EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	19
	α-Lactalbumin in Milk	6-17-09 + 6-24-09	

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
Jason Larson	Kristin + Von		Biochem Lab

Title: Isolation and Characterization of alpha-Lactalbumin

Reference: Experiment #4 in Modern Exp. Biochem 3rd
slides on black board

Synopsis: Analyze the protein content of milk by attempting to extract α-Lactalbumin & other proteins. Extraction is done via centrifuge & gel filtration w/ Sephadex G-50. Analysis of proteins extracted is performed by a gel electrophoresis, Bradford assay at 595 and UV spectrophotometric characterization @ A_{260}, 280 & 290. The results were compared to milk whey, dilute skim milk and a α-lactalbumin standard.

SDS-PAGE

what type of information was being determined from each characterization method?

Procedure (-½)

— Column Filtration - fraction Range

add 10-15mL Tris Buffer
fill column w/ 300-350
well mixed G-50
fill to ~30cm

find bed volume when settled

drain tris until meniscus is just above gel.

add 2mL dye (cobalt chloride/blue dextran)
drain dye into bed.
add thick layer of tris,
connect Sep. funnel to top filled w/ tris.
drain column noting volumes that all blue & all red exit

Observations

≃ 325mL

h = 39.4cm d = 2.8cm

$V = \pi\left(\frac{d}{2}\right)^2 h$ $V = 242.6\ cm^3$

4% of 242.6 = 9.704 cm^3

a bit thicker than some

blue = Void Volume = 80.0mL

red = elution Volume = 68.00 mL

SIGNATURE	DATE	WITNESS/TA	DATE

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	
	α-Lactalbumin in Milk	6-17-09 + 6-24-09	20

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
Jason Larsen	Kristin + Von		Biochem Lab

Procedure

Milk whey —

using same column, drain meniscus of TRIS to just above sel,

Add .4% of bed volume to column

drain to void volume, ~ 80 or so mL

start collecting 2mL fractions, up through elution volume

measure fractions @ A 280 (UV) looking for the 2 highest peaks that are split up,

these 2 highest values were stored in freezer for 1 week.

Analysis Procedure (week 2)

measure $A_{260, 280, 290}$ of both fractions

perform 240 – 340 scan of each + standard ALB

Gel electrophoresis

Samples to be prepared: 2 groups fraction 1 + 2, dilute Skim milk, crude milk whey, α lactalbumin std.

not prepared: Molecular weight marker std. (M3913)

Observations

– only used 9.0 mL instead of 9.7

– we drained ~12 mL, 68, in microcentrifuge tubes,

in all, collected 66 2mL fractions,

fr 1 was 0.525

fr 63 = 0.562

Report the absorbances for all of the fractions measured

ok

SIGNATURE	DATE	WITNESS/TA	DATE

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	21
	Quantification of [illegible] in Milk	5-17-09 - 6-24-09	

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
[illegible]	[illegible]		[illegible]

Procedure

Prepare each Sample:
100 µL of sample
+ 8 µL 25% SDS
+ 6 µL mercapto ethanol
into a micro centrifuge.

boil 3 min to denature
Cool to RT
add 100 µL of 2x Protein loading dye.

Load samples into premade Vertical gels,
- prefilled w/running buffer.
- remove bubbles from wells,
- add 10 µL of each sample.

run until dye front nears bottom,
stain w/ Coomassie blue overnight.
add destaining Solution
65% H_2O, 30% methanol, 5% HAc.
until clear.

} Melissa Bush performed,

Bradford Assay A595 -dilute w/DI water to 1mL.
Produce std curve using γ-globulin, 0.10 mg/mL,
Beers law plot,
$R^2 \geq 0.985$

Analyze crude whey, Fraction 1 & 2
within the range of std. Curve,
Varying mL of Sample until on
the Curve.

Observations

SIGNATURE	DATE	WITNESS/TA	DATE

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.

Fraction measures @ A280

fraction #	Absorbance	#	Abs
1	0.525	34	0.043
2	0.474	35	
3		36	0.042
4	0.420	37	
5		38	0.042
6	0.377	39	
7		40	0.042
8	0.333	41	
9		42	0.049
10	0.287	43	
11		44	0.061
12	0.240	45	
13		46	0.081
14	0.199	47	
15		48	0.117
16	0.167	49	
17		50	0.165
18	0.139	51	
19		52	0.229
20	0.115	53	
21		54	0.305
22	0.101	55	
23		56	0.393
24	0.085	57	
25		58	0.471
26	0.073	59	
27		60	0.532
28	0.063	61	0.553
29		62	
30	0.057	63	0.562
31		64	0.554
32	0.050	65	0.535
33		66	0.510

Lanes left to right (See attached)

1 = MW marker
2 = Fraction # 2, other group
3 = Fraction #1, ~~other~~ group
4 = " " #1 our group
5 = Fraction # 2 ours
6 = MW marker
7 = milk whey
8 = Stim
9 = α-lactal bumin.

Bradford Assay #5

Standards mL	A_{595}	mg of protein
0.10	0.164	0.010
0.20	0.222	0.020
0.30	0.268	0.030
0.35	0.282	0.035

	mL	A_{595}	mg
Fraction #1	0.1	0.282	0.01
#2	0.725	0.148	0.00725
milk whey	0.01 mL	0.268	0.01

$y = 4.7729x + 0.1206$

$R^2 = 0.9878$

Fr 1 = $0.282 = 4.7729x + 0.1206$, $= 0.338$ mg

Fr 2 = $0.148 = 4.7729x + 0.1206$, $= 0.00574$ mg

milk whey: $0.268 = 4.7729x + 0.1206 = 0.0309$ mg

this abs is outside of the range of our Std? how did we miss that?

SIGNATURE	DATE	WITNESS/TA	DATE

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.

electro phoresis results,
see excel print out attached.

$y = -0.21x + 5.0861$ Std line equation used

UV spectrophotometric Results:

Fraction #1 $A_{260} = 0.3041$
$A_{280} = 0.4934$
$A_{290} = 0.3566$

Protein Conc mg/mL $= 1.55 A_{280} - 0.76 A_{260}$

$1.55(0.4934) - 0.76(0.3041) = 0.5336_5$ mg/mL
$= 0.05336_5$ g/100mL

$\frac{A_{280}}{A_{290}} = \frac{0.4934}{0.3566} = 1.383_6$ (pure α-lactalbumin = 1.301)

$\epsilon = \frac{A_{280}}{(1.0\,cm)\ conc\ g/100ml} = \frac{0.4934}{(1)(0.05336_5)} = 9.25$ (compare to 20.1, pure α-lactalbumin std)

Fraction #2 $A_{260} = 0.3511$ | $A_{280} = 0.4668$ |
$A_{290} = 0.3692$

Protein conc $= 1.55(0.4668) - 0.76(0.3511) = 0.4567_0$ mg/mL forgot to use divider
0.04567_0 g/100mL

A $\frac{0.4668}{0.3692} = 1.264_4$

$\epsilon \ \frac{.4668}{1.0(0.0456_7)} = 10.22$

mg of fraction
0.010
0.020
0.030
0.035

mg
0.01
0.02
0.001

$y = 4.7729x + 0.1206$
$R^2 = 0.9878$

Fr 1 $0.282 = 4.7729x + 0.1206$, $= 0.338$ mg
Fr 2 $0.14 = 4.7729x + 0.1206$ $= 0.00574$ mg
milk whey: $0.26 = 4.7729x + 0.1206 = 0.0209$ mg

this also is outside of the range of our Std ... did we miss that?

14.5/15

SIGNATURE	DATE	WITNESS/TA	DATE

NOTE: INSERT BACK COVER UNDER COPY SHEET BEFORE WRITING

Conclussions/Discussion

The ultimate goal of this experiment was to purify protein(s) (hopefully α-lactalbumin) from a milk sample. Several steps were performed on the samples prior to them being delivered to the students as part of the initial purification process. Non-fat milk cells were ruptured and their cell contents were centrifuged at high speeds. The clear supernatant that contained the proteins was then placed in a Sephadex (G50) gel filtration column. The fractions collected were measured to find the highest absorbance points, which should represent the highest protein concentrations.

Several analysis methods were performed on the two seemingly most purified protein samples to see if we could determine what protein we obtained, and how effective our purification methods were. The first was a graphical analysis using UV spectrophotometer results. The graphs obtained (attached) did fit the general structure anticipated, with both the standard and fraction 1 having two peaks, and fraction 2 (#63) only having 1, although it does not drop off as sharply as expected. The 2 peaks in fraction 1 are centered around 280 and 290 nm. Absorbance at 280 nm is typical of proteins. The general shapes do suggest that we may indeed have α-lactalbumin in Fraction #1, but perhaps something else in Fraction #2.

Further analysis of the UV results allows us to calculate the ratio of A280 to A290 and estimate the concentration of protein contained in the samples. Fraction 1 is estimated to .5226 mg/ml (.05336 g/100ml). Fraction was estimated at 0.4567 mg/ml (.04567 g/100ml). The ratios obtained are 1.384 for fraction 1 and 1.264 for fraction 2. Pure α-lactalbumin is known to have a ratio of 1.301. That fraction 1 has a higher ratio suggests there was a stronger peak at A280 than A290, and the opposite is true for fraction 2, which is lower than the pure sample.

Using the calculated concentrations we are able to calculate absorbance coefficient. The absorbance coefficient of pure α-lactalbumin is 20.1. For fraction 1 we obtained 9.25, and fraction two was 10.22. Both of these values are substantially lower than the standard. Either the A280 absorbance is smaller than expected in pure (which when compared to the graphs, this does not seem to be the case) or the concentration of protein within our sample is larger than expected, which seems more likely. The higher concentration may be due to contaminating proteins also contained within the samples.

The Bradford Assay provides another measure of protein concentration in mg/mL. A standard curve was developed by measuring a standard gamma globulin at A595. The absorbencies of our two fractions and milk whey was then compared and plugged into the line equation from the standard graph. The results determined that the concentration of fraction one is 3.38 mg/mL and fraction 2 was .00792 mg/mL. These numbers are quite different than what was estimated from the UV results above, with fraction 1 being estimated roughly 7 times higher in the Bradford Assay, and 160 times lower. The milk whey was estimated to be 3.09 mg/mL.

The final analysis was a gel electrophoresis of our samples, milk why, skim milk, pure α-lactalbumin and a standard molecular weight marker. Our samples were ran alongside a second groups, and they appeared nearly identical to ours, with fraction 1's having two bands and fraction 2 having no bands. Note that most of the lanes contained a faint line around 0.9 cm (~79,000 Da). This was perhaps some contamination from the skim milk or milk whey, which had the darkest bands at this level. See the attached tables and graph for results. The lack of any bands in fraction two suggest that there were no proteins in the samples.

The α-lactalbumin standard had a single, dark band at 10,867 Da. A similar weight band was found in the fraction 1's, skim and milk whey, suggesting that α-lactalbumin is present in all of these samples. The samples, mlk whey and skim milk also all had very dark, broad bands around 15,500 Da. This is perhaps a second protein present (which would explain the higher concentrations found in the Bradford assay for our fraction 1 than expected), in a higher concentration than α-lactalbumin. Skim and milk whey both had several other bands present of larger weight proteins, which shows the progressive effect of the purification process, where the milk whey had less than skim, and our fraction's having less than milk whey.

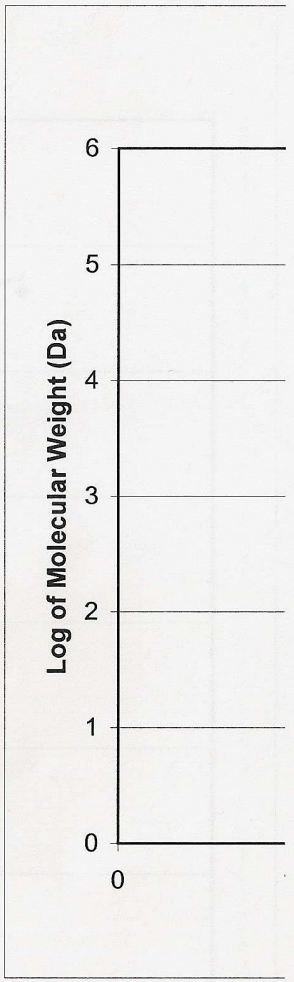
Taken together, the results did suggest that α-lactalbumin was isolated, along with a second protein, in our first fraction obtained during the column separation. The amount obtained is a bit more difficult to guess, as the Bradford assay and UV results gave starkly different values. Perhaps the Bradford assay is more accurate for the overall protein concentration obtained, at 3.38 mg/mL. A second protein is clearly present in our fraction, and is perhaps in higher concentration given the dark, think band produced around 15,500 Da. When compared to the milk whey and skim milk samples in the gel electrophoresis, it is clear that our purification methods were very effective, up to a point. The relatively similar sizes of the two proteins, however, may mean that the purification techniques were unable to differentiate between the two. It is interesting to note that the known MW of α-lactalbumin is 14,200, and our equation results in the weight being closer to 11,000 Da. This difference appears to be due to the line obtained not being as linear as possible, and so the equation may not be entirely accurate.

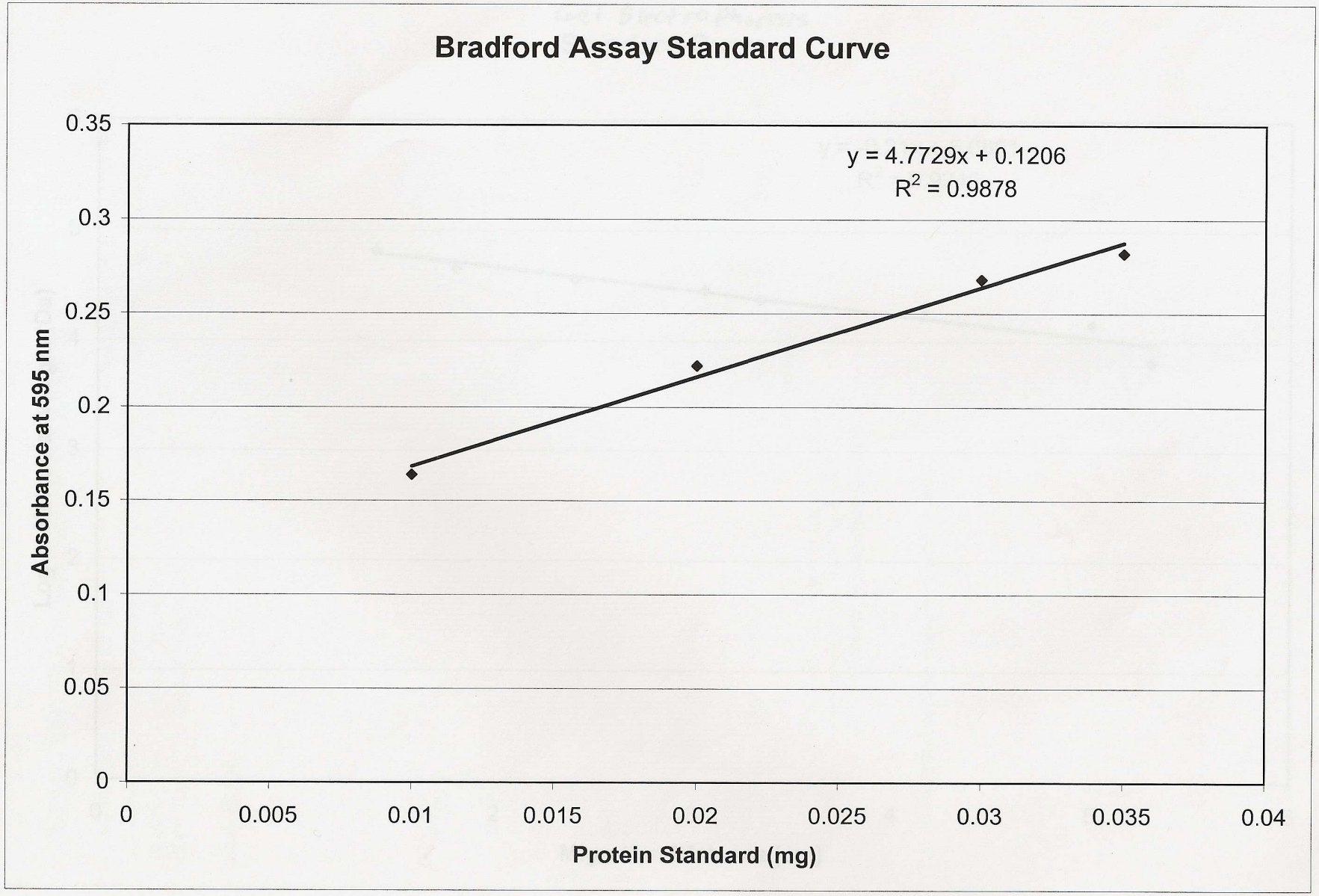
Log10 A# y=-.21(F#)+5.0861 Antilog G#

Std's weight (Da)	Distance (cm)	Log of Std Weights
66000	1.4	4.819544
45000	1.8	4.653213
36000	2.4	4.556303
29000	3.05	4.462398
24000	3.33	4.380211
20000	4.15	4.30103
14200	5	4.152288
6500	5.3	3.812913

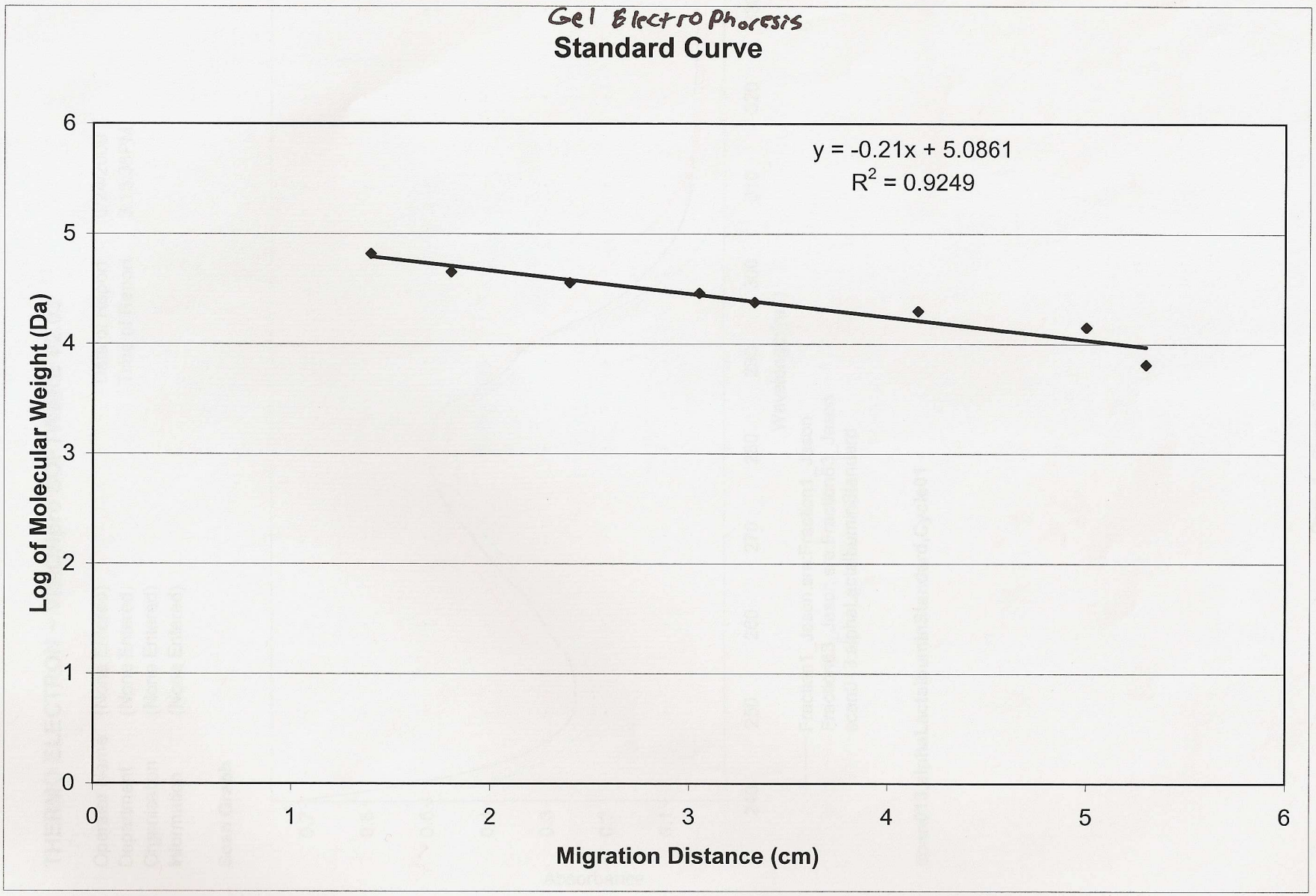
Include sample ID's with fractions

Lane #	Distance (cm)		Weight of protein at this line (Da)
3	0.9	4.8971	78904
	4.25	4.1936	15617
	4.95	4.0466	11133
4	0.9	4.8971	78904
	4.25	4.1936	15617
	4.95	4.0466	11133
7	0.7	4.9391	86916
	1	4.8761	75180
	4.27	4.1894	15467
	4.95	4.0466	11133
8	0.2	5.0441	110688
	0.7	4.9391	86916
	0.98	4.8803	75910
	3.1	4.4351	27233
	3.45	4.3616	22993
	4.3	4.1831	15244
	4.5	4.1411	13839
	5	4.0361	10867
	5.2	3.9941	9865
9	5	4.0361	10867




Bradford Assay Standard Curve
y = 4.7729x + 0.1206
R2 = 0.9878
Absorbance at 595 nm
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.005
0.01
0.015
0.02
0.025
0.03
0.035
0.04
Protein Standard (mg)

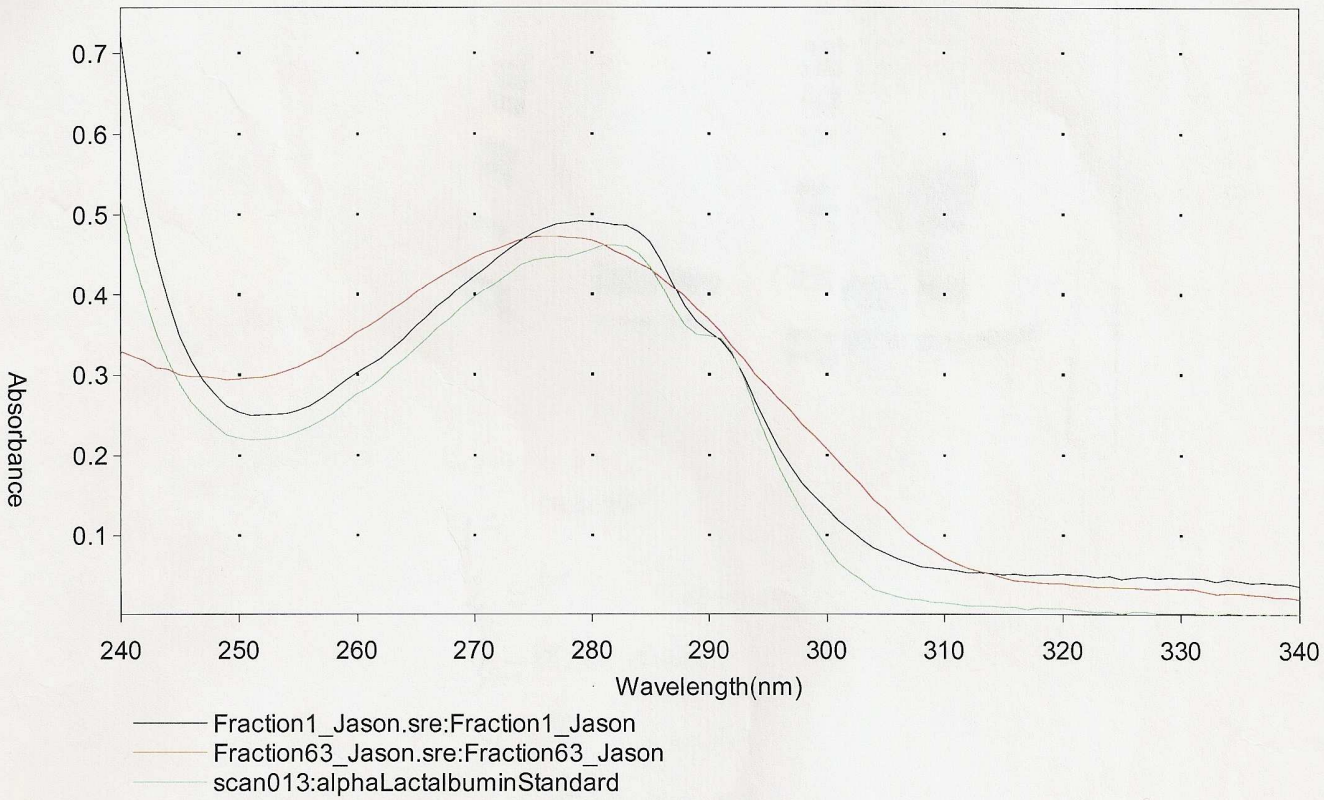
Gel Electrophoresis



THERMO ELECTRON ~ VISIONpro SOFTWARE V2.03

Operator Name	(None Entered)	Date of Report	6/24/2009
Department	(None Entered)	Time of Report	3:15:38PM
Organisation	(None Entered)		
Information	(None Entered)		

Scan Graph



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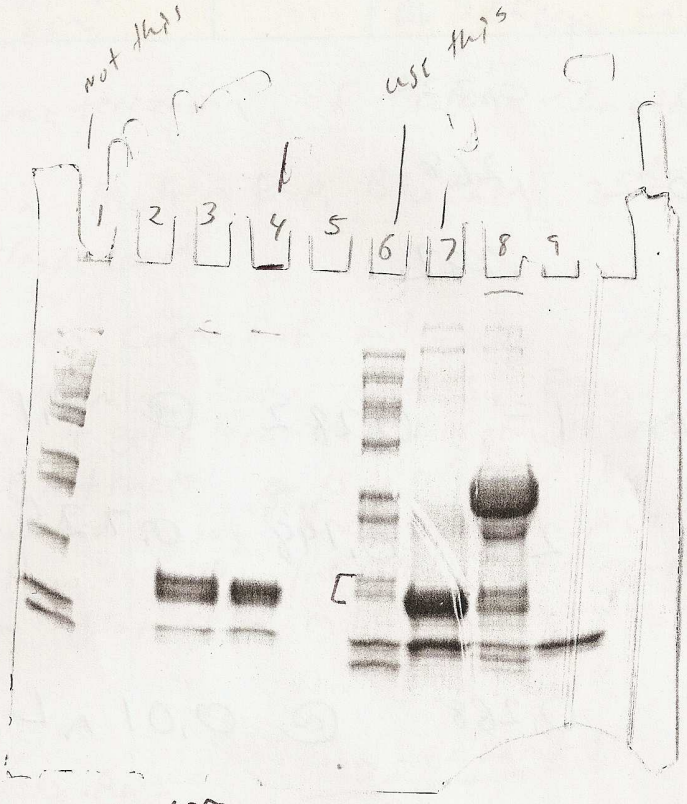

1 = marker

2 = fr 2 other

3 = fr 1 other

4 = fr 1 ours

5 = fr 2 ours

6 marker

7 milk whey

8 skim

9 α lactalbumin

2

0