

## Electrochemical detection for high-performance liquid chromatography using a Kel-F wax–graphite electrode

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(First received October 3rd, 1989; revised manuscript received April 24th, 1990)

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### ABSTRACT

A carbon paste electrode using Kel-F wax as the binder was characterized for electrochemical detection in high-performance liquid chromatography. The resulting electrode was stable in solutions with high organic content and possessed good reproducibility and detection limit. The amperometric response of the Kel-F wax carbon paste electrode by flow injection analysis was about 26% higher than that of Kel-F oil carbon paste electrode in an acetonitrile–water (70:30) solution. This represented a two-fold improvement over the Nujol oil carbon paste electrode. Cyclic voltammetric data indicated the greater response of the Kel-F wax carbon paste electrode in high-acetonitrile-containing solutions was due to a higher surface area caused in part by solvent swelling of the electrode. Three unconjugated estrogens were separated and detected satisfactorily at the pg level using this Kel-F wax carbon paste electrode.

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### INTRODUCTION

The most common electrodes used for electrochemical detection in high-performance liquid chromatography (HPLC) are based on various types of carbon such as glassy carbon, pyrolytic graphite, and numerous kinds of carbon paste<sup>1–5</sup>. Carbon paste electrodes (CPEs) can be advantageous because they are cheap, easy to prepare and replace, and modifiable to suit the required electrode potential. These CPEs, however, suffer from several drawbacks. The most severe limitation of the CPE is the tendency for the binder (such as Nujol) to dissolve in solutions containing an appreciable content of organic solvent. To overcome this problem, a variety of composite electrodes such as graphite–Kel-F particle<sup>6</sup> and graphite–epoxy electrodes<sup>7</sup> have been developed.

In our previous work<sup>8</sup>, we have compared the performance of Nujol and Kel-F oil-based CPEs. The response of identically prepared Kel-F and Nujol oil CPEs to estriol was evaluated by flow injection analysis (FIA) using mobile phases of various acetonitrile content. An enhancement factor of about 1.7 over the response of the Nujol oil CPE was obtained for the Kel-F oil CPE as the acetonitrile content in the

mobile phase was increased from 0 to 80%. As determined by chronocoulometry, the active surface area of the CPEs increased when immersed in a mixed acetonitrile–water solution.

We report here the use of Kel-F wax as a binder in a CPE for electrochemical detectors in HPLC. Recent preliminary data suggested that Kel-F wax is superior to Kel-F oil in several respects such as solvent stability and lower residual current. Both characterization of the Kel-F wax CPE and applicability of the CPE for HPLC detection of three estrogens will be presented.

## EXPERIMENTAL

### *Chemicals*

Kel-F oil (No. 700) and Kel-F wax were obtained from Halocarbon (Hackensack, NJ, U.S.A.) and from Alltech (Arlington Heights, IL, U.S.A.), respectively. The molecular weight of the oil was about 800–1000 while that for the wax was undetermined since it is a mixture of two higher-molecular-weight Kel-F polymers. The graphite particles (Fisher Scientific, Fairlawn, NJ, U.S.A.), determined to be about 10 to 25  $\mu\text{m}$  by light microscopy, were treated successively with hexane, chloroform, acetone, water, 6 *M* nitric acid and finally water until a neutral pH before drying overnight in a vacuum oven at 110°C. The estrogens were purchased from Sigma (St. Louis, MO, U.S.A.) All chemicals were at least reagent grade; the acetonitrile was HPLC grade. Triply distilled water was obtained from a Barnstead Nanopure distillation unit (Sybron/Barnstead, Boston, MA, U.S.A.).

### *Apparatus*

The HPLC arrangement was composed of a Beckman Model 110A pump modified with a SSI pulse dampener (Scientific Systems, State College, Pa, U.S.A.), a Rheodyne Model 7010 injector (Rheodyne, Berkeley, CA, U.S.A.) with a 20- $\mu\text{l}$  sample loop, a 5- $\mu\text{m}$  Lichrospher 100 RP-18 column with dimensions of 20 cm  $\times$  4.6 mm I.D. (EM Science, Cherry Hill, NJ, U.S.A.) and a BAS (West Lafayette, IN, U.S.A.) Model LC-3A or a Waters (Milford, MA, U.S.A.) 460 electrochemical detector. The electrochemical cell consisted of a stainless-steel auxiliary cell half, a plastic Kel-F cell half, and an Ag/AgCl reference electrode. Peaks were generated on a Linear Model 1201 (Linear Instruments, Reno, NV, U.S.A.) strip-chart recorder or a Model C-R6A Chromatopac integrator (Shimadzu, Columbia, MD, U.S.A.).

Cyclic voltammetry and chronocoulometry measurements were performed using a BAS-100 electrochemical analyzer. The electrochemical cell was composed of a CPE, a Pt wire counter electrode and a saturated Ag/AgCl reference electrode. Cyclic voltammograms were generated at the scan rate of 100 mV/s. For the chronocoulometry, the potential was stepped from 0 to 600 mV data pulse width of 250 ms. The active surface area of the CPE was calculated from the slope of a plot of charge  $Q$  (coulombs) versus  $t^{\frac{1}{2}}$ (ms) based on the Cottrell equation

$$Q = \frac{2nFAC_0D_0^{\frac{1}{2}}}{\pi^{\frac{1}{2}}} (t^{\frac{1}{2}})$$

where  $n$  = number of electrons transferred,  $F$  = Faraday constant,  $A$  = surface area,  $C_0$  = concentration and  $D_0$  = diffusion coefficient.

The diffusion coefficient value of  $7.6 \cdot 10^{-6}$  cm<sup>2</sup>/s for K<sub>3</sub>Fe(CN)<sub>6</sub> in 1 M KCl solution was used<sup>9</sup>. For the solution containing 70% acetonitrile, the  $D_0$  value of  $12 \cdot 10^{-6}$  cm<sup>2</sup>/s was appropriate as calculated previously<sup>8</sup>.

### Procedure

Kel-F oil and wax carbon pastes were prepared in batches by combining graphite powder (pre-dried at 110°C) and the binder in the weight ratios of 1.25. The required amount of Kel-F wax was weighed into a beaker and was heated on a hot plate at low heat until the wax had melted. The graphite powder was added slowly with stirring. The mixture was allowed to cool and then mixed more thoroughly with a mortar and pestle. Small amounts of the paste were then transferred to the flow cell cavity with a spatula and packed by applying pressure with a small brass rod. The electrode surface was smoothed by polishing it on glazed white paper<sup>10</sup>. Electrode repacking was done by replacing approximately one-fifth of the previously used carbon paste. Equilibration of a new CPE was carried out for at least 8 h in 0.025 M phosphate buffer containing 30% acetonitrile, pH 3.2. Oxidation of the estrogens was performed at +0.95 V vs. Ag/AgCl and the flow-rate was maintained at 1 ml/min. Each data point was generated from at least five injections.

The extent of swelling of the Kel-F wax by an organic solvent was carried out as described in our previous work<sup>8</sup>. Determinations were done in triplicate.

## RESULTS AND DISCUSSION

Our primary interest is to develop a stable, easily prepared CPE with a wide range of potential limits. Three estrogens which require high applied potentials for oxidation were chosen to be the test solutes. Using a glassy carbon electrode, a required applied potential of 1.15 V vs. Ag/AgCl has been reported for estriol<sup>11</sup>. Hydrodynamic voltammograms of repetitive injections of 60 ng estriol, 76 ng estradiol, and 116 ng estrone are shown in Fig. 1. A potential higher than +1.15 V vs. Ag/AgCl must be applied in order to reach the limiting current plateau. A + 0.95 V applied oxidation potential was chosen for the estrogens. At higher applied potentials, oxidation of the mobile phase can occur which results in high background currents. The optimum operating potential for any compound is a compromise between minimizing the background current and maximizing the limiting current.

Fig. 2 illustrates the stability of identically prepared Kel-F oil and wax CPEs used in solution with 70% acetonitrile content. The response to 10 ppm estriol decreases by about 15% and 3% for Kel-F oil and wax, respectively, over a 9-h period. The Kel-F wax CPE is less soluble in this organic solvent and thus, is more suitable for use as a HPLC detector. Also, the response of the Kel-F wax CPE is about 26% higher than that of Kel-F oil CPE (Fig. 3). For the five repetitive measurements, relative standard deviations (R.S.D.) of less than 5% and 3% were calculated for the Kel-F oil and wax CPEs, respectively. The peak width of the Kel-F wax CPE is also about 10% narrower than the Kel-F oil CPE indicating better mass transfer of the solute in and out of the electrode. Better reproducibility and detection limit of the Kel-F wax CPE are expected because of the lower noise (Fig. 3). From baseline data (after 8 h of

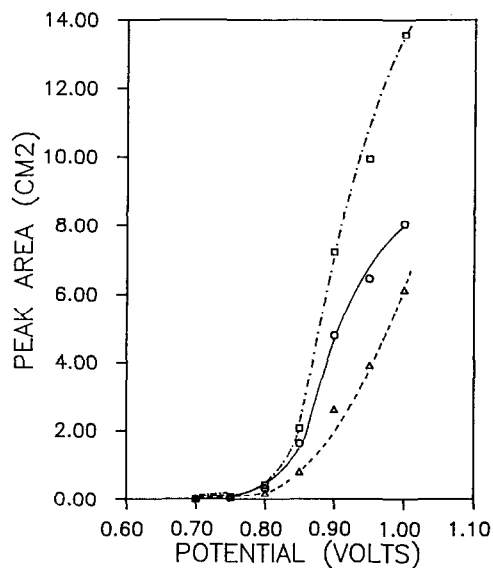


Fig. 1. Hydrodynamic voltammograms for estriol ( $\Delta$ ), estradiol ( $\circ$ ) and estrone ( $\square$ ).

equilibration), values of 0.5 nA and 0.3 nA were observed for Kel-F oil and wax CPEs, in buffer containing 70% acetonitrile, respectively. Compared to the standard Nujol CPE<sup>8</sup>, the Kel-F wax CPE has better than a two-fold response.

The Kel-F wax CPE response was found to increase directly with the amount of organic solvent with a moderate solvent polarity parameter ( $\epsilon^0$ )<sup>12</sup> such as acetonitrile ( $\epsilon^0 = 0.65$ ) (Fig. 4). Methanol which possesses a high  $\epsilon^0$  value of 0.95 did not improve the response of the Kel-F wax electrode. Similar results were found for the Kel-F oil CPE<sup>8</sup>. It should be noted, however, that the Kel-F wax CPE is more stable in methanol

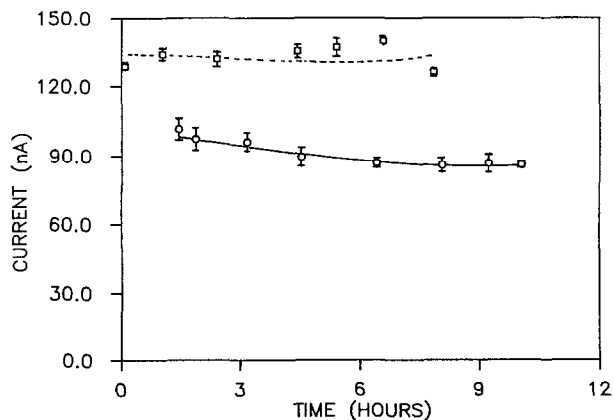


Fig. 2. Lifetimes of Kel-F oil ( $\circ$ ) and Kel-F wax ( $\square$ ) CPEs in 0.025 M phosphate buffer containing 70% acetonitrile, pH 3.2.

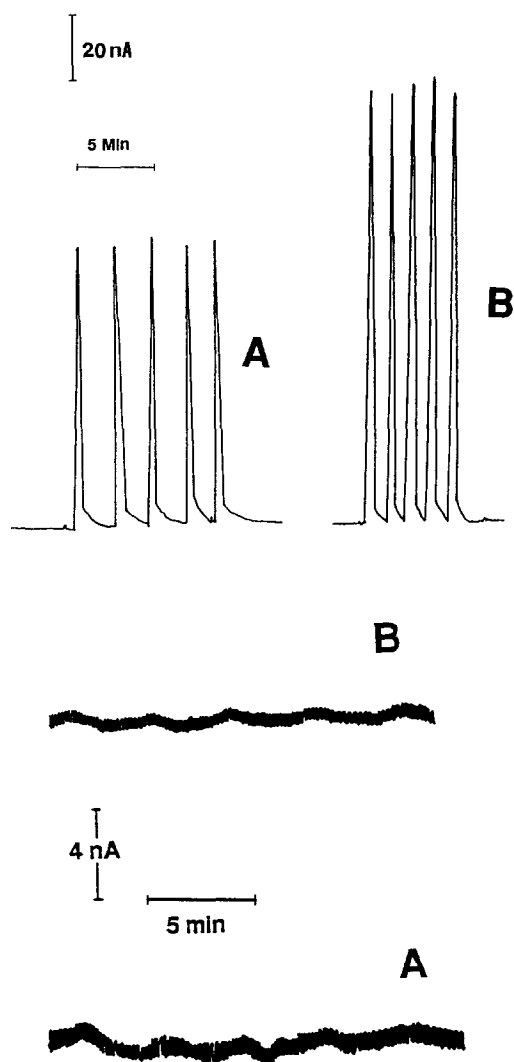


Fig. 3. Five repetitive measurements of 10 ppm estriol with Kel-F oil (A) and Kel-F wax (B) CPEs and baseline noise of CPEs after 8 h of equilibration in 0.025 *M* phosphate buffer containing 70% acetonitrile, pH 3.2.

than the Kel-F oil CPE. The extent of swelling of Kel-F wax measured as percent change in weight in acetonitrile (1.13%) and methanol (0.04%) supports the data in Fig. 4.

For comparison purposes, cyclic voltammetry and chronocoulometry measurements of the Kel-F oil CPE electrode and Kel-F wax CPE in both aqueous and 70% acetonitrile solutions were carried out. These data are tabulated in Table I. The R.S.D. for the active surface area for five different electrodes was about 7.6%. If untreated graphite was used, the precision was about two times worse. The current

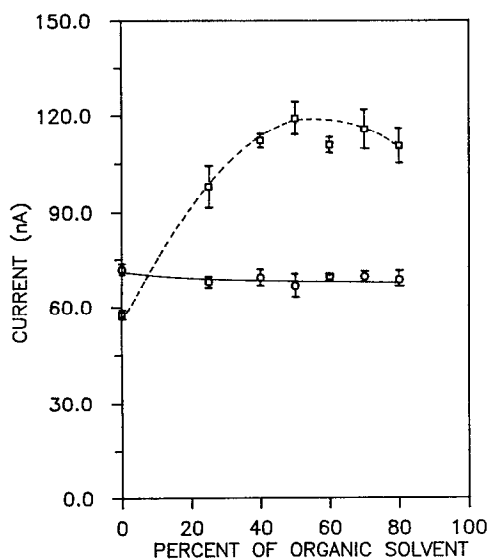


Fig. 4. The effect of organic solvents on the response of the Kel-F wax CPE. □ = Acetonitrile; ○ = methanol.

signals of both electrodes in aqueous solution are comparable but improve in a 70% acetonitrile solution. The peak current ratio (anodic/cathodic) values of close to 1 indicate reversibility although the peak potential separation values of about 100 mV indicate non-Nernstian behavior. The active surface area has increased about four-fold for both electrodes when immersed in a 70% acetonitrile solution after 8 h of equilibration. Without some equilibration time, the current signals and surface areas of both CPEs are comparable to those found in aqueous solution. This increase in

TABLE I

CYCLIC VOLTAMMETRIC AND CHRONOCOULOMETRIC DATA FOR KEL-F OIL AND KEL-F WAX CPEs

All electrodes were equilibrated in its respective solvent for 8 h.

| Electrode<br>( $n=5$ ) | Solvents  | Average<br>cathodic<br>current<br>( $\mu A$ ) | Peak<br>current ratio<br>( $\frac{ipa}{ipc}$ ) | Average<br>peak potential<br>separation (mV) | Average<br>active surface<br>area ( $cm^2$ ) |
|------------------------|---|---|--|--|--|
| Kel-F oil              | 0.264 mM $K_3Fe(CN)_6$<br>in acetonitrile-water<br>(70:30) (0.1 M KCl)<br>(solvent A) | 4.51  | 1.05   | 120  | 0.128  |
| Kel-F oil              | 0.264 mM $K_3Fe(CN)_6$<br>in 0.1 M KCl (solvent B)                                    | 5.80  | 1.00   | 65.9   | 0.453  |
| Kel-F wax              | A   | 4.56  | 1.07   | 113.2  | 0.122  |
| Kel-F wax              | B   | 6.83  | 1.22   | 86.7   | 0.501  |

active surface area is likely due in part to the swelling of both polymers by the organic solvent. The ratio of the acetonitrile active surface area data of the two CPEs relates to the 26% increase in response of the Kel-F wax CPE over the Kel-F oil CPE under hydrodynamic voltammetry conditions. The increase in active surface area is not directly proportional to the increase in current, however.

Three estrogens were separated satisfactorily as shown in Fig. 5A. The order of elution was estriol, estradiol and estrone with retention times of 3.0, 8.6, and 11.8 min, respectively. Comparable detection limits as those obtained by Hayashi *et al.*<sup>13</sup> were observed with our system. Based on a signal-to-noise, ratio of 3, the limit of detections are 60, 76 and 116 pg for estriol, estradiol and estrone, respectively (Fig. 5B). Since  $\alpha$ -naphthol is eluted at the same time as estradiol with the specified mobile phase, it is

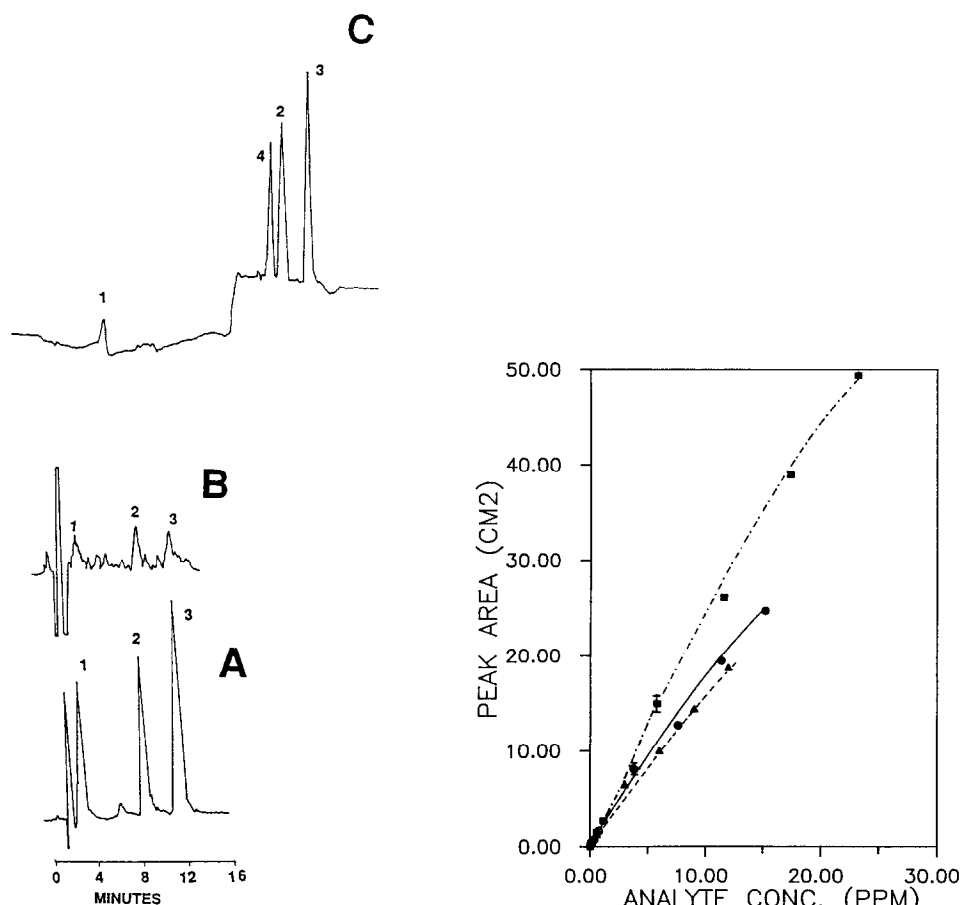


Fig. 5. (A) Separation of estriol (1), estradiol (2) and estrone (3) using 0.025 M phosphate buffer with 50% acetonitrile, pH 3.2. (B) Chromatogram showing the detection limits for estriol, estradiol, and estrone. (C) Separation of the three unconjugated estrogens with  $\alpha$ -naphthol (4) as an internal standard. The organic content of the mobile phase was stepped from 30 to 50% acetonitrile.

Fig. 6. Calibration curves for estriol ( $\blacktriangle$ ), estradiol ( $\bullet$ ) and estrone ( $\blacksquare$ ).

used as an external standard to take into account the slight variation of the electrode response due to the electrode surface area. It is possible to use  $\alpha$ -naphthol as an internal standard with the use of step gradient elution, as demonstrated in Fig. 5C.

Calibration curves for the estrogens are depicted in Fig. 6. An external standard,  $\alpha$ -naphthol, was injected before each calibration curve. A linear relationship was obtained for the three estrogens up to about 125 ng. The R.S.D. values involved in five repetitive area measurements for the three estrogens are less than 6%. The reproducibility of the Kel-F wax electrode between batches is within 8%. The lifetime of the electrode is satisfactory (at least about five months) when it is stored in a dry environment.

Comparison of response of the Kel-F wax CPE to that of a glassy carbon electrode for the oxidation of NADH to NAD<sup>+</sup> as a function of time has been made. The signal generated by the Kel-F wax CPE was about 1.5  $\times$  higher and more stable. Over a 12-h period of repetitive 20- $\mu$ l injections of 1.5 mM NADH, the signal decreased almost 50% for the glassy carbon CPE but only 10% for the Kel-F wax CPE. Electrode fouling due to dimerization of NAD<sup>+</sup> is a well-known problem for glassy carbon but may not be as pronounced for the Kel-F wax CPE.

The results of this study show that the Kel-F wax CPE is suitable for use in HPLC because of its stability in organic solvents enhanced response, and lower background current. Because Kel-F polymers can be chemically modified<sup>14-16</sup>, the fabrication of CPEs for specific HPLC applications may be feasible.

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