BPL_IEC_operation script with FMPy

The key library FMPy is installed.

After the installation a small application BPL_IEC_operation is loaded and run. You can continue with this example if you like.

```
!lsb_release -a # Actual VM Ubuntu version used by Google
```

```
No LSB modules are available.
Distributor ID: Ubuntu
Description:    Ubuntu 22.04.4 LTS
Release:        22.04
Codename:       jammy
```

```
!python --version
```

```
Python 3.11.11
```

```
!pip install fmpy
```

```
Collecting fmpy
  Downloading FMPy-0.3.22-py3-none-any.whl.metadata (1.9 kB)
Requirement already satisfied: attrs in /usr/local/lib/python3.11/dist-packag
Requirement already satisfied: Jinja2 in /usr/local/lib/python3.11/dist-packa
Collecting lark (from fmpy)
  Downloading lark-1.2.2-py3-none-any.whl.metadata (1.8 kB)
Requirement already satisfied: lxml in /usr/local/lib/python3.11/dist-packages
Requirement already satisfied: msgpack in /usr/local/lib/python3.11/dist-packa
Requirement already satisfied: numpy in /usr/local/lib/python3.11/dist-packag
Requirement already satisfied: MarkupSafe>=2.0 in /usr/local/lib/python3.11/d
Downloading FMPy-0.3.22-py3-none-any.whl (4.9 MB)
   ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━ 4.9/4.9 MB 22.8 MB/s eta 0:00:00
Downloading lark-1.2.2-py3-none-any.whl (111 kB)
   ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━ 111.0/111.0 kB 5.6 MB/s eta 0:00:0
Installing collected packages: lark, fmpy
Successfully installed fmpy-0.3.22 lark-1.2.2
```

Now specific installation and the run simulations. Start with connecting to Github. Then upload the two files:

- FMU - BPL_IEC_operation_linux_om_me.fmu
- Setup-file - BPL_IEC_operation_fmpy_explore.py

```
%%bash
git clone https://github.com/janpeter19/CONF_2023_08_NPCW24
```

```
Cloning into 'CONF_2023_08_NPCW24'...
```

```
%cd CONF_2023_08_NPCW24
```

```
/content/CONF_2023_08_NPCW24
```

BPL_IEC_operation

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In this notebook we show operation of a typical ion-exchange chromatography step. The impact of pH is also illustrated.

The model is based on the simplified model [1].

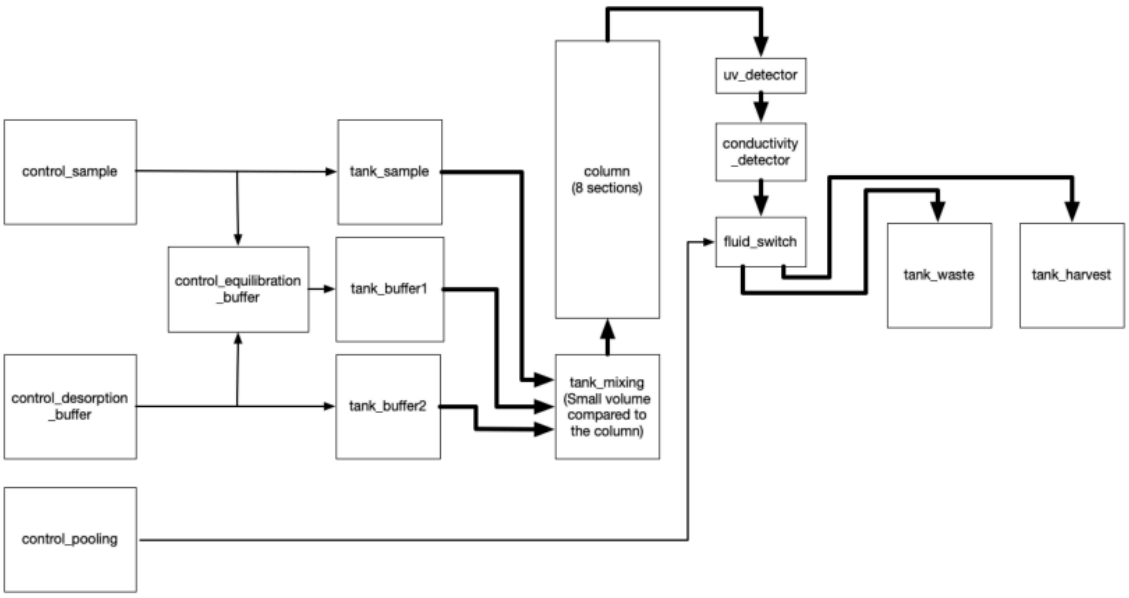
```
run -i BPL_IEC_fmpy_explore.py
```

```
Linux - run FMU pre-comiled OpenModelica 1.21.0

    Model for bioreactor has been setup. Key commands:
     - par()       - change of parameters and initial values
     - init()      - change initial values only
     - simu()      - simulate and plot
     - newplot()   - make a new plot
     - show()      - show plot from previous simulation
     - disp()      - display parameters and initial values from the last simulati
     - describe()  - describe culture, broth, parameters, variables with values/u

    Note that both disp() and describe() takes values from the last simulation
    and the command process_diagram() brings up the main configuration

    Brief information about a command by help(), eg help(simu)
    Key system information is listed with the command system_info()
```

```
plt.rcParams['figure.figsize'] = [30/2.54, 24/2.54]
```

```
process_diagram()
```

```
No processDiagram.png file in the FMU, but try the file on disk.
```



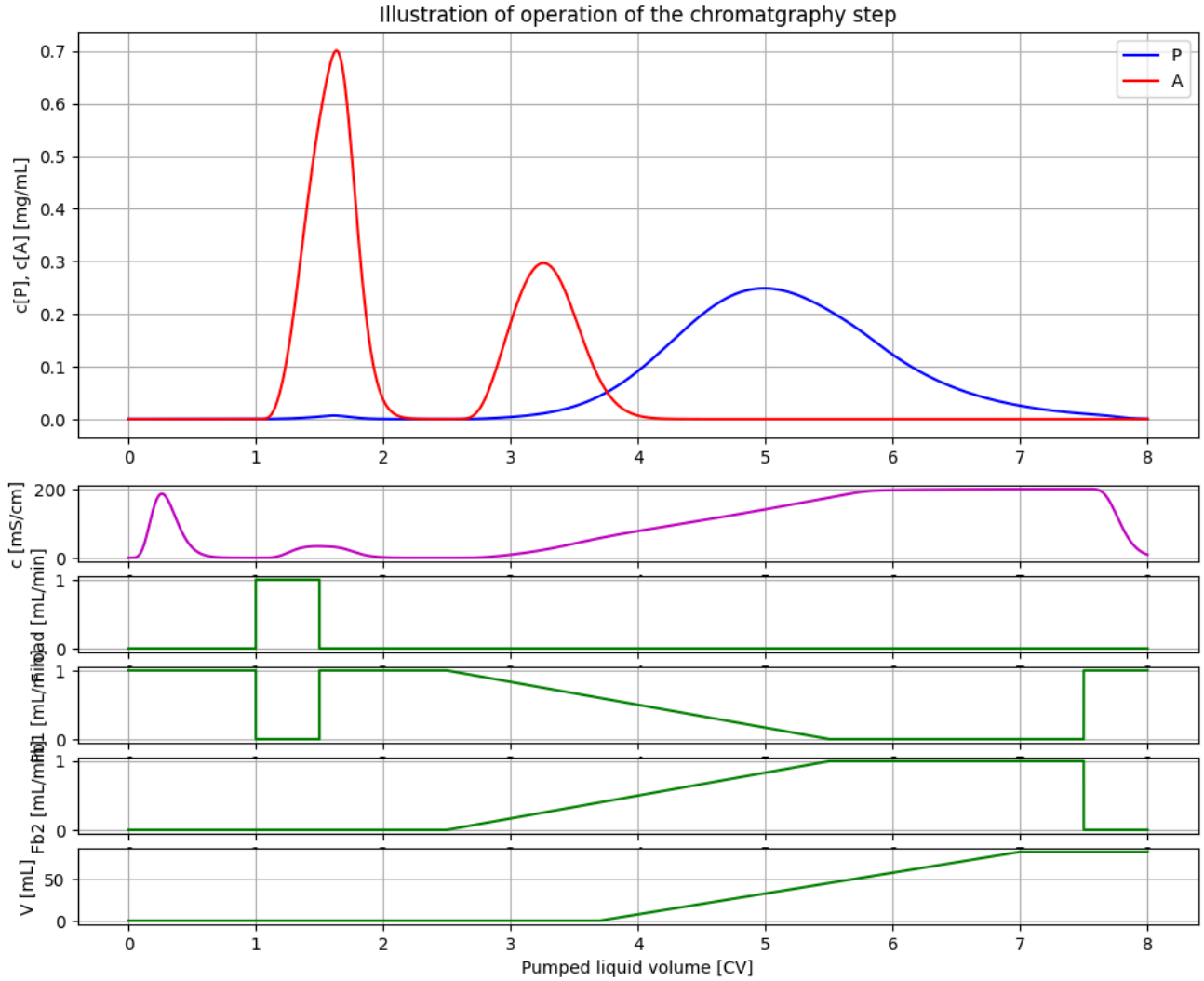
1 Typical parameters an ion exchange chromatography column step

```
# From  given colunn height (h) diameter (d) and linear flow rate (lfr)
# actual column volume (V) and volume flow rate (VFR) are calculated below.

from numpy import pi
h = 20.0
d = 1.261
a = pi*(d/2)**2
V = h*a
print('V =', np.round(V,1), '[mL]')

lfr = 48
VFR = a*lfr/60
print('VFR =', np.round(VFR,1), '[mL/min]')
```

```
V = 25.0 [mL]
VFR = 1.0 [mL/min]
```

```
# Sample concentration product P_in and antagonist A_in
par(P_in = 1.0)
par(A_in = 1.0)
par(E_in = 0.0)
```

```
# Column properties are described by the size and binding capacity of the resin Q
par(height = h)
par(diameter = d)
par(Q_av = 6.0)

# Resin parameters - default values used

# Remaining salt koncentration in the column from prvious batch and eliminated du
init(E_0 = 50)

# Salt concentration of the desorption buffer
par(E_in_desorption_buffer = 8.0)

# Flow rate rate through the
par(LFR=lfr)

# Switching points during operation are conveniently described in terms of multip
CV_ekv = 1.0
CV_ads = 0.5
CV_wash = 1.0
CV_desorb = 3.0
CV_start_pool = 1.2
CV_stop_pool = 4.5
CV_wash2 = 2.5
par(scale_volume=True, start_adsorption=CV_ekv*V, stop_adsorption=(CV_ekv+CV_ads)
par(start_desorption=(CV_ekv+CV_ads+CV_wash)*V, stationary_desorption=(CV_ekv+CV_
par(stop_desorption=7.5*V)
par(start_pooling=(CV_ekv+CV_ads+CV_wash+CV_start_pool)*V, stop_pooling=(CV_ekv+C

# Simulation and plot of results
newplot(title='Illustration of operation of the chromatgraphy step', plotType='El
simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)
```



Comments of steps of operations: 1) Time: 0-1 hours - equilibration. Just to illustrate the equilibration process the first part of the column is given an initial value of salt concentration. 2) Time: 1-1.5 hours - sample is loaded on the column. The product P is adsorbed to the columne and just a small amount passes through and goes to the waste. The antagonist A is much less adsrobed. 3) Time: 1.5-2.5 hours - washing 1. The column comes to equilibrium and both antagonist and product comes down to low levels. 4) Time: 2.5-5.5 hours - desorption. A linear gradient of increaseing salt concentration is applied. First the antagonist and later the product comes out. 5) Time: 5.5-7.5 hours - washing 2 The The column has constant salt concentration

and stationary desorption. 6) Time: 3.7-7.0 hours - pooling of product. The start- and stop of pooling are chosen with trade-off between maximizing the product pooled and minimize the amount of antagonist in the pooling. 7) Time: 7.5-8.0 hours - desorption stopped and salt is washed out and preparation of the next batch to come.

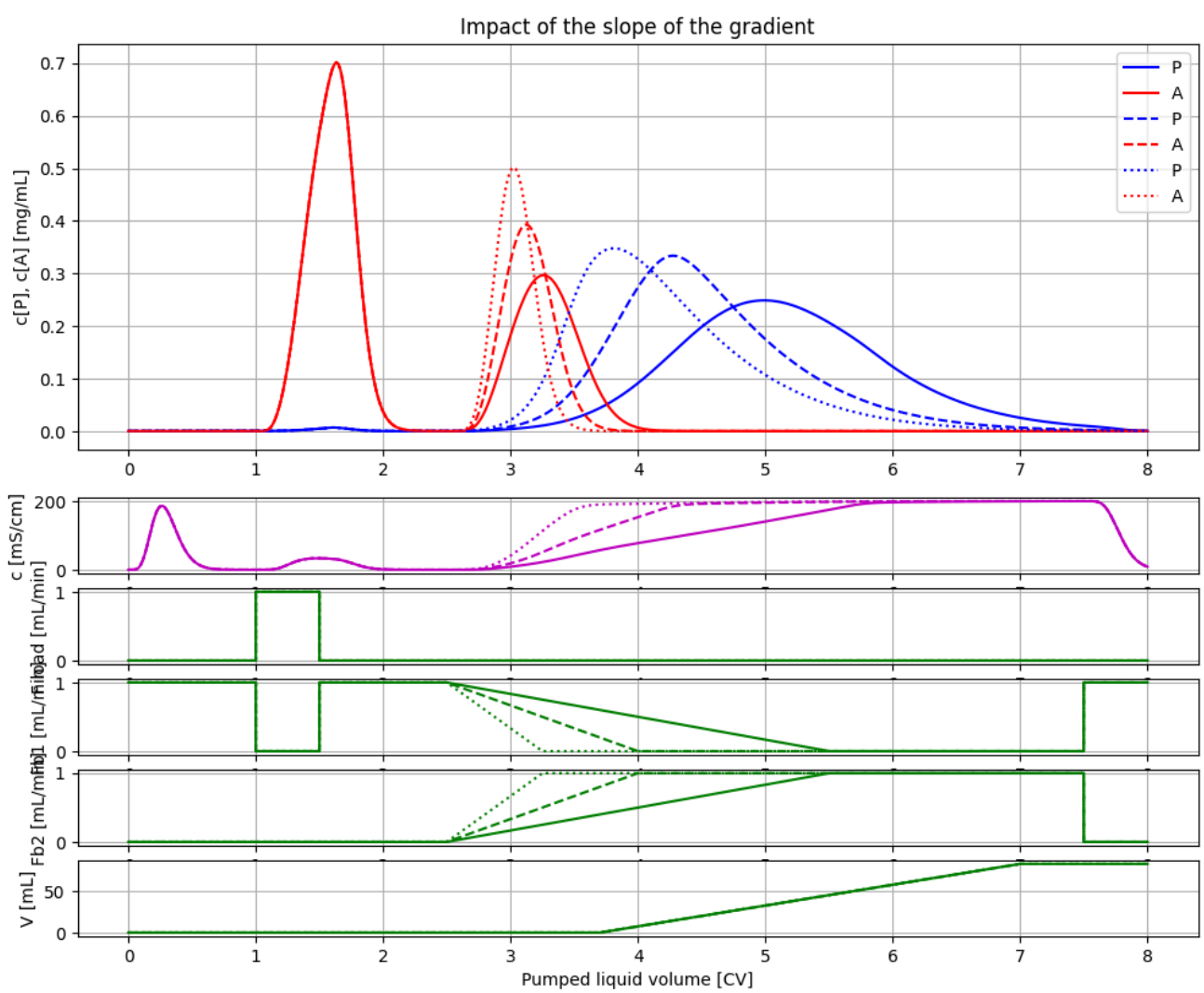
Note that step 4 and 5 is parallel to step 6.

```
# Check mass-balance of P and A
P_mass = model_get('tank_harvest.m[1]') + model_get('tank_waste.m[1]')
A_mass = model_get('tank_harvest.m[2]') + model_get('tank_waste.m[2]')
print('P_mass [mg] =', P_mass)
print('A_mass [mg] =', A_mass)
```

```
P_mass [mg] = 12.422130344708323
A_mass [mg] = 12.488781164505506
```

2 The impact of the slope of the desorption gradient

```
# Simulations showing the impact of change of slope of the desorption gradient
newplot(title='Impact of the slope of the gradient', plotType='Elution-conductivi

# Same gradienet as before
par(start_desorption=(CV_ekv+CV_ads+CV_wash)*V, stationary_desorption=(CV_ekv+ CV
par(stop_desorption=7.5*V)
simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)

# Gradeint finishes after 0.5 of the volume
par(stationary_desorption = (CV_ekv + CV_ads + CV_wash + 0.5*CV_desorb)*V )
simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)

# Fradient finishes after 0.25 of the volume
par(stationary_desorption = (CV_ekv + CV_ads + CV_wash + 0.25*CV_desorb)*V )
simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)
```

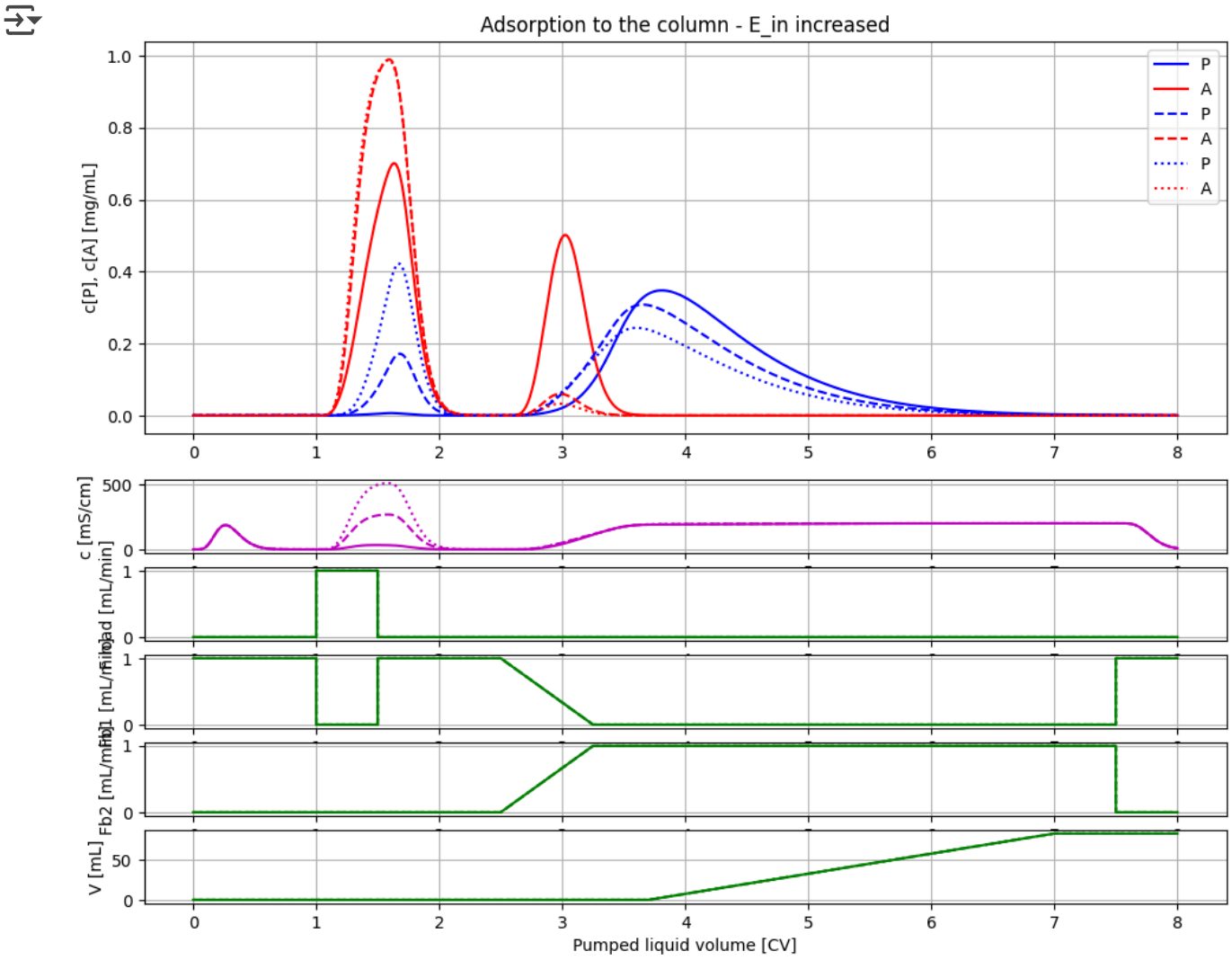


3 The impact of salt concentration in the sample

These values should be compared with the expected value 12.5 mg, i.e. half a column volume with sample concentration 1 mg/L. The difference is due to numerical errors during simulation.

```
# Let us investigate the impact of increasing salt concetration in the sample E_i

# Simulate and plot the results
newplot(title='Adsorption to the column - E_in increased', plotType='Elution-cond
```

```
for value in [0, 10, 20]:
    par(E_in=value)
    simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)

# Restore default values
par(k2=0.05, k4=0.3, E_in=0)
```



Note, that increased salt concentration in the sample affect binding of both proteins. During adsorption less is bound. During desoprtion less product P can be harvested but the fraction of

antagonist A may be lowered. Thus, some product is lost but the quality in terms of purity is improved.

4 The impact of change of binding strength due to pH

There are many factors that contribute to the binding strength. A most important factor is the pH-value of the resin and the characteristic iso-electric point of the protein. The binding strenght can be seen as proportional to the difference.

The binding strength of the resin is described by the quotient KP=k1/k2 for the protein P and similarly KA=k3/k4 for the protein A.

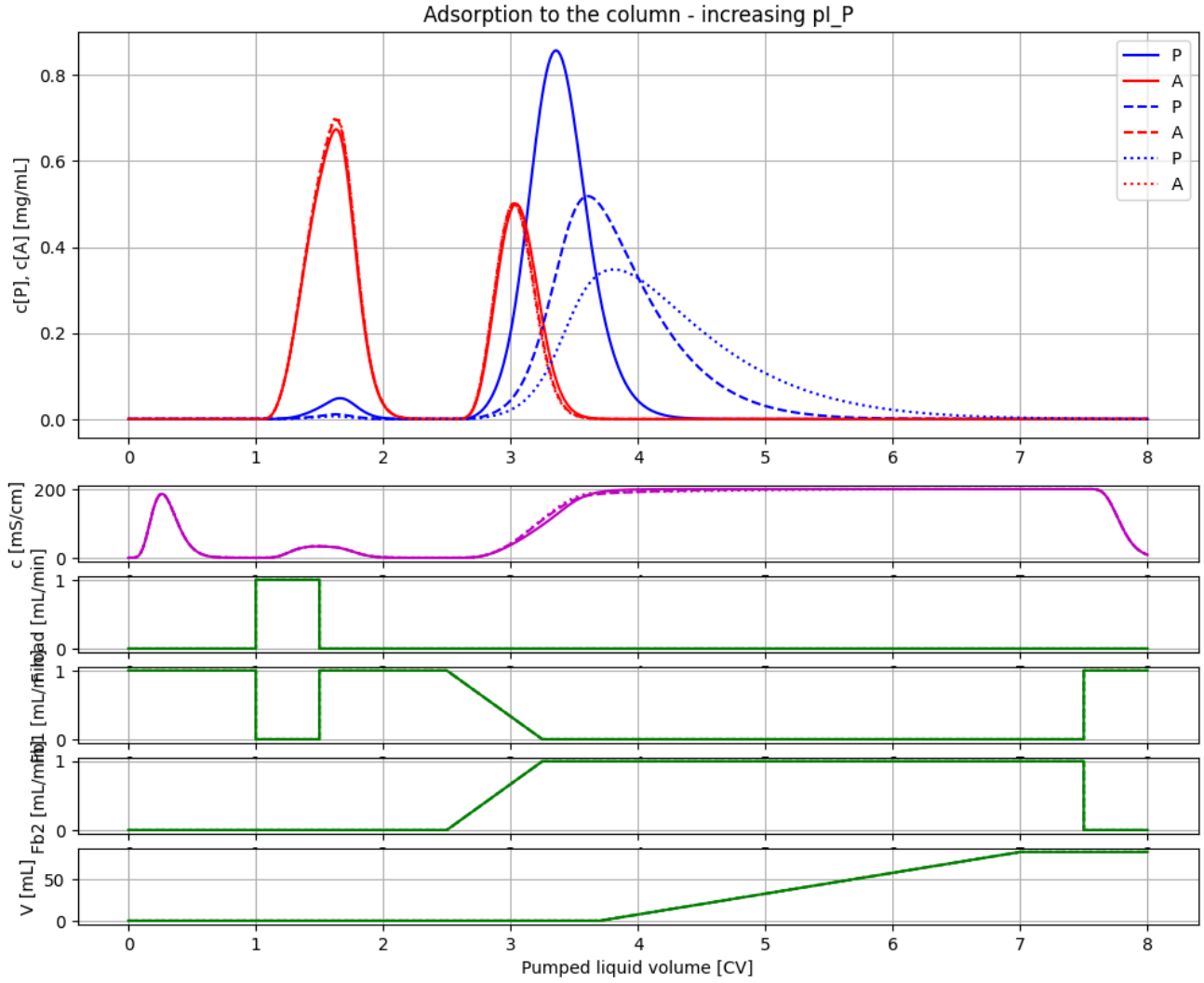
Below a few help-functions that describe this idea of the pH difference and its impact on binding strength in terms of the parameters k1, k2, k3, and k4 of the protein-resin interaction.

```
# Define function that describe the proportionality of binding strength ot
# the pH difference of the iso-electric point and the resin

def KP_pH_sensitivity(pI_P=8.0, pH_resin=7.0):
    K_P_nom = 0.0
    coeff_pH = 6.0
    return K_P_nom + coeff_pH*(pI_P-pH_resin)

def KA_pH_sensitivity(pI_A=7.1667, pH_resin=7.0):
    K_A_nom = 0.0
    coeff_pH = 1.0
    return K_A_nom + coeff_pH*(pI_A-pH_resin)

def par_pH(pI_P=8.0, pI_A=7.1667, pH_resin=7.0, TP=3.33, TA=20.0):
    if (pI_P > pH_resin) & (pI_A > pH_resin):
        par(k2 = 1/(TP*KP_pH_sensitivity(pI_P=pI_P, pH_resin=pH_resin)))
        par(k4 = 1/(TA*KA_pH_sensitivity(pI_A=pI_A, pH_resin=pH_resin)))
    else:
        print('Both pI_P > pH_resin and pI_A > pH_resin must hold - no parameter


# The default parameters of the column
disp('column')
```

```
diameter : 1.261
height : 20.0
x_m : 0.3
k1 : 0.3
k2 : 0.05
k3 : 0.05
k4 : 0.3
Q_av : 6.0
E_0 : 50
```

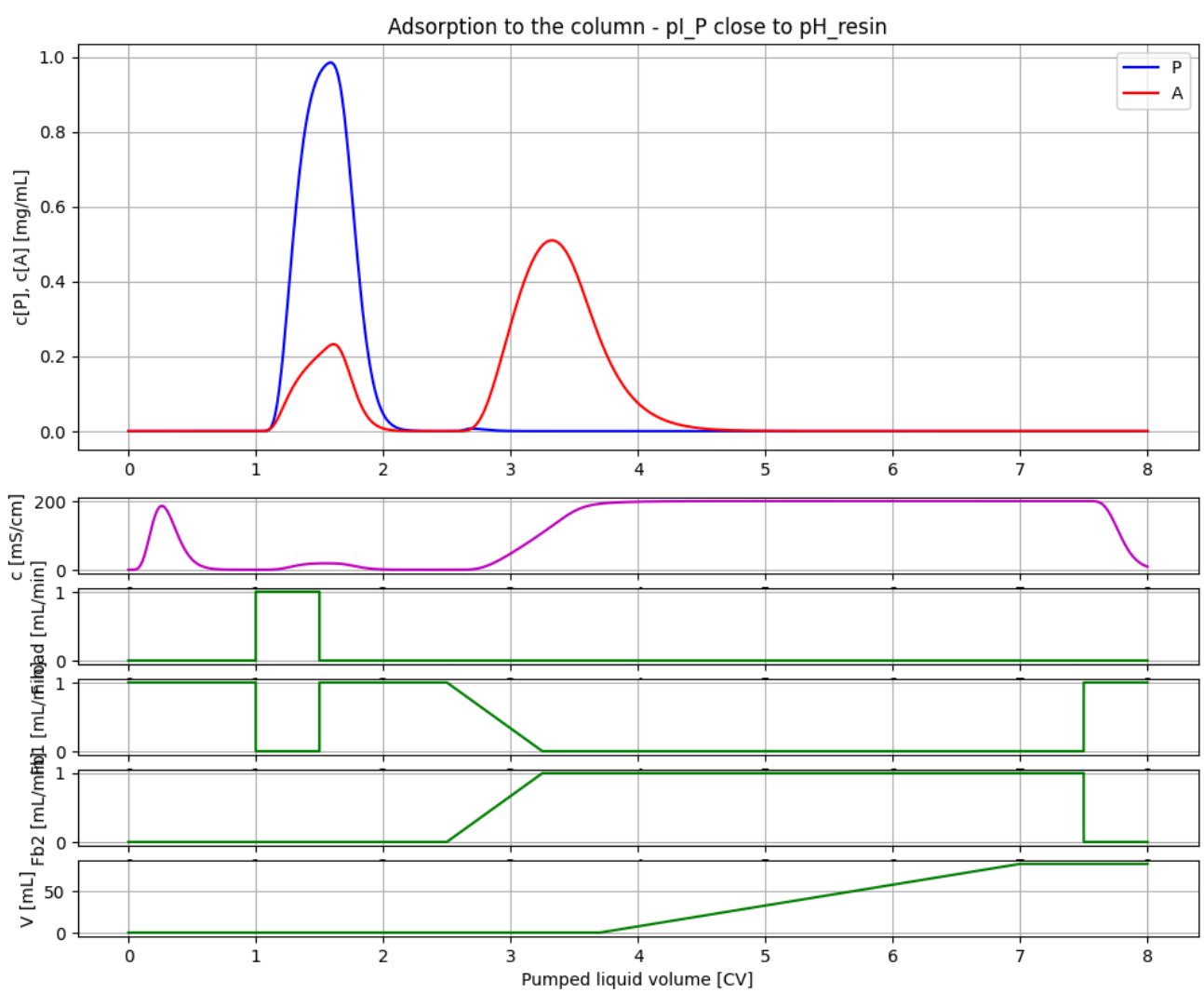
```
# Let us investigate the impact of change of the iso-electric pH for protein P
```

```
# Simulate and plot the results
newplot(title='Adsorption to the column - increasing pI_P', plotType='Elution-con

for value in [7.2, 7.6, 8.0]:
    par_pH(pI_P=value, pI_A=7.1667, pH_resin=7.0)
    simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)

# Restore default values
par(k2 = 0.05, k4 = 0.3)
```

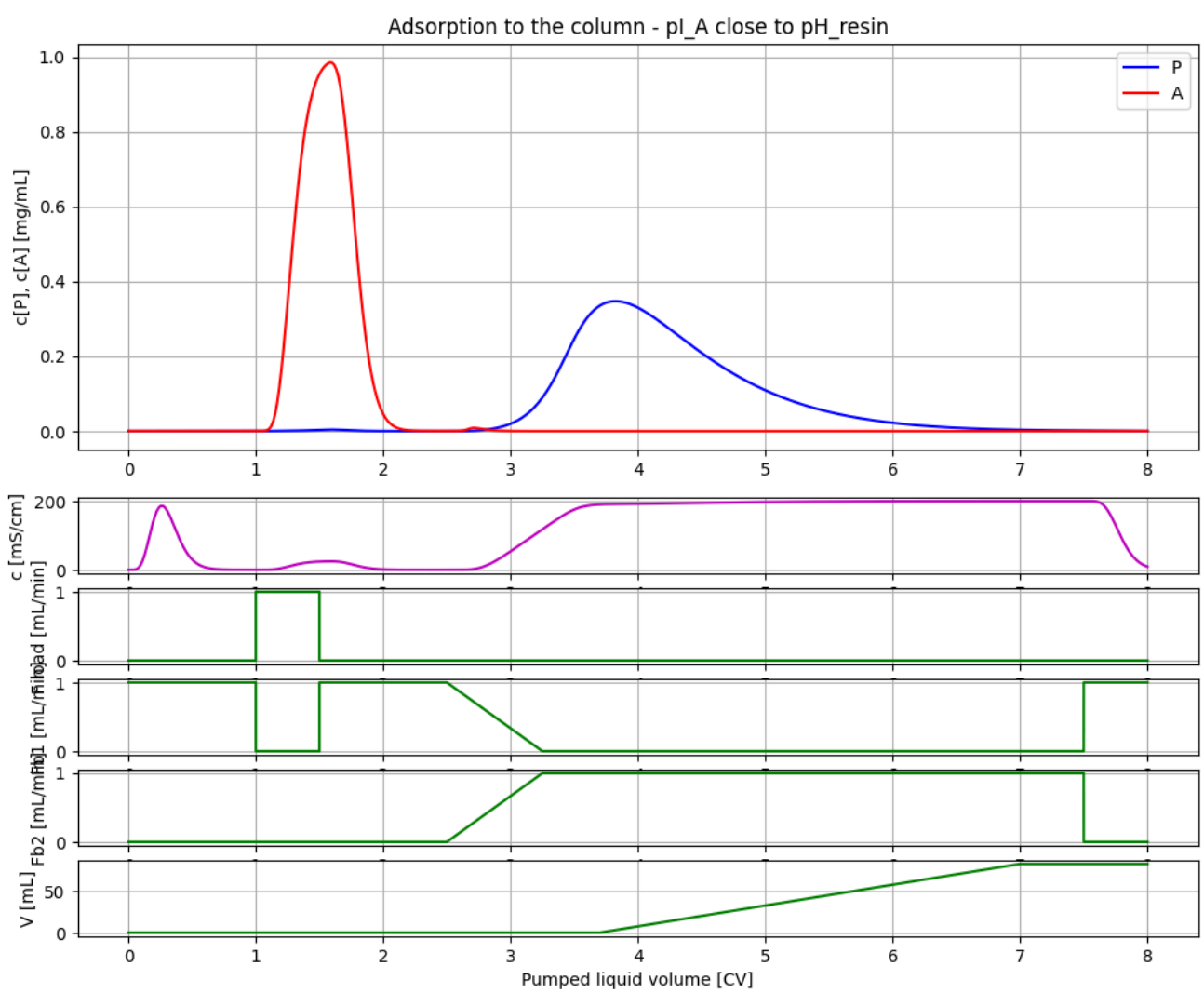


Note, with increasing pI_P the binding of P increase which leads less loss of product during adsorption. During desorption the peak height is lower with increasing binding strenght, but the total amoiunt of product P that can be harvested is higher, due to the smaller loss during adsorption.

```
# Let us investigate the impact of pI_P close to pH_resin

# Simulate and plot the results
newplot(title='Adsorption to the column - pI_P close to pH_resin', plotType='Elut

for value in [7.0001]:
    par_pH(pI_P=value, pI_A=8)
    simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)

# Restore default values
par(k2=0.05, k4=0.3)
```

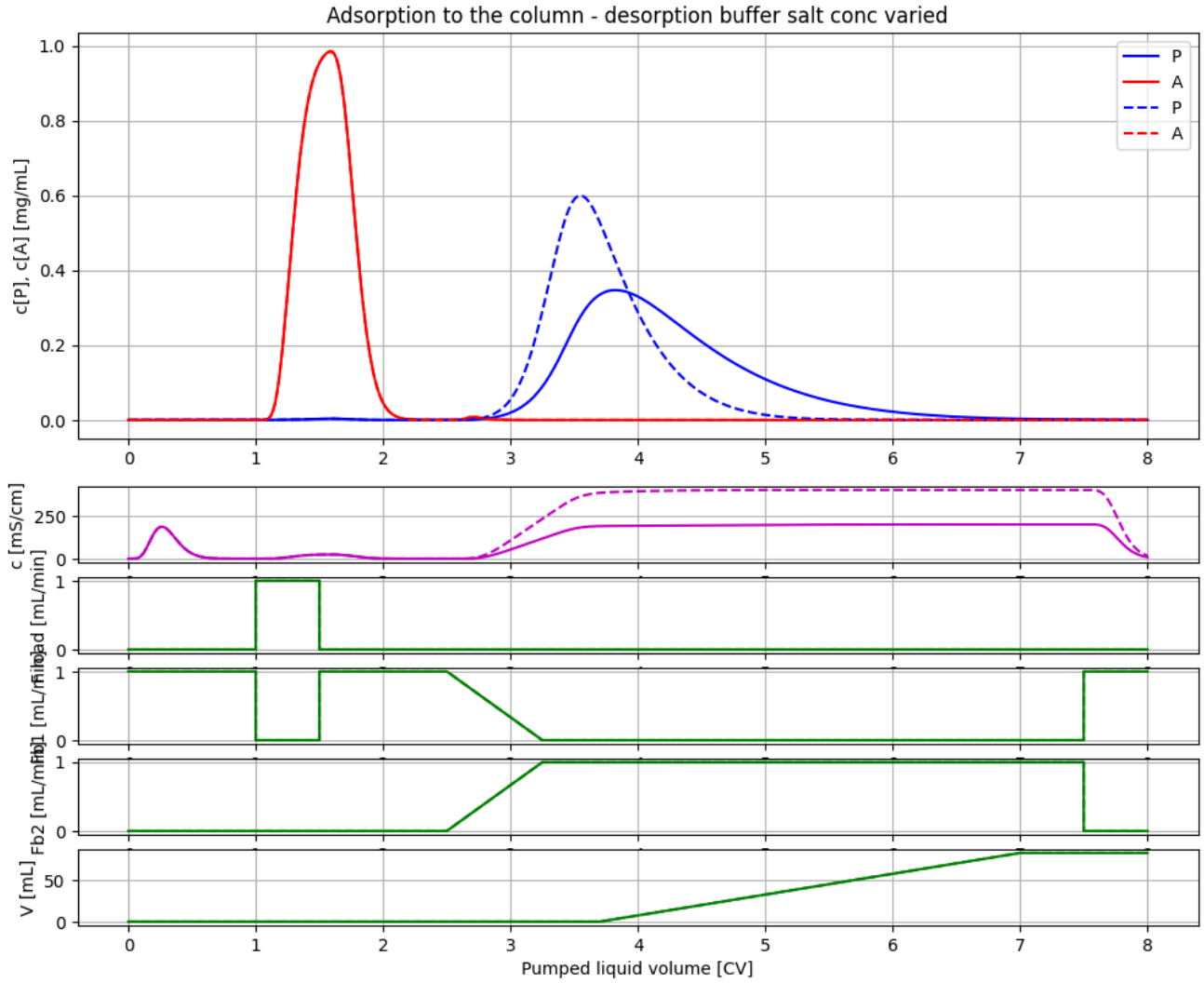



```
# Let us investigate the impact of pI_A close to pH_resin

# Simulate and plot the results
newplot(title='Adsorption to the column - pI_A close to pH_resin', plotType='Elut

for value in [7.001]:
    par_pH(pI_P=8.0, pI_A=value)
    simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)

# Restore default values
par(k2=0.05, k4=0.3)
```




```
# Let us also investigate the impact of salt concentration of the desorptions buf

# Simulate and plot the results
newplot(title='Adsorption to the column – desorption buffer salt conc varied', pl

for value in [8.0, 16.0]:
    par(E_in_desorption_buffer=value)
    par_pH(pI_P=8.0, pI_A=7.001, pH_resin=7.0)
    simu((CV_ekv+CV_ads+CV_wash+CV_desorb+CV_wash2)*V/VFR)

# Restore default values
```

```
par(E_in_desorption_buffer=8.0)
par(k2=0.05, k4=0.3)
```



5 Breakthrough curve often used during process development

```
# Experiment to check column capacity Q_av often called breakthrough curve
par(P_in=1, A_in=0, E_in=0)
init(E_0 = 0)
par(Q_av=6.0)
```

```
par(scale_volume=True, start_adsorption=1*V, stop_adsorption=4.01*V)
par(start_desorption=10*V, stationary_desorption=10.5*V, stop_desorption=11*V)
par(start_pooling=11*V, stop_pooling=12*V)

newplot(title='Impact of variation in column capacity Q_av', plotType='Elution-c
for value in [1, 2, 3, 6]: par(Q_av=value); simu(4.0*V/VFR)

# Linje för 10% UV
ax1.plot([0,4], [0.1,0.1],'k--')

# Restore default parameters
par(Q_av=6.0, A_in=1.0)
```