

Minerals in Milk – The forgotten fraction

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My Background

- Educated at Birmingham Univ. Chem.Eng.
- 38 years at Reading University
- Physical properties of foods
- Food processing operations
- Pilot plant activities (UHT pilot plant)
- Supervised over 30 PhD students and 150 BSc and MSc students
- Fascinated by milk, biological fluid

Milk Chemical Composition-Complexity

- Protein - caseins 2.6% ;

main caseins : α_{s1} , α_{s2} , β , γ , and κ

Casein micelle size - in micelles of size range 30 - 300 nm

Whey proteins - 0.6% - main one is β lactoglobulin (0.3%)

denaturation starts about 60°C. Denatured whey protein associates with the casein micelle.

- Minerals (salts) (0.6 to 0.7%): Ca Mg, P, citrate, Na, K and Cl

- Calcium total 30 mM

micellar 20 mM ; soluble 10 mM

of which ionic calcium is 2mM (between 6 and 7%)

- vitamins (water and fat soluble)

- Trace nutrients

- Complete food (ascorbic acid)

- When milk is heated, we need to consider its effects on all these compounds

The overall chemical properties of raw cow's milk over one year

Compositional Properties	Mean±SD	Range	Seasonal variation
pH	6.79±0.35	6.73 - 6.87	SP and W > SM and A
Ca ²⁺ (mM)	2.05±0.25	1.68 - 2.55	NS
Total Solids (%)	12.78±0.29	12.31 - 13.31	A > SM
Protein (%)	3.29±0.16	2.89 - 3.56	SP > SM and A
Total casein (%)	2.36±0.09	2.08 - 2.52	SP > SM and A
Fat (%)	4.08±0.36	3.62 - 4.77	A > SP, SM and W
Lactose (%)	4.59±0.44	4.52 - 4.69	NS
Ash (%)	0.71±0.14	0.53 - 1.03	NS
Total Ca (mM)	29.29±1.78	24.53 - 31.53	NS
Total Mg (mM)	5.11±0.34	4.21 - 5.81	NS
Total Citrate (mM)	9.04±0.53	8.22 - 10.09	NS
Total P (mM)	27.52±2.12	22.58 - 33.57	NS
Urea (mM)	3.95±0.40	5.44 - 2.65	NS
Somatic Cell Count (cells ml ⁻¹ x 10 ³)	155±63	65 - 357	W > SP, SM and A

Salt Composition of Milk

Constituent	Mean (mg/100 g)	Range (mg / 100 g)	Mean (mM)	Percent Diffusible
Sodium	58	47-77	25.2	
Potassium	140	113-171	35.8	
Calcium	118	111-120	29.5	31
Magnesium	12	11-13	4.9	65
Amines			~1.5	
Phosphorus	74	61-79	23.9	53
InorganicP _i	63	52-70	20.4	53
Ester	11	8-13	3.5	
Chloride	104	90-127	29.3	
Citrate	176	166-192	9.2	90
Carbonate			~2.0	
Sulphate			~1.0	
Organic acids			~2.0	

Source: White and Davies (1958)

Total minerals ~0.7%

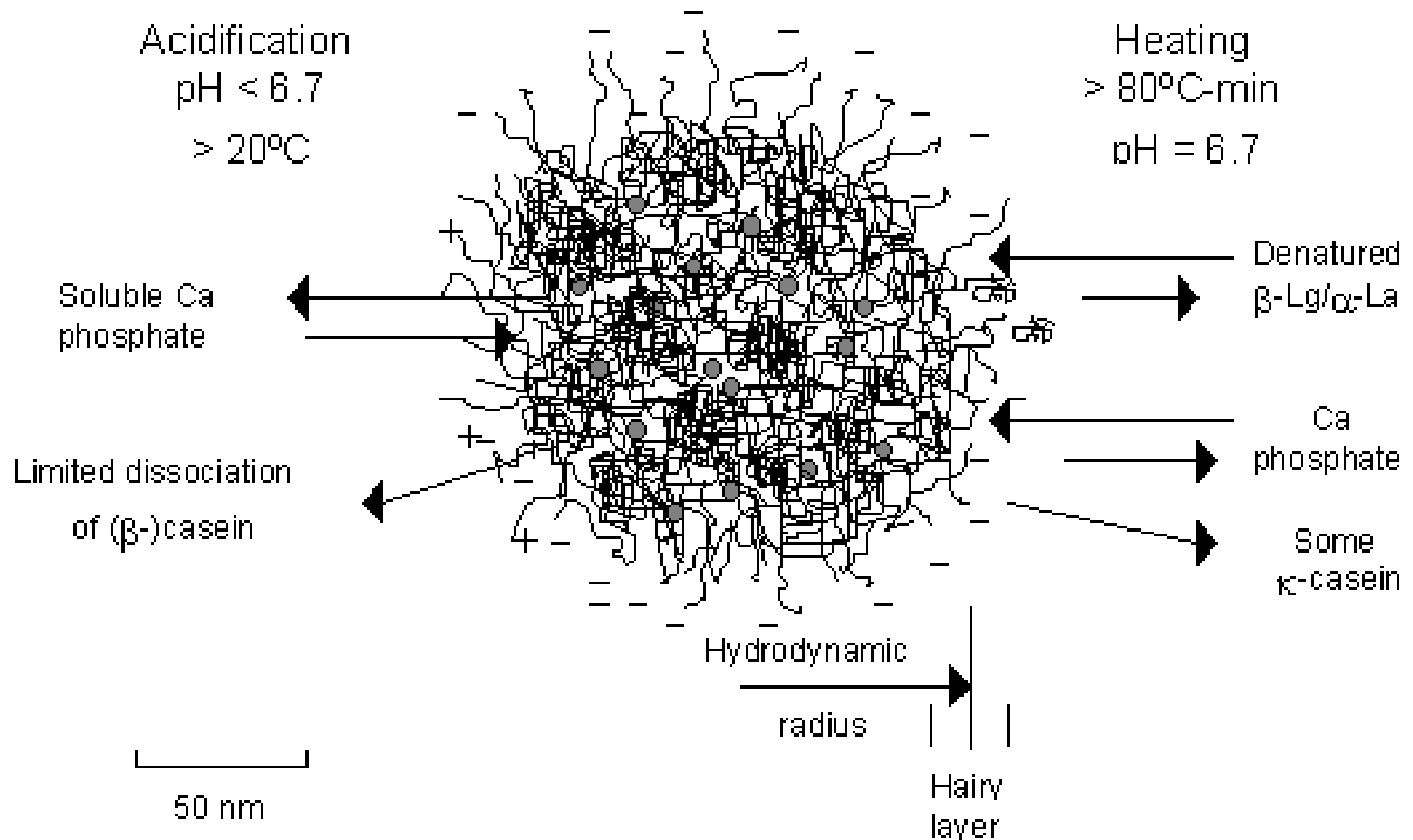
**Average values for citrate, phosphorus, magnesium, calcium,
and ionic calcium for individual cows milk**

Citrate	Phosphorus	Magnesium	Calcium	Ca²⁺
7.8	27.8	4.11	29.3	1.77
(4.8-13.2)	(18.4-38.4)	(3.37-5.31)	(21.5-41.3)	(1.05-3.43)

All results are given in mM (mmol/L)

Ranges are shown in parentheses

One model for the casein micelle



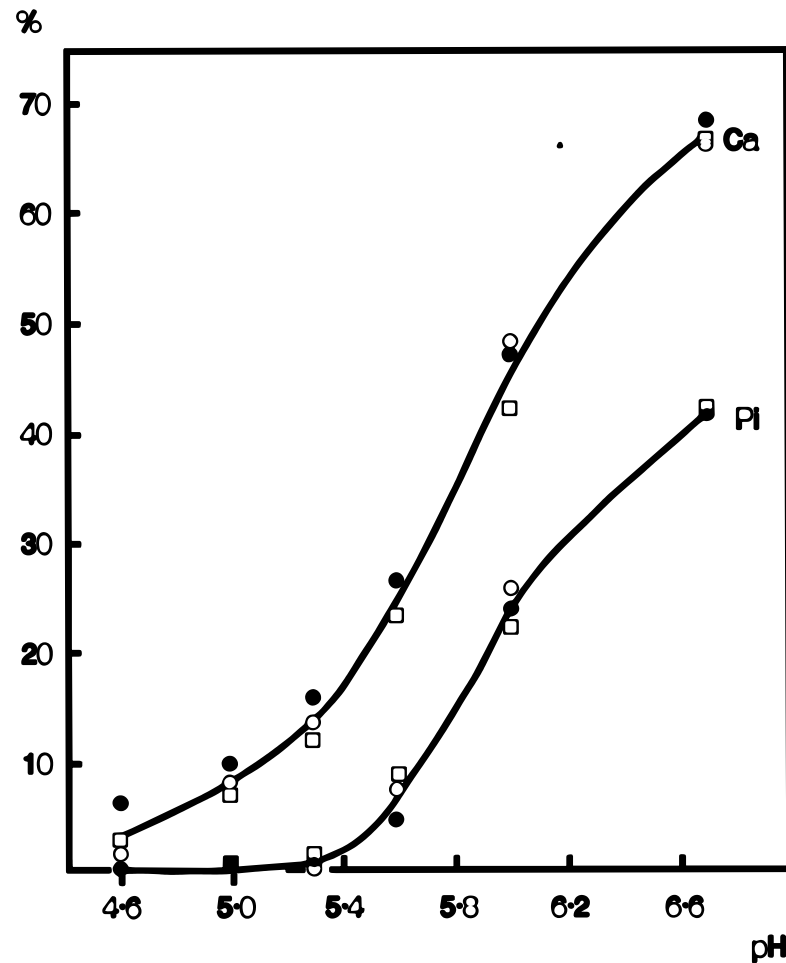


UF permeate from milk

- Note that this contains low molecular weight compounds which are not associated with the casein micelle
- Why is it yellow/green?
- What is its calcium concentration?
- What will happen if it is heated to 60 C?
- What will happen to it when it cools down?
- What is its lactose concentration?
- What are the uses for this permeate?

Note: Calcium phosphate becomes less soluble as temperature increases

Percentage of Component associated with the casein micelle at different values of pH



Reversibility

- Reducing pH of milk will result in a repositioning of some of the Ca and P.
- If pH is recovered, how will this affect the quality of the milk and the products made from that milk
- Will this process convey any advantages on the products produced.
- For example, ionic calcium is higher in pH restored samples.
- This leads to a reduction in heat stability and a decrease in rennet coagulation time.

Main cations in milk

K^+	Na^+	Ca^{2+}	H^+
31-43 mM	17-28 mM	1-3 mM	< 0.001 mM

Note: any change in the K : Na ratio may affect the flavour of the product

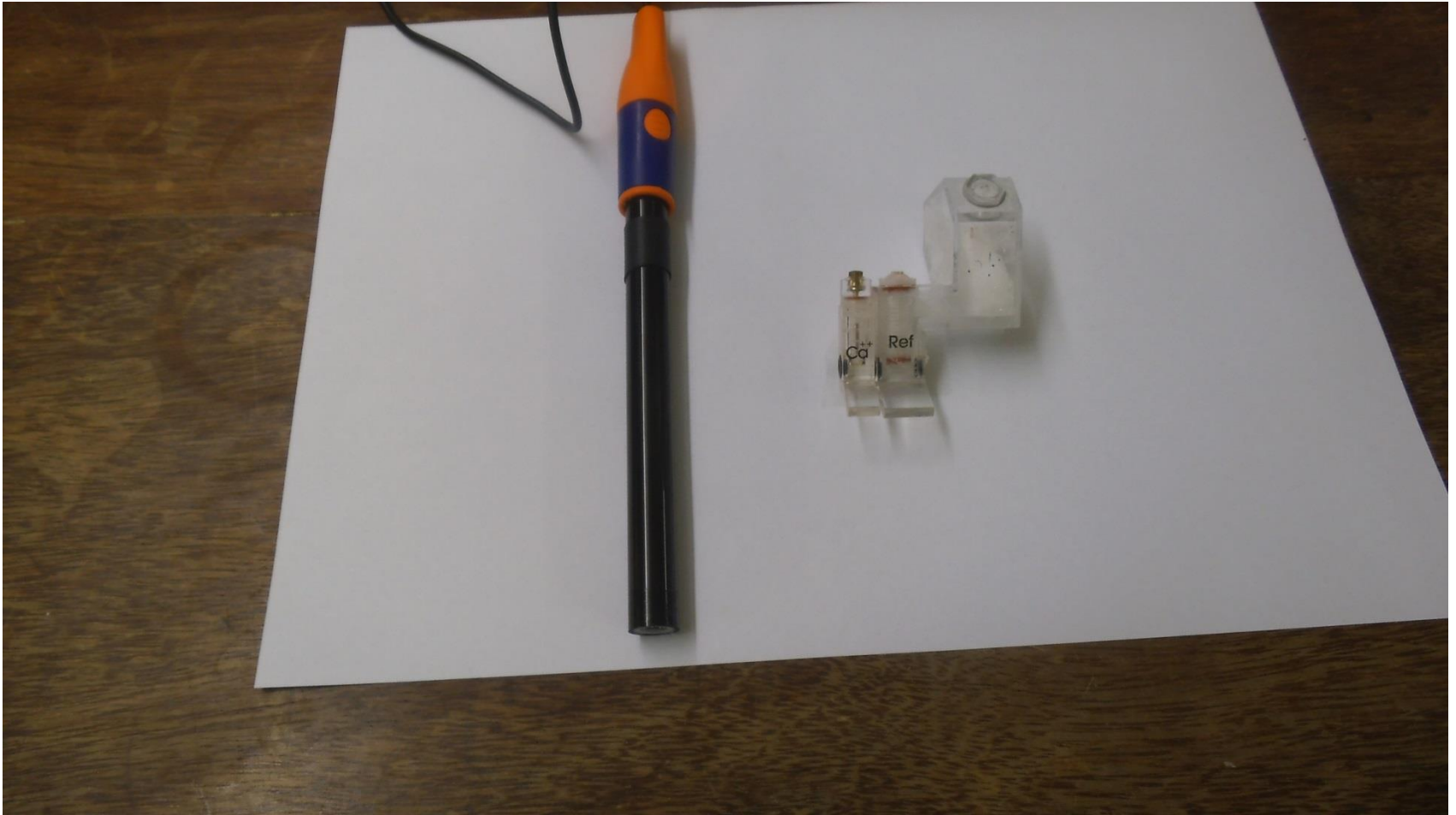
Total calcium and ionic calcium in milk of different species (average values)

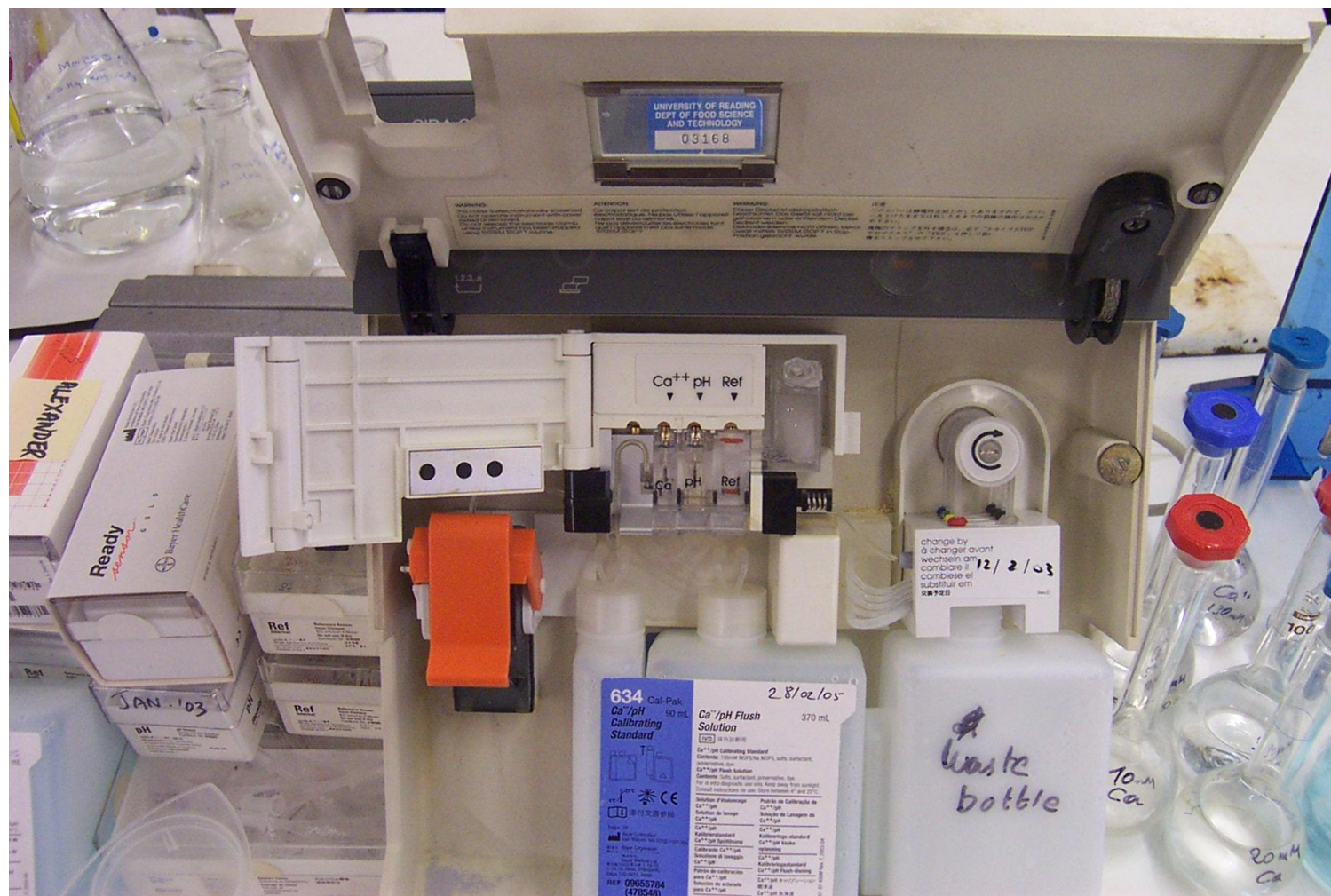
	Total Calcium (mM)	Ionic calcium (mM)
Human	7.5	3.0
Cow	30	1.80
Goat	34	2.67
Sheep	55	2.01
Mouse	71	?
Human Blood	2.05-2.5	1.1 – 1.32

Measuring ionic calcium in milk

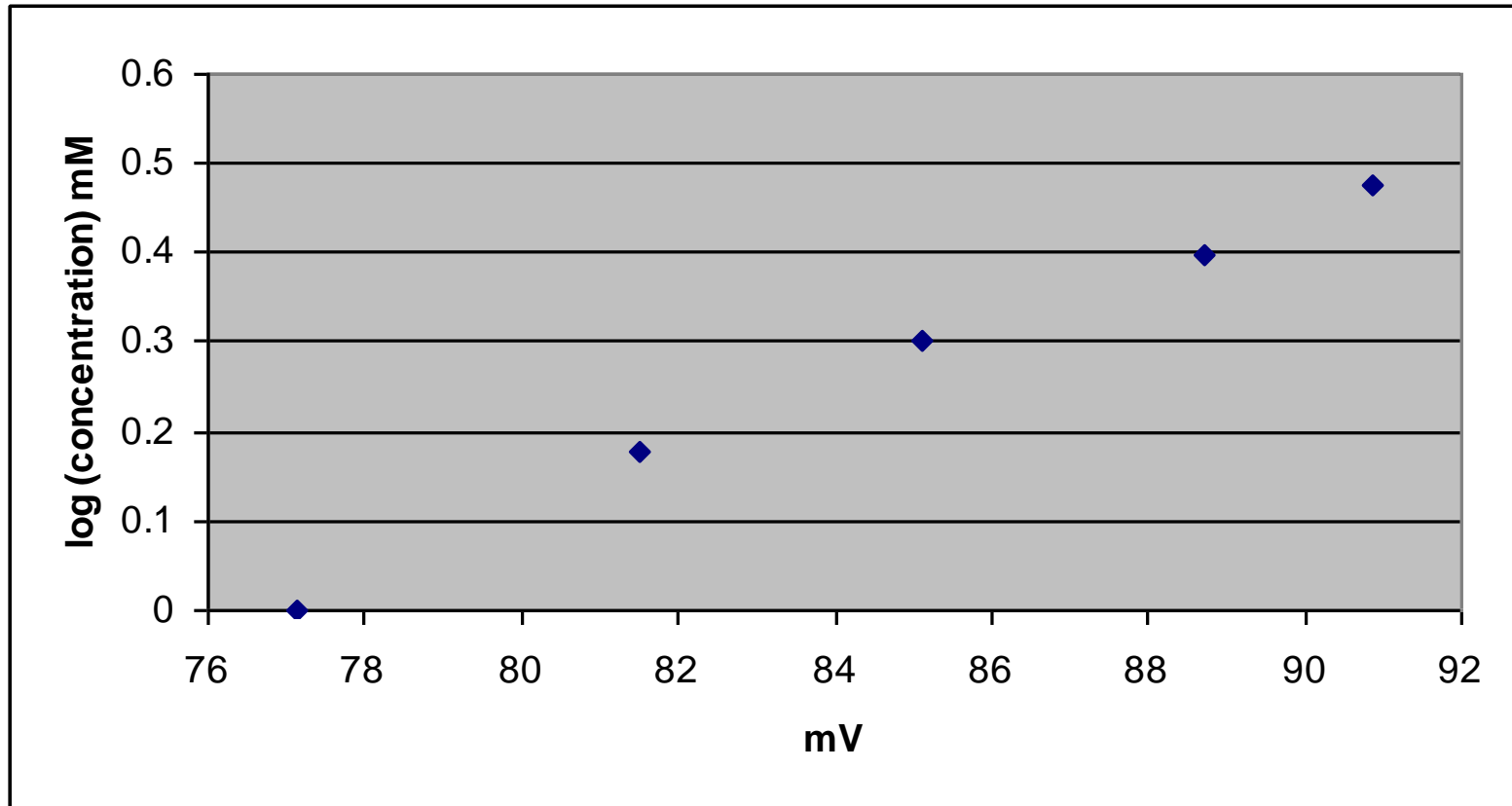
- **Ion-exchange** equilibration, Christianson et al. (1954).
- **Murexide** complexing agent, Tessier and Rose (1958); White and Davis (1958).
- Use of an **ion selective electrode** designed for measuring calcium in blood (measures activity).
- Development of **standards** with pH (6.7) and ionic strength (81mM) similar to milk
- **Calibration** of the system prior to use daily: (with five standards, over range 0.25 to 3.00 mM)

Immersion type and flow-through electrodes





Calibration for ionic calcium meter



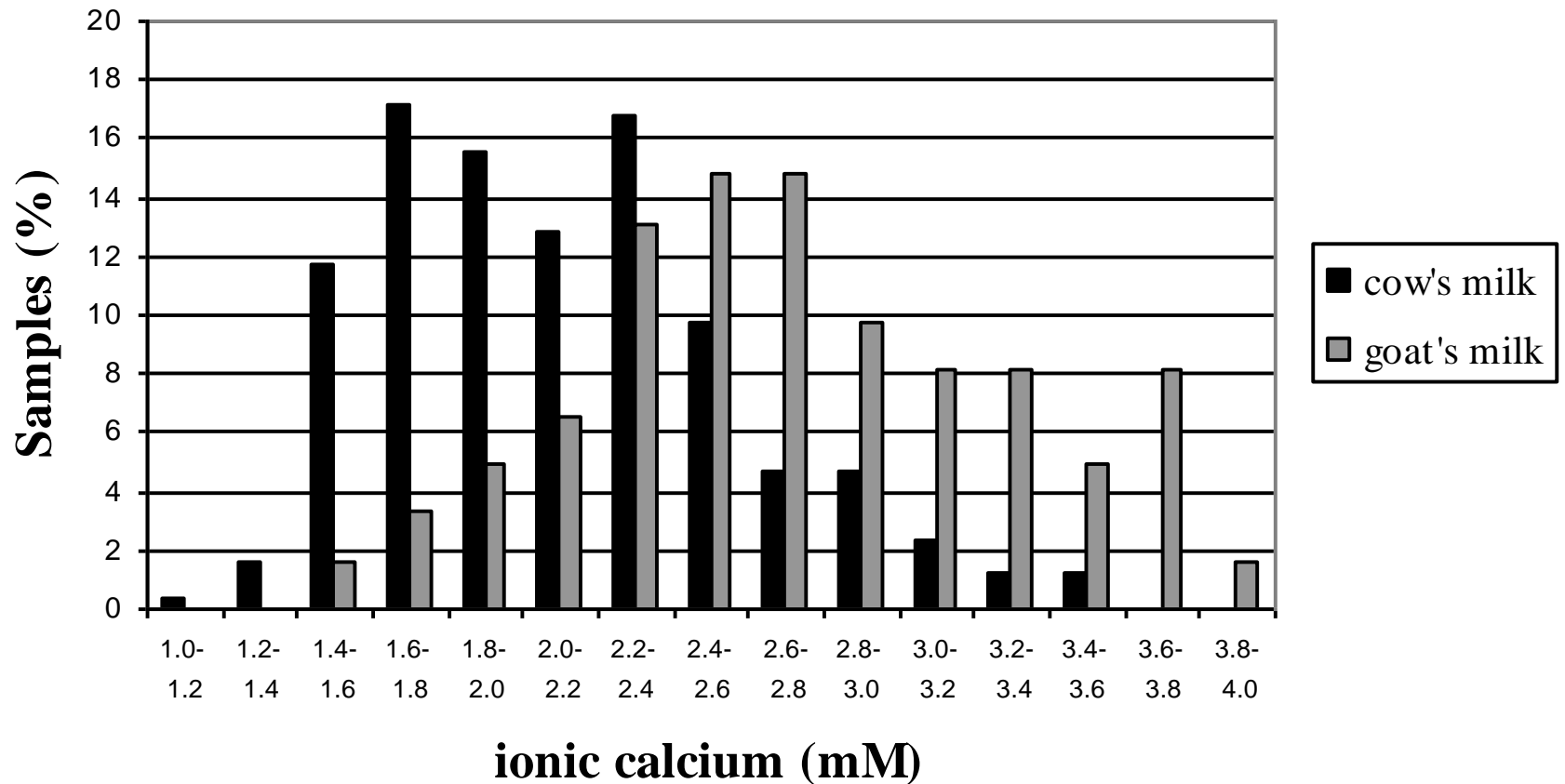
Guidelines for reliable results

- Immersion probe or flow-through probes
- Divalent ions: ~ 29 mV change for 10 fold change in concentration
- 8 to 9 mV per doubling of concentration
- Important to check performance and reproducibility of the electrode
- Calibrate every day prior to use.

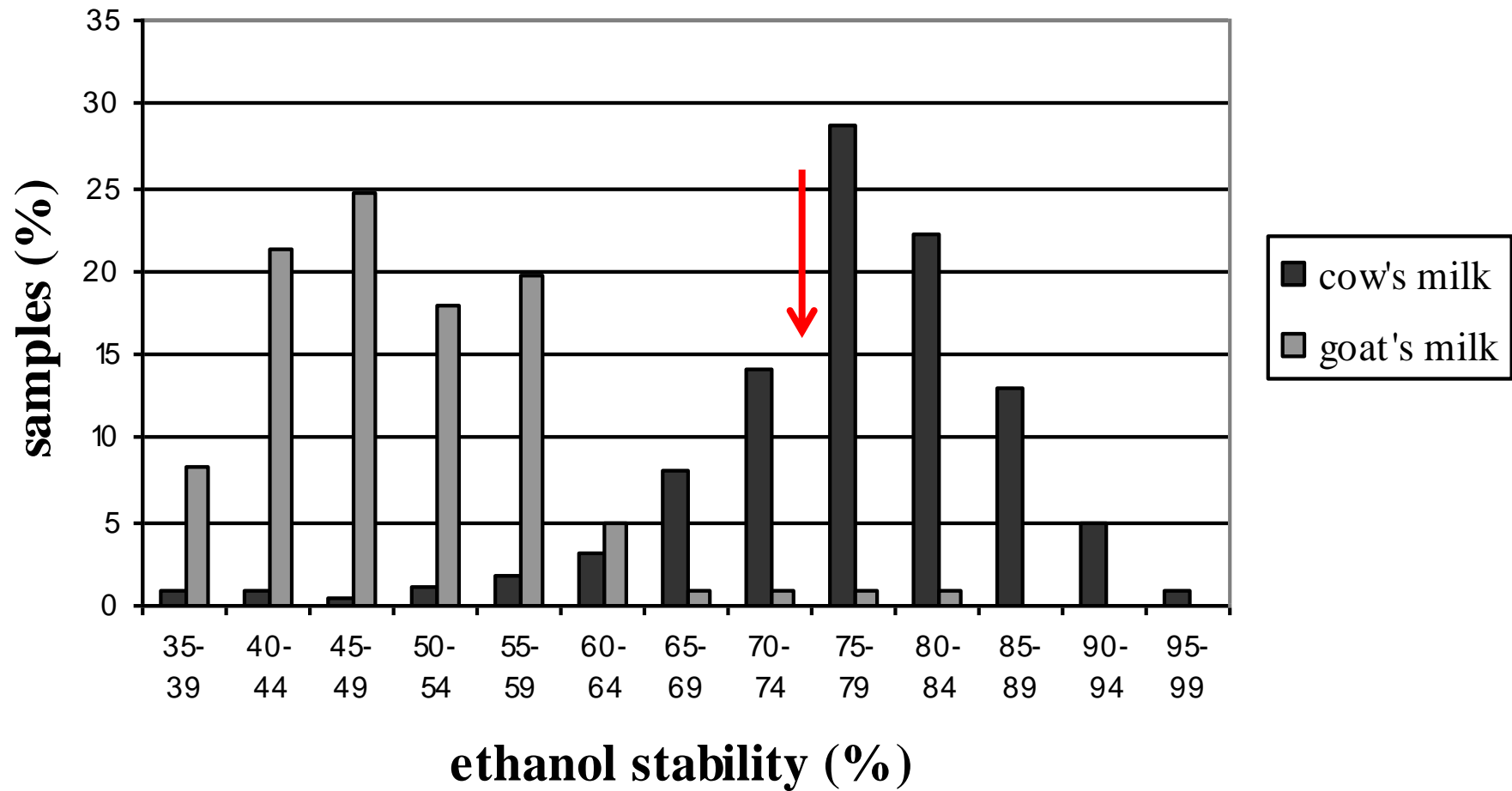
Ionic calcium and alcohol stability for 10 different cows over a complete lactation period

Cow No	Ionic Calcium (mM)		Alcohol stability (%)	
	Mean	sd	Mean	sd
6848	1.53	0.22	84%	5%
227	1.87	0.19	87%	7%
6737	1.92	0.29	79%	7%
6789	1.97	0.41	79%	7%
6653	2.14	0.44	75%	10%
6930	2.23	0.65	78%	12%
36	2.30	0.53	79%	6%
6790	2.38	0.56	72%	11%
6747	2.40	0.38	76%	5%
6596	2.67	0.57	68%	7%

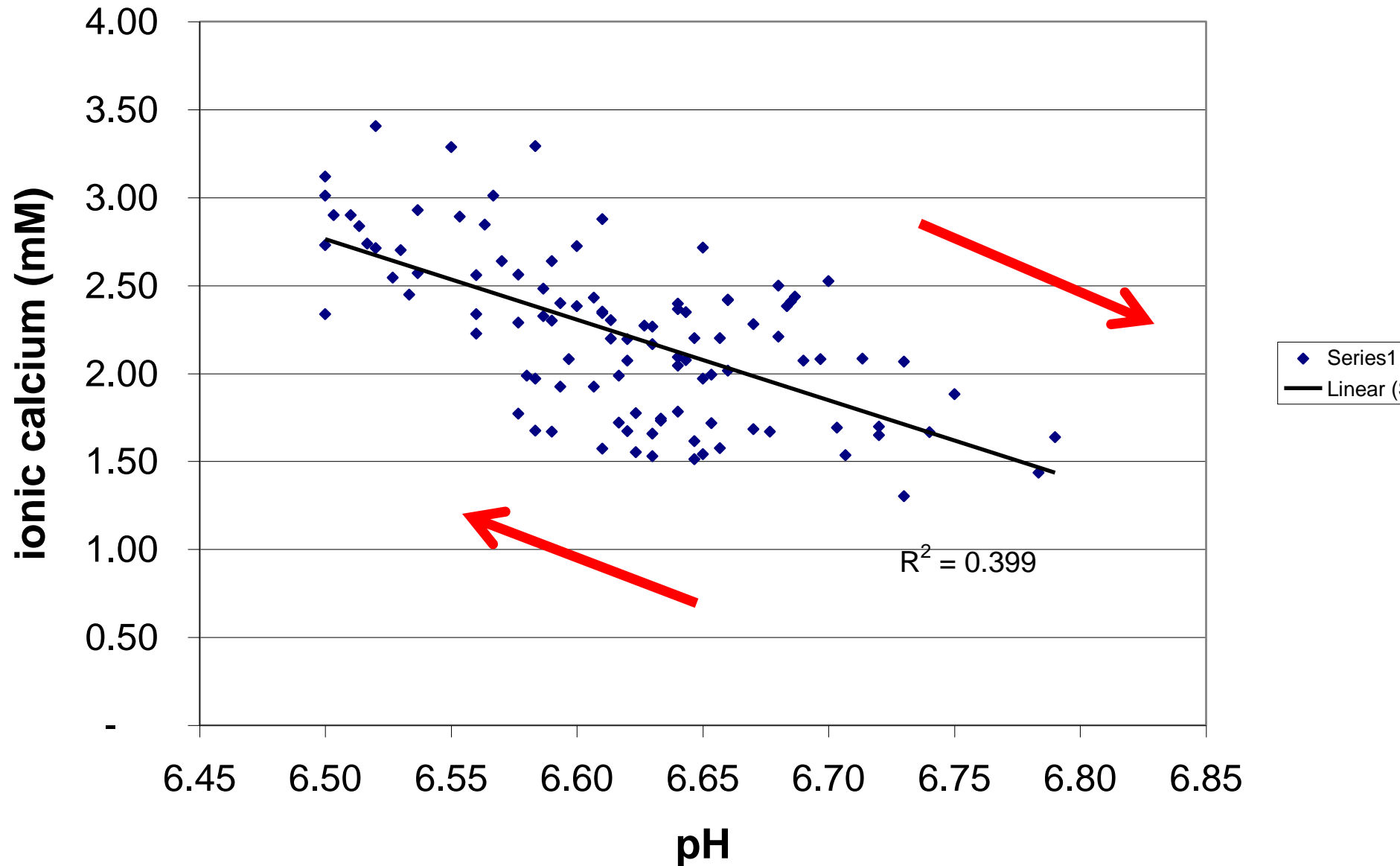
Distribution of ionic calcium in cow's and goat's milk



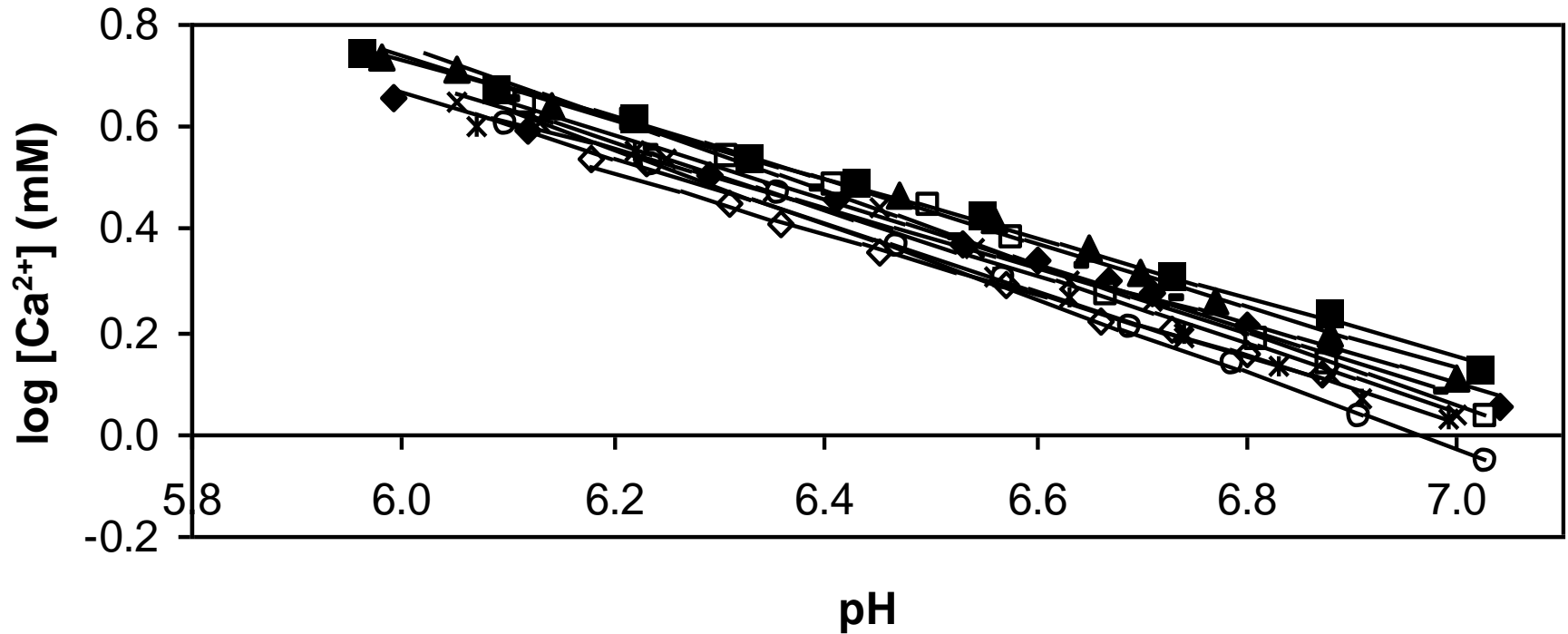
Distribution of ethanol stability in cow's and goat's milk



Relationship between pH and ionic calcium for milk samples from individual cows



Ionic calcium against adjusted pH for several cows



◆ 6928, pH 6.71

□ 7191, pH 6.67

▲ 6818, pH 6.70

× 26, pH 6.71

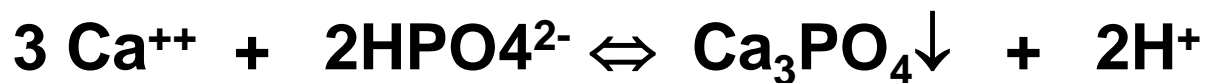
✱ 46, pH 6.56

○ 42, pH 6.69

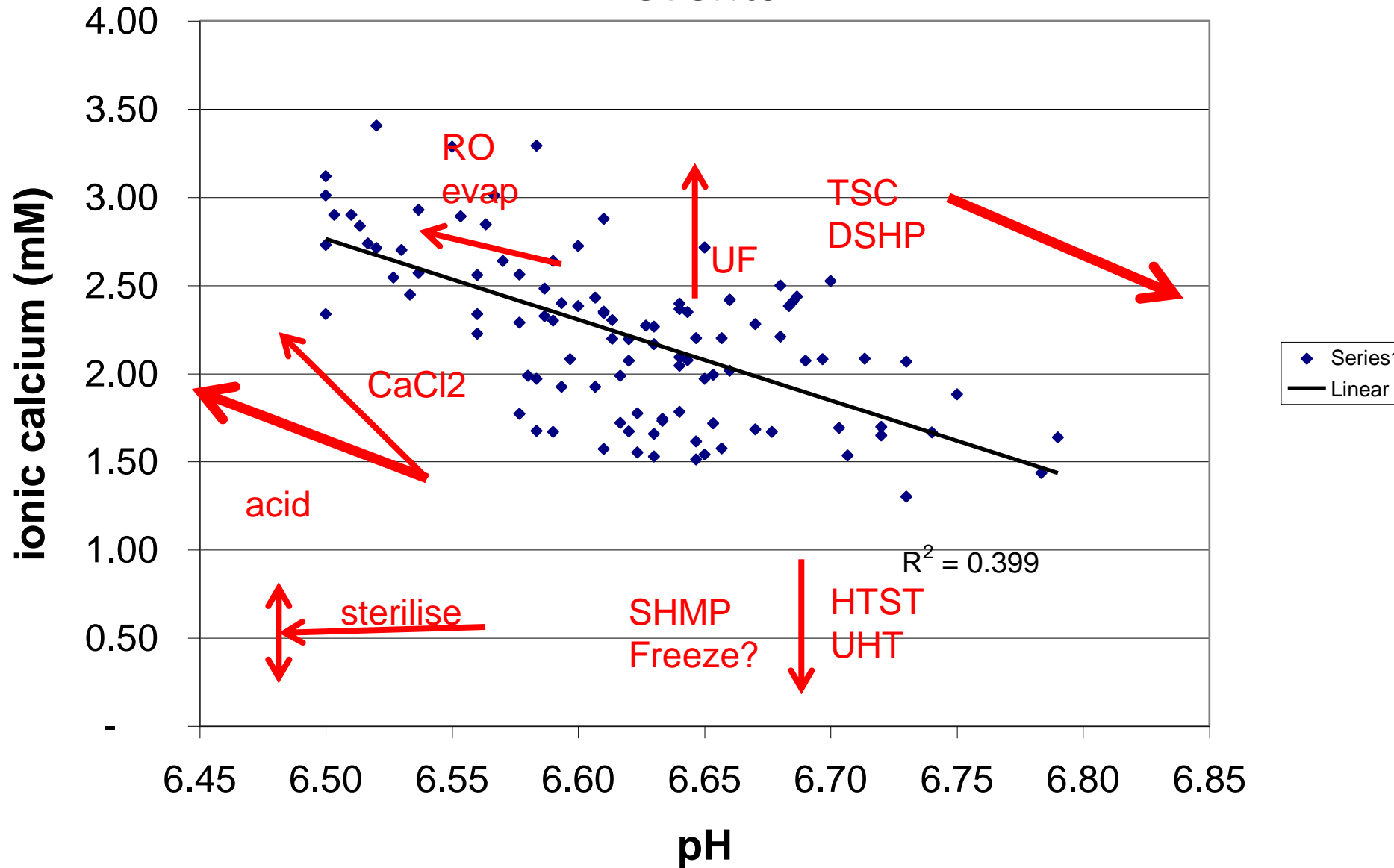
■ 57, pH 6.73

● 58, pH 6.78

◇ bulk, pH 6.66



Changes in pH and ionic calcium caused by different events



Addition of different stabilisers, followed by in-can sterilisation at 120 C for 15 min

control

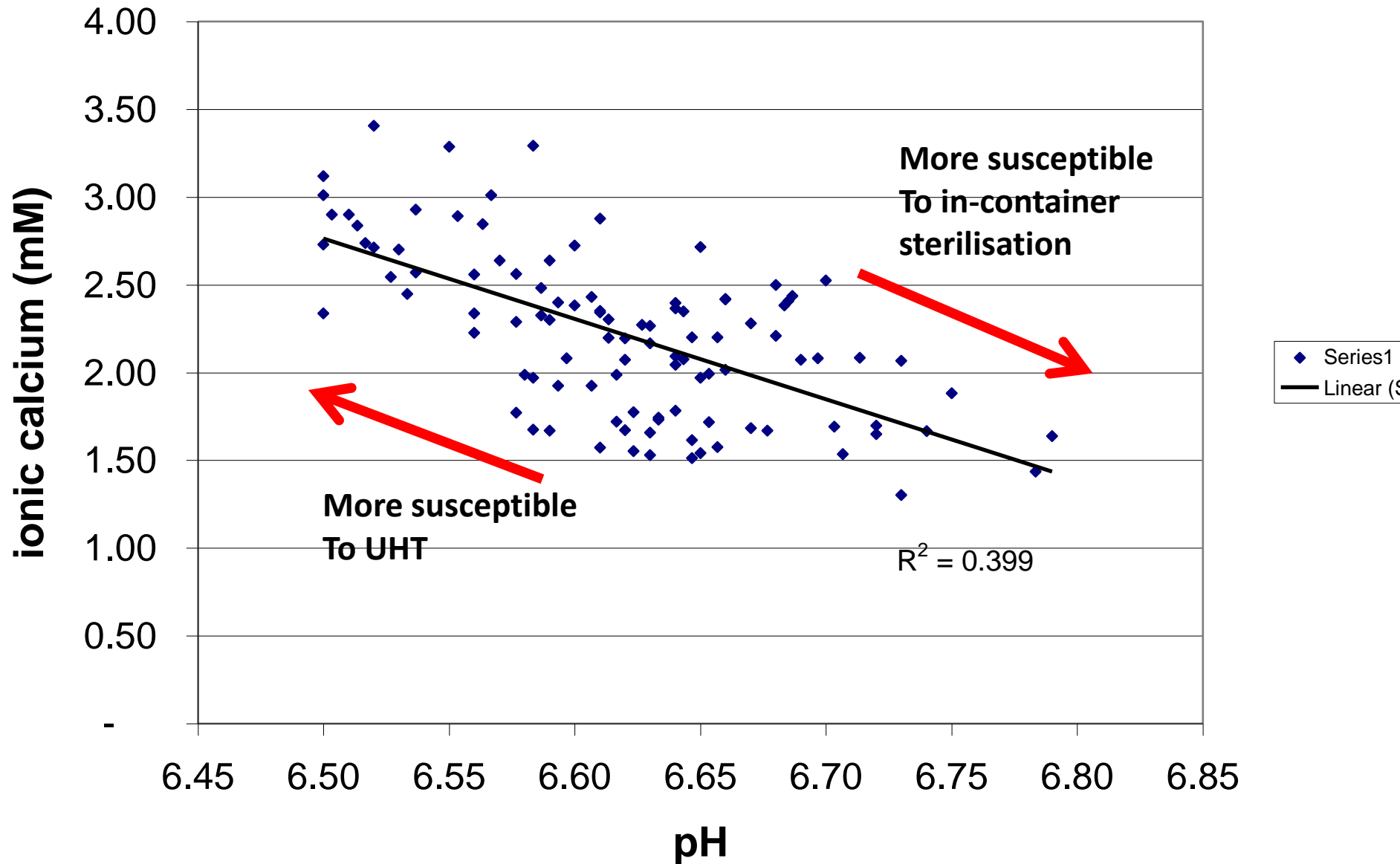


These differences arise from the relatively small changes in pH and ionic calcium resulting from stabiliser addition.



**Sterilised evaporated milk
samples with different
stabilisers**

Summary



Can we measure pH and ionic calcium at high temperatures?

What is the pH and ionic calcium of milk at 140°C?

Separate milk permeate or dialysate at various temperatures and measure it at room temperature.

Measurement of pH and ionic calcium at high temperatures by dialysis

Visking dialysis tubing, containing water

Milk to water ratio > 10:1

Dialyse against milk in sealed cans during the
sterilisation procedure

Remove dialysis bag as quickly as possible

Let it cool to room temperature

Analyse dialysate at room temperature.

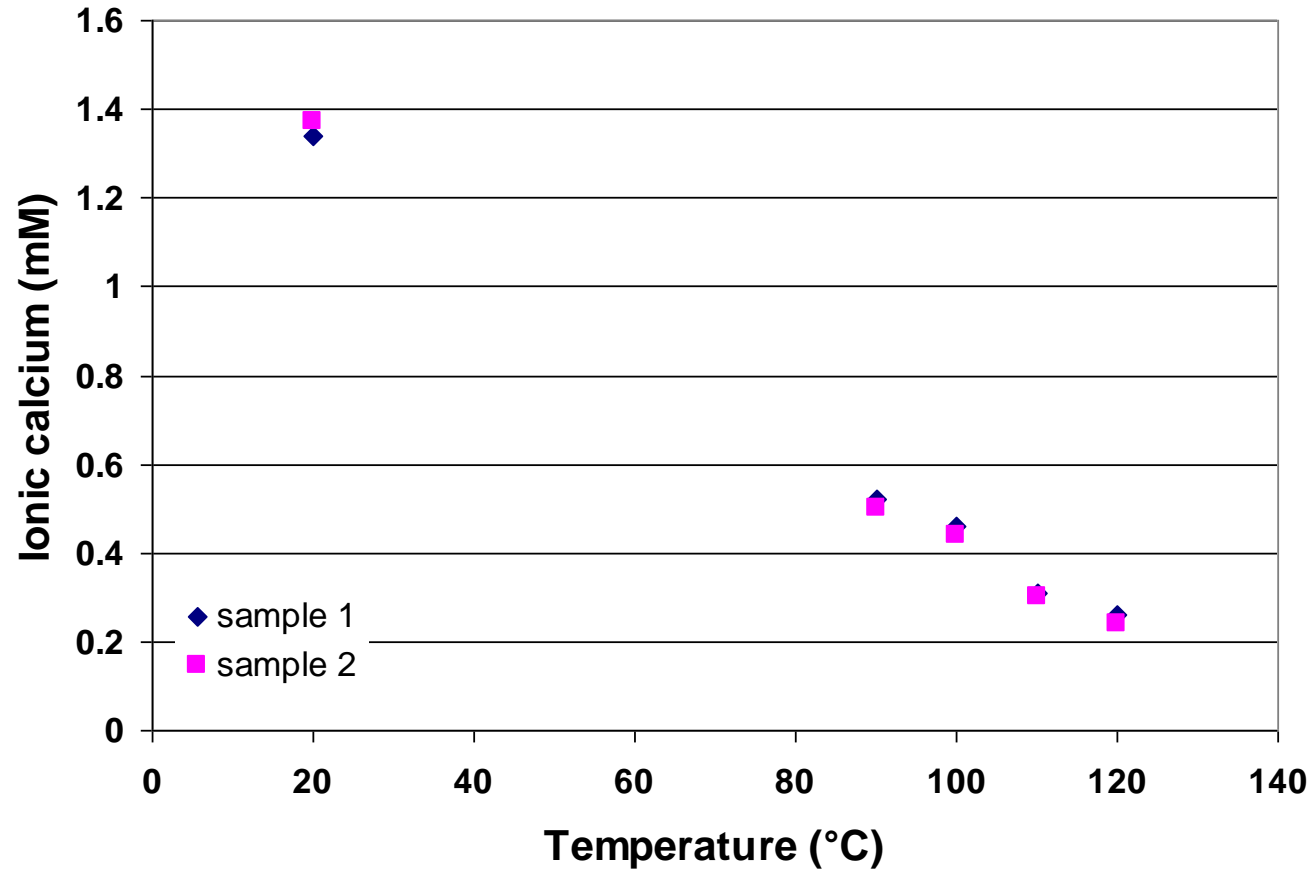
In this way it is possible to measure the pH and ionic
calcium values at sterilisation conditions.

MWCO = 12,000

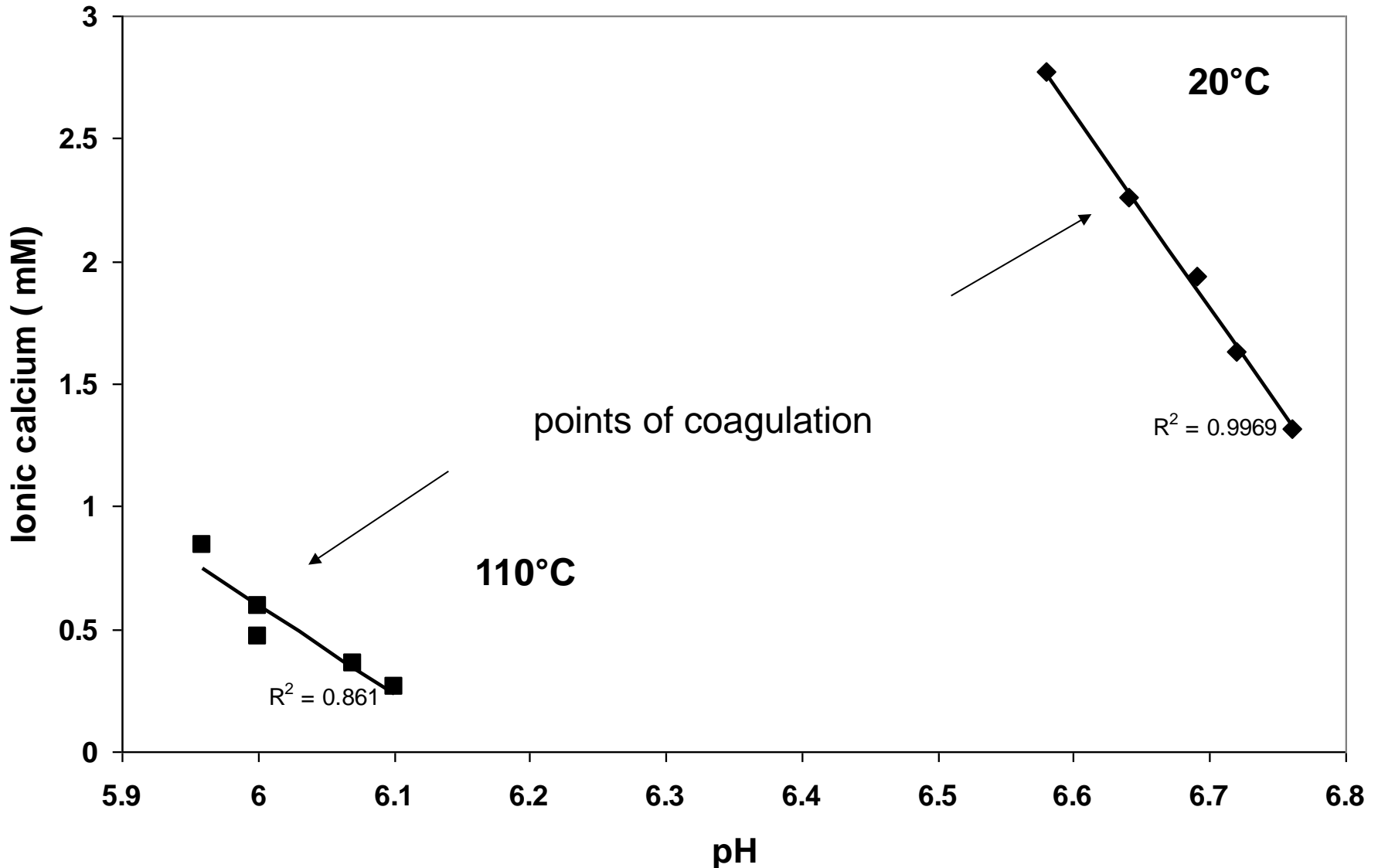
control



Ionic calcium at high temperatures measured by dialysis



Ionic calcium against pH at 20°C and 110°C for milk with added calcium chloride



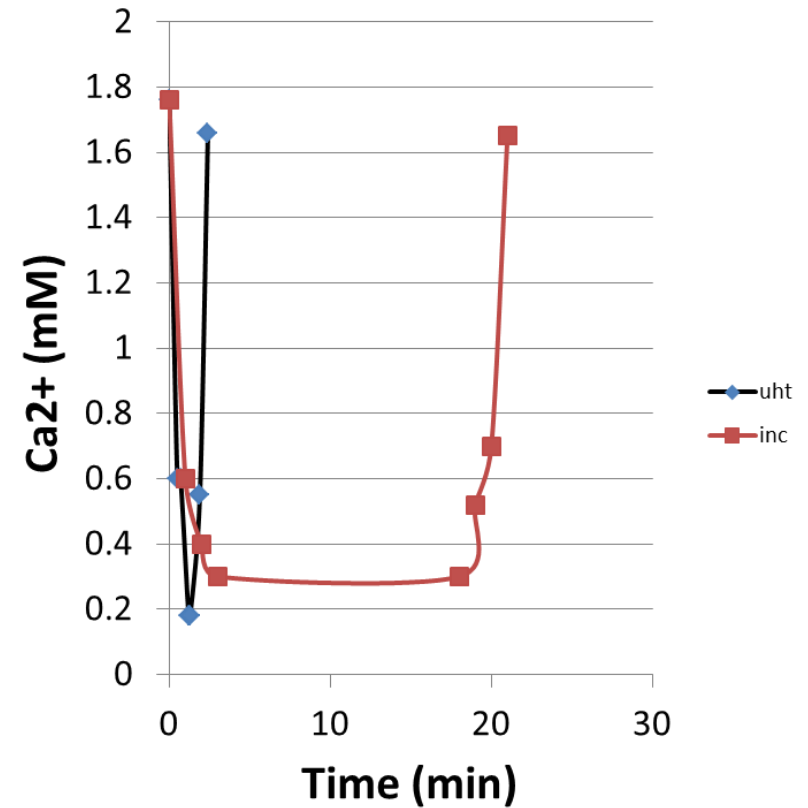
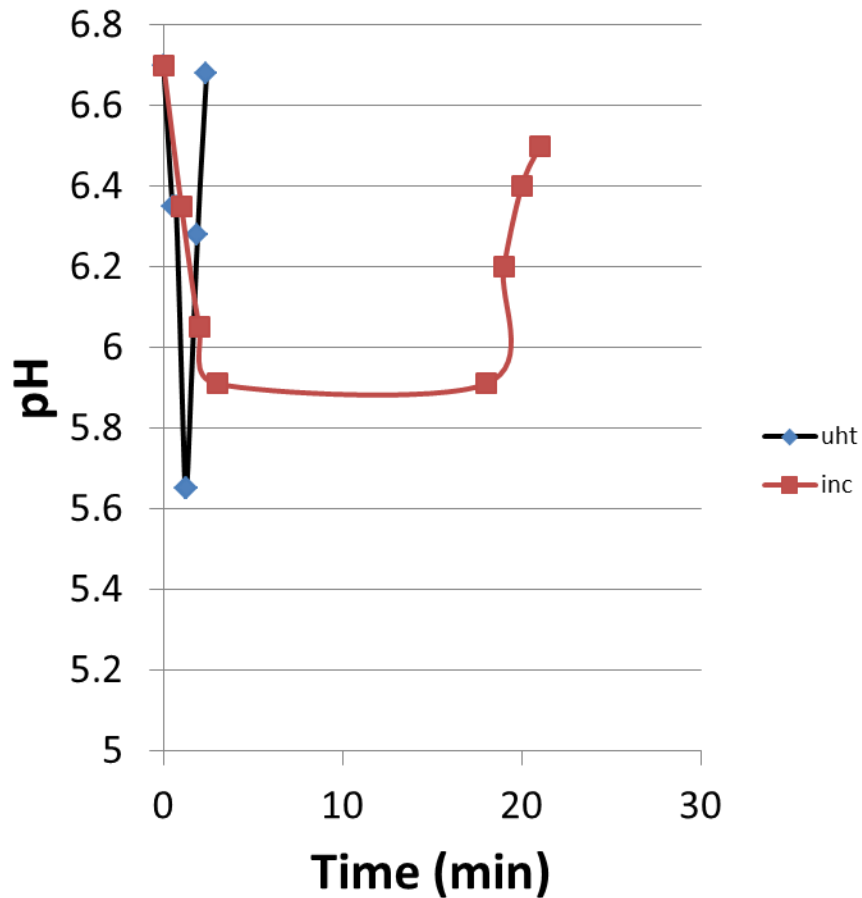
UF Experimental Set-Up



pH and ionic calcium in UF permeates at different temperatures

Samples	pH	Ca ²⁺ (mM)
Milk	6.70±0.04	1.76±0.55
Permeate at 80°C	6.41±0.04	0.57±0.19
Permeate at 120°C	5.91±0.03	0.29±0.03
Permeate at 140°C	5.65±0.04	0.19±0.04

Changes in pH and ionic calcium during UHT and in-container sterilisation



Manipulation of Minerals composition

- Nanofiltration
- Ultrafiltration and diafiltration:
- Note that this will preferentially concentrate minerals associated with the casein micelle
- Ion exchange/electrodialysis
- Supplementation/ recent work on Fe, Se and other metals.
- Dialysis/ analytical

Some calcium salts

- Calcium carbonates: E170
- Calcium lactate: E327
- Calcium citrates E333
- Calcium phosphates E341
- Calcium chloride E509
- Calcium hydroxide E526
- Calcium gluconate E578
-
- Calcium acetate (E263); calcium tartrate (E354); calcium sulphate (E516); calcium oxide (E529), calcium ferrocyanide (E538), Calcium silicate (E552), calcium diglutamate (E623), calcium guanylate (E628), Calcium inosinate (E362); calcium 5'-ribonucleotides (E634)
- Calcium DISODIUM ethylene diamine tetra acetate (E385)

Iodine in milk

- Dairy Council – Iodine in dairying
- Milk and dairy products are the biggest contributor of iodine in the diet.
- Growth/brain development
- 22% of teenage girls and 10% women are deficient in iodine
- Milk and dairy supply:
 - 64 % intake 1-3 years
 - 54% intake 4-10 years
 - 40% intake 11-18 years
 - 33% intake 19-64 years
 - 37% intake >65 years
- National diet and nutritional survey
 - Effect of milk hygiene and processing on iodine concentration of organic and conventional winter milk at retail- implications for nutrition – Payling et al (2015) food Chem 327-330

Selenium in milk

- Selenium Deficiency
- Whilst severe deficiency is rare, it has been acknowledged for some time that people in certain countries have actually had Selenium deficient diets for many years.
-
- In the UK, the current average daily intake is only 30-40ug/day compared to the recommended 55ug/day, and even higher if you are a teenage girl or if you are currently breastfeeding.
-
- **Nemi Dairy**- Selenium enriched milk / cows fed organic seleniium
- Selenium is important as it contributes to
- The normal function of the immune system and thyroid function.
- The protection of cells from oxidative stress.
- Normal spermatogenesis (male fertility).
- The maintenance of normal hair and nails.

Radionucleotides

- All foods (in fact all living matter) is slightly radioactive.
- We are used to living with the consequences of natural levels of radioactivity; in water and in soil.
- Measurement of radioactivity: Becquerel/ kg
- Types of radioactive decay/ relative harmful effects
- Survey of levels in milk and milk products/
- Chernobyl and nuclear plant in Japan:--
- Contamination of milk supply
- Strategies for reducing radionucleotides in the feed
- Ion exchange and membrane techniques have been explored for removing them from milk.
- Some interesting questions: what constitutes a safe level

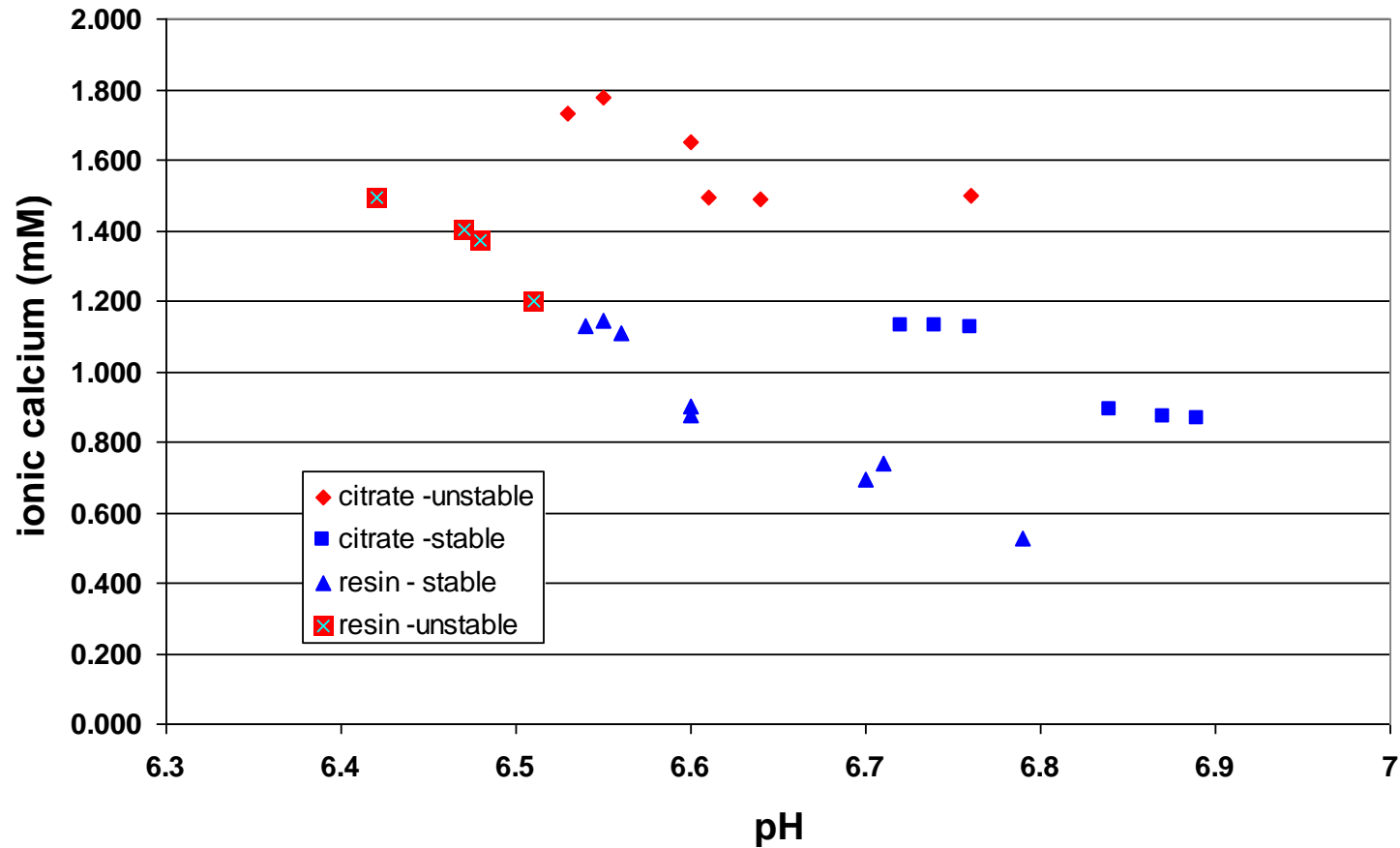
Analysis of minerals

- Papers from 50 years ago/
- Total minerals- ashing around 525 C.
- Titrations/ EDTA/ hardness of water/ use for milk and milk products: quick and convenient
- EGTA:
- Colorimetric determinations for measuring P and citrate
- Ashing:-- use of flame photometry and atomic absorption???
- Inductively coupled plasma (ICP) emission spectroscopy is gaining in popularity
- Dionex/ cations and anions
- Selective ion electrodes:

Effects of reducing ionic calcium on heat stability of **low-heat** skim milk powders

- Milk was treated by addition of TSC or a cation exchange resin prior to evaporation and drying
- The trial was repeated three times.
- Powders were reconstituted to 25% TS
- All control milks showed poor heat stability
- Reduction in ionic calcium was accompanied by an increase in pH and improvement in heat stability
- Browning became a problem if pH increased too much

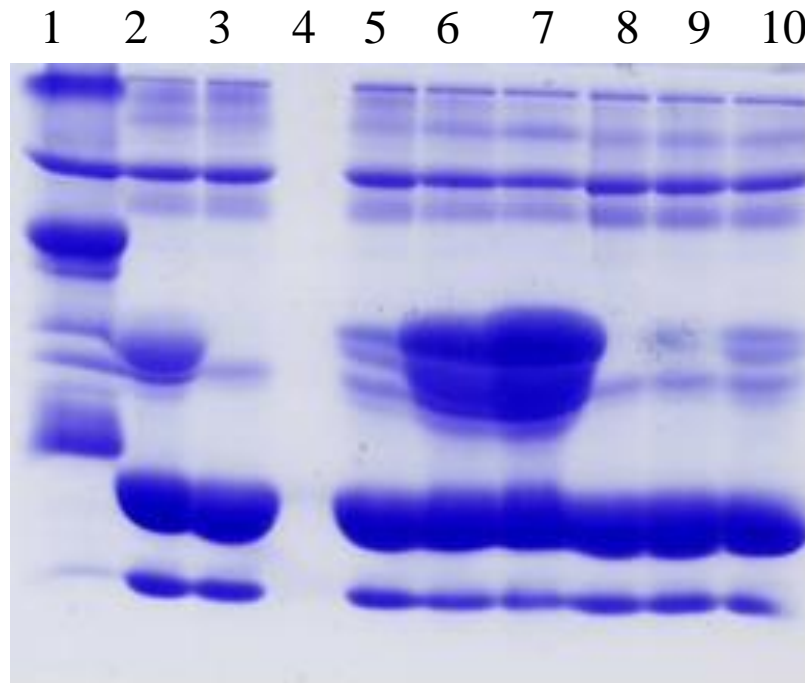
Heat stable and unstable products



Dialysis using membrane with MWCO of 1 million

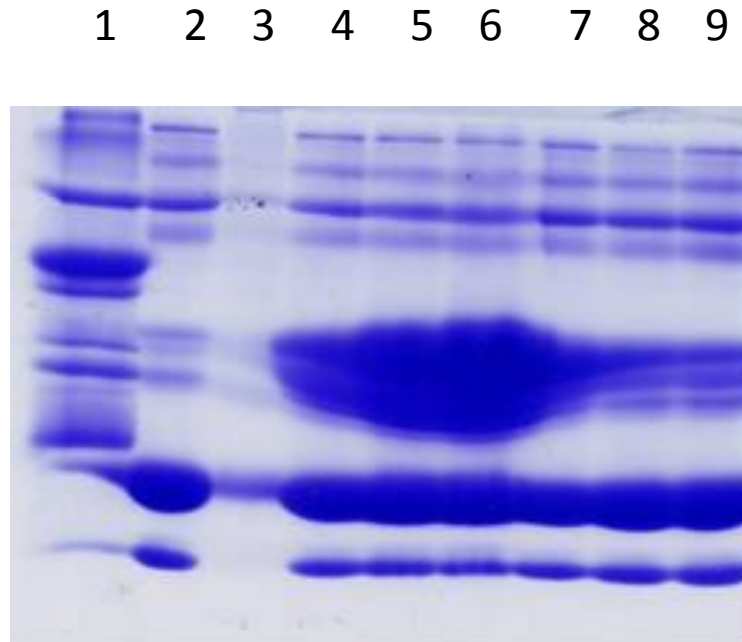
- This permits the separation of soluble casein from micellar casein.
- Unlike ultracentrifugation, it permits this to be done at high temperatures.
- Preliminary experiments have shown that TSC and to a lesser extent DSHP will give rise to an increase in soluble calcium
- This could be one explanation for the poor heat stability shown when TSC and DSHP are added to sterilised milk.

Dialysis of milk with different levels of TSC and DSHP



Lane 1: Marker 3913; Lane 2: Dialysate of milk at 4°C; Lane 3: Dialysate of milk at 20°C; Lane 4: Dialysate of milk at 100°C; Lane 5: Dialysate of milk + 10 mM TSC at 20°C; Lane 6: Dialysate of milk + 20 mM TSC at 20°C; Lane 7: Dialysate of milk + 30 mM TSC at 20°C; Lane 8: Dialysate of milk + 10 mM DSHP at 20°C; Lane 9: Dialysate of milk + 20 mM DSHP at 20°C; Lane 10: Dialysate of milk + 30 mM DSHP at 20°C

UC of milk different levels of TSC and DSHP



Lane 1: Marker 3913; Lane 2: UC of milk at 20°C; Lane 3: UC of milk at 100°C; Lane 4: UC of milk + 10 mM TSC at 20°C; Lane 5: UC of milk + 20 mM TSC at 20°C; Lane 6: UC of milk + 30 mM TSC at 20°C; Lane 7: UC of milk + 10 mM DSHP at 20°C; Lane 8: UC of milk + 20 mM DSHP at 20°C; Lane 9: UC of milk + 30 mM DSHP at 20°C