

# UV spectroscopy와 Linear analysis를 이용한 Coffee의 조성 분석

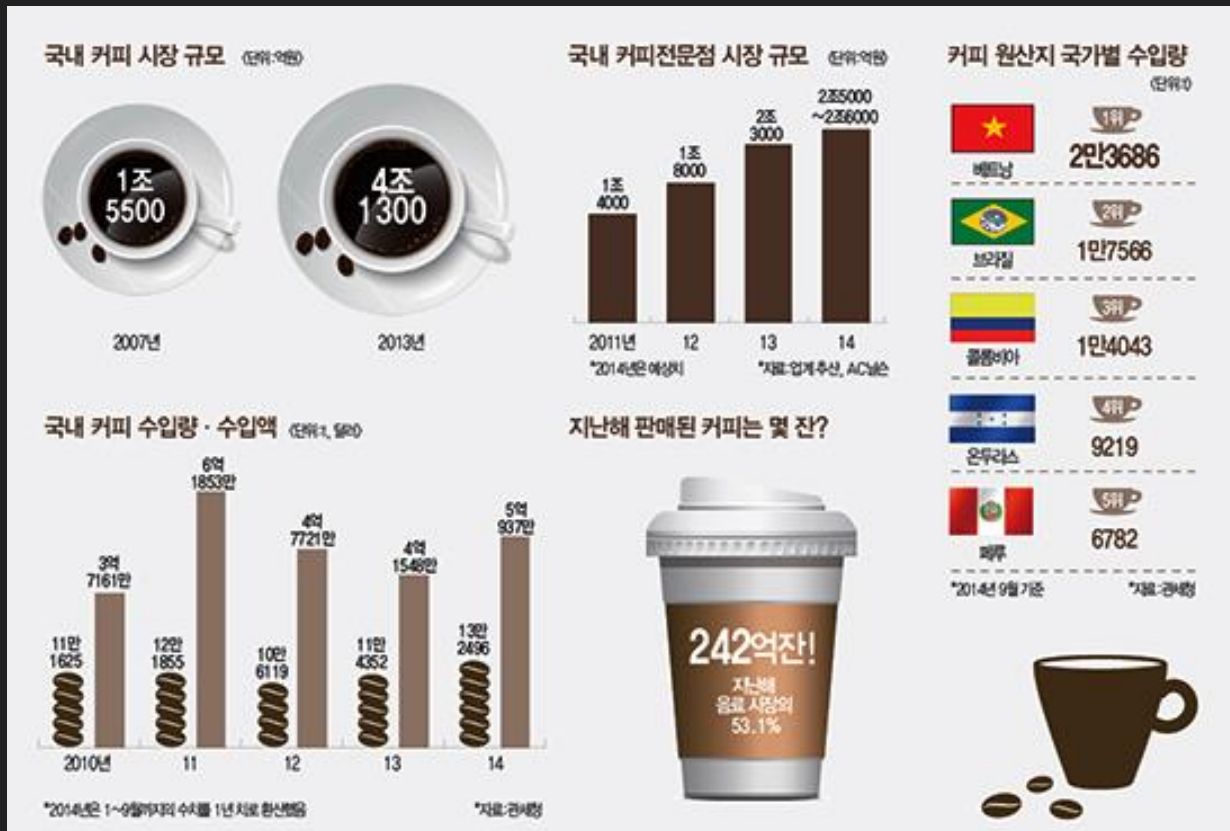
커피 속의 화학 / KAIST Coffee Club Kaldi

조장 : 박보현(생명화학공학/화학 14)

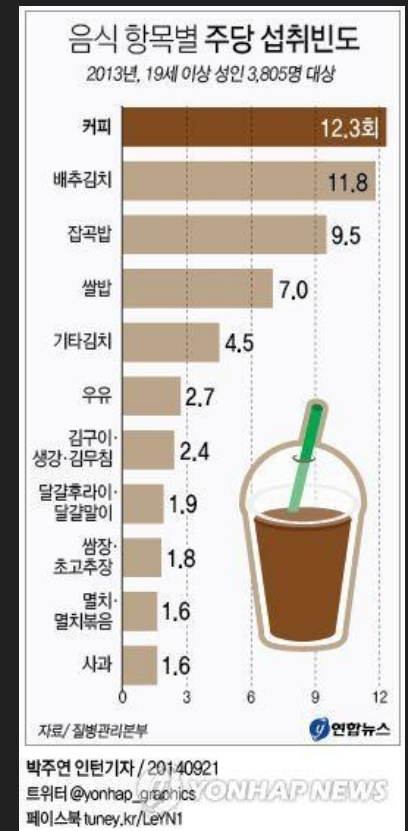
발표자 : 김수빈(생명화학공학/화학 14)

조원 : 신원우(화학과 15), 권승호(생명화학공학 15)

# 쌀밥보다 자주 먹는 커피



R[1]



R[2]

# 커피의 정량적 분석 필요

대중적인 커피의 인기에 비해 학술적인 연구가 활발히 이루어지지 않음

작년, 캘리포니아의 UCD(University of California Davis) -> 커피를 중점적으로 연구하는 'Coffee Lab' 공식 오픈

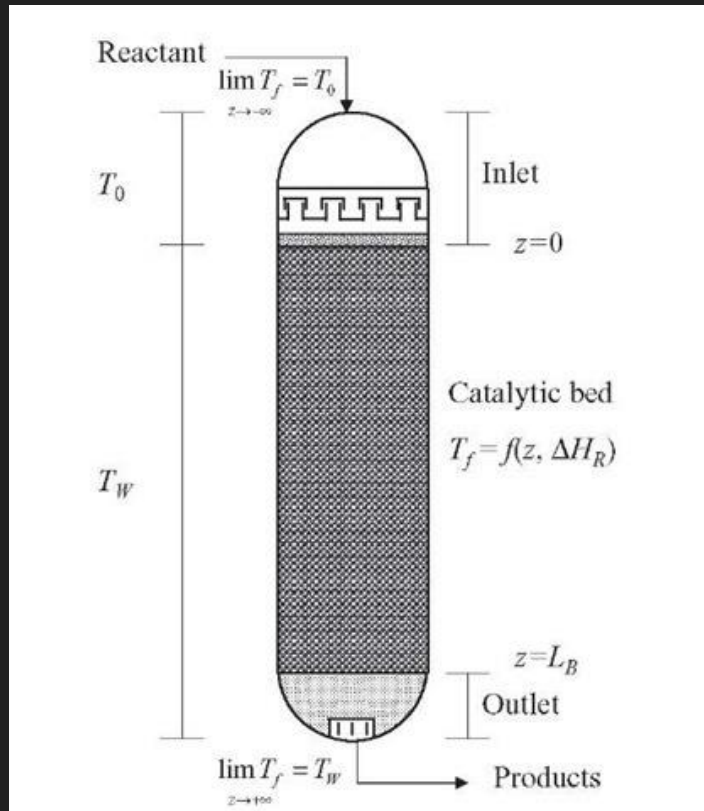
커피의 관한 연구결과는 계속 바뀌어 가고 있다

따라서, 커피에 대한 구체적인 정량 분석 연구가 필요한 상황

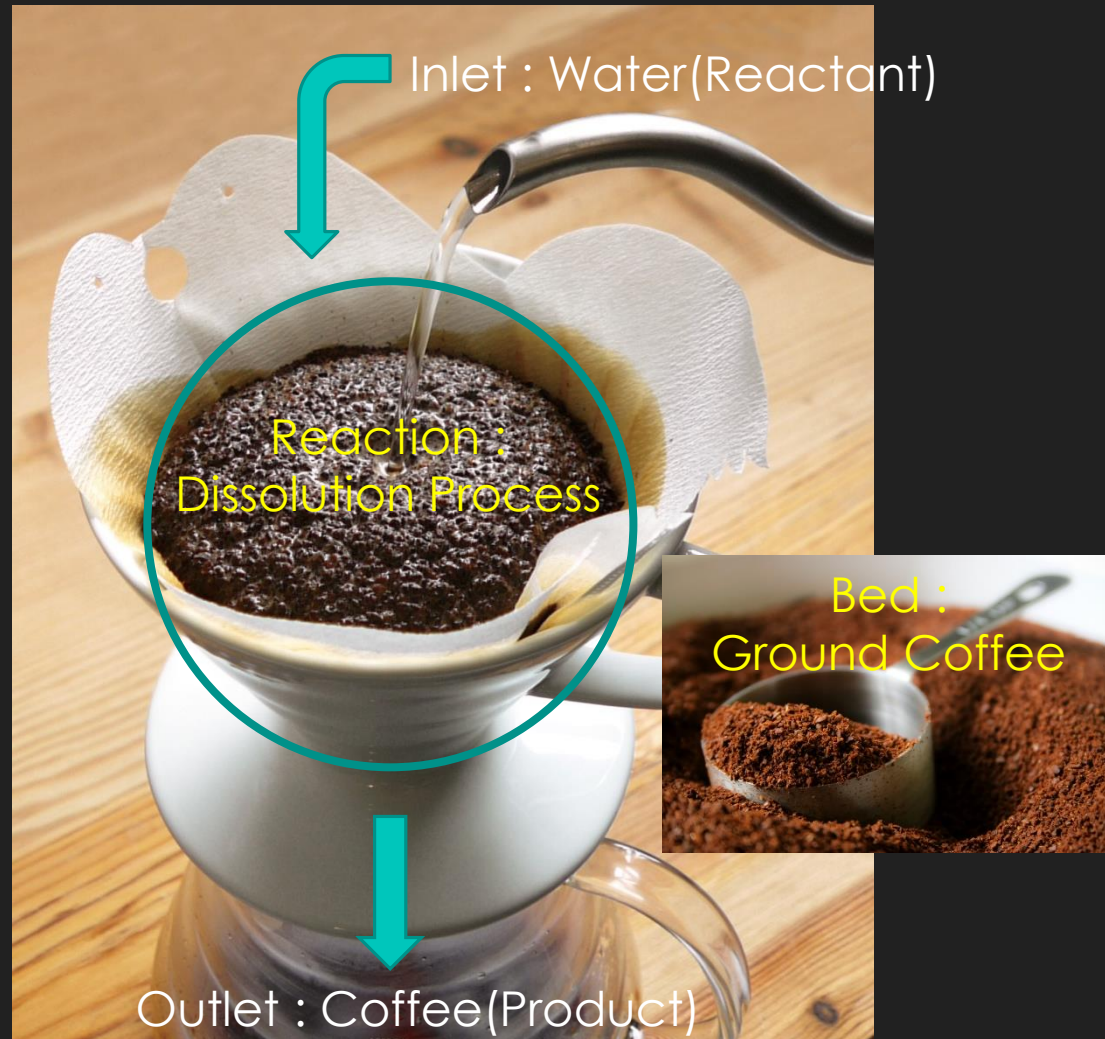




# Packed Bed Reactor: Coffee



Conventional  
Packed Bed Reactor  
R[4]



# Our goal

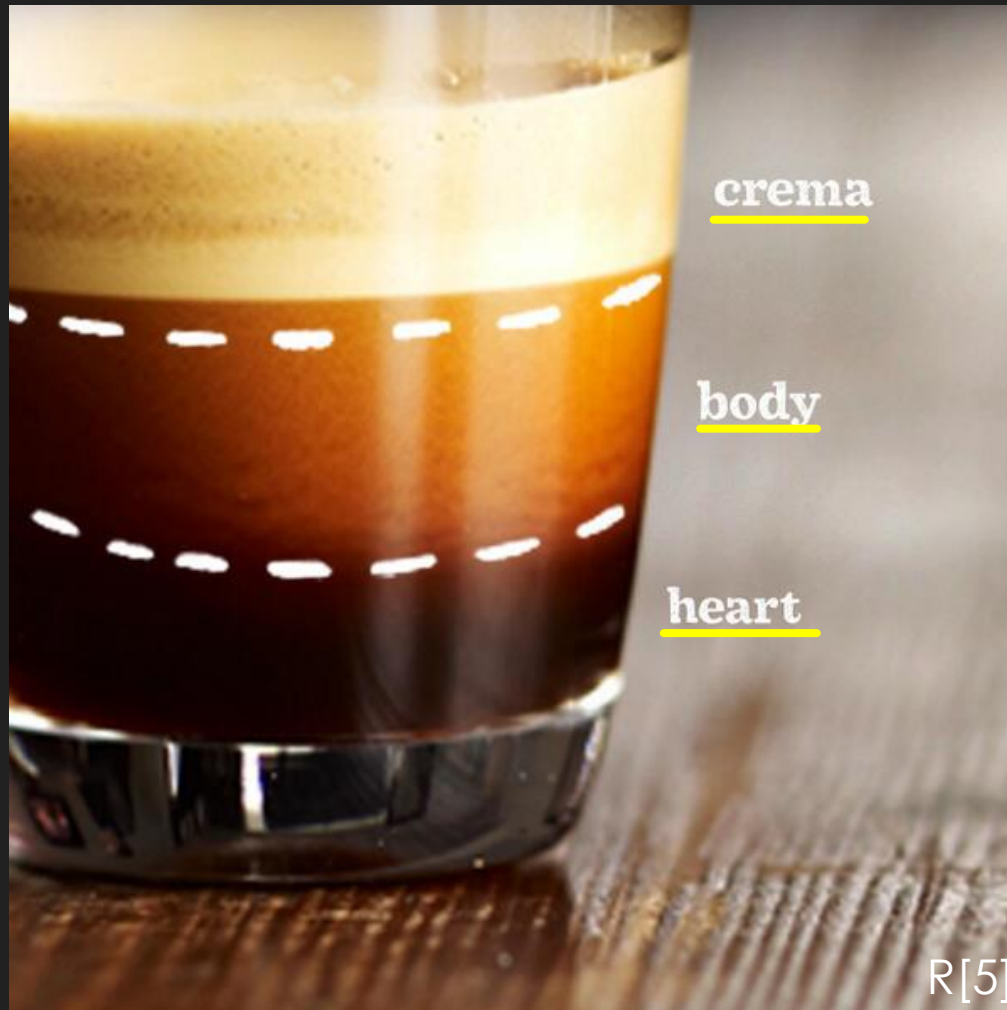
- Previous Research → 복잡한 process, 정확한 성분분석 제대로 이루어지지 않음

- Our goal : UV spectroscopy와 linear analysis 이용,



한번에 커피의 주요 성분들을 분석할 수 있는 방법 고안

# 분석에 쓰일 커피 선별



-> Espresso  
: UV분석 불가

But, Drip & Dutch  
: UV분석 가능

Water soluble  
molecule only

# Composition of Coffee



Typical chemical composition per 100 mL coffee brew

Caffeine[7],[9],[10], CGA(Chlorogenic Acid)[7],[8],[9],  
Trigonelline, and Niacine[9] are selected compounds for analysis

# Overview of Our method

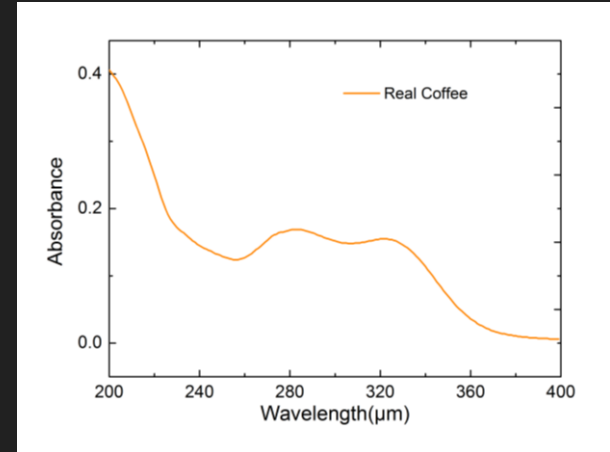


R[11]

1/1000  
dilution



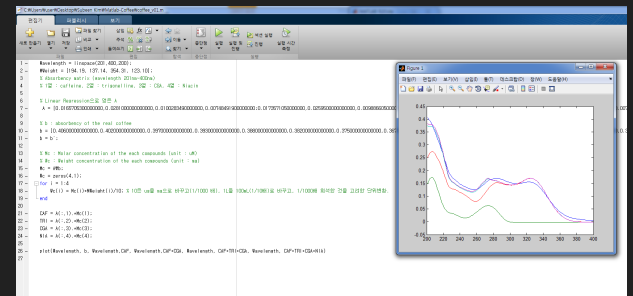
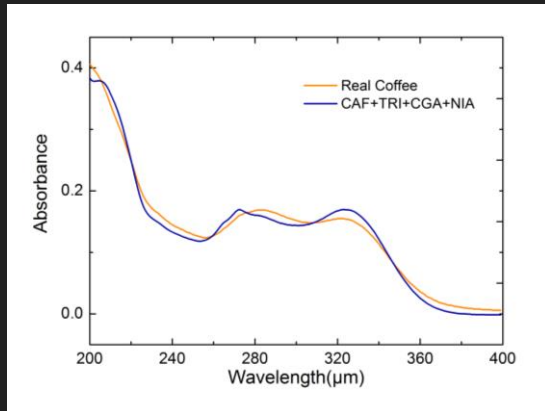
Measure  
the UV  
spectrum



Obtaining the  
Concentration using  
MATLAB

$$\begin{pmatrix} A_{\lambda_1} \\ A_{\lambda_2} \\ \vdots \\ A_{\lambda_n} \end{pmatrix} = b \begin{pmatrix} \epsilon_{1,\lambda_1} & \epsilon_{2,\lambda_1} & \epsilon_{3,\lambda_1} & \epsilon_{4,\lambda_1} \\ \epsilon_{1,\lambda_2} & \epsilon_{2,\lambda_2} & \epsilon_{3,\lambda_2} & \epsilon_{4,\lambda_2} \\ \vdots & \vdots & \vdots & \vdots \\ \epsilon_{1,\lambda_n} & \epsilon_{2,\lambda_n} & \epsilon_{3,\lambda_n} & \epsilon_{4,\lambda_n} \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \end{pmatrix}$$

Get  
Concentration data  
Regenerated Curve  
(Verification)





# Beer lambert's law

Solution  
containing  
single  
compound

*Beer-lambert's law*

$$A = \log \frac{P}{P_0} = \epsilon bc$$

$A$  : absorbance(흡광도)  
 $P$  : intensity(빛의 세기)  
 $\epsilon$  : molar absorptivity  
 $b$  : cell length  
 $c$  : concentration

Solution  
containing  
two  
compounds

*Two simultaneous equations  
combined with Beer-lambert's law*

$$A_{\lambda_1} = \epsilon_{1,\lambda_1} bc_1 + \epsilon_{2,\lambda_1} bc_2$$

$$A_{\lambda_2} = \epsilon_{1,\lambda_2} bc_1 + \epsilon_{2,\lambda_2} bc_2$$

# Beer-lambert's law

Solution  
containing  
multiple  
compounds

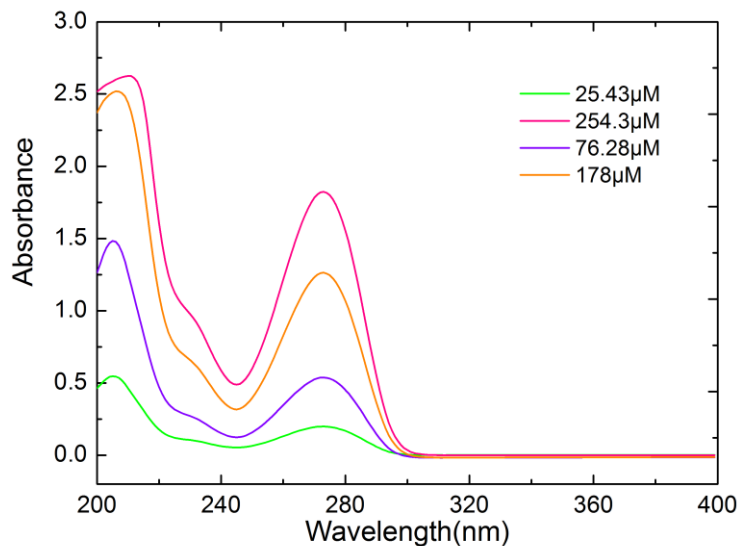
$$\mathbf{A} = b \mathbf{E} \mathbf{X}$$

$$\begin{pmatrix} A_{\lambda_1} \\ A_{\lambda_2} \\ \vdots \\ A_{\lambda_n} \end{pmatrix} = b \begin{pmatrix} \epsilon_{1,\lambda_1} & \epsilon_{2,\lambda_1} & \epsilon_{3,\lambda_1} & \epsilon_{4,\lambda_1} \\ \epsilon_{1,\lambda_2} & \epsilon_{2,\lambda_2} & \epsilon_{3,\lambda_2} & \epsilon_{4,\lambda_2} \\ \vdots & \vdots & \vdots & \vdots \\ \epsilon_{1,\lambda_n} & \epsilon_{2,\lambda_n} & \epsilon_{3,\lambda_n} & \epsilon_{4,\lambda_n} \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \end{pmatrix}$$

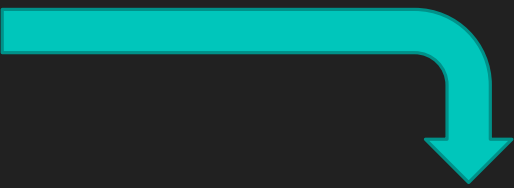
- $A_{\lambda_k}$  : absorbance at  $\lambda_k$
- $b$  : beam path length(cm) or cell length(cm)
- $\epsilon_{i,\lambda_k}$  : compound  $i$ 의  $\lambda_k$ 에서 molar absorptance(몰 흡광 계수,  $M^{-1}cm^{-1}$ )
- $c_i$  : compound  $i$ 의 몰 농도 (M)

**GOAL : E & A를 구하여 Matrix를 풀면 → X를 얻을 수 있다.**

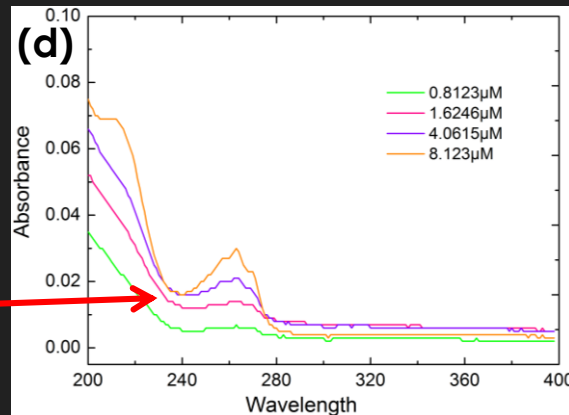
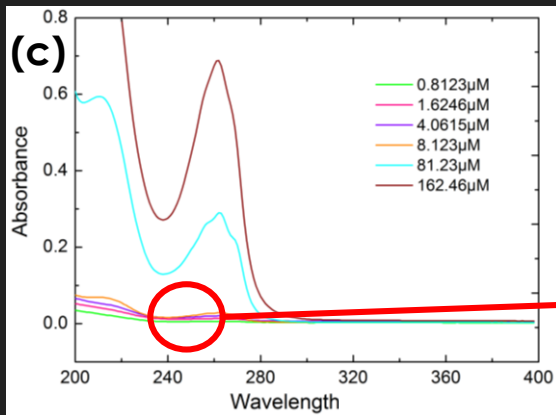
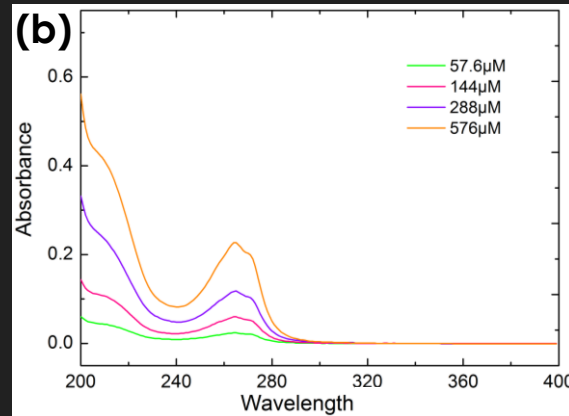
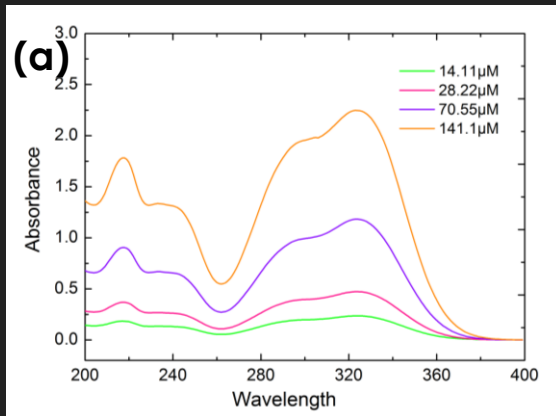
# Obtaining matrix E & Linearity



Caffeine UV spectrum


$$E = \begin{pmatrix} \epsilon_{1,\lambda_1} & \epsilon_{2,\lambda_1} & \epsilon_{3,\lambda_1} & \epsilon_{4,\lambda_1} \\ \epsilon_{1,\lambda_2} & \epsilon_{2,\lambda_2} & \epsilon_{3,\lambda_2} & \epsilon_{4,\lambda_2} \\ \vdots & \vdots & \vdots & \vdots \\ \epsilon_{1,\lambda_n} & \epsilon_{2,\lambda_n} & \epsilon_{3,\lambda_n} & \epsilon_{4,\lambda_n} \end{pmatrix}$$

# Obtaining matrix E & Linearity



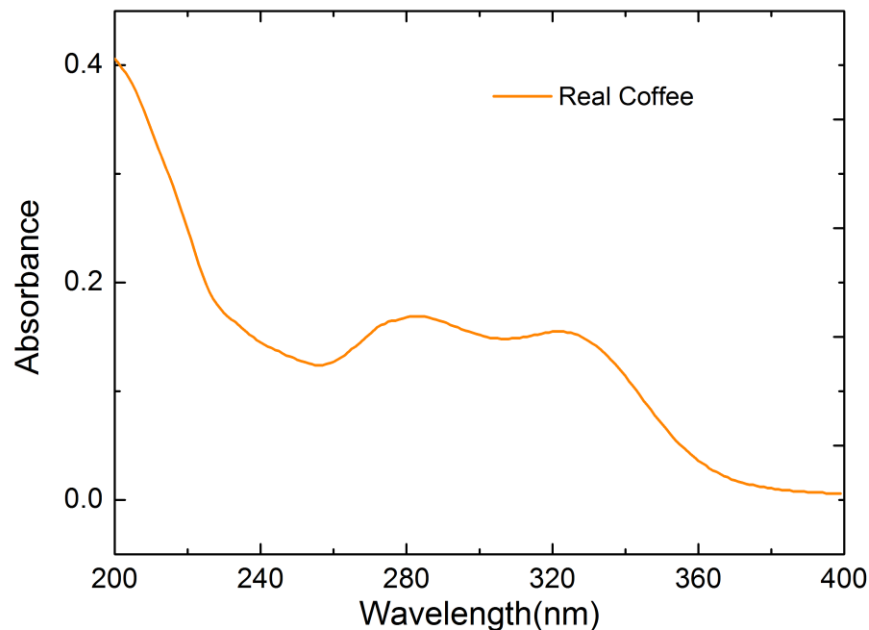
<Coffee 내 주 화합물의  
UV peak>  
(a) CGA(Chlorogenic acid)  
(b) Trigonelline  
(c), (d) Niacin



$$\begin{pmatrix} \epsilon_{1,\lambda_1} & \epsilon_{2,\lambda_1} & \epsilon_{3,\lambda_1} & \epsilon_{4,\lambda_1} \\ \epsilon_{1,\lambda_2} & \epsilon_{2,\lambda_2} & \epsilon_{3,\lambda_2} & \epsilon_{4,\lambda_2} \\ \vdots & \vdots & \vdots & \vdots \\ \epsilon_{1,\lambda_n} & \epsilon_{2,\lambda_n} & \epsilon_{3,\lambda_n} & \epsilon_{4,\lambda_n} \end{pmatrix}$$



# UV spectrum of the Real Coffee



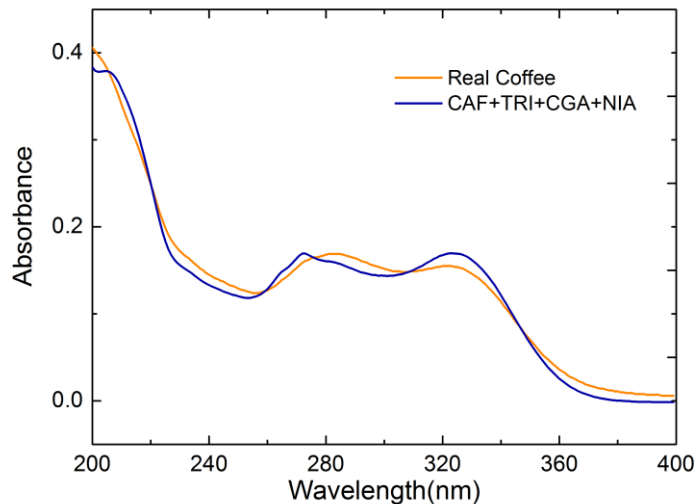
- Obtaining Absorbance value at each wavelength

$$\begin{pmatrix} A_{\lambda_1} \\ A_{\lambda_2} \\ \vdots \\ A_{\lambda_n} \end{pmatrix}$$

- Now we can solve the given Matrix!

1/1000 diluted hand-drip coffee

# Concentration of compounds from Linear analysis



$$\begin{pmatrix} A_{\lambda_1} \\ A_{\lambda_2} \\ \vdots \\ A_{\lambda_n} \end{pmatrix} = b \begin{pmatrix} \epsilon_{1,\lambda_1} & \epsilon_{2,\lambda_1} & \epsilon_{3,\lambda_1} & \epsilon_{4,\lambda_1} \\ \epsilon_{1,\lambda_2} & \epsilon_{2,\lambda_2} & \epsilon_{3,\lambda_2} & \epsilon_{4,\lambda_2} \\ \vdots & \vdots & \vdots & \vdots \\ \epsilon_{1,\lambda_n} & \epsilon_{2,\lambda_n} & \epsilon_{3,\lambda_n} & \epsilon_{4,\lambda_n} \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \end{pmatrix}$$

○ Molar Concentration (in 100mL coffee)

Caffeine : 8.9219mM

Chlorogenic Acids : 10.2536mM

Trigonelline : 4.44196mM

Niacin : 1.1668mM

○ Mass Concentration (in 100mL coffee)

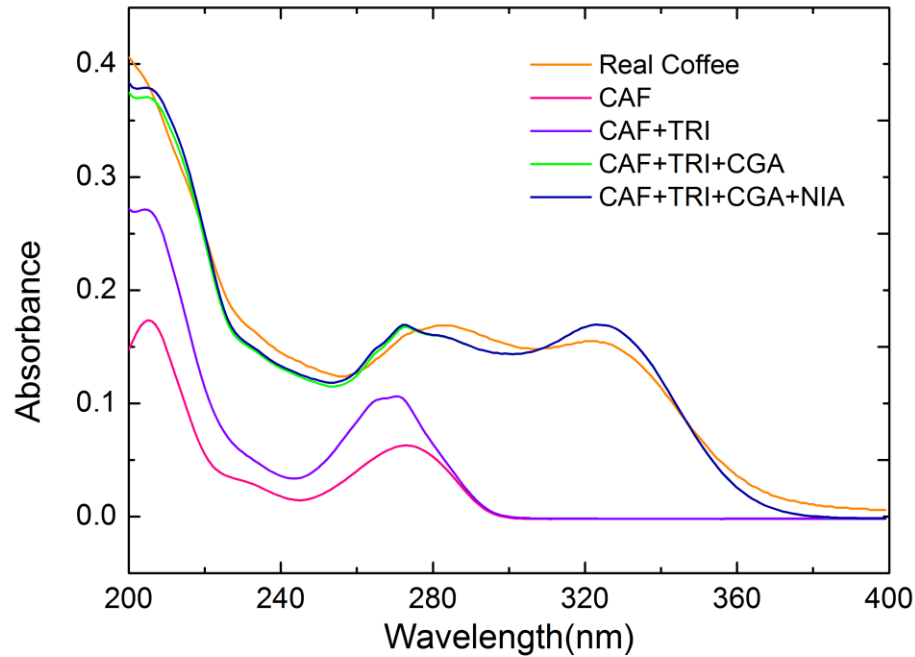
Caffeine : 173.2542mg

Chlorogenic Acids : 60.6106mg

Trigonelline : 363.2937mg

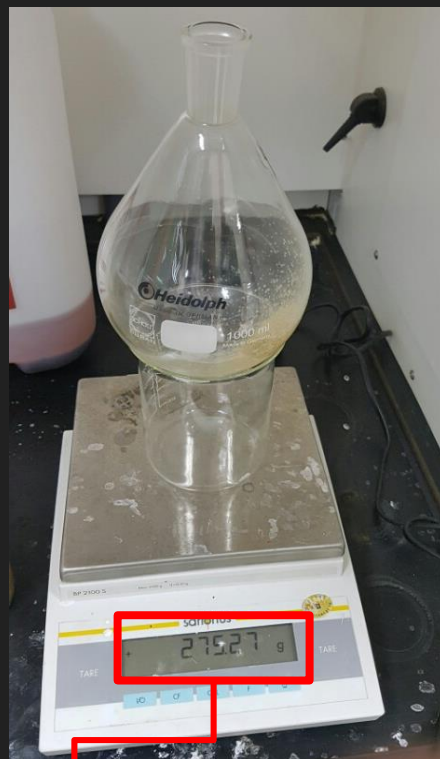
Niacin : 14.3627mg

# Concentration of compounds from Linear analysis



- Can regenerate curve from our concentration data
- Each compounds show their own contributions on UV absorbance

# Results – 근사법의 타당성 검증



R[12], [13]

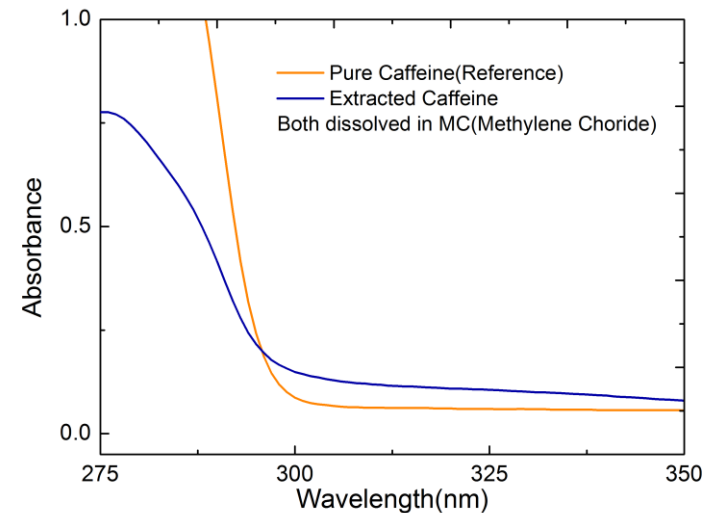
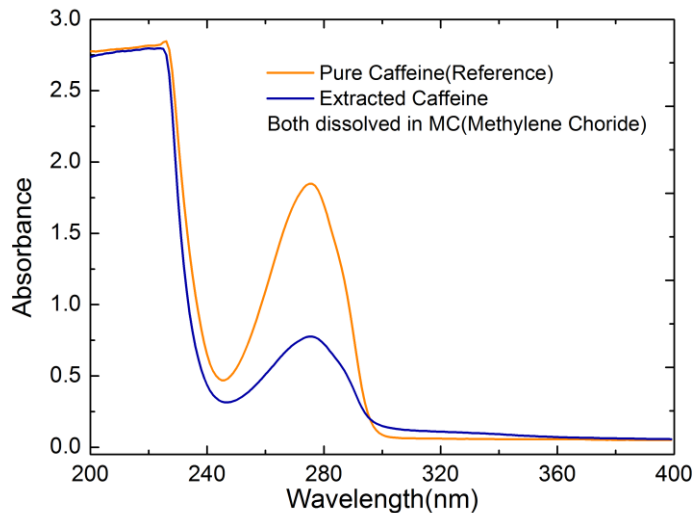
$$-275.15 + 275.27 = 0.12 \text{ g}$$

Sample에서 caffeine을 추출하여 compound의 실제 함량과 추정치를 비교하였다.

- ①  $\text{CaCO}_3$ 로 tannin group adsorption
- ② Methylene Chloride(MC)로 caffeine extraction
- ③  $\text{MgSO}_4$ 로 dehydrating
- ④ Solvent evaporation
- ⑤ Vacuum drying
- ⑥ Yield를 측정

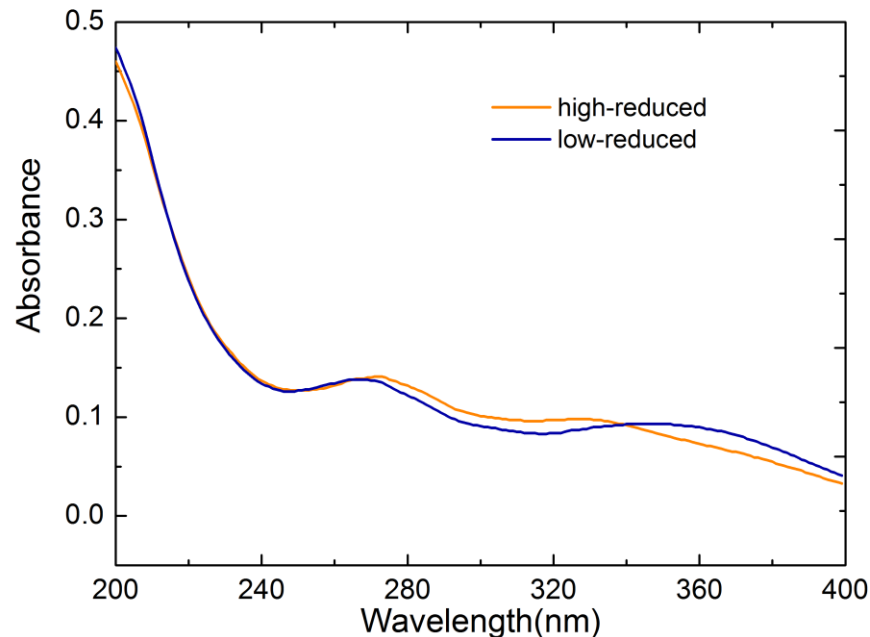


# Results – 근사법의 타당성 검증



- 수득물의 질량 : 120 mg of 100 mL sample
- MC와 물의  $K_{\text{ext}} = 14.35$  (308K) <sub>R[12]</sub>
- 실제 caffeine의 양 : **128.4mg = 6.61 mM**
- 본 근사법으로 추정한 caffeine 양 : **153.96mg = 7.93 mM**
- 따라서 본 근사법이 오차범위 **16.6%**에서 타당함을 알 수 있다.

# Results – 추출 온도에 따른 조성비



- 저온에서 caffeine, CGA, trigonelline, niacin 각각의 농도는 **7.651, 6.647, 4.515, 1.096 (mM)**, 고온에서는 **7.928, 7.228, 4.737, 0.9368 (mM)**로 나타났다.
- 저온과 고온에서의 compound 조성비 차이를 감지할 수 있었다.

# Conclusion

- ① 커피와 UV/vis spectrophotometer만 있으면 그 커피에 들어 있는 caffeine, CGA, trigonelline, niacin의 농도를 알 수 있다. 이들 네 compound는 커피를 구성하는 주요 물질이기에 그 의미가 더 크다.
- ② 분석 결과는 잘 알려진 Caffeine 추출 및 양 측정 방법과 비교하였을 때 높은 신뢰도를 보인다.
- ③ 실제로 커피의 추출 온도를 달리하여 네 compound의 조성비를 관찰한 결과 유의미한 분석이 가능하였다.

# Future Plan

- Coffee 내의 Compound 수 증가를 통한 Library 구축
  - 더욱 정밀한 data analysis가 가능할 것으로 보임
- Matlab code를 program으로 시장에 선보일 수 있도록 발전
  - 만들어진 커피를 쉽게 분석하고, roasting/dripping 과정에 반영
  - 분자 커피 시장 개척 및 합성 화합물로 만드는 커피 시장 개척
- Coffee의 volatile 성분(향 성분) 에 대해서도 analysis 방법 구축
  - Coffee의 좋은 향을 재현할 수 있는 커피



# References

- 1) <http://news.mk.co.kr/v2/economy/view.php?year=2014&no=1405692>
- 2) <http://www.yonhapnews.co.kr/bulletin/2014/09/19/0200000000AKR20140919148500017.HTML>
- 3) <http://coffeetv.co.kr/?p=7311>
- 4) <http://what-when-how.com/petroleum-refining/deviation-from-an-ideal-flow-pattern-part-1-petroleum-refining/>
- 5) <https://twitter.com/starbucks/status/317332821175844865>
- 6) <https://en.wikipedia.org/wiki/Coffee>
- 7) Farah, A., Donangelo, C. M. Phenolic compounds in coffee. *Braz. J. Plant Physiol.* 2006, **18**, 23–36.
- 8) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden. *J.Sci. Food Agric.* 1999, **79**, 362–372.

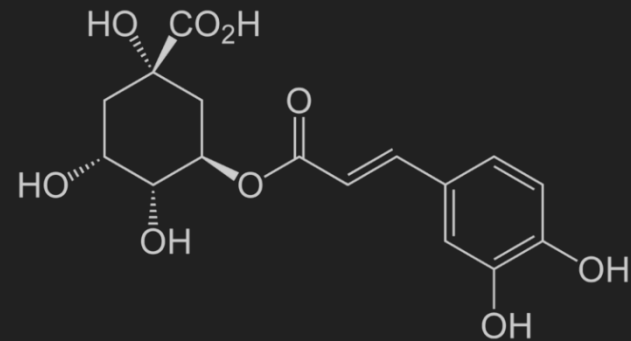
# References

- 9) Salazar-Martinez, E., Willett, W. C., Ascherio, A., Manson, J. E., Leitzmann, M. F., Stampfer, M. J., Hu, F. B. Coffee consumption and risk for type 2 diabetes mellitus. *Ann. Intern. Med.* 2004, **140**, 1–8.
- 10) Olthof, M. R., Hollman, P. C., Zock, P. L., Katan, M. B. Consumption of high dose of chlorogenic acid present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *Am. J. Clin. Nutr.* 2001, **73**, 532–538.
- 11) <http://www.hdfondos.eu/imagen/163185/cafe-taza-de-bebida-caliente>
- 12) Lat. Am. appl. res. vol.40 no.3 Bahía Blanca jul. 2010
- 13) *J. Chem. Educ.*, 1996, 73 (6), p 556
- 14) ESI for food & function 2013

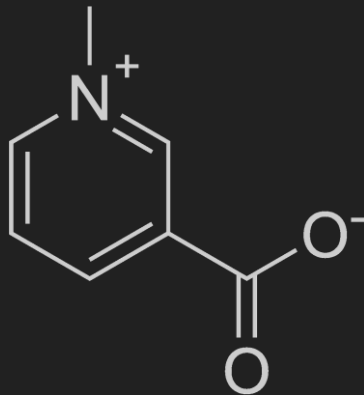
# Molecular structure of compounds



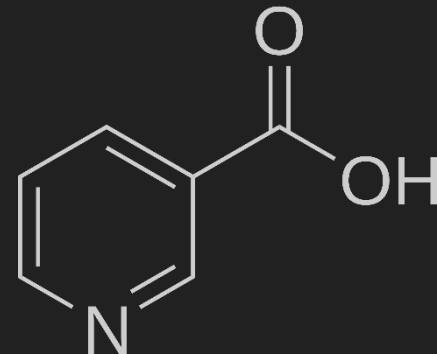
Caffeine



Chlorogenic acid  
(CGA)

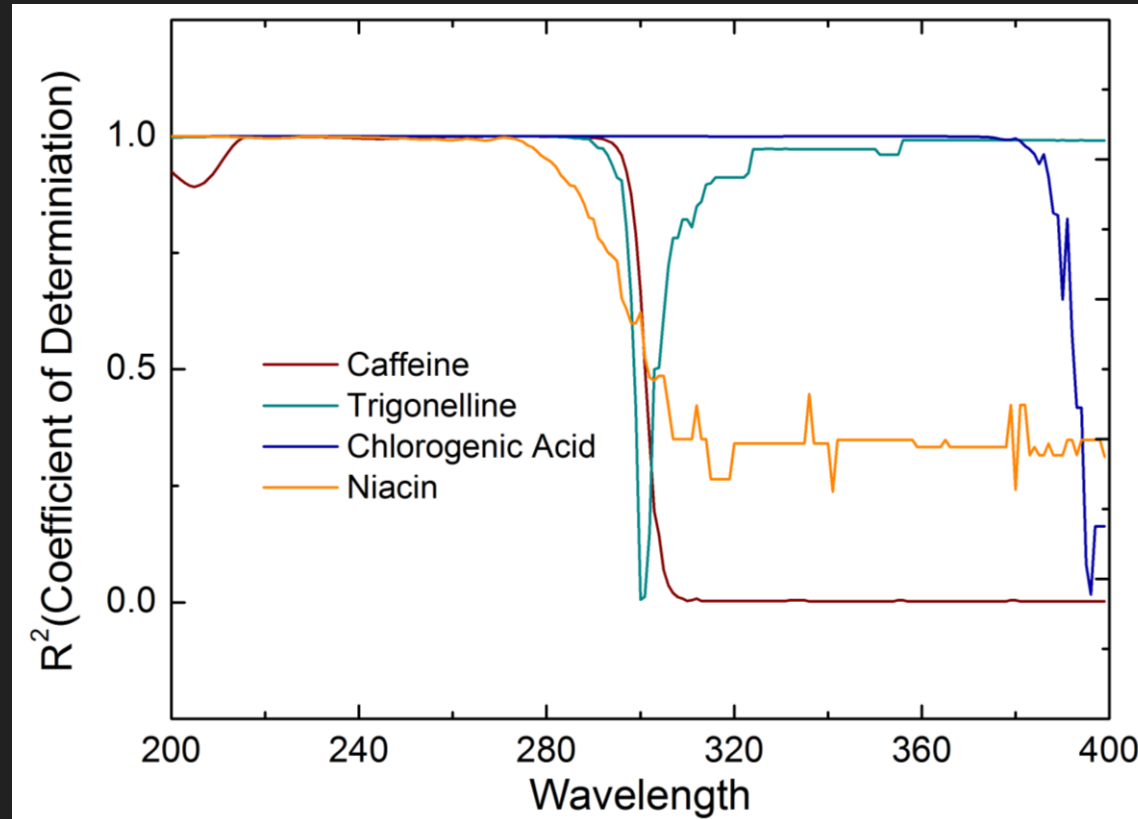


Trigonelline



Niacin

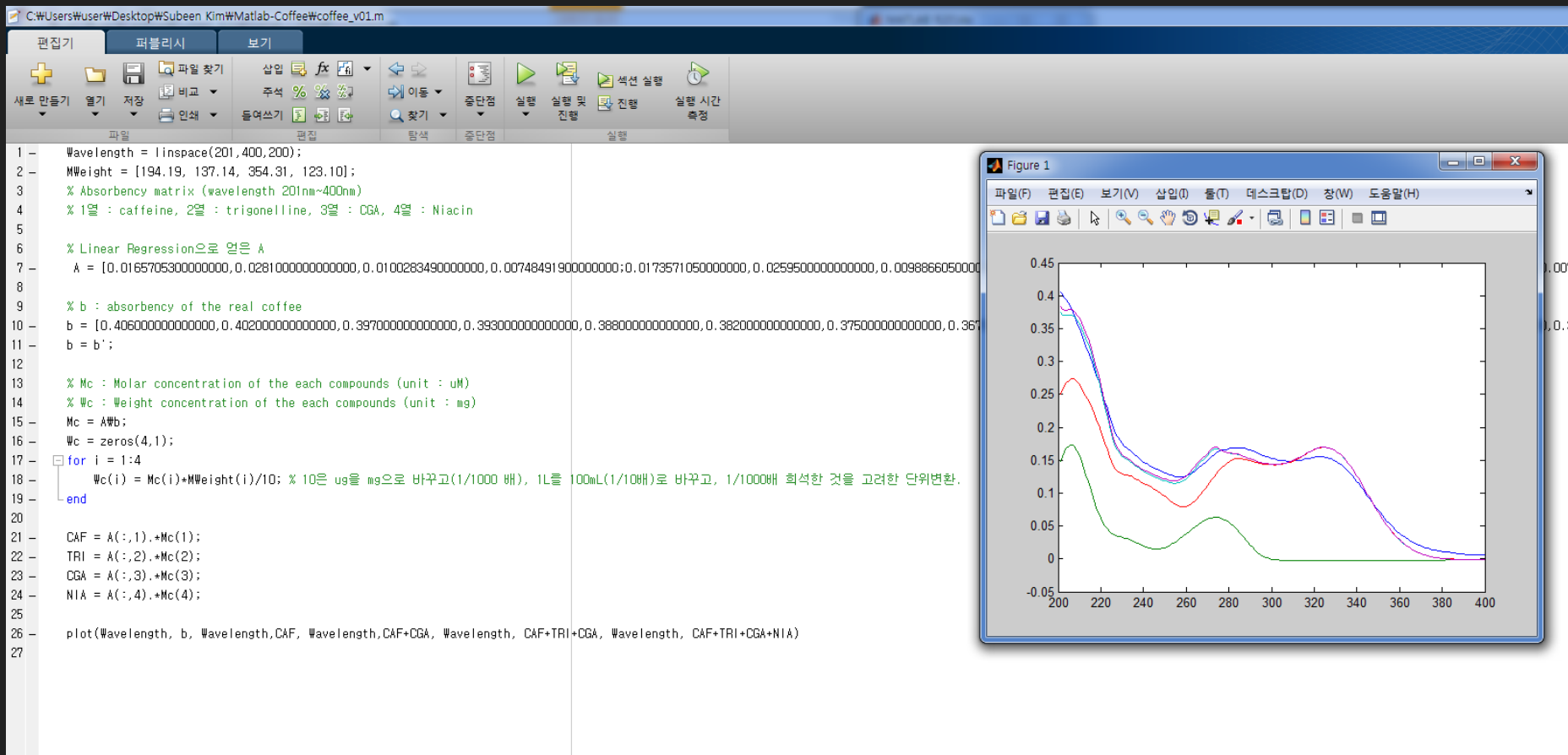
# Linearity of molar absorbance



Linear Regression을 통하여 네 compound에서  $R^2$  (Coefficient of determination) 값이 공통적으로 1에 가까운 나오는 wavelength만을 선별하였음.

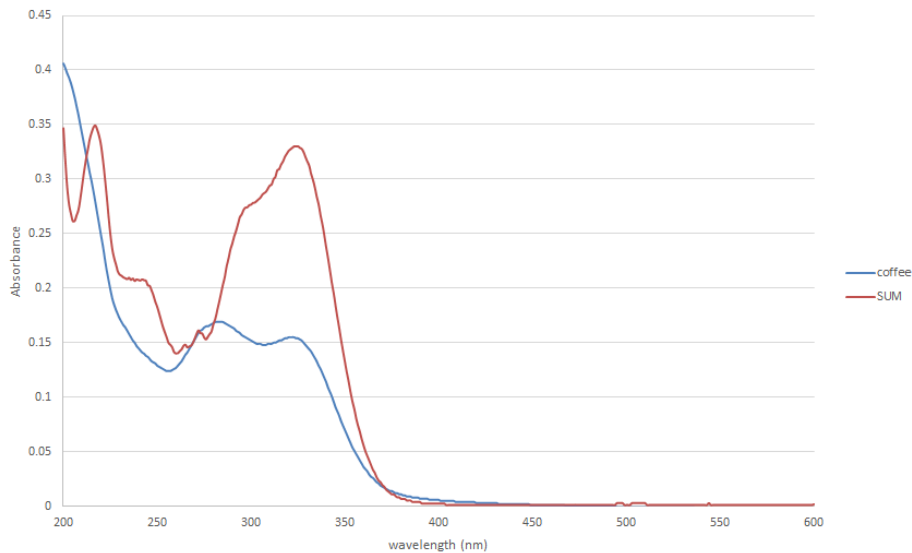


# Matlab Code & Result

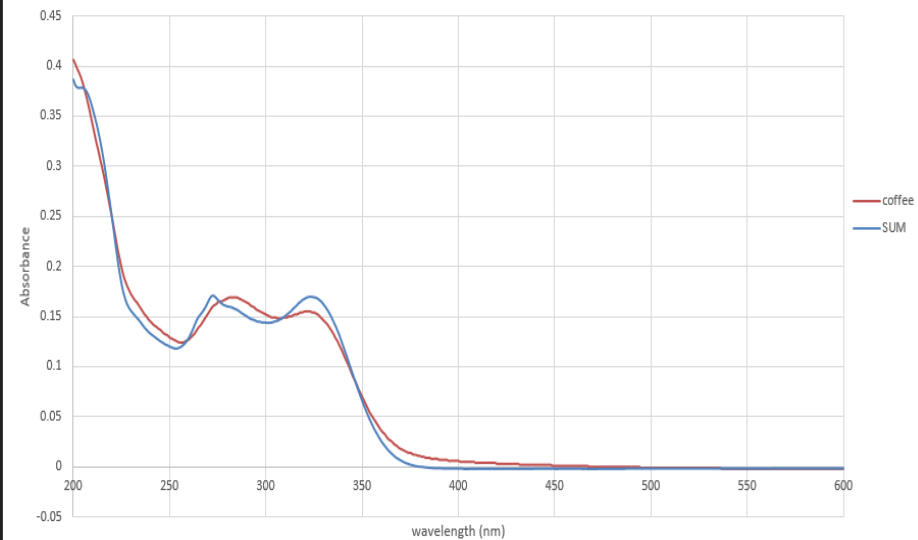


# Backup slide – 표본 파장대 범위 설정(왜 n=201로 했냐?)

n=3일 때 근사 결과값과 실제 coffee의 흡광도 graph



n=401일 때 근사 결과값과 실제 coffee의 흡광도 graph



- n값이 커질수록 E\*X값과 커피의 실제 흡광도 사이의 오차가 작아졌다.

# UV Spectrum of Melanoidins

