Analysis of a sticky impurity: lactose phosphate: a contaminant of lactose

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Lactose & its derivatives

Role of lactose-phosphate in lactose crystallisation

- Lactose phosphate: strong inhibition effect on lactose crystal growth (Visser 1980, 1984 and 1988)

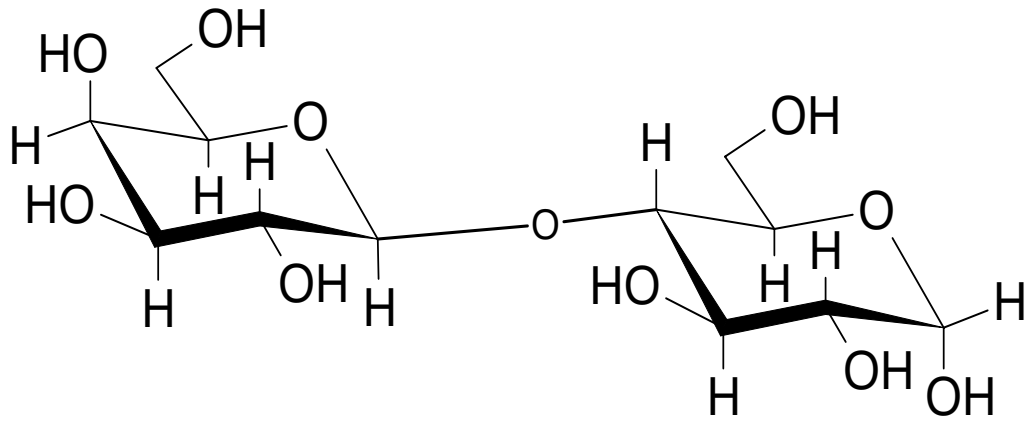
- Pharmaceutical grade lactose contaminated by 270 to 400ppm lactose phosphate; preferentially integrated in the crystals; can not be washed off.

  → Impact for industrial crystallisation:
  Poor control of particle size, size distributions and yield.

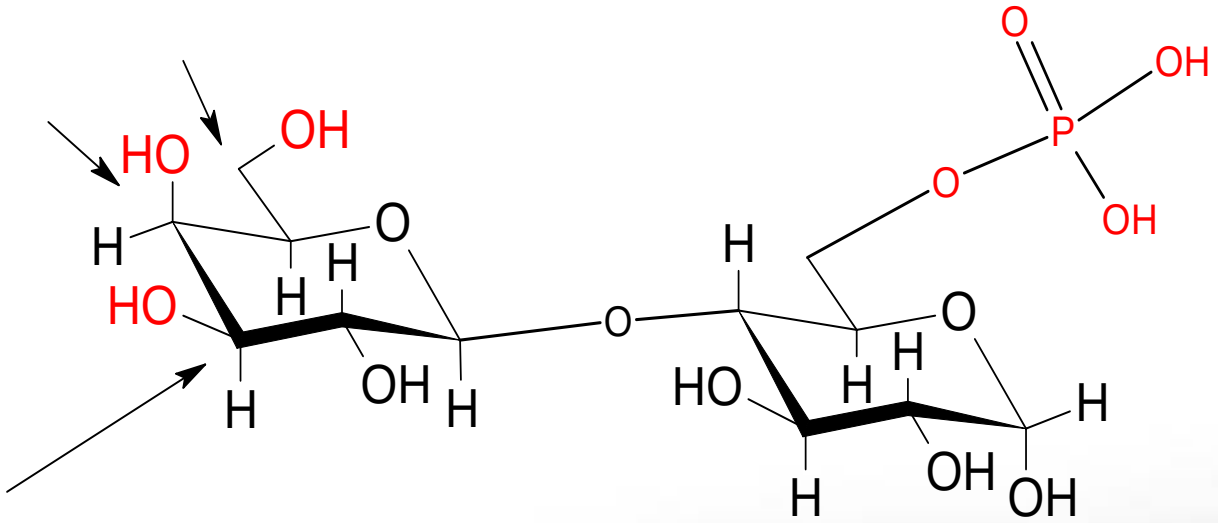
- New IEL lactose is free of lactose phosphate and other impurities; used as the control in this work.

- Study also relevant to pre-crystallisation of spray-dried lactose and dairy powders.

alpha-lactose



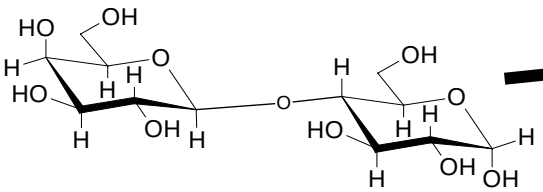
lactose-phosphate



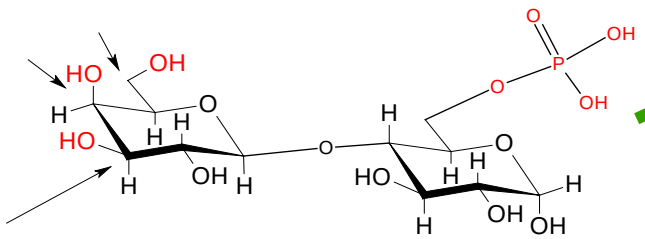
Aim: *to control lactose processing in a way relevant to the factory scale; to understand the impact of seeding and impurities on lactose crystallisation kinetics.*

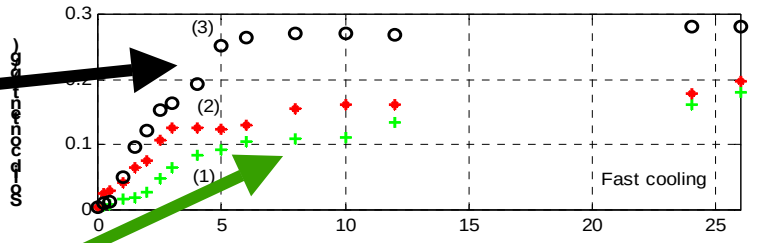
Crystallisation inhibition by lactose phosphate

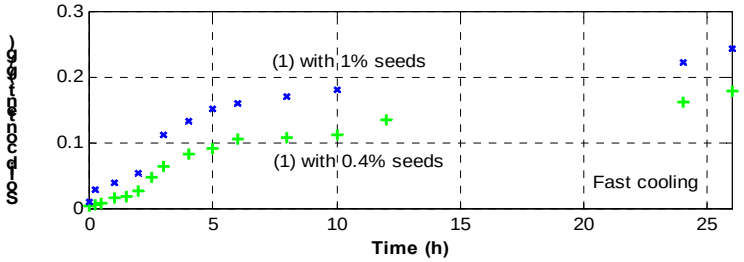
alpha-lactose

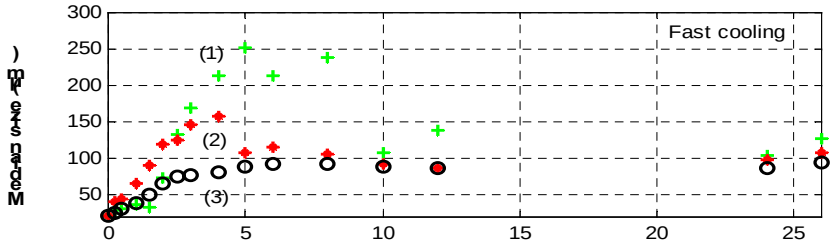


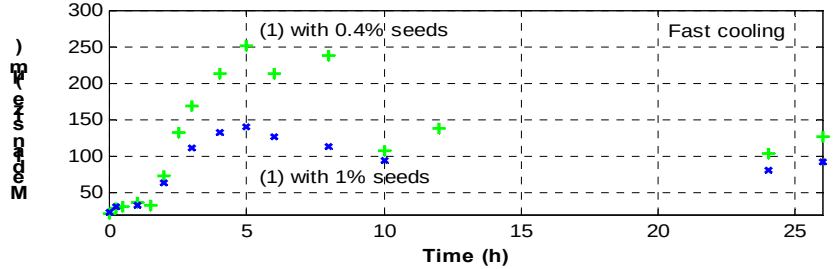
lactose-phosphate











Analysis of the different forms of phosphorus in lactose

- Total phosphorus (P):
  - ICP-AES
  - Spectrophotometric method (FIL-IDF 42B, 1990)
- Inorganic phosphorus (P_i):
  - Spectrophotometric method (Chen, 1956; FIL-IDF 42B, 1990)
  - Same spectrophotometric method under milder conditions (Lowry et al., 1953; Chen, 1956)
  - Flow Injection Analysis (FIA) (automated version)
- Organic phosphorus/lactose phosphate:
  - Indirect: difference between P and P_i
  - Direct: CE

Total P content of dairy powders measured by ICP-AES

Mineral (ppm)	Skim milk powder	Whey powder	Edible grade lactose	Pharma grade β-lactose	Pharma grade 1_1	Pharma grade 1_2	Pharma grade 2	Ultra-pure lactose
Ca	11917	6046	1000	22	76	1	12	4
K	17334	14107	124	72	18	34	4	0
Mg	1047	1058	129	4	3	2	1	0
Na	3611	4534	138	72	2	21	3.1	0
P	**10834**	**6550**	**438**	**37**	**35**	**26**	**19**	**0**
Sulphated ash (%)	9.54	7.12	0.320	0.0500	0.0300	0.0200	0.0100	0

- Concentrations in mg/kg powder (ppm), n= 2
- Large variations of total phosphorus content depending on manufacturer and batch of lactose
- Similar results found with spectrophotometric method (FIL-IDF 42B, 1990)

Determination of the inorganic phosphorus content of lactose powders

- Reference methods: Fiske & Subbarow, 1925. Modified by Lowry, 1946, 1953, and Chen et al., 1956, FIL-IDF42B, 1990.

- Principle: sulfuric acid + ascorbic acid + ammonium molybdate added to sample, incubated at 38°C for 2 hours, develops blue colour.

- The inorganic phosphate ions and molybdate ions form molybdophosphoric acid. This acid is reduced to phosphomolybdenum blue, the concentration of which is measured by spectrophotometry.

- The blue colour produced is proportional to the inorganic phosphorus concentration.

Inorganic phosphorus content of dairy powders (n = 10)

Sample	IDF (ppm)	pH 4 (ppm)	FIA (ppm)
Skim milk powder	2226 ± 98	2012 ± 73	2195 ± 64
Whey powder	1850 ± 45	1833 ± 42	1840 ± 28
Edible grade lactose	145 ± 4.8	103 ± 4.1	137 ± 3.5
Pharmaceutical grade β-lactose	2.40 ± 0.3	1.95 ± 0.4	2.25 ± 0.2
Pharmaceutical grade α-lactose 1 Batch 2	2.01 ± 0.1	1.85 ± 0.1	2.05 ± 0.07
Pharmaceutical grade α-lactose 1 Batch 1	1.78 ± 0.1	1.50 ± 0.1	1.77 ± 0.05
Pharmaceutical grade α-lactose 2	1.10 ± 0.1	1.10 ± 0.2	1.10 ± 0
Ultra-pure lactose	No blue colour formation		

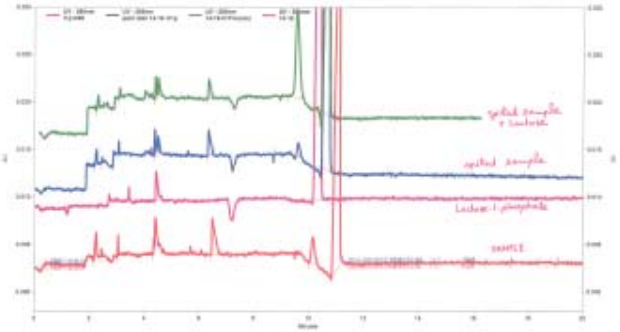
ppm: mg phosphorus per kg powder

IDF: FIL-IDF42B, 1990, pH = 0.85

pH 4: Lowry et al., 1953

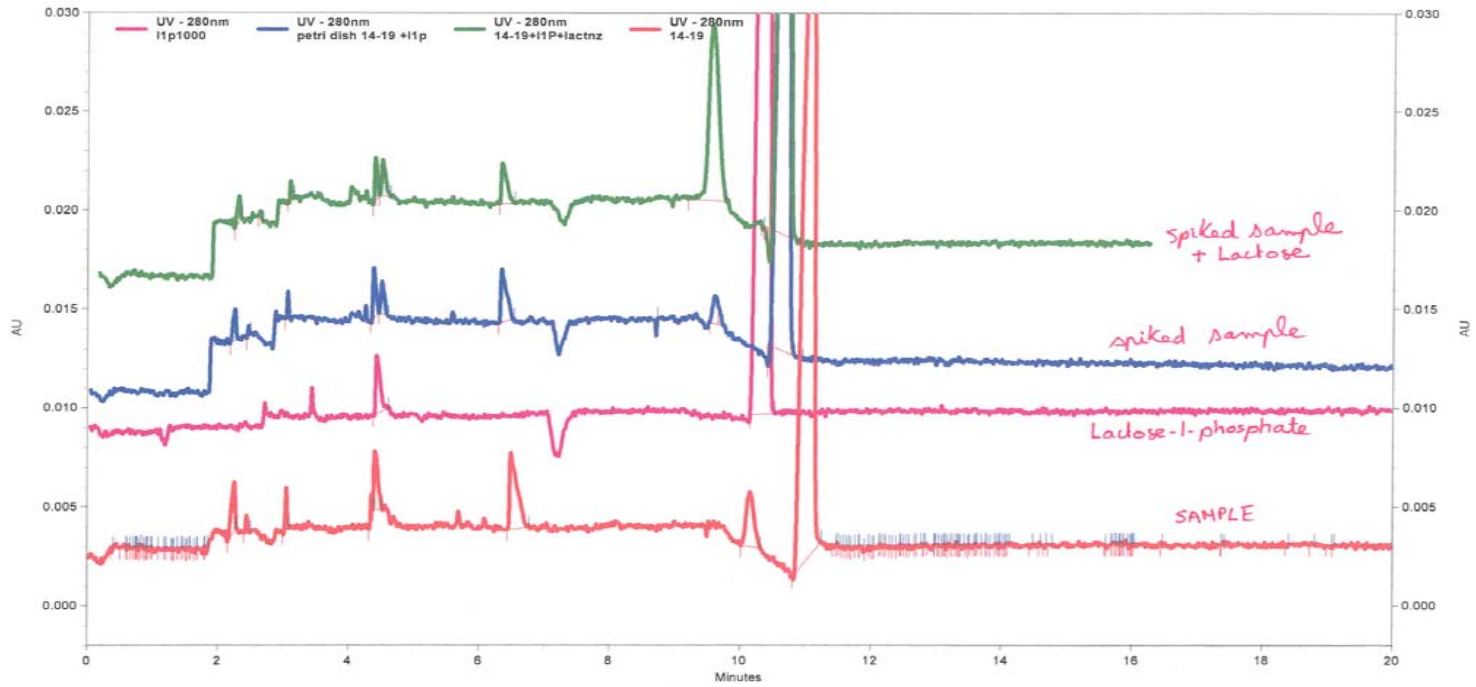
FIA: Flow Injection Analysis, automatic spectrophotometric method

Direct analysis of lactose phosphate in dairy products by capillary electrophoresis (CE)



- Method for analysis of anionic compounds (Soga and Imaizumi, 2001; Izco et al., 2003).
- Direct detection of lactose-phosphate, Pi and carbohydrates present in samples in one analysis.
- Method applied to lactose, cheese, whey and milk

Development of a direct method of analysis of dairy powders by CE

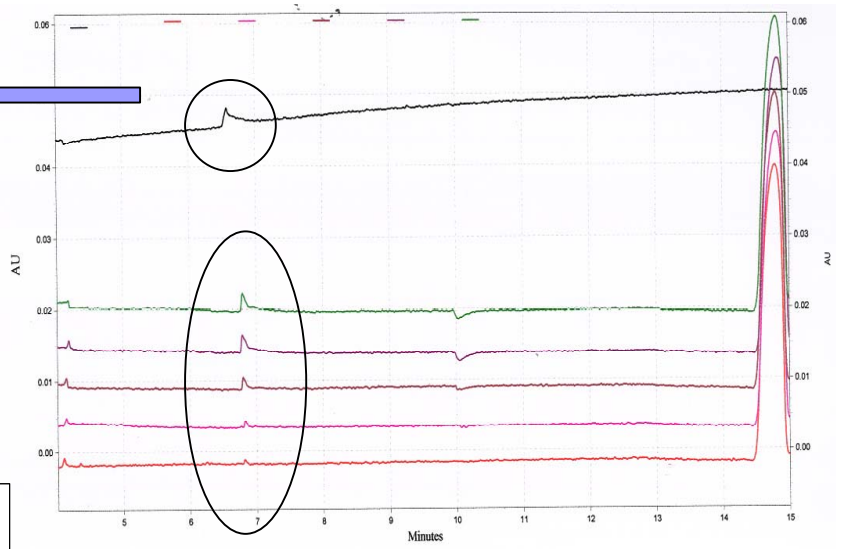


- Uses indirect UV detection mode with 20mM PDC + 0.5mM CTAB as background electrolyte, optimisation of temperature, pH and injection pressure

- Can analyse lactose phosphate in the presence of lactose and other organic compounds without pre-treatment or risk of overloading

Calibration and validation

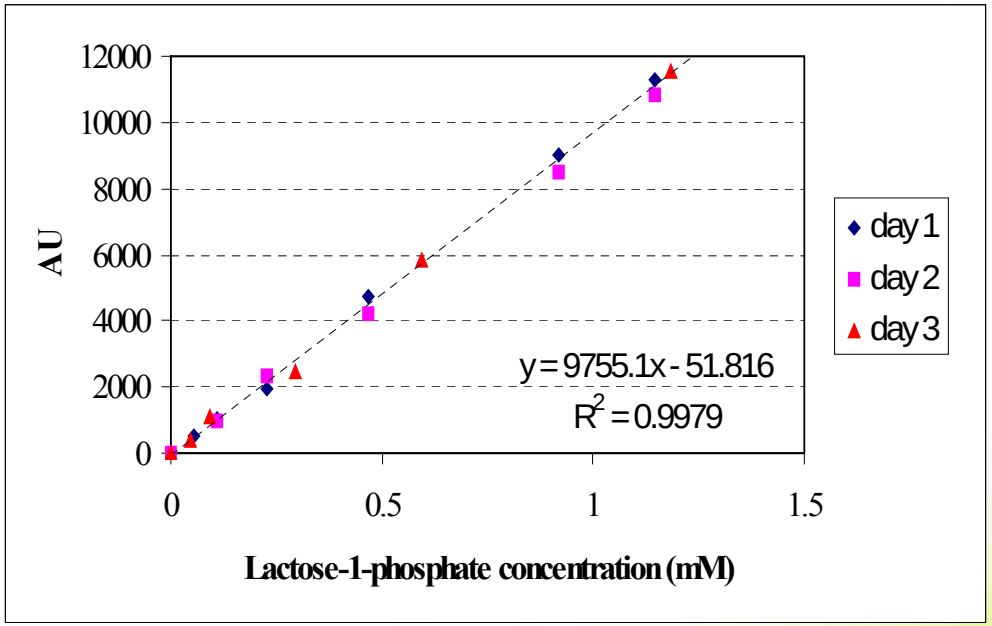
Lactose phosphate from lactose



Lactose-1-phosphate standards

Lactose-1-phosphate standards (0.9 to 2.4mM)

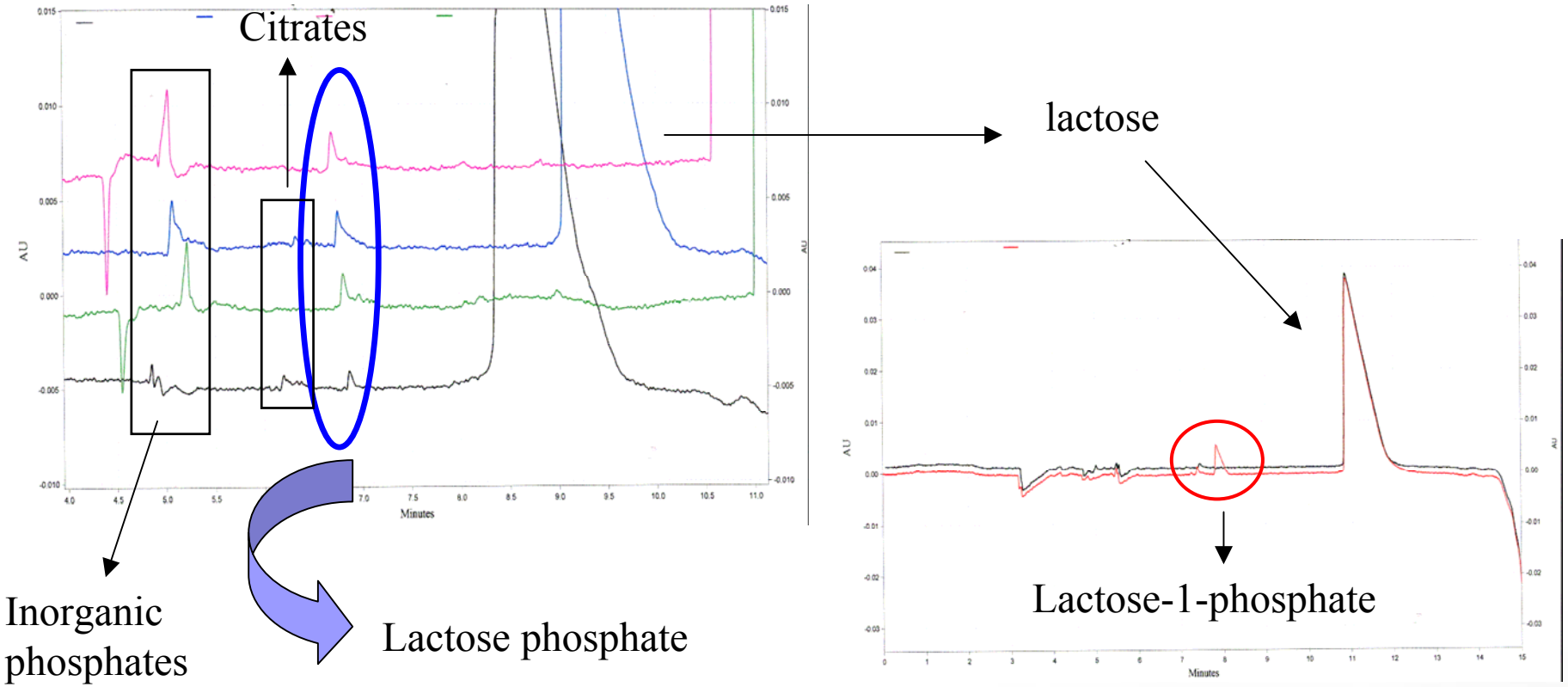
Small intra-day (0.5%) and day to day (2.7%)variations



Linearity, precision and accuracy all within the range of similar studies

(Castro et al. 1989; Izco et al., 2003)

Analysis of lactose phosphate in lactose powders



Commercial pharmaceutical grade lactose powders

IEL lactose

1. IEL lactose free of lactose phosphate
2. Quantification of lactose phosphate in lactose samples using CE

Determination of the lactose phosphate content of lactose powders by CE

Sample	Lactose phosphate (ppm)
Edible grade lactose	368 ± 6
Pharmaceutical grade β-lactose	250 ± 4.8
Pharmaceutical grade α-lactose 1 Batch 2	252 ± 3.9
Pharmaceutical grade α-lactose 1 Batch 1	204 ± 3.7
Pharmaceutical grade α-lactose 2	144 ± 2.5
Ultra-pure lactose	No detectable peak

Results expressed as means ± standard deviations, n = 10

ppm: mg lactose phosphate per kg lactose powder

Results are lower than in Visser (1988) estimation of lactose phosphate in batches of pharmaceutical grade lactose (: 270 to 400 ppm)

Determination of the organic phosphorus content of lactose powders by difference

Sample	P^a (ppm)	P_i^a (ppm)	P_o^a (ppm)	Lactose phosphate[b] (ppm)
Edible grade lactose	438 -	103 =	335	4556
Pharmaceutical grade β-lactose	37	1.9	35	476
Pharmaceutical grade α-lactose 1 Batch 2	35	1.8	33	449
Pharmaceutical grade α-lactose 1 Batch 1	26	1.5	24	326
Pharmaceutical grade α-lactose 2	19	1.1	18	245
Ultra-pure lactose	No blue colour formation			

To be compared to the value found by the direct method

368

250

252

204

144

[a] All these organic phosphorus values are in mg of phosphorus per kg of lactose
expressed in mg of phosphorus per kg of lactose
Part of the organic phosphorus present in lactose samples is not lactose phosphate as was previously assumed

[b] expressed in mg of lactose phosphate per kg of lactose
Other sources of organic phosphorus exist

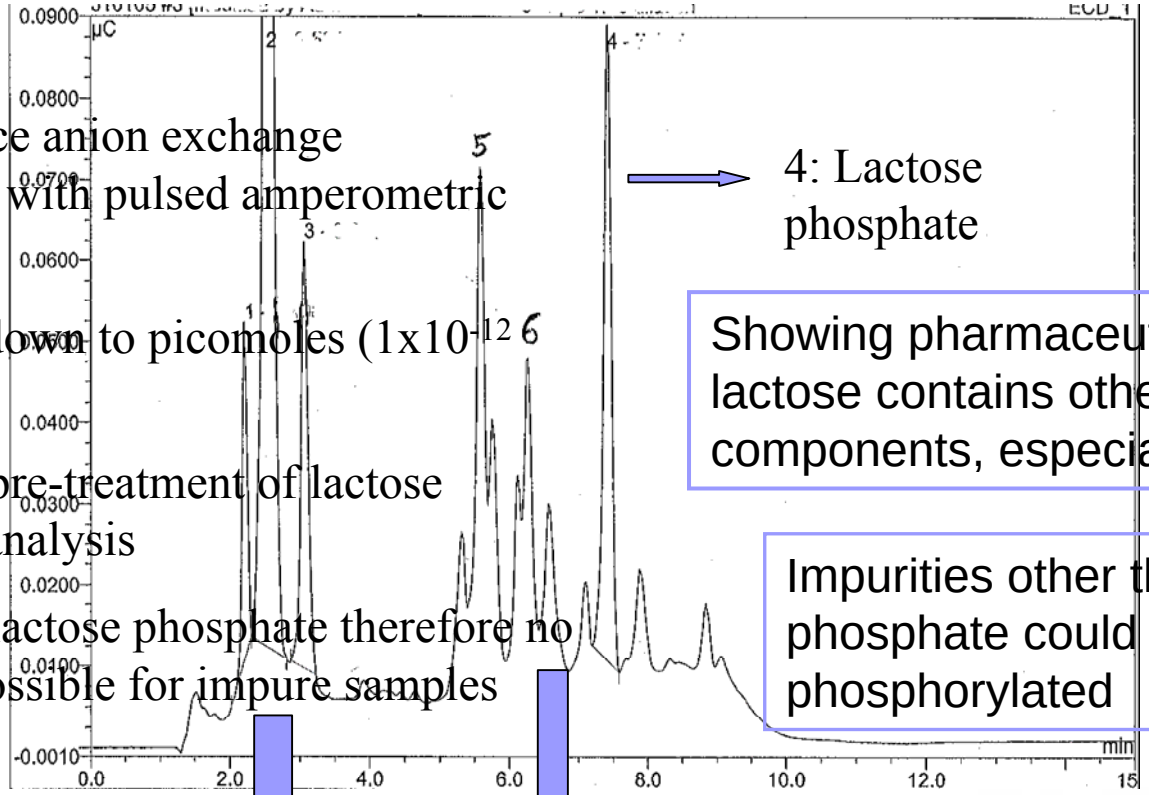
Analysis of lactose phosphate extracted from lactose by HPAEC-PAD

High performance anion exchange chromatography with pulsed amperometric detection

Very sensitive, down to picomoles ($1x10^{-12}$ moles)

Need extensive pre-treatment of lactose samples before analysis

Causing loss of lactose phosphate therefore no quantification possible for impure samples



4: Lactose phosphate

Showing pharmaceutical grade lactose contains other acidic components, especially GOS

Impurities other than lactose phosphate could also be phosphorylated

1,2 and 3:

Unknown peaks

5: N-acetylneuraminic acid

6: KDN

Both sialic acid analogues

Summary

- Analysis of the different forms of phosphorus in lactose powders

1. Suitability of the spectrophotometric method to measure total and inorganic phosphorus, in the absence of organic phosphates

2. Lactose phosphate can not be quantified indirectly

3. Lactose phosphate can be analysed in lactose and other dairy products by CE

4. Lactose phosphate is only one of several acidic contaminants present in traces (picomole order) in pharmaceutical grade lactose

→ CE was used as a tool to monitor lactose phosphate integration

During seeded batch isothermal and cooling crystallisations

(Lifran et al.,Powder technology (2006), doi:10.1016/j.powtec.2006.11.010).

Acknowledgements

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Thank You For Your Attention