

✓ 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==0.3.20
```

```

Collecting fmpy==0.3.20
  Downloading FMPy-0.3.20-py3-none-any.whl.metadata (2.0 kB)
Requirement already satisfied: attrs in /usr/local/lib/python3.11/dist-packages (from fmpy==0.3.20) (25.1.0)
Requirement already satisfied: Jinja2 in /usr/local/lib/python3.11/dist-packages (from fmpy==0.3.20) (3.1.2)
Collecting lark (from fmpy==0.3.20)
  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 (from fmpy==0.3.20) (5.3.1)
Requirement already satisfied: msgpack in /usr/local/lib/python3.11/dist-packages (from fmpy==0.3.20) (1.0.8)
Requirement already satisfied: numpy in /usr/local/lib/python3.11/dist-packages (from fmpy==0.3.20) (1.26.4)
Requirement already satisfied: MarkupSafe>=2.0 in /usr/local/lib/python3.11/dist-packages (from Jinja2->fmpy==0.3.20) (2.1.5)
Downloading FMPy-0.3.20-py3-none-any.whl (6.7 MB)
 6.7/6.7 MB 18.1 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 0:00:00
Installing collected packages: lark, fmpy
Successfully installed fmpy-0.3.20 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/BPL_IEC_operation
```

```
Cloning into 'BPL_IEC_operation'...
```

```
%cd BPL_IEC_operation
```

```
/content/BPL_IEC_operation
```

✓ 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-compiled OpenModelica
```

```

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 simulation
- describe()  - describe culture, broth, parameters, variables with values/units

```

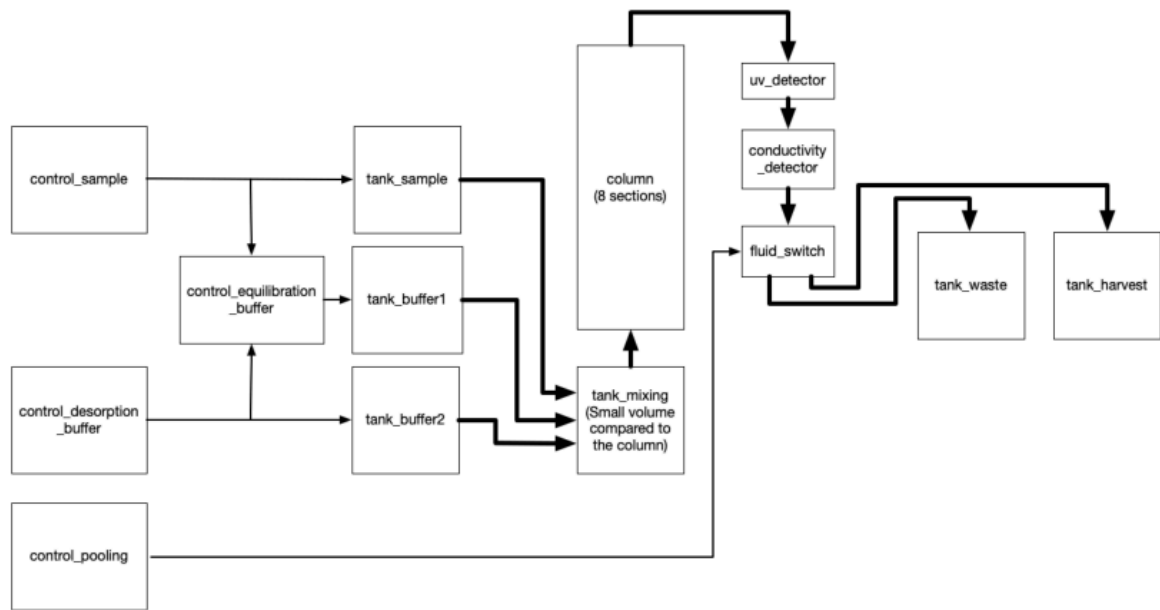
Note that both `disp()` and `describe()` takes values from the last simulation and the command `process_diagram()` brings up the main configuration

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]
```

```
# The process diagram is made outside Modelica to illustrate the configuration
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 column 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_av
par(height = h)

```

```

par(diameter = d)
par(Q_av = 6.0)

# Resin parameters - default values used

# Remaining salt concentration in the column from previous batch and eliminated during the initial equilibration
init(E_start = 50)

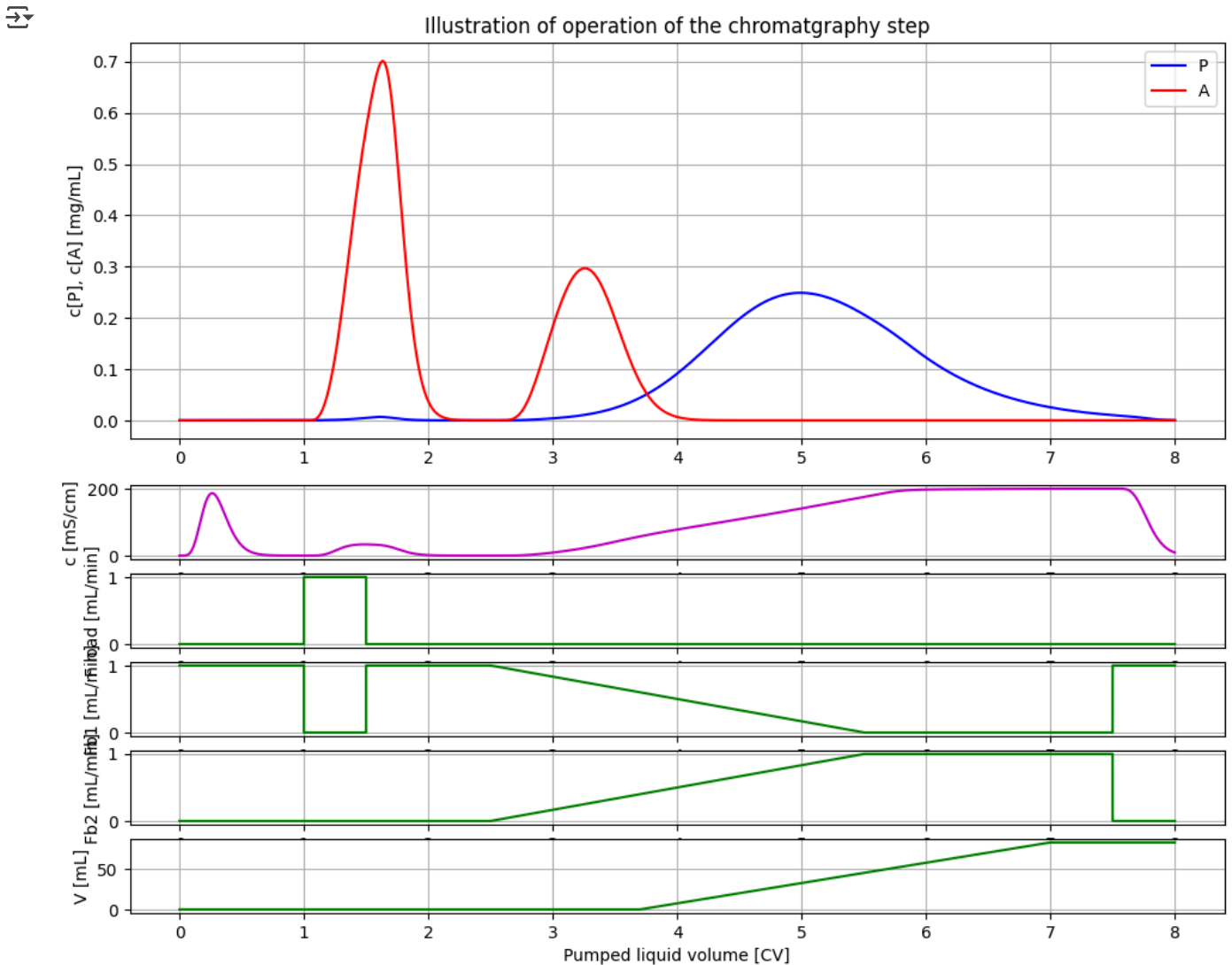
# Salt concentration of the desorption buffer
par(E_in_desorption_buffer = 8.0)

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

# Switching points during operation are conveniently described in terms of multiples of the column volume V
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)*V)
par(start_desorption=(CV_ekv+CV_ads+CV_wash)*V, stationary_desorption=(CV_ekv+CV_ads+CV_wash+CV_desorb)*V)
par(stop_desorption=7.5*V)
par(start_pooling=(CV_ekv+CV_ads+CV_wash+CV_start_pool)*V, stop_pooling=(CV_ekv+CV_ads+CV_wash+CV_stop_pool)*V)

# Simulation and plot of results
newplot(title='Illustration of operation of the chromatography step', plotType='Elution-conductivity-vs-CV-combi
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 column and just a small amount passes through and goes to the waste. The antagonist A is much less adsorbed. 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 increasing salt concentration is applied. First the antagonist and later the product comes out. 5) Time: 5.5-7.5 hours - washing 2. 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] = 12.422130344957237
  A_mass [mg] = 12.488781164503445
```

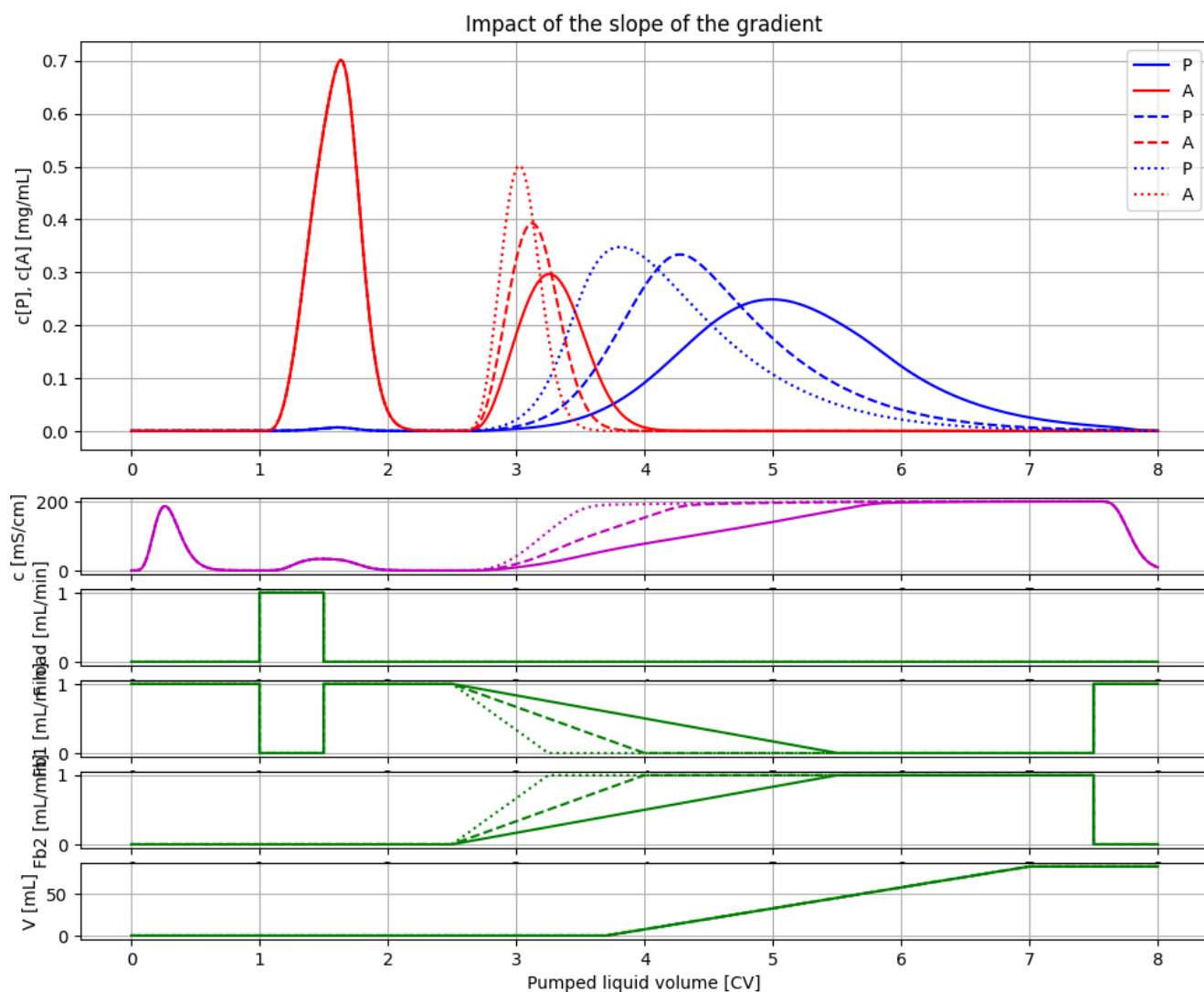
✓ 2 The impact of the slope of the desorption gradient

```
# Simulations showing the impact of change of slope of the desorption gradient
newplot(title='Impact of the slope of the gradient', plotType='Elution-conductivity-vs-CV-combined-all')

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

# Gradient 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)

# Gradient 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

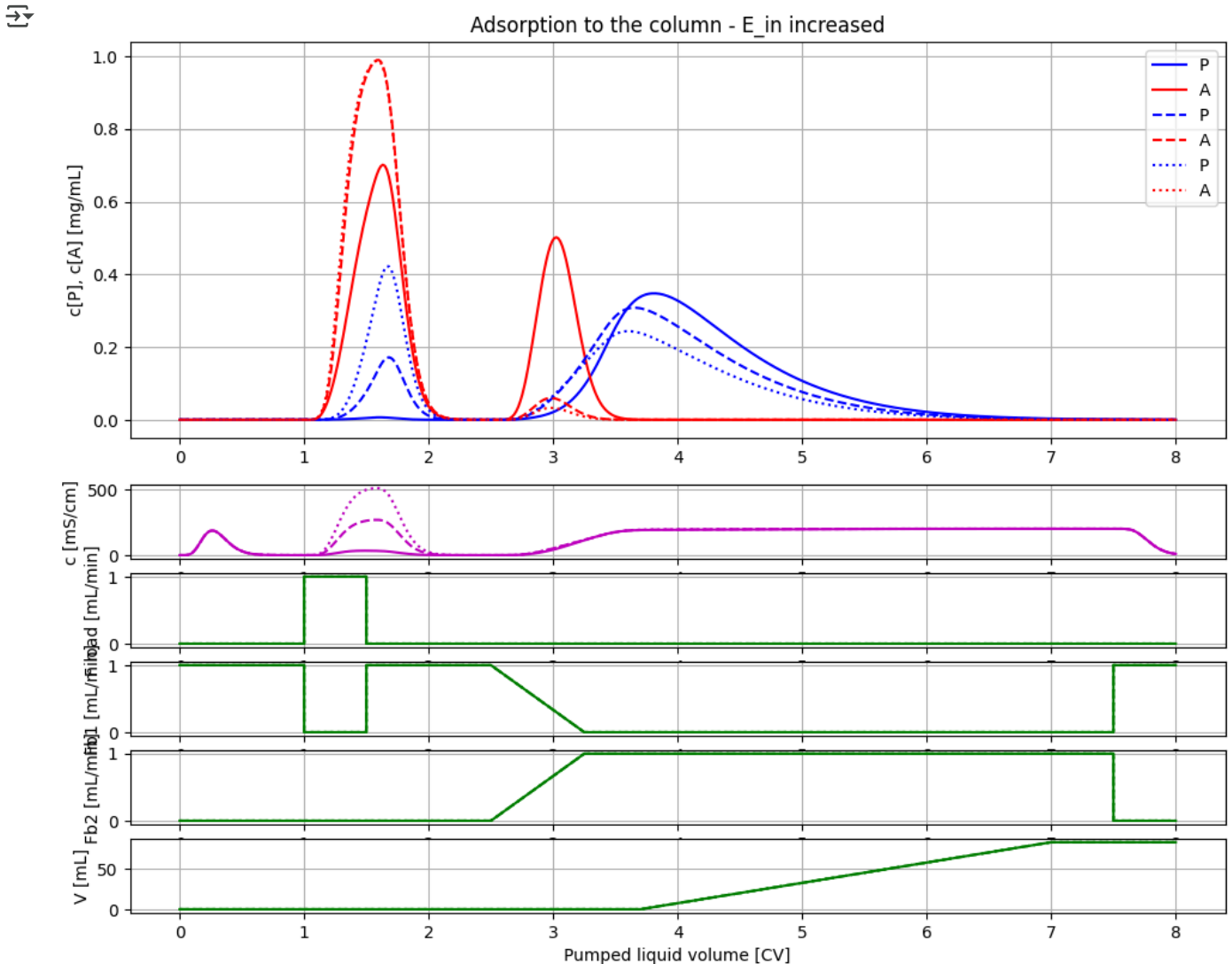
These values should be compared with the expected value 12.5 mg, i.e. half a column volume with sample concentration 1 mg/L. The difference is due to numerical errors during simulation.

```
# Let us investigate the impact of increasing salt concentration in the sample E_in

# Simulate and plot the results
newplot(title='Adsorption to the column - E_in increased', plotType='Elution-conductivity-vs-CV-combined-all')

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 desorption 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 $K_P = k_1/k_2$ for the protein P and similarly $K_A = k_3/k_4$ 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 k_1 , k_2 , k_3 , and k_4 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 change made')

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

↗ diameter : 7.136
height : 20.0
x_m : 0.3
k1 : 0.3
k2 : 0.05
k3 : 0.05
k4 : 0.3
Q_av : 3.0
E_start : 0.0

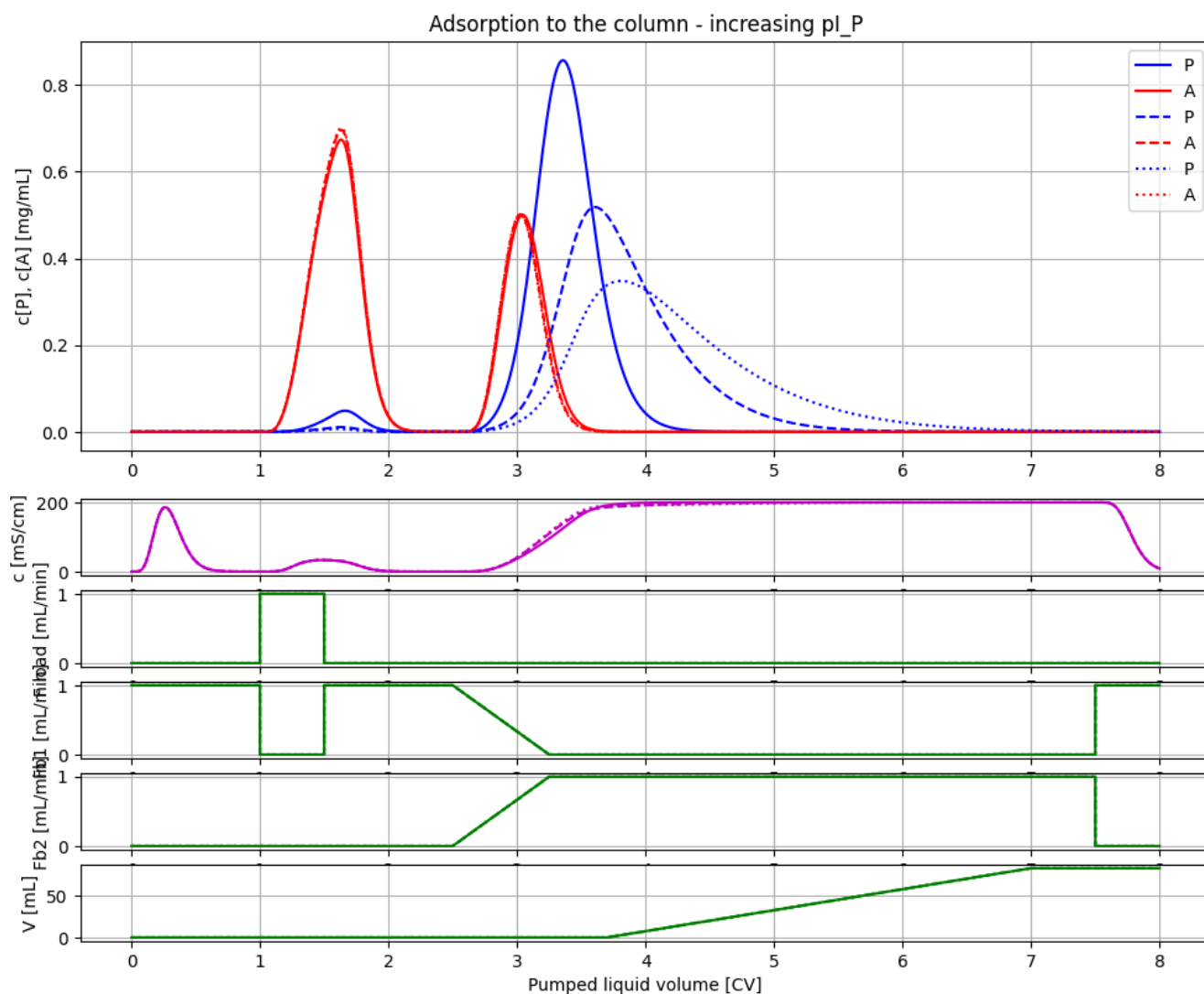
# Let us investigate the impact of change of the iso-electric pH for protein P

# Simulate and plot the results
newplot(title='Adsorption to the column - increasing pI_P', plotType='Elution-conductivity-vs-CV-combined-all')

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='Elution-conductivity-vs-CV-combine
```

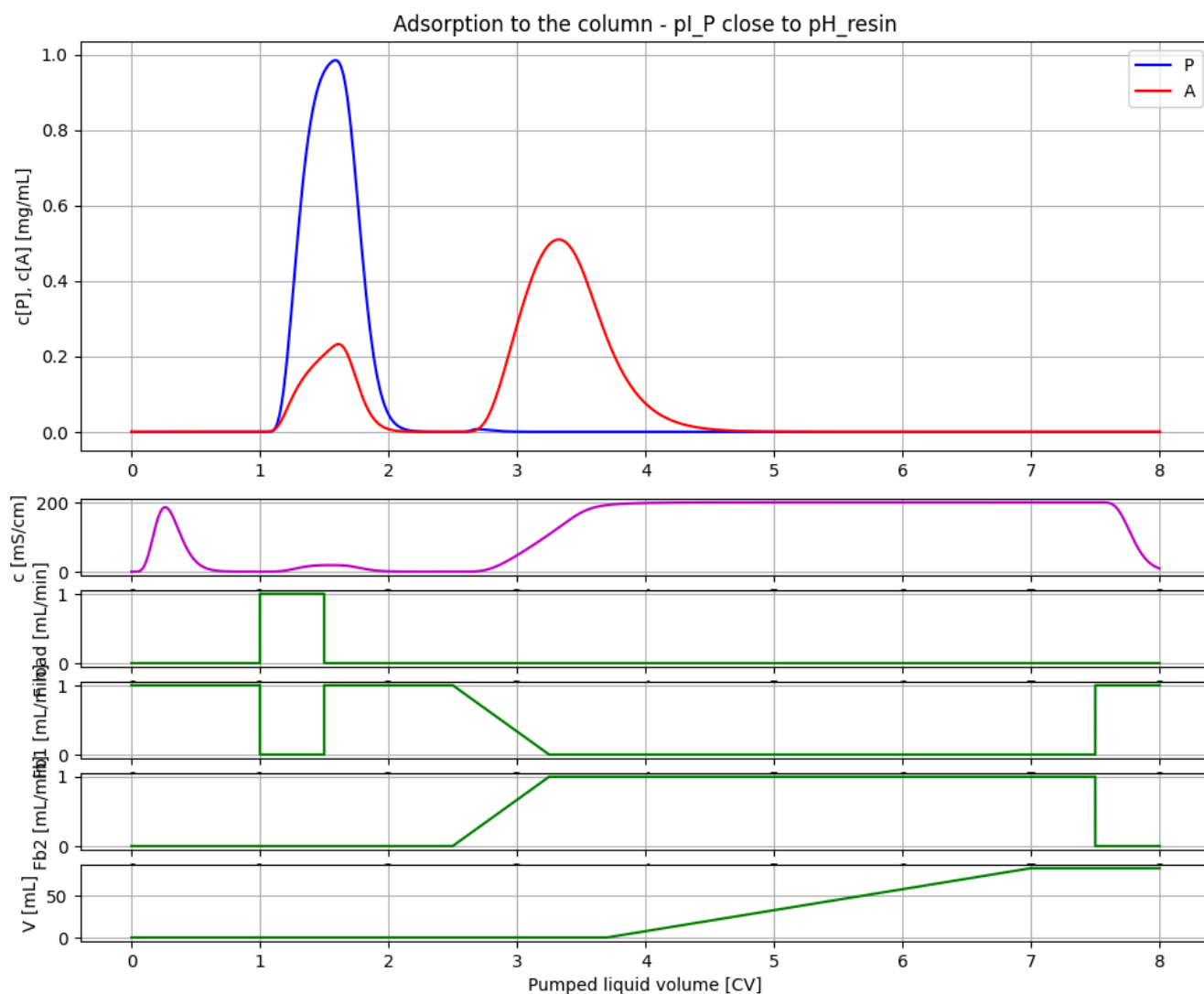
```
for value in [7.0001]:
```

```
    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='Elution-conductivity-vs-CV-combine
```

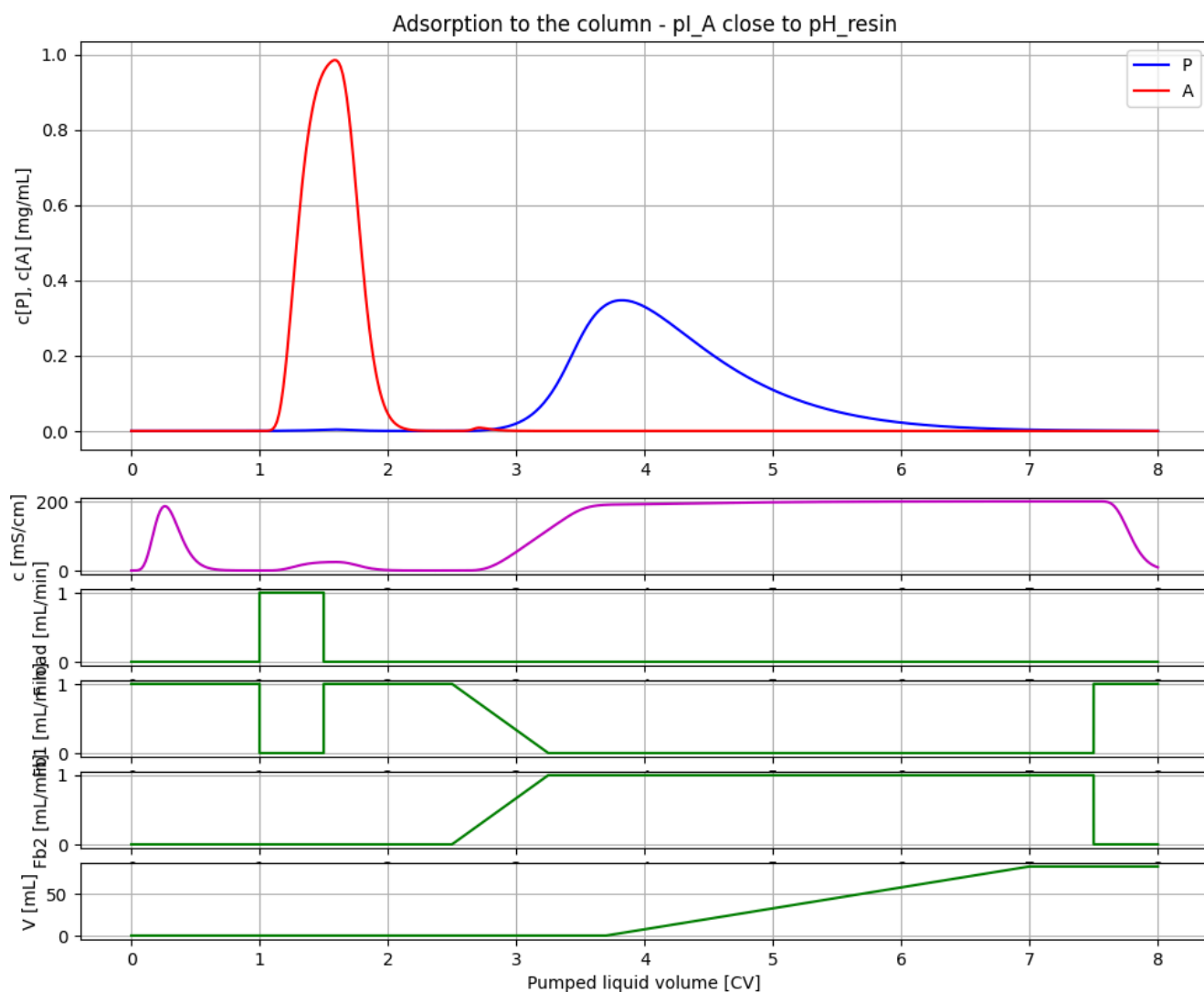
```
for value in [7.001]:
```

```
    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 buffer
```

```
# Simulate and plot the results
```

```
newplot(title='Adsorption to the column - desorption buffer salt conc varied', plotType='Elution-conductivity-\
```

```
for value in [8.0, 16.0]:
```

```
    par(E_in_desorption_buffer=value)
```

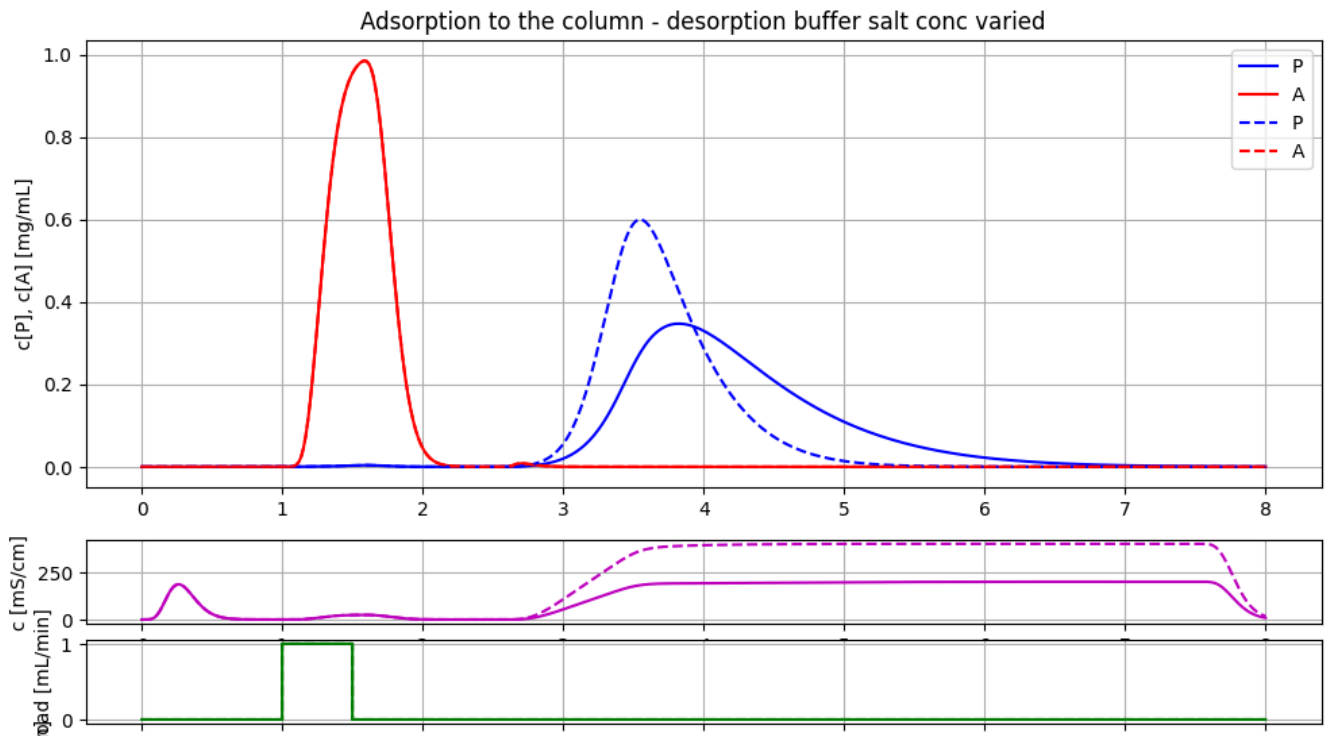
```
    par_pH(pI_P=8.0, pI_A=7.001, pH_resin=7.0)
```

```
    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_start = 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-conductivity-vs-CV-combined-all'
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)
```

