1 Cyclic voltammetry Simulation

Cyclic voltammetry (CV) is a popular electrochemical technique commonly employed to investigate the reduction and oxidation processes of molecular species [1]. CV is also invaluable to study electron transfer-initiated chemical reactions, which includes catalysis.[2] In order to evaluate the proper parameters for the Electrochemical Impedance Spectroscopy (EIS) measurement such as perturbation potential and formal (bias) potential it can be helpful to obtain best possible conditions.

This cyclic voltammetry simulation couples a one-electron electrochemical reduction with a subsequent chemical reaction of the reduced species, as below:

$$O + e^- \underset{k_b}{\overset{k_f}{\rightleftharpoons}} R \xrightarrow{k_c} Z \qquad [3] \tag{1}$$

In this simulation (see figure 1), we model three mechanistic processes: the electrochemical reaction (charge transfer), the chemical reaction, and diffusion. This type of coupled electrochemical-chemical process is often called an EC reaction. [3]

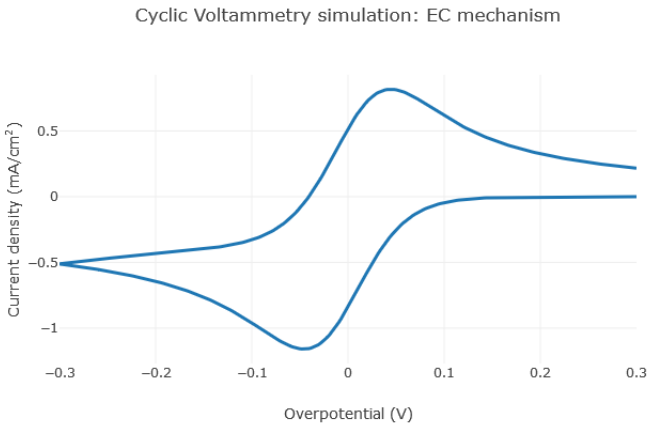

Figure 1: CV model of a bare electrode

The x-axis represents a parameter that is imposed on the system, here the applied potential E, while the y-axis is the response, here the resulting (exchange) current (density) passed.

Table 1: Essential paramters for CV Simulation.[4][5],[6],[1]

Symbol	Description	Value
C_0	initial concentration of O	$0.05\frac{mol}{cm^3}$
D	diffusion coefficient of both O and R	$3.09e-06\frac{cm^2}{s}$
η_i	initial overpotential	$0.3V$
η_f	final overotential	$-0.3V$
ν	scan rate	$0.1\frac{V}{s}$
α	charge tranfer coefficient	0.5
k^0	electrochmical rate constant	$0.0114\frac{cm}{s}$
k_c	chemical rate constant	$1e-03\frac{1}{s}$

The simulation was realised through the online simulation tool using a MATLAB script (`https://petermattia.com/cyclic_voltammetry_simulation/index.html`)

The formal potential is often estimated with the experimentally determined $E_{1/2}$ value (average potential between the two current peaks)[1]. The peaks are considered as the equilibrium of the electrochemical reaction. This equilibrium is described by the Nernst equation. It provides a powerful way to predict how a system will respond to a change of concentration. [1]

The Nernst equation relates the potential of an electrochemical cell (E) to the standard potential of a species (E_0) and the relative activities of the oxidized (Oxi) and reduced (Red) analyte in the system at equilibrium [7].

$$E_{Ferricyanide/Ferrocyanide} = E_0 - \frac{RT}{nF}\ln\left(\frac{Oxi}{Red}\right) = E_0 - \frac{RT}{nF}\ln\left(\frac{[Fe(CN)_6^{4-}]}{[Fe(CN)_6]^{3-}}\right) \tag{2}$$

2 Electrochemical Impedance Spectroscopy Simulation with COMSOL

Randles model

The Simplified Randles cell is one of most common cell models.[8] It includes a solution resistance, a double layer capacitor and a charge transfer (or polarization resistance)[9]. The double-layer capacitance is in parallel with the charge-transfer resistance. In addition to being a useful model in its own right, the simplified Randles Cell is the starting point for other more complex models.

The equivalent circuit for a Simplified Randles Cell is shown in Figure 2.

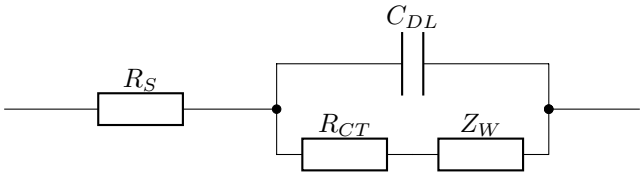

Figure 2: Randles cell model: Equivalent circuit with mixed kinetic and charge-transfer control

2.1 Charge-transfer resistance - R_{CT}

The charge transfer resistance is formed by a single, kinetically-controlled electrochemical reaction during equilibrium. Consider a redox reaction including a metal substrate and an electrolyte. The metal can electrolytically dissolve into the electrolyte, according to,

$$Me \Leftrightarrow Me^{n+} + ne^- \tag{3}$$

or

$$Red \Leftrightarrow Ox + ne^-$$

In the previous reaction (eq. 3), electrons enter the metal and metal ions diffuse into the electrolyte. This implies that charge is being transferred [10].

This charge transfer reaction has a particular speed, which depends on the kind of reaction, the temperature, the concentration of the reaction products and the potential. This certain resistance can be calculated through:

$$R_{CT} = \frac{R \cdot T}{n \cdot F \cdot i_0} \qquad [4] \tag{4}$$

From this equation the exchange current density i_0 can be calculated when R_{CT} is known. The exchange current i_0 is given by the equation:

$$i_0 = nFAk_0C_{ox} \qquad [4] \tag{5}$$

or can be read off figure 1. Combining eq. 4 and eq. 5 lead to:

$$k_0 = \frac{RT}{n^2F^2R_{CT}AC} \qquad [4] \tag{6}$$

A typical value for R_{CT} would be 1kΩ [4].

Symbol	Description
R	gas constant
T	temperature
n	number of electrons involved
i_0	exchange current density
F	Faradays constant

2.2 Warburg element - Z_W

Diffusion also causes an impedance called a Warburg impedance [11]. This impedance depends on the frequency of the potential perturbation. At high frequencies (up 100kHz), the Warburg impedance is small since diffusing reactants don't have to move very far. At low frequencies (down to 0.01 Hz), the reactants have to diffuse farther, increasing the Warburg-impedance. [12] The equation for the "infinite" Warburg impedance is:

$$Z_w = \frac{\sigma}{\sqrt{\omega}} + \frac{\sigma}{j\sqrt{\omega}} \qquad [11] \tag{7}$$

On a Nyquist Plot the Warburg element appears as a diagonal line with a slope of 45°. The phase response of the Warburg impedance exhibits a phase shift of 45°.[11]

In eq. 8, σ the Warburg coefficient is defined as:

$$\sigma = \frac{RT}{n^2F^2A\sqrt{2}}\left(\frac{1}{C_O^*\sqrt{D_0}} + \frac{1}{C_R^*\sqrt{D_R}}\right) \qquad [11] \tag{8}$$

Symbol	Description
ω	radial frequency
D_O	diffusion coefficient of the oxidant
D_R	diffusion coefficient of the reductant
A	surface area of the electrode
n	number of electrons involved
C_O^*	concentration of oxidant in the bulk
C_R^*	concentration of reductant in the bulk

2.3 Double layer capacitor - C_{DL}

The most ordinary capacitor is formed when two conducting plates are separated by a non-conducting media, called the dielectric [13]. If a voltage is applied on these two plates, the electric field is formed in between which value depends on the dielectric. The value of the capacitance also depends on the size of the plates, the distance between the plates and the properties of the dielectric. The relationship is given by:

$$C_{DL} = \frac{\epsilon_r \cdot \epsilon_0 \cdot A}{d} = 58.436pF \qquad [13] \tag{9}$$

(see also appendix 4.4)

Symbol	Description	Value
ϵ_r	dielectric constant (relative electrical permittivity)	4-8 (organic coating)
ϵ_0	permittivity of free space	$8.854.. \cdot 10^{-12}\frac{F}{m}$
A	surface area at one plate	11 mm^2
d	distance between two plates	$\approx 10\mu m$ (approx. size of spores)

2.4 Solution (Electrolyt) resistance - R_S

The resistance of the electrolyt often plays an important role in the impedance of an electrochemical cell. A modern three electrode potentiostat compensates for the solution resistance between the counter electrode and reference electrode. Therefore, any solution resistance between these two electrodes must be considered when modelling an electrochemical cell[14].

The resistance of an ionic solution depends on the ionic concentration, type of ions, temperature, and the geometry of the area in which current is carried. In a bounded area with area A, and length, l, carrying a uniform current, the resistance is defined by

$$R_S = \frac{\rho \cdot l}{A} \qquad [14] \tag{10}$$

where ρ is the solution resistivity. Usually, the solution resistance is not calculated from ionic conductances. Instead, you calculate it when you fit experimental EIS data to a model. [15]

2.5 COMSOL - Simulation

Symbol	Description	Value	Source
A_{el}	Electrode area	11[mm^2]	datasheet
c_bulk_ox	Bulk concentration	5[mol/m^3]	[6]
c_bulk_red	Bulk concentration	5[mol/m^3]	[6]
C_{DL}	Double layer interfacial capacitance	0.00053[uF/cm^2]	eq. 9
D_{ox}	Diffusion coefficient	3.09e-6[cm$^2/s$]	[5]
D_{red}	Diffusion coefficient	3.09e-6[cm$^2/s$]	[5]
$freq_{max}$	Maximum frequency	100000[Hz]	
$freq_{max}$	Minimum frequency	0.1[Hz]	
i_0	Exchange current density	6[A/m^2]	fig.1, eq. 5
k_0	Heterogeneous rate constant	0.0115[cm/s]	eq. 6
L_{el}	Electrolyte length	$x_{diff_{max}} * 10$ =3.1362E-4 m	
$log_{freq_{max}}$	Log of max frequency	$log_{10}(freq_{max}[1/Hz])$	
$log_{freq_{min}}$	Log of min frequency	$log_{10}(freq_{min}[1/Hz])$	
V_{app}	Applied perturbation potential	0.01 V	[6]
$x_{diff_{max}}$	Mean diffusion layer thickness at min. freq.	$\sqrt{\frac{D_{ox}}{\pi * freq_{min}}}$=3.1362E-5 m	
$x_{diff_{min}}$	Mean diffusion layer thickness at max. freq.	$\sqrt{\frac{D_{ox}}{\pi * freq_{max}}}$= 3.1362E-8 m	

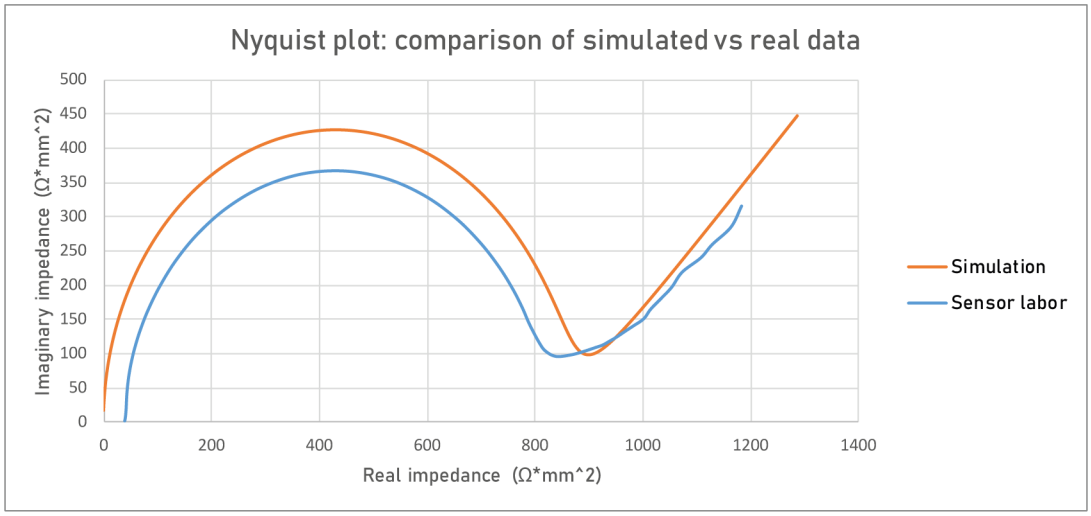

Figure 3: COMSOL simulation is similar to the data from sensor labor

3 Phages Spore Binding

The adsorption of a bacteriophage is generally represented by the equation

$$P + B \xrightarrow{k} PB \qquad [16] \tag{11}$$

where B is the bacterial concentration and P is the free phage concentration also called phage titer.
Taking into account the latest findings, phage adsorption occurs in at least two steps, a reversible interaction being followed by an irreversible reaction. According to such a hypothesis, equation 11 should be adapted into

$$P + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (PB)' \xrightarrow{k_3} PB \qquad [16] \tag{12}$$

where PB is the irreversibly adsorbed phage–bacterium complex.

In general, studies have demonstrated that this mechanism obeys a first order observed rate of reaction where the concentration of the host as an available binding entity remains constant. In this case, the virus concentration decreases exponentially and the rate of adsorption can be described by the rate function

$$r_{ads} = kBP \qquad [17] \tag{13}$$

where k is the adsorption rate constant.

Experiments on the adsorption of phage to living and heat killed *Staphylococcus aureus* in excess bacterial concentrations have led to propose the following pseudo 1st order model to describe the decrease of free phage concentration over time

$$\frac{dP}{dt} = kBP \qquad [17] \tag{14}$$

If all collisions between phage and bacteria lead to irreversible attachment,the maximum value of k is given by

$$k = 2\pi \cdot (h + r) \cdot D_{diff} \qquad [17] \tag{15}$$

Table 2: List of common honeybee pathogens and associated trivial names[18]

Symbol	Description	Value
r	radius of a cylinder "equivalent" to the bacterium P. larvae	$0.25\mu m$
h	height of cylinder "equivalent" to the bacterium P. larvae	1.5-6 μm
D_{diff}	diffusion coefficient of P. larvae	$2.4 \cdot 10^{-8} \frac{cm^2}{s}$
k	adsorption rate constant	
r_{ads}	rate function	
P	phage titer	
B	bacterial concentration	
$[R]_s$	surface concentration	$[[R]_s]$ = molecules/μm^2

If applied on our circumstances equation 15 leads to:

$$k_{min} = 2.6 \cdot 10^{-11} \frac{ml}{s} \tag{16}$$

(see also appendix 4.1)

3.1 Calculation of the effective collision time in the process of phage adsorption

The collision time is defined as the average period of time during which a phage particle remains within a distance ρ from the surface of a bacteria. During this time τ, the particle is said to be in the vicinity of the bacteria. The bacterial surface is assimilated to a plane, and we consider an axis with its origin and direction as shown in Figure 4. It should be noted that certain assumption have been made, referring to the phage-bacteria relation. Meaning that the phages are attached to the electrode surface while the bacteria is floating in the redox solution. Now that this is clarified, let us call $\bar{x}$ the absolute value of the projection on the x-axis of a phage particle after a time t caused by the natural displacement through Brownian motion. The average value of $\bar{x}$ is given by the equation $\bar{x}^2 = 2t \cdot D_{diff}$, where D_{diff} is the diffusion coefficient of the particle. After the average time $t = \frac{x'^2}{2D}$, half of the particles which had a positive abscissa x' will have a negative abscissa, i.e. will have left the vicinity of the bacterium. After an average time $t = \frac{(2\rho - x')^2}{2D}$ the other half of the particles which were at the abscissa x', will have also left the vicinity of the bacterium after bouncing on the bacterial surface. A particle of abscissa x' will therefore take on average a time of

$$\frac{1}{2}\frac{x'^2}{2D} + \frac{1}{2}\frac{(2\rho - x')^2}{2D} \qquad [16] \tag{17}$$

to leave the vicinity of the bacterium. This average time is then integrated for all values of x' between 0 and ρ, assuming an homogeneous distribution of the particles. The collision time is therefore

$$\tau = \int_0^\rho \left[\frac{1}{2}\frac{x'^2}{2D} + \frac{1}{2}\frac{(2\rho - x')^2}{2D}\right]\frac{dx'}{\rho} = 6.25ms[16]$$

(see also appendix 4.2)

The steric factor is defined as the fraction of the collision time during which the phage tail is oriented towards the surface in such a way that it can interact with the receptor. Suppose that a particle is located in a space of distance y from the surface, this will happen $\frac{\theta}{\pi}$ fraction of the time (see figure 4). Since $\theta = \arccos\frac{y}{\rho}$, the steric factor, always assuming an homogeneous distribution of particles, will be calculated by

$$\frac{1}{\pi}\int_0^\rho \left(\arccos\frac{y}{\rho}\right)\frac{dy}{\rho} = \frac{1}{\pi} \qquad [16]$$

(see also appendix 4.3)

The effective collision time, defined as the product of the steric factor by the collision time, is therefore

$$\tau_{eff} = \frac{1}{\pi}\cdot\frac{2\rho^2}{3D} = \frac{1}{\pi}\cdot 6.25ms = 1.989ms \approx 2ms \qquad [16] \tag{18}$$

3.2 The probability of phage-receptor interactions during the effective collision time

The probability of association between the tip of the phage tail and a receptor molecule should be proportional to $[R]_s$, the surface density of receptor and to k_a the association rate constant between phage and receptor, assumed to be the same in vivo as it is in vitro. Therefore, if [P] is the concentration of phage which is: (i)located within ρ of the bacterial surface, (ii) oriented properly as defined above and (iii) no yet bound to receptor, one can specify eq. 14:

$$\frac{d[P]}{[P]} = -\frac{k_a}{l}[R]_s dt. \qquad [16] \tag{19}$$

In this equation l is a constant which has the dimension of a distance which is related to the sizes of the receptor molecule and of the tip of the phage tail, to the flexibility of the phage tail and to the rotational diffusion coefficient of the phage head. The probability that a phage has reacted with receptor after an effective collision time τ_{eff} is therefore

$$\epsilon = 1 - \exp -\frac{k_a[R]_s\tau_{eff}}{l} \qquad [16] \tag{20}$$

which we will call the adsorption efficiency ϵ. This term, normally determined experimentally, is defined by the fraction of the phage population able to adsorb to a host cell over the course of the experiments (in short, the fast adsorbing subpopulation). The experimental determined adsorption efficiency is replaced by the reaction probability of the phages.

$$\frac{dP}{dt} = -kB[P - P_0(1-\epsilon)] \qquad [17] \tag{21}$$

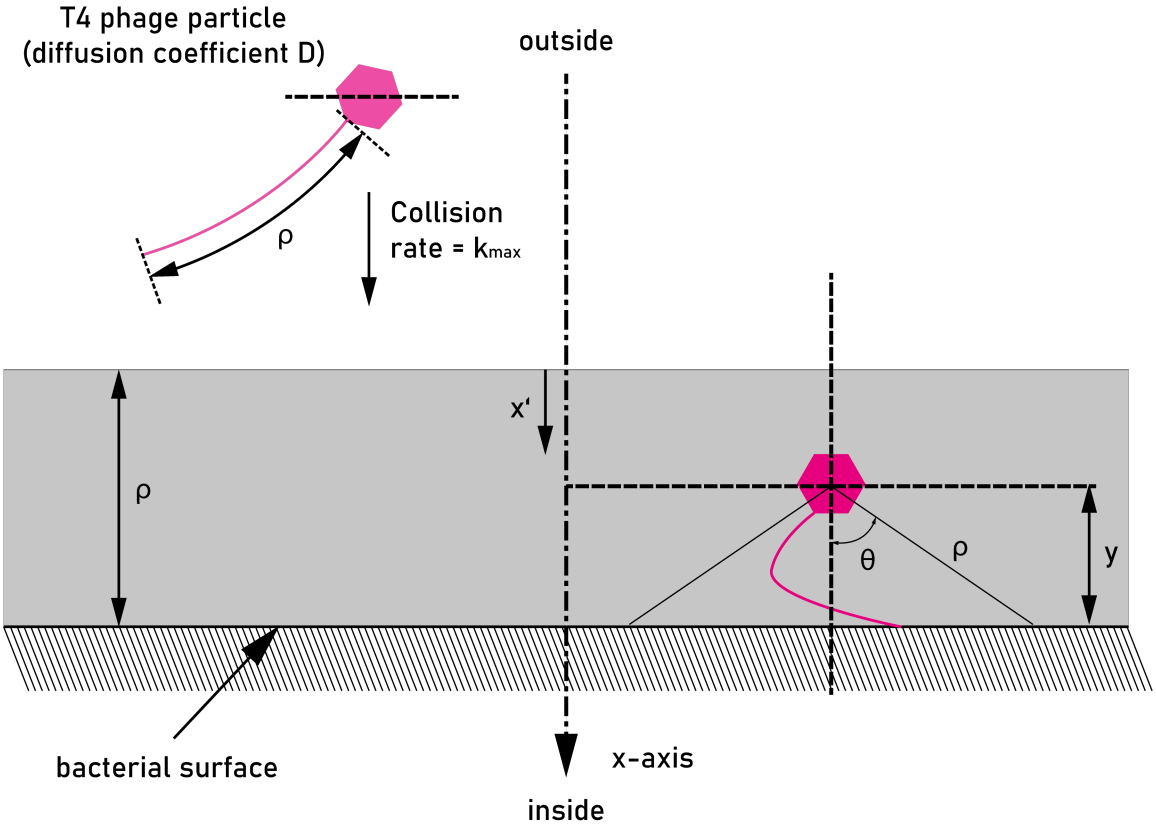

Figure 4: Schematic representation of phage adsorption [16]

4 Appendix

4.1 Calculation of the adsorption rate constant

$$
\begin{aligned}
k &= 2\pi \cdot (h + r) \cdot D_{diff} \\
k_{min} &= 2\pi \cdot (1.5\mu m + 0.25\mu m) \cdot 2.4 \cdot 10^{-8} \frac{cm^2}{s} \\
k_{min} &= 2\pi \cdot 1.75\mu m \cdot 2.4 \cdot 10^{-8} \frac{cm^2}{s} \\
k_{min} &= 26.389 \cdot \mu m \cdot 10^{-8} \frac{cm^2}{s} \\
k_{min} &= 26.389 \cdot 10^{-4} \cdot 10^{-8} \frac{cm^3}{s} \\
k_{min} &= 26.389 \cdot 10^{-12} \frac{cm^3}{s} \\
k_{min} &= 2.6 \cdot 10^{-11} \frac{ml}{s}
\end{aligned}
$$

4.2 Calculation of the collision time

$$
\begin{aligned}
\tau &= \int_0^{\rho} \left[\frac{1}{2} \frac{z^2}{2D} + \frac{1}{2} \frac{(2\rho - z)^2}{2D} \right] \frac{dz}{\rho} = \\
&= \frac{1}{\rho} \left[\left[\frac{z^3}{12D} \right]_0^{\rho} + \int_0^{\rho} \frac{1}{4D} \cdot (4\rho^2 - 4\rho z + z^2) dz \right] = \\
&= \frac{1}{\rho} \left[\frac{\rho^3}{12D} + \left[\frac{1}{4D} \cdot (4\rho^2 z - 2\rho z^2 + \frac{z^3}{3}) \right]_0^{\rho} \right] = \\
&= \frac{\rho^2}{12D} + \frac{\rho^2}{D} - \frac{\rho^2}{2D} + \frac{\rho^2}{12D} = \frac{2}{3} \frac{\rho^2}{D} = \\
&= \frac{2}{3} \frac{(150nm)^2}{2.4 \cdot 10^{-8} \frac{cm^2}{s}} = \frac{45 \cdot 10^3 \cdot 10^{-14} cm^2}{7.2 \cdot 10^{-8} \frac{cm^2}{s}} = 6.25ms
\end{aligned}
$$

4.3 Calculation of the steric factor

$$
\begin{aligned}
&\frac{1}{\pi} \int_0^{\rho} \left(\arccos \frac{y}{\rho} \right) \frac{dy}{\rho} = \\
&= \frac{1}{\rho\pi} \cdot \left[y \cdot \arccos \frac{y}{\rho} - \rho \sqrt{1 - \frac{y^2}{\rho^2}} \right]_0^{\rho} = \\
&= \frac{1}{\rho\pi} \left[\left[\rho \cdot \arccos \frac{\rho}{\rho} - \rho \sqrt{1 - \frac{\rho^2}{\rho^2}} \right] - \left[0 - \rho \sqrt{1 - 0} \right] \right] = \frac{1}{\pi}
\end{aligned}
$$

4.4 Calculation of C_{DL}

$$
C_{DL} = \frac{\epsilon_r \cdot \epsilon_0 \cdot A}{d} = \frac{8.854 \cdot 10^{-12} \cdot 6 \cdot 11 \cdot 10^{-6}}{10 \cdot 10^{-6}} = 5,8436 \cdot 10^{-11} F
$$

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