

# Quasi-continuous temperature-controlled protein chromatography in downstream processing of antibodies

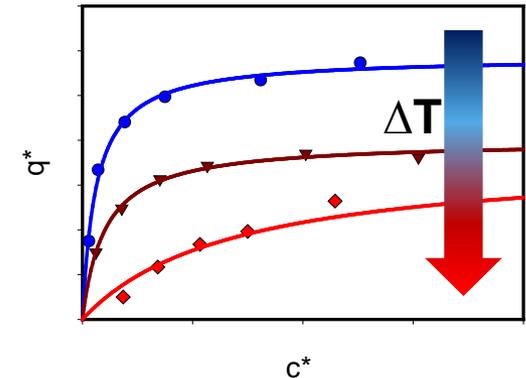
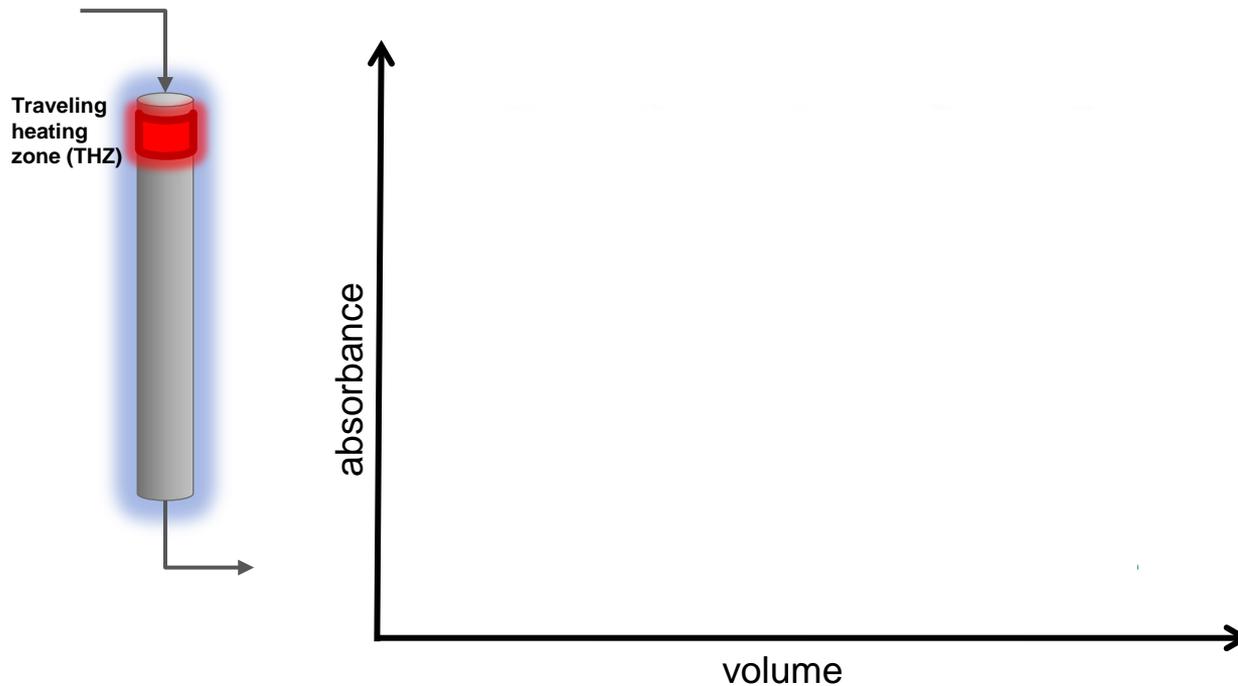
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# Principle: Continuous concentration

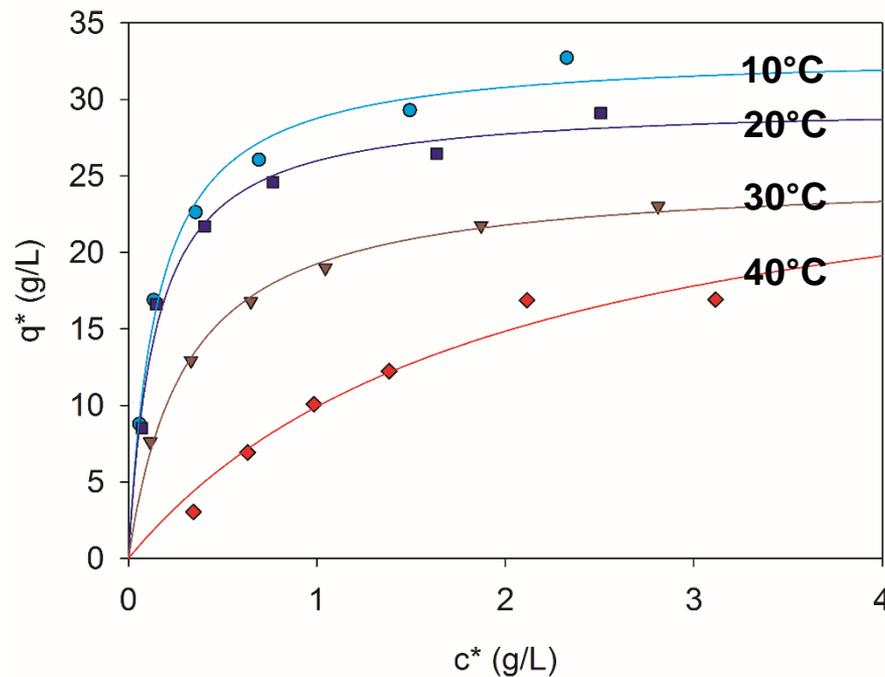
## Principle

- Constant feed, no elution- regeneration-, equilibration steps
- Repetitive movement of the THZ before breakthrough



# Temperature dependent batch adsorption

- Byzen Pro<sup>®</sup> (Nomadic Bioscience)
- Monoclonal IgG

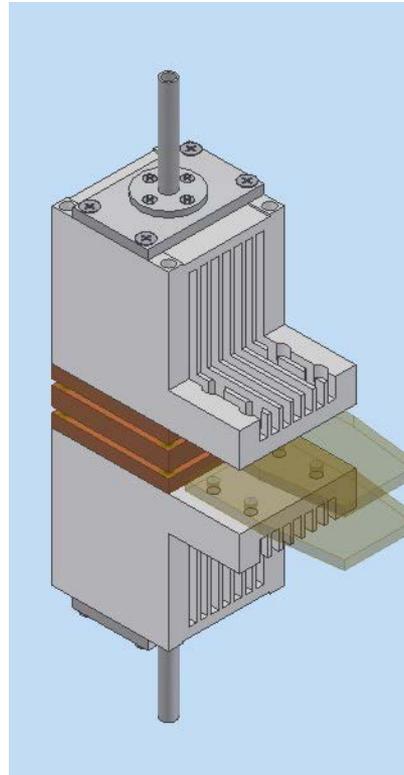


- $q^*$ : gram antibody per L settled resin

# Single column quasi-continuous chromatography

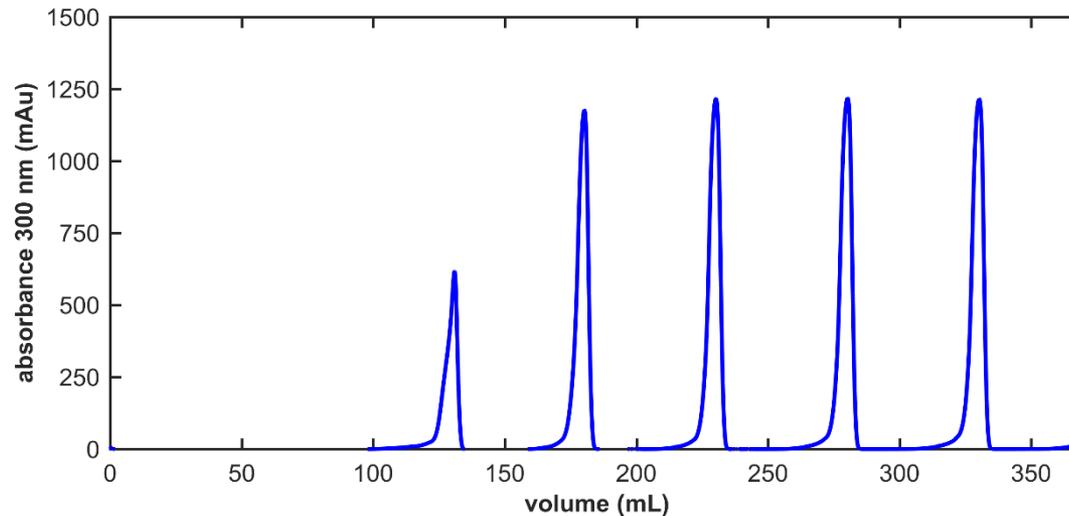
## Constructive implementation

- Fixed-bed column
- surrounded by a movable temperature zone
- Advantage: sharp elution peaks and higher product concentration



# 1 Continuous concentration

- **Commercially available protein A resin Byzen Pro (Nomadic Bioscience, JPN)**
- Feed: 1.0 g/L pH 8, 150 mM NaCl



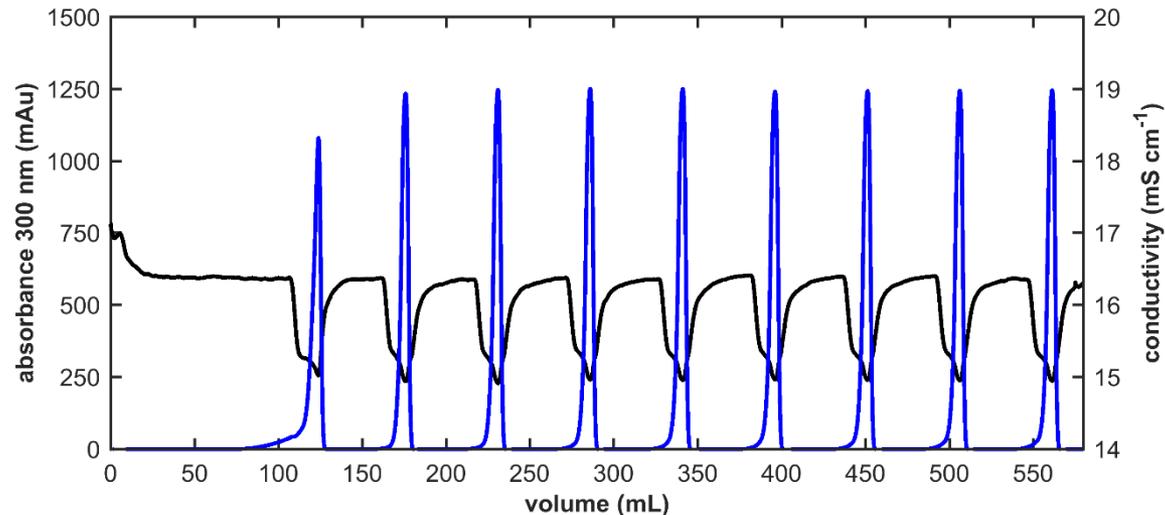
T(heating zone)  
= 40 °C  
ambient temperature  
= 15°C  
  
~ 6 CV/h

## Average performance during stationary phase

- Concentration: 5 -fold
- Yield : 93 % yield

## 2 Continuous buffer exchange

- Feed: 1.3 g/L mAb in 20 mM phosphate buffer pH 8, 150 mM NaCl
- Exchange buffer: 20 mM HEPES buffer pH 8, 150 mM NaCl

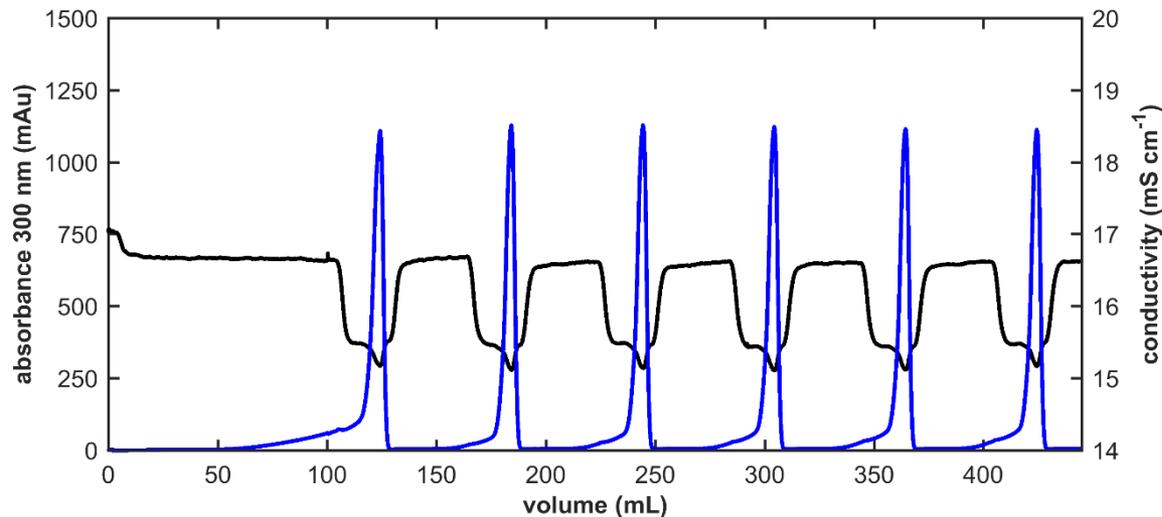


### Average performance during stationary phase

- buffer exchange: 239-fold
- Concentration: 3.7-fold
- Yield : 94.4% yield

## 2. Continuous buffer exchange

- Feed: 1.4 g/L mAb in 20 mM phosphate buffer pH 8, 150 mM NaCl
- Exchange buffer: 20 mM HEPES buffer pH 8, 150 mM NaCl

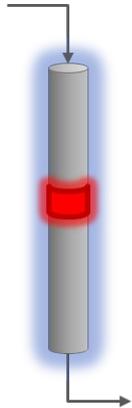


### Average performance during stationary phase

- buffer exchange: 7027 (+-1396.1) -fold

## 2. Continuous buffer exchange

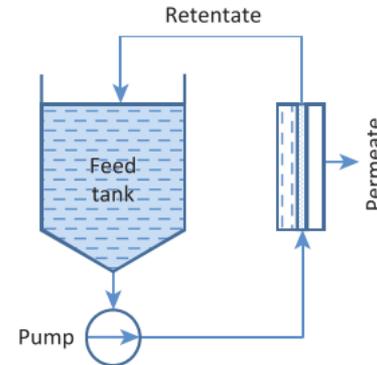
### THZR



- THZR: >2000-fold buffer exchange
- ➔ ratio buffer to product: ~ 2

vs.

### TFF Tangential flow filtration



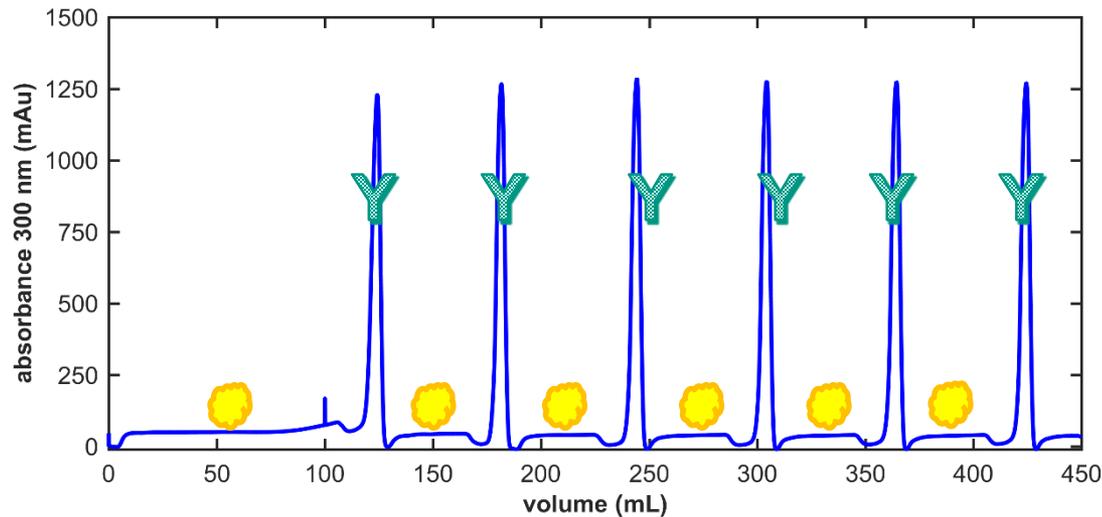
- TFF: for 2000-fold buffer exchange
- ➔ ratio buffer to product: ~ 7.0

➔ continuous mode possible

➔ **requires less than a third of exchange buffer**

# 3. Continuous capture

- Feed: 1.4 g/L mAb (pH 8, 150 mM NaCl)  
+1.0 g/L BSA



**Y** mAbs concentrated in the peak fraction

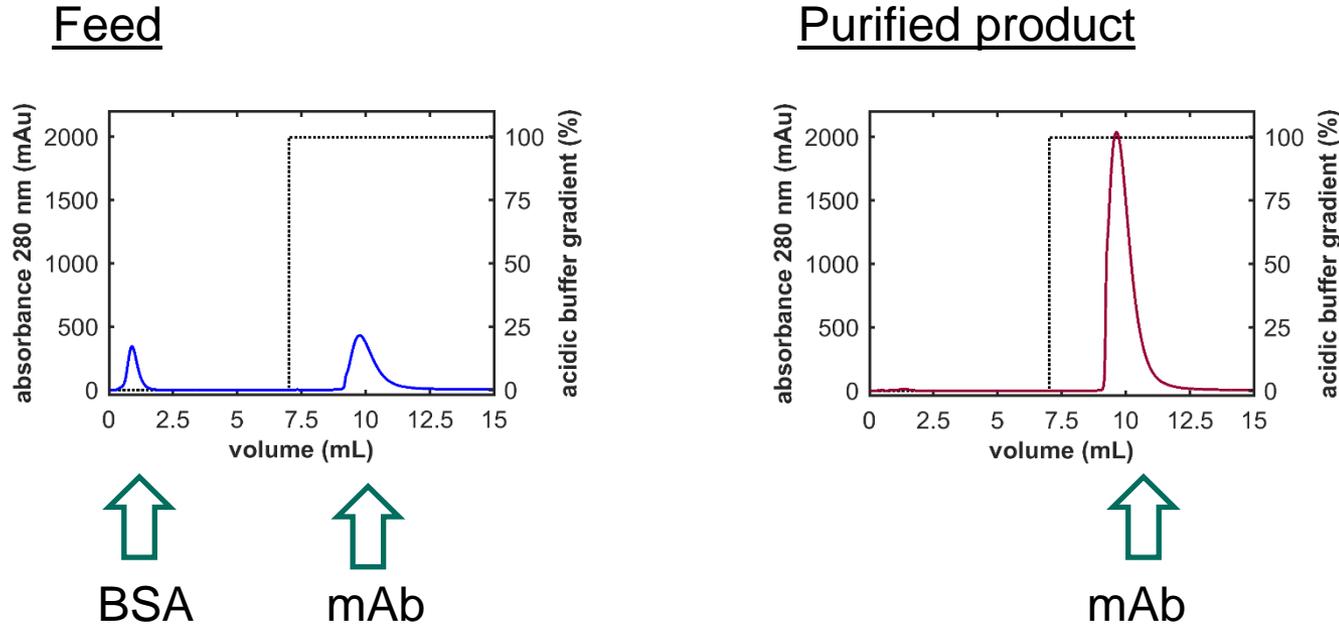
**●** contaminants in the breakthrough

## Average performance during stationary phase

- Concentration: 4-fold
- Yield : 89 %

# 3. Continuous capture

- Protein G analytics



- ➔ Protein G analytics show high purity of concentrated mAb
- ➔ Besides the application for buffer exchange, the TCZR has potential as continuous process step for mAb capture

# 4. Modeling and Simulation

CADET simulation software was extended with a moveable temperature profile  
→ THZR processes can be simulated and preliminary calculation can be done

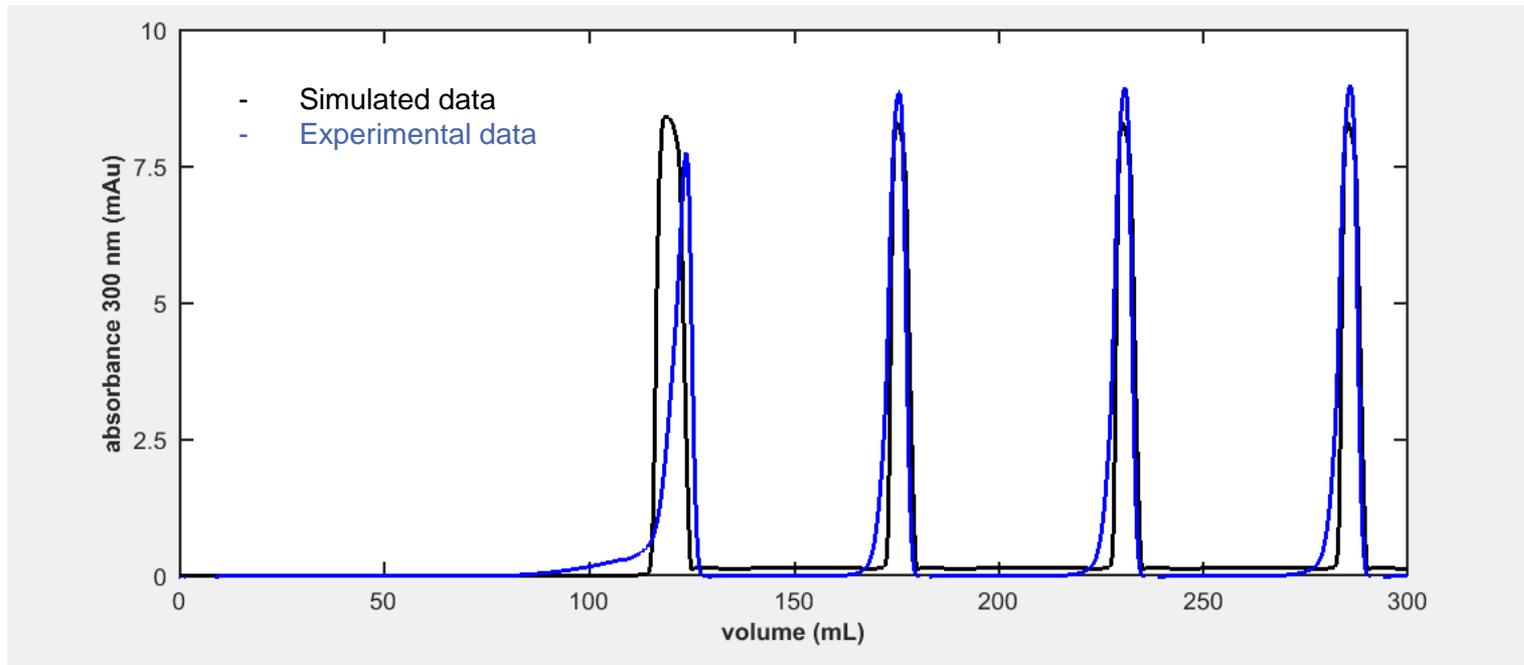


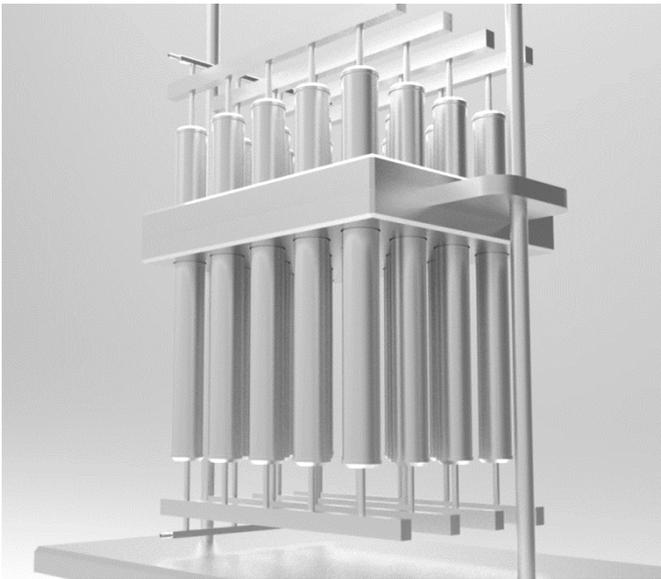
Fig.: Preliminary calculation of a buffer exchange process

## Outlook:

### TCZR Technical feasibility (Scenario 75 L/h)

- Heat transfer is the limiting factor for scaled TCZR designs
- CFD calculations show that the path for heat transfer should not exceed approx. 2cm -> new column designs required.

#### Option 1: Parallel operation of multiple simple columns



#### Option 2: Annular gap column

Max. cooling  
capacity required:  
1.75 kW