

Supplementary Material

Imidazolium-based ionic liquids support biosimilar flavin electron transfer

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Contents

| | |
|---|----|
| 1. Materials and Methods: | 2 |
| 1.1 Chemicals and Reagents | 2 |
| 1.2 Analysis and Measurement | 2 |
| 1.3 Electrochemical Cell Setup..... | 2 |
| 1.4 Flavin Mononucleotide Solubility Assay..... | 2 |
| 1.5 Cyclic Voltammetry | 3 |
| 1.6 Chronoamperometry | 3 |
| 1.7 Electrochemical Impedance Spectroscopy (EIS)..... | 3 |
| 1.8 Electrolyte Conductivity | 3 |
| 2. Supplemental Figures: | 4 |
| 3. Supplementary Tables | 12 |
| 4. Supplementary References | 13 |

1. Materials and Methods:

1.1 Chemicals and Reagents

All chemicals were reagent grade and used without further purification unless otherwise stated. 1-ethyl-3-methylimidazolium tetrafluoroborate (98%) was purchased from TCI. Riboflavin-5-phosphate sodium salt dihydrate, alternatively referred to as flavin mononucleotide (FMN) throughout text, (98%) and 1,4-Piperazin-bis-(ethanesulfonic acid) (PIPES, 98.5% for biochemistry) and was purchased from ThermoScientific. Agarose (molecular biology grade) and potassium chloride were purchased from Fischer BioReagents. PIPES buffer was prepared by dissolving PIPES in 1 liter of milliQ water to achieve a 50mM solution that was then adjusted to pH 7.6 with sodium hydroxide.

1.2 Analysis and Measurement

Electrochemical measurements (cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy) were performed on a Reference 600+™ Potentiostat/Galvanostat/ZRA (Gamry Instruments, USA). UV-visible absorbance data was measured using a NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Scientific, USA). Conductivity measurements were made using a SevenCompact™ Duo S213 Benchtop pH/mV/Conductivity Meter (Mettler-Toledo, USA).

1.3 Electrochemical Cell Setup

All electrochemical measurements were made using the same 3-electrode cell setup in 750uL of solution in a 2mL Eppendorf tube. Glassy carbon electrodes (GCEs, Part # AFED050P040GC, Pine Research) were used as the working electrode; all GCEs used were polished prior to making any measurements. The reference electrode was a gel-tipped reference electrode¹ constructed by placing an Ag/AgCl in 3M KCl (CH Instruments, Austin, TX) reference electrode into a 200uL micropipette tip filled with a 2 wt% solution of agarose dissolved in 3M KCl. The agarose solution was allowed to set for ~5 hours, the tip was trimmed slightly to expose the gel medium and then stored in a 3M KCl solution until use. Platinum wire was used as the counter electrode.

1.4 Flavin Mononucleotide Solubility Assay

Identical solutions of varying FMN concentrations (2.5mM – 12.5mM) were prepared in both 300mM KCl and 300mM [Emim][BF₄] (all in 50mM PIPES buffer at pH 7.6). UV-vis absorbance at 445 nm was measured for the different FMN concentrations in triplicate. These values were then used to construct a concentration vs. absorbance calibration curve (Figure S2a & b). Then, fully saturated solutions of FMN in both KCl and [Emim][BF₄] were prepared similar to the previous solutions. Absorbance at 445 nm of a 1:10 dilution of the fully saturated solutions was measured (in triplicate) and final concentrations were determined by calculating based on the linear fit of the appropriate calibration curve. The 1:10 dilution was chosen to achieve an absorbance within the linear range established by the calibration curve.

1.5 Cyclic Voltammetry

All cyclic voltammetry (CV) measurements were performed on 5mM FMN solutions in 50mM PIPES buffer (pH 7.6) with varied concentrations of KCl or [Emim][BF₄] as the electrolyte species. Electrolyte concentrations were 100mM, 300mM, and 500mM. All CV measurements were made scanning the potential range of -0.8V and 0.0V vs. Ag/AgCl. CV measurements were taken for varied scan rates from 2mV/s up to 1V/s. Peak currents, midpoint potential, charge transfer, and other key values were determined using the Gamry Echem Analyst software.

1.6 Chronoamperometry

Chronoamperometry measurements were taken of 5mM FMN solutions in either 300mM KCl or 300mM [Emim][BF₄] in 50mM PIPES buffer (pH 7.6). A step potential of -0.7V was applied for 30 minutes and the resulting changes in current were recorded.

1.7 Electrochemical Impedance Spectroscopy (EIS)

Potentiostatic electrochemical impedance spectroscopy (P-EIS) measurements were made of 5mM FMN solutions in either 300mM KCl or 300mM [Emim][BF₄] in 50mM PIPES buffer (pH 7.6) using the same 3-electrode setup previously described. Measurements were made versus a reference potential determined by taking the midpoint potential from a 200mV/s cyclic voltammetry scan between -0.8V and 0V vs Ag/AgCl. P-EIS measurements were taken in frequency range of 100000 Hz to 0.10 Hz with a sinusoidal frequency of 10 mV/s and repeated in triplicate. P-EIS results were fit to the CPE with Diffusion model using the Gamry Echem Analyst software.

1.8 Electrolyte Conductivity

Electrolyte solutions were prepared in triplicate, dissolving either potassium chloride or [Emim][BF₄] in PIPES buffer (pH 7.6) to obtain solutions with a 300mM concentration of the chosen electrolyte. A SevenCompact™ Duo S213 Benchtop pH/mV/Conductivity Meter (Mettler-Toledo, USA) was calibrated using the 12.88 mS/cm calibration standard solution. Then conductivity at room temperature for each solution was recorded, washing the probe with DI water between measurements.

2. Supplemental Figures:

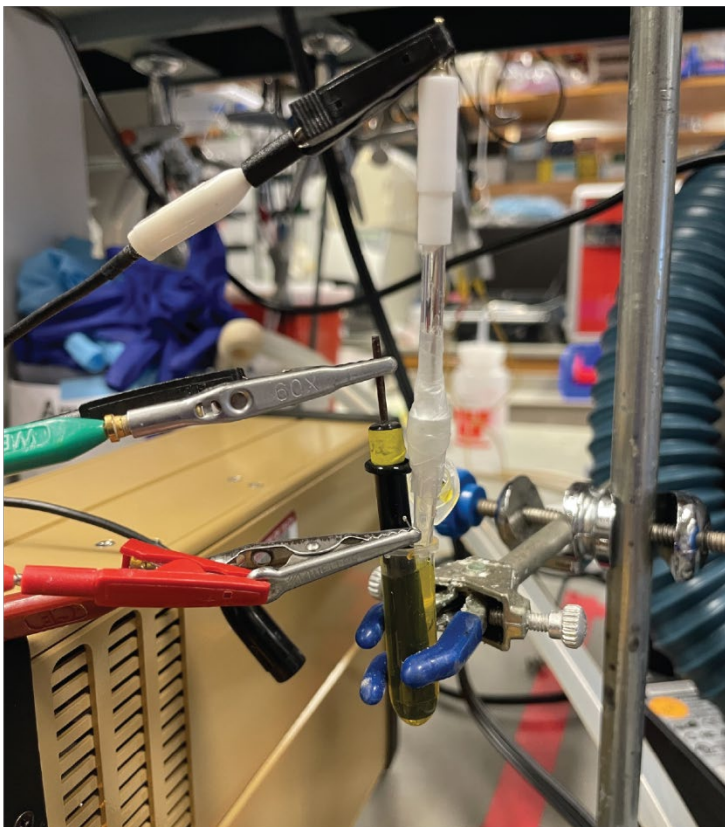


Figure S1. Image of electrochemical setup utilized for all CV, CA, and EIS experiments with a glassy carbon working electrode, platinum wire counter electrode, and gel-tipped Ag/AgCl reference electrode.

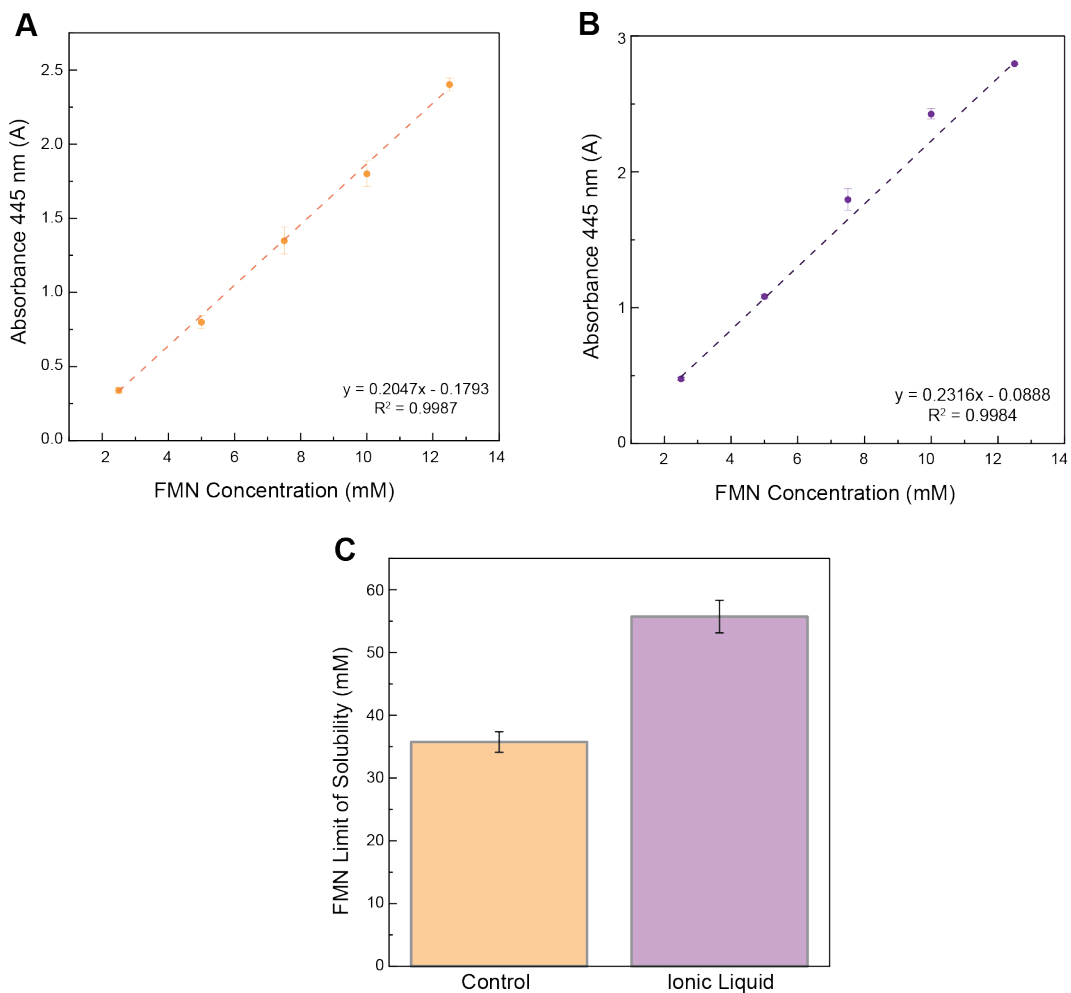


Figure S2. FMN limit of solubility derived from UV-Vis absorption data. A) FMN concentration in 300mM KCl electrolyte vs. absorbance at 445nm calibration curve with linear fit equation; B) FMN concentration in 300mM IL electrolyte vs. absorbance at 445nm calibration curve with linear fit equation; C) FMN limit of solubility in mM for KCl control compared to IL electrolyte determined using the linear fits of 2a and 2b respectively. All measurements were done in triplicate (n=3) with standard deviation shown with error bars.

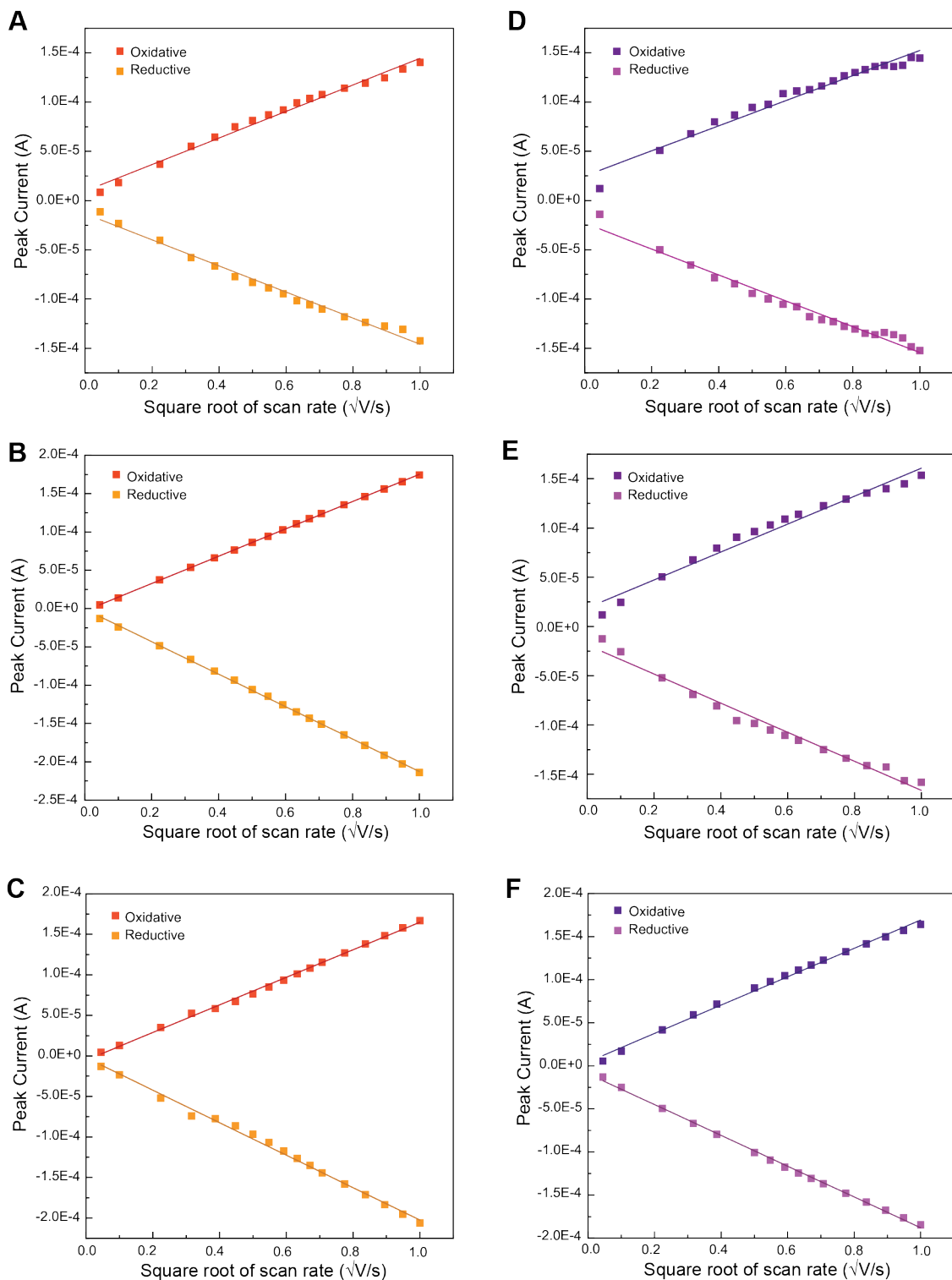


Figure S3. Triplicates of Randles-Sevcik plots derived from variable scan rate CV data. A-C are Randles-Sevcik plots with linear fits for the inorganic control (5mM FMN in 300mM KCl and 50mM PIPES buffer) solutions. D-F is the same but replacing KCl with [Emim][BF₄] IL.

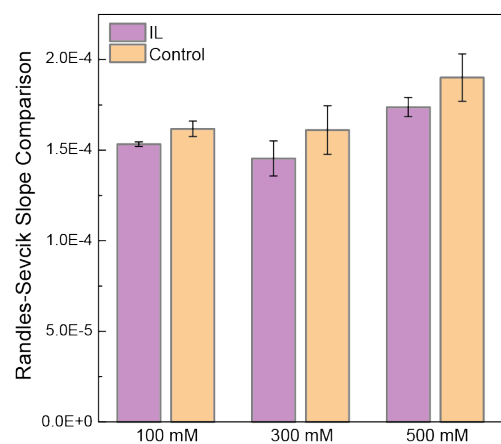


Figure S4. Slope comparison between IL and KCl standard of Randles-Sevcik plots from variable scan rate CV data for 100mM, 300mM, and 500mM electrolyte solutions.

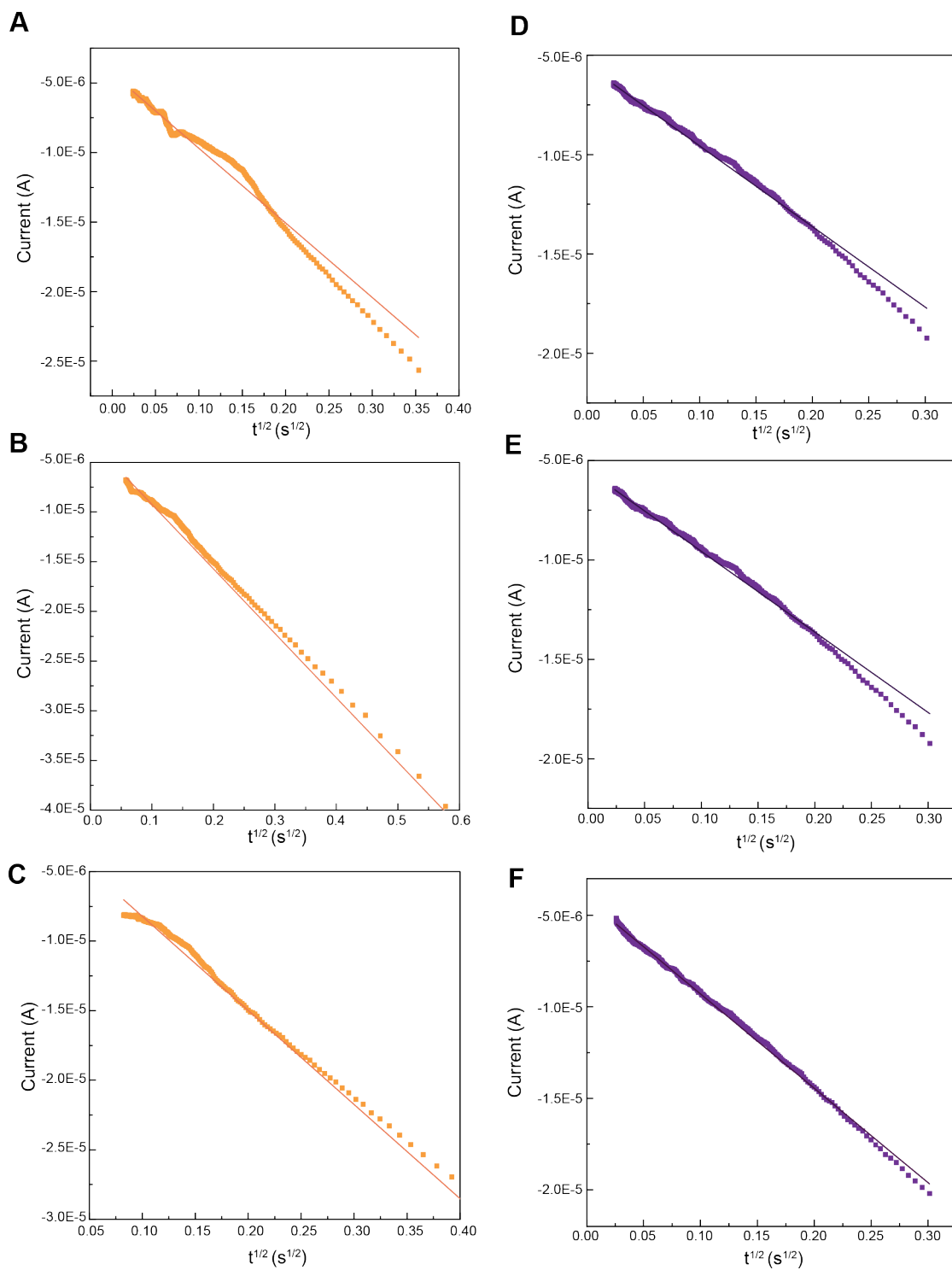


Figure S5. Cottrell equation linear fit to chronoamperometry data. A-C are CA data with Cottrell linear fit for inorganic electrolyte control (5mM FMN in 300mM KCl and 50mM PIPES buffer). D-F is the same but replacing KCl with [Emim][BF₄] IL.

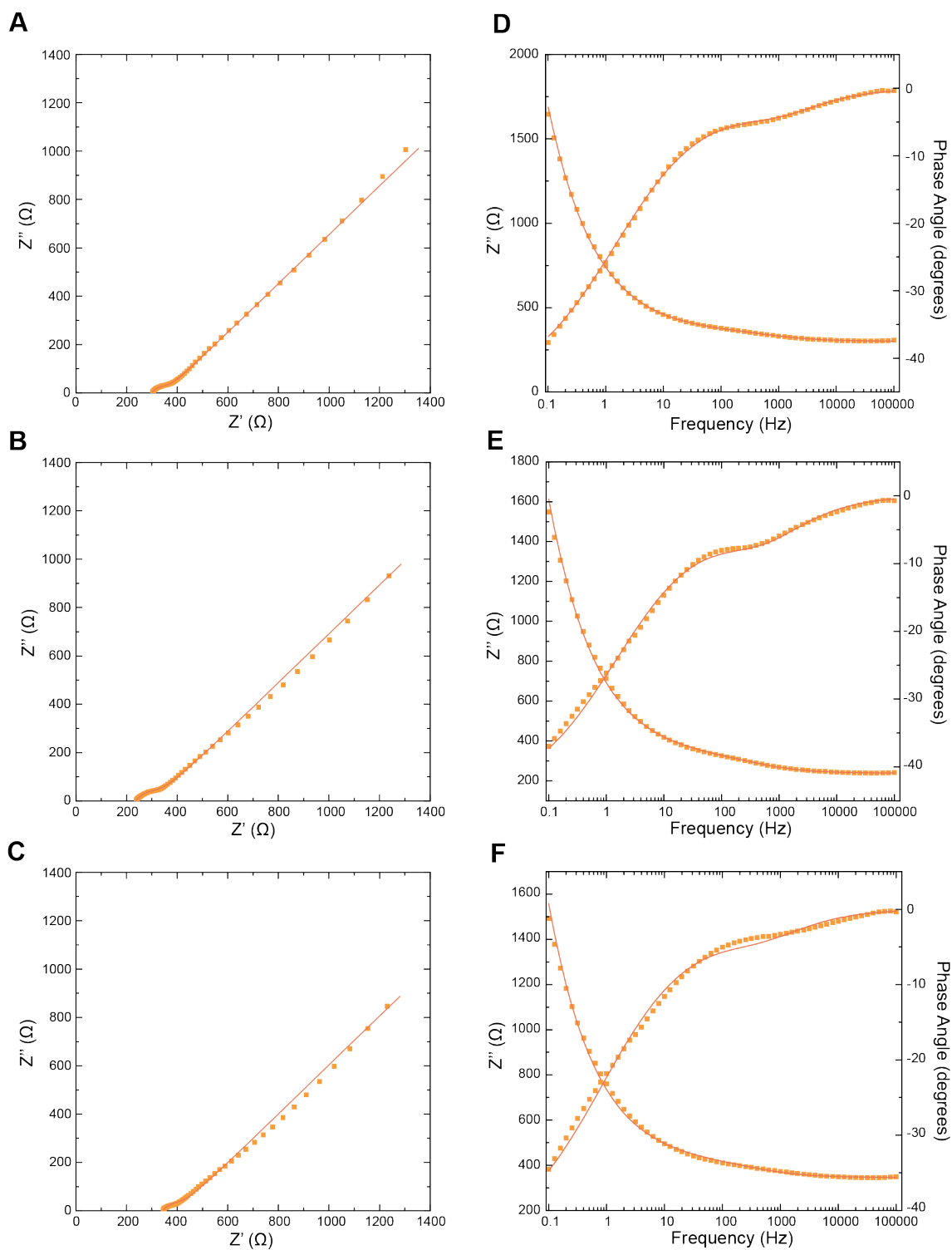


Figure S6. EIS data and CPE with Diffusion model fits for 5mM FMN in 300mM KCl and 50mM PIPES buffer. A-C are Nyquist plots with the model fit. D-F are the corresponding Bode plots.

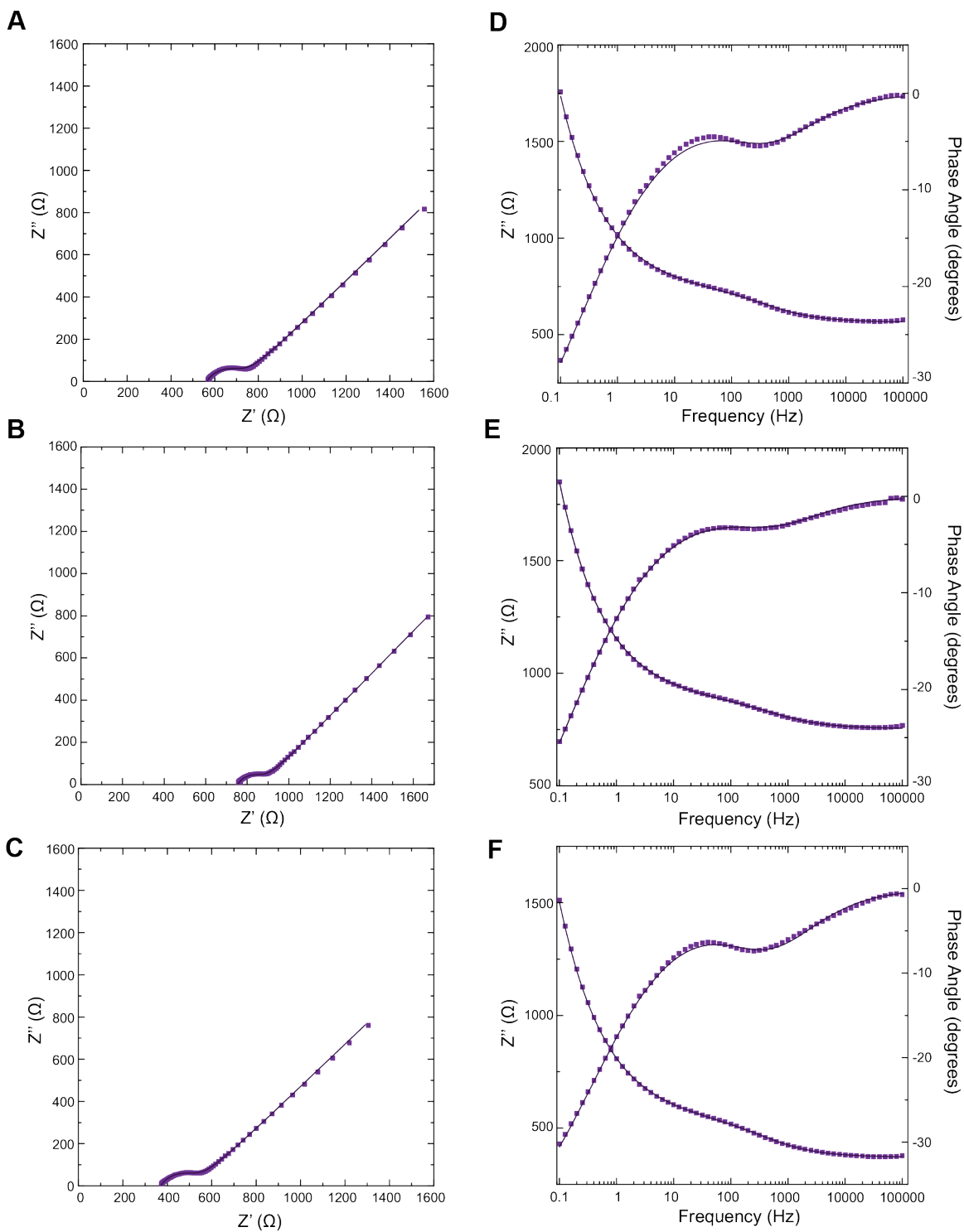


Figure S7. EIS data and CPE with Diffusion model fits for 5mM FMN in 300mM IL and 50mM PIPES buffer. A-C are Nyquist plots with the model fit. D-F are the corresponding Bode plots.

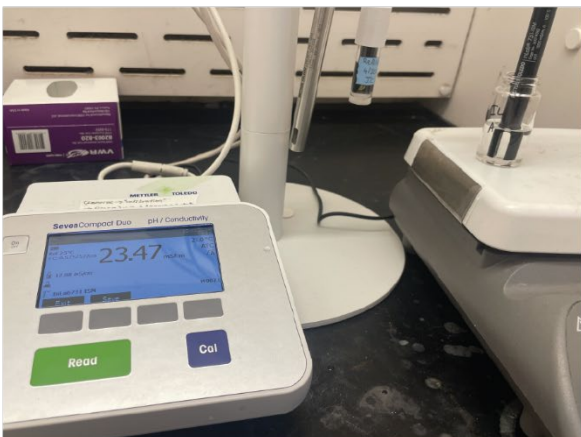
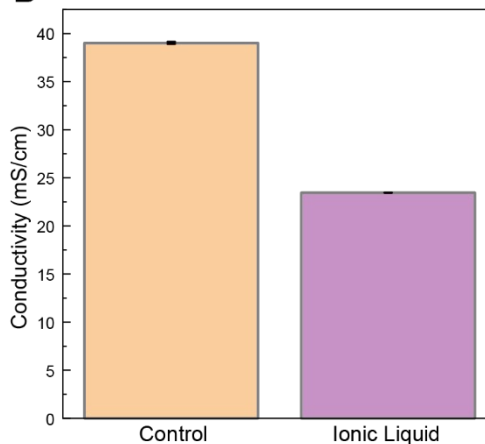
A**B**

Figure S8. Electrolyte conductivity measurements. A) Image of conductivity meter measuring an IL solution. B) Bar graph comparing average conductivity of 300mM KCl electrolyte and 300mM IL electrolyte, both in PIPES buffer. Error bars represent standard deviation of n=3 triplicate measurements.

3. Supplementary Tables

| Circuit element | IL | Control |
|-----------------|-----------------------------------|------------------------------------|
| R_s | $565.2 \pm 92.91 \Omega$ | $291.0 \pm 55.53 \Omega$ |
| Y_0 | $2.09E-05 \pm 7.1E-06 S*s^\alpha$ | $2.94E-05 \pm 1.70E-05 S*s^\alpha$ |
| α | 0.721 ± 0.0424 | 0.7899 ± 0.1144 |
| R_{ct} | $157 \pm 24.9 \Omega$ | $72.2 \pm 13.85 \Omega$ |
| W_d | $1.12E-3 \pm 3.21E-5 S*s^{1/2}$ | $9.1E-04 \pm 4.44E-05 S*s^{1/2}$ |

Table S1. Calculated values of circuit elements from fitting EIS data to the Randles equivalent cell with a constant phase element (CPE with diffusion). Error shown is standard deviation of three replicates seen in Figure S6 and S7.

4. Supplementary References

- (1) Hassel, A. W.; Fushimi, K.; Seo, M. An Agar-Based silver/silver Chloride Reference Electrode for Use in Micro-Electrochemistry. *Electrochemistry Communications* **1999**.