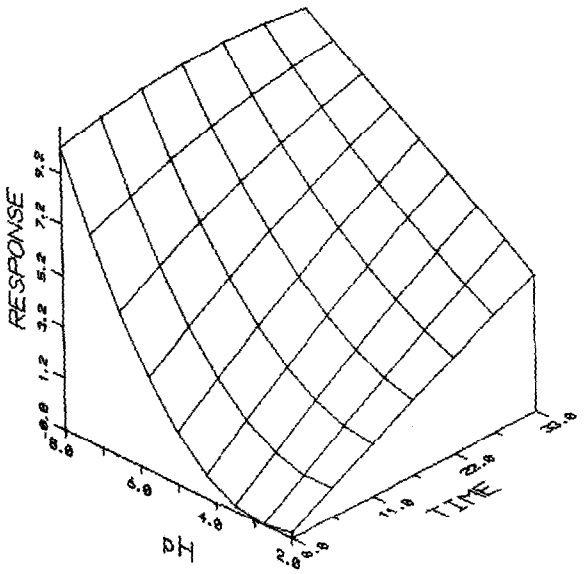

Fig. 7. Reduced third-order regression model response surface as a function of irradiation time and mobile phase pH for hydrochlorothiazide. Time in s.

produces an amperometric response only on irradiation and the response for hydrochlorothiazide remains unchanged under these conditions. The chromatograms for chlorthalidone and clonidine are given in Fig. 9. Irradiation results in an amperometric response for chlorthalidone and a 390% increase in the response for clonidine. A cyano-bonded phase was used in this study because it provided good separations for mixtures containing compounds of widely different lipophilicity while maintaining shorter analysis times than C_{18}-bonded phase columns.

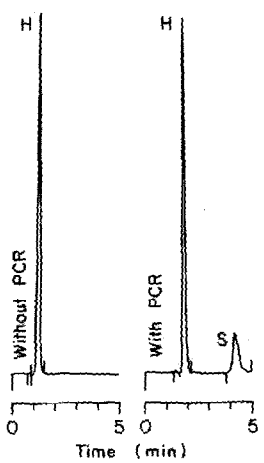

Fig. 8. Chromatograms of a solution containing hydrochlorothiazide (H) and spironolactone (S) without the on-line photochemical reactor (PCR) and with PCR and 33.4-s irradiation coil. Conditions: methanol–0.1 *M* NaH_2PO_4 pH 8.0 (45:55), 1.0 ml/min, Zorbax CN cartridge column, BAS glassy carbon electrode, + 1.0 *vs.* Ag/AgCl, 20 nA range.

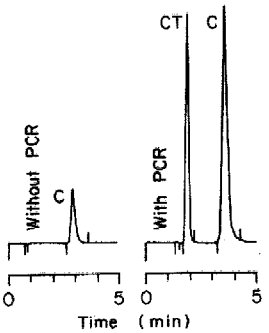

Fig. 9. Chromatograms of a solution containing chlorthalidone (CT) and clonidine (C) without the on-line photochemical reactor (PCR) and with PCR and 33.4-s irradiation coil. Conditions as in Fig. 8.

Photochemical reactor design

Because there are no commercially available PCRs for HPLC–photochemical reaction detection, a large variety of individual reactor designs have appeared in the literature. These designs employ irradiation sources ranging from high-intensity mercury or xenon arc lamps to medium- and low-pressure mercury or zinc lamps. The irradiation coils have been made of either quartz or PTFE and a number of different coil geometries have been proposed.

Although the majority of previous reactor designs have employed medium- and high-pressure lamps, a low-pressure mercury discharge lamp was selected for use. The factors involved in the lamp selection included the desire to decrease the size, complexity and cost of the photoreactor relative to existing designs. Low-pressure mercury lamps have a number of advantages for use in photochemical reactors. They generate much less heat and infrared radiation than high-pressure arc lamps and therefore do not require complicated liquid cooling systems. Air cooling is usually sufficient. High temperatures within the irradiation coil can result in unwanted side reactions such as polymerization. Temperature fluctuations can decrease system reproducibility. Low-pressure lamps have a very stable output over long periods and lamp life is longer also contributing to system reproducibility. Compared to high-pressure arc lamps, these lamps emit a higher percentage of ultraviolet light and very low heat loss. 85% of the radiation is emitted at 254 nm, a wavelength useful for many photochemical reactions[20]. Because low-pressure lamps do not require d.c. power or high current ratings, the power supplies are small and inexpensive. Finally, use of high-pressure sources can shorten the lifetime of PTFE irradiation coils which become brittle on prolonged exposure to high-intensity UV light. The particular lamp selected was chosen because it provided a large irradiated cylindrical surface (23 cm long × 0.95 cm diameter) onto which the irradiation coil could be directly placed for efficient transfer of radiant energy to the coil. The irradiation coils can be quickly changed by sliding off one coil and sliding on another. If size, cost and complexity are not considerations, the decision to incorporate either a high-pressure arc lamp or low pressure discharge lamp in a PCR is ultimately dependent on the reaction kinetics of the particular system under study. Both the intensity of the source and the length of irradiation time influence the concentrations of intermediate short-lived radicals and excited molecules[2]. These concentrations in turn influence the concentrations of the

various photoproducts that result from the multiple reaction pathways that often exist in photochemical reactions. The desired detectable species is produced in highest yield using the appropriate combination of high or low intensity and long or short irradiation time and is dependent on the analyte-specific kinetics. On a more pragmatic level, neither the kinetics nor the reaction products need be known as long as the post-column photochemical reaction is reproducible and results in enhanced sensitivity or selectivity of detection. However, elucidation of the electroactive photochemical reaction products is currently being investigated because knowledge of the mechanism and structural requirements will aid in optimization procedures and allow extension to other analytes.

The advantages of PTFE reaction coils in photochemical reaction detectors were reported by Scholten *et al.*[21]. PTFE provides good transparency in the 200–300 nm region and is readily available in a variety of internal and external diameters. PTFE tubing is less expensive, less fragile and easier to connect to systems than quartz. Knitted PTFE open tubular reactors exhibit minimal dispersion in comparison to coiled reactors of identical dimensions due to the deformed flow pattern they produce within the tubing. The geometry used in the irradiation coils in this study is a modification of a design published by Engelhardt and Neue[22]. Their original design was knitted on a device with four pins that produces a coil with four distinguishable sides. Our coils were knitted on a device with six pins placed on the corners of a regular hexagon. The six-sided design was chosen in order to maximize the surface area of the coil exposed to the UV lamp. The coils require no external support and slide directly onto the surface of the lamp in a "sleeve-like" manner.

All the components of the PCR fit within a relatively small box. The bottom and rear panels of the reactor box were made from 3-mm perforated masonite ("peg board") to provide improved ventilation and to facilitate the mounting of the lamp and cooling fan and the routing of irradiation coil entrance and exit tubing and electrical connections to the lamp and fan. The remaining sides were fabricated from thin gauge sheet steel.

Reproducibility of the HPLC–hν–ED system as measured by repeatability of injection ($n = 6$) was determined by making replicate injections of a working standard solution containing hydrochlorothiazide and spironolactone. Relative standard deviations were $\pm 0.9\%$ for hydrochlorothiazide and $\pm 1.7\%$ for spironolactone.

CONCLUSIONS

An improved procedure for optimization in HPLC–hν–ED has been presented that more accurately represents the dynamic analytical conditions than static off-line methods or dynamic methods that vary flow-rates. The factorial design allows the entire response surface to be constructed and provides a better representation of the effect of experimental conditions than simplex or univariate techniques. Graphical representation of response surfaces indicates visually the best condititons and the interaction between parameters. The ruggedness of experimental conditions can be judged by whether an optimum occurs on a narrow ridge or a wide plateau.

Stepwise multiple linear regression was found to be a useful statistical technique for developing mathematical models for analytical response phenomena. It produces models which adequately describe the experimental data while minimizing the num-

ber of terms and maximizing the degrees of freedom for estimation of the error in the model. If the model equation maximizes within the experimental parameter space, mathematical analysis can be used to predict the optimum experimental conditions.

The results of this optimization study will be used in the development of analytical methods for these four drugs in dosage forms and in biological fluids. It is anticipated that the high selectivity of HPLC–hν–ED will be of particular value in the determination of pharmaceuticals in biological fluids. The high selectivity of this technique results from the conditions that the analyte: (1) possesses a specific retention time, (2) absorbs radiation from the UV source, and (3) undergoes a photoreaction to produce an electroactive species which is oxidized at the specific electrode and operating potential. The probability that an interfering component will meet all three of these criteria is quite low.

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