

# New Method for Selective, Sensitive, and Fast Analysis of Organic Acids

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## ABSTRACT

Pulsed Amperometric Detection (PAD) with disposable platinum electrodes following a separation with hydrolytically stable silica-based or polymeric columns open up new possibilities of chromatographic organic acid analysis. Acclaim<sup>®</sup> organic acid columns are silica-based reversed-phase columns which can be used in 100% aqueous acidic mobile phases as low as pH 2. Polymeric columns from the IonPac<sup>®</sup> series allow mobile phase in a broad pH range from 0 to 14. Using an optimized PAD waveform under acidic conditions, new disposable platinum electrodes deliver a stable detection performance for organic acids. The PAD with disposable platinum electrodes can be up to 30 times more sensitive than UV detection between 200 and 210 nm. We have carried out a comprehensive method development including optimization of waveform potentials and concentrations of acidic mobile phases. This presentation describes analytical performance (detection limits and reproducibility) not only for aliphatic mono carboxylic acids but also for aliphatic di-carboxylic acids, hydroxy carboxylic acids, and unsaturated carboxylic acids.

## INTRODUCTION

Electrochemical detection (ED) is a widely used detection method in liquid chromatography (LC and HPLC), capillary electrophoresis and flow-injection analysis. Recent reports indicate an increasing use of ED due to its high sensitivity, ease of microfabrication, and acceptable cost. Pulsed Amperometric Detection (PAD) is considered to be more reliable and significantly more reproducible than traditional DC amperometric detection techniques. PAD has been applied to a number of electro-active analytes already. Most important applications of PAD, so far, have been for alcohols and carbohydrates.

We have developed a robust process of microfabrication for disposable thin film electrodes of various types, such as Pt, Au, and Ag. We have also developed optimized PAD waveforms for use with such disposable electrodes. The thin film electrodes offer a predictable stability of response, fast equilibration, and an excellent electrode-to-electrode reproducibility.

In this presentation, we describe a development of an optimized PAD waveform for direct detection of organic acids. As a working electrode, we utilize the newly developed disposable thin-film Pt electrodes. The Limits of Detection (LOD) achievable with PAD detection have been compared with those by single-wavelength UV detection.

## EXPERIMENTAL

The chromatography was performed using a Dionex ICS-3000 system (Dionex, Sunnyvale, CA). The ICS-3000 system consisted of a DP gradient pump with on-line degas, an AS autosampler (injection loop, 20  $\mu$ L), a temperature controlled column compartment and an ED electrochemical detector. The separations were carried out at 30 °C. The eluent, a 0.10 M solution of methanesulfonic acid (MSA), was pumped at a flow rate of 0.20 mL/min through an IonPac ICE-AS1 (4  $\times$  250 mm) column. A three-electrode thin-layer amperometric detection cell was equipped with a disposable platinum electrode. The surface area of the platinum working electrode was 0.785 mm<sup>2</sup>. A reference electrode (Ag/AgCl) was placed downstream from the thin-layer channel. The system control, data analysis and data processing were carried out with the help of Chromeleon<sup>®</sup> software from Dionex. All chromatographic results reported here were generated using experimental conditions described above, unless specified otherwise.

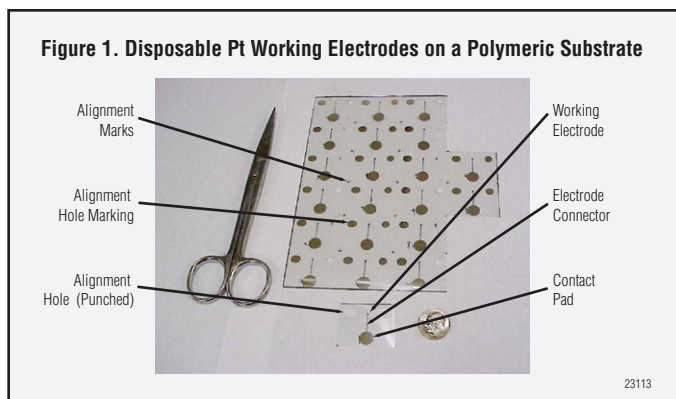
## RESULTS AND DISCUSSION

### Fabrication of Disposable Electrodes

The polymeric substrates were cleaned, dried, placed on a stainless steel supporting plate, covered with a stainless steel mask defining the shape of working electrode, of contact lead and of contact pad. The assembly, consisting of ground plate, polymeric film, and stainless steel mask, was placed into a radio frequency sputtering chamber. The chamber was then evacuated to  $\sim 10^{-7}$  Torr. A layer of ca. 500 Å titanium was sputtered first to promote adhesion of the platinum film to the polymeric film. Following that, a second layer of ca. 10,000 Å platinum was sputtered onto the titanium layer using a 99.99% Pt target.

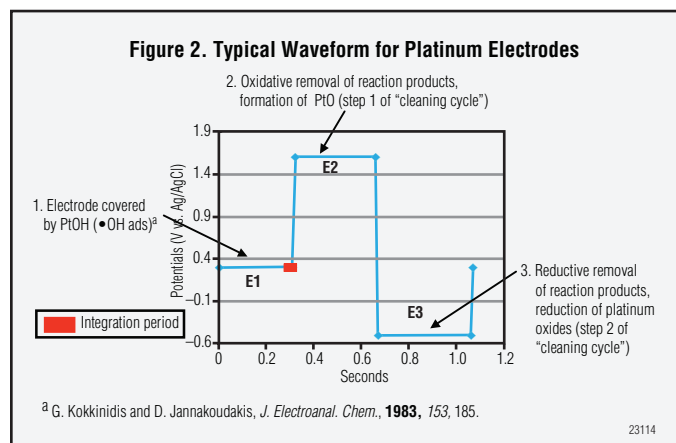
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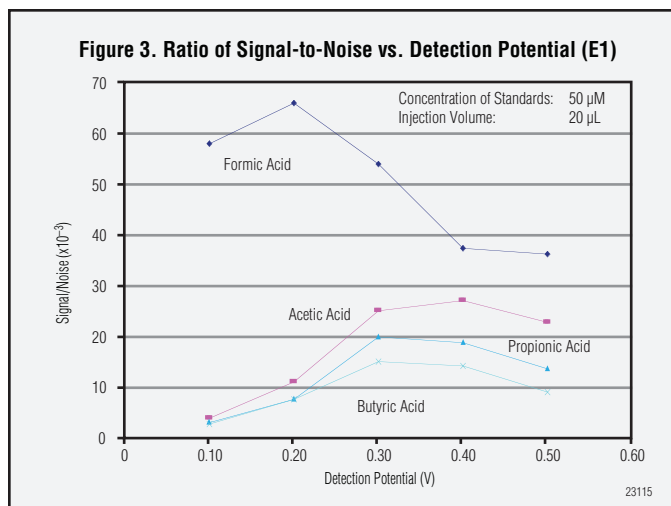
## Pulsed Amperometric Detection (PAD)

PAD uses a multi-step potential waveform incorporating a signal acquisition by current integration as well as a specified period of time for anodic and cathodic cleaning potentials. In a triple-potential waveform, current integration occurs during a first potential, usually at the end of the time period to minimize the contribution by non-Faradaic currents. The potential is then stepped up to a higher potential achieving oxidative cleaning of the electrode surface. Subsequently, a third potential, which is lower than the first and second potentials, is applied for electrode reconditioning by cathodic dissolution of the surface oxide formed during the first and/or second potentials of the waveform.



## Waveform Optimization

The E1 potential was varied between 0.10 and 0.50 V vs Ag/AgCl in 0.10 V increments. The E2 and E3 potentials were kept constant at 1.15 and -0.30 V respectively. The resulting series of response results is represented as a plot of signal/noise versus detection potential in Figure 3. The potential of 0.30 V is the optimal detection potential for all of the organic acids evaluated in study.



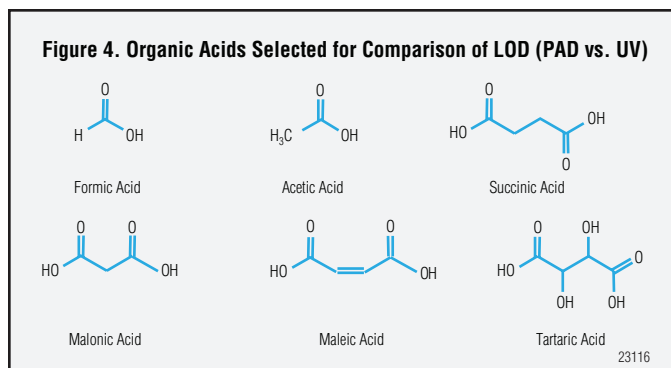
**Table 1. Optimized Waveform**

Time (s)	Potential (V)*	Integration
0.00	0.30	
0.29	0.30	Start
0.31	0.30	End
0.32	1.15	
0.66	1.15	
0.67	-0.30	
1.06	-0.30	
1.07	0.30	

\* vs. Ag/AgCl

## Limits of Detection

Limits of Detection were determined as three multiples of noise in μM units using an injection volume of 10 μL. For the comparison of detection limits, we carried out separations at 30 °C with an eluent containing 5 mM methanesulfonic acid (MSA). The flow rate and column were 0.60 mL/min and Acclaim OA silica-based (5 μm, 4.6 × 250 mm) respectively. The UV detection was performed at 210 nm using an AD20 detector (Dionex). All the other conditions for both UV and ED detections were same as described in Experimental.



**Table 2. Comparison of Limits of Detection ( $\mu\text{M}$ ) by PAD and UV**

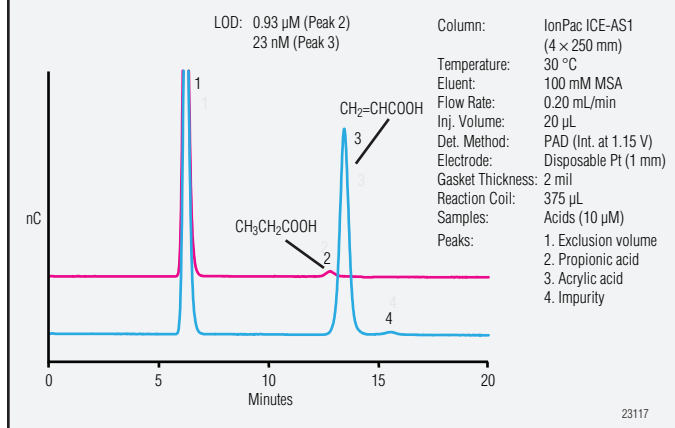
	Formic Acid	Acetic Acid	Malonic Acid	Succinic Acid	Tartaric Acid	Maleic Acid
UV	25.63	24.31	28.77	16.50	0.30	1.09
ED	0.97	2.97	1.58	0.77	0.04	0.21
LOD Ratio of UV/ED	26.42	8.18	18.21	21.43	7.50	5.19

**Table 3. Dependence of LOD on Eluent Concentration: PAD and IonPac ICE-AS 1 Column**

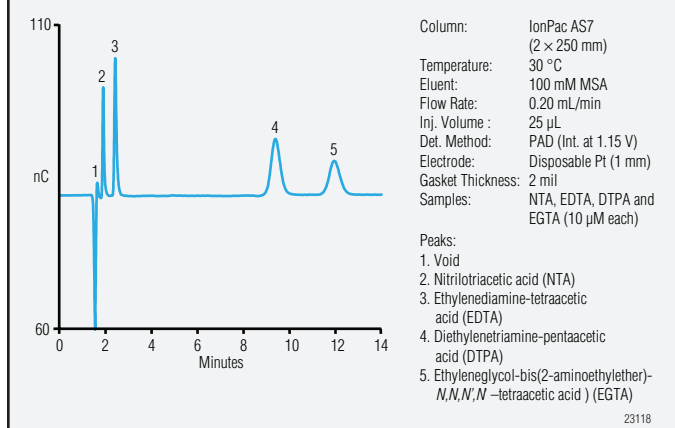
MSA Conc. (mM)	Formic Acid ( $\mu\text{M}$ )	Acetic Acid ( $\mu\text{M}$ )	Propionic Acid ( $\mu\text{M}$ )	Butyric Acid ( $\mu\text{M}$ )
25	25.58	53.48	36.71	46.27
50	9.30	15.88	22.73	35.81
100	2.78	5.98	7.54	9.96
200	2.55	22.01	22.56	20.09

The optimal concentration of MSA is at 100 mM for all tested aliphatic organic acids.

**Figure 5. Example Chromatograms of Organic Acids**



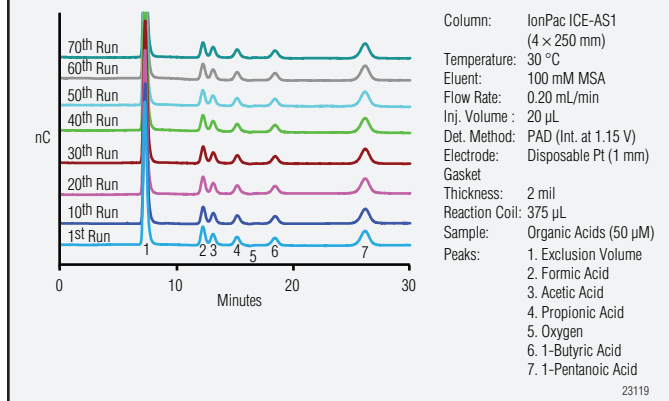
**Figure 6. Example Chromatogram of Carboxylic Chelates**



## Reproducibility

The reproducibility was evaluated by 70 consecutive injections of a mixture of five aliphatic acids over a 48-hr period. The chromatograms from the 1<sup>st</sup>, 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup>, 40<sup>th</sup>, 50<sup>th</sup>, 60<sup>th</sup>, and 70<sup>th</sup> runs are shown in Figure 7. The relative standard deviations of peak areas for all organic acids in the 70 chromatograms are in the range of 2.1–7.4% (Table 4).

**Figure 7. Reproducibility Evaluation: Organic Acids**



**Table 4. Reproducibility: 70 Injections, 2 Days**

	Formic Acid	Acetic Acid	Propionic Acid	Butyric Acid	Pentanoic Acid
RSD (%)	7.44	3.22	5.34	2.93	2.07

## CONCLUSIONS

- Significantly better Limits of Detection (LOD) with ED than with UV
- Direct detection of organic acids (no derivatization)
- Unsaturated carboxylic acids are detected with a higher sensitivity than saturated carboxylic acids
- Achieved good reproducibility (% RSD areas) < 7.5%
- Developed a fabrication process for manufacturing of disposable Pt electrodes
- Optimized a waveform for disposable Pt electrodes

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