Acrylamide in heated potatoe products
Analytics and formation routes

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Analytical properties of acrylamide (1)

- High polarity
- Very good soluble in water, alcohols, acetone and acetonitrile
- Slightly soluble in ethylacetate, CH_2Cl_2, dietylether
- Insoluble in Hexane and other alkanes
- Low, but significant volatility

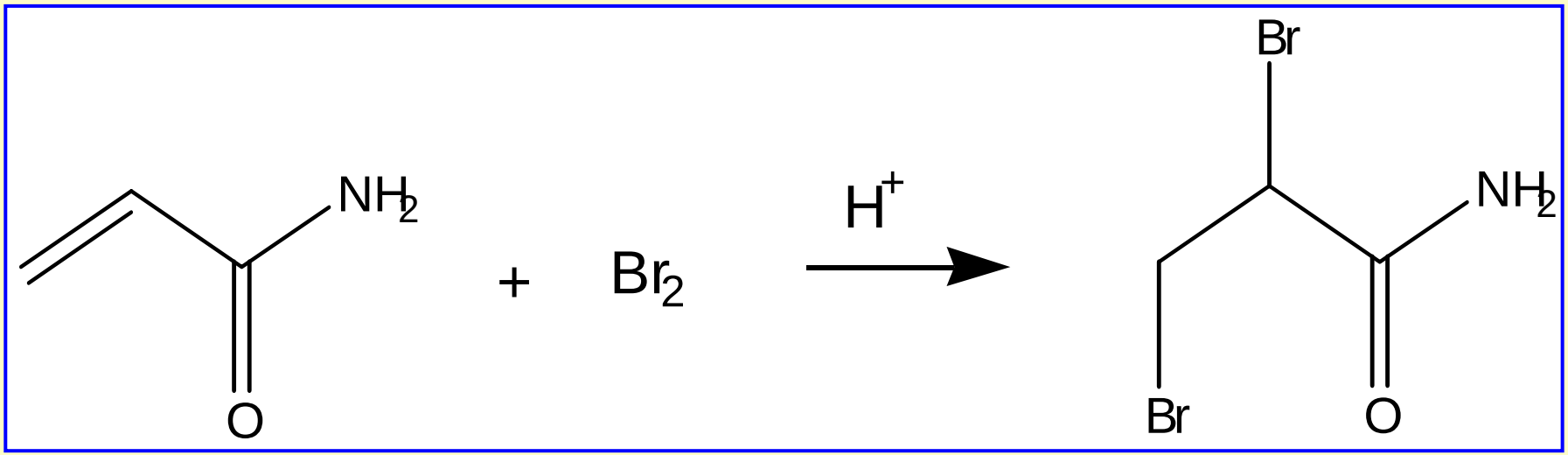
Analytical properties of acrylamide (2)

- Very low retention on reversed phases like RP-18
- No significant UV-absorption above 220 nm
- Reactive C=C – double bond
- Fast additon of Br_2 or R–SH
- Amide-group is protonated by medium and strong acids

Acrylamide – detection methods

- **LC-MS-MS (ESI)** without derivatisation
- **GC-MS** after **bromination**
- **GC-MS without derivatisation**
  Ionisation by electron impact or chemical ionisation (CH_4, C_4H_{10} or NH_3)
- **LC-MS (ESI)** after derivatisation with mercaptobenzoic acid

GC-MS-method with bromination (1)



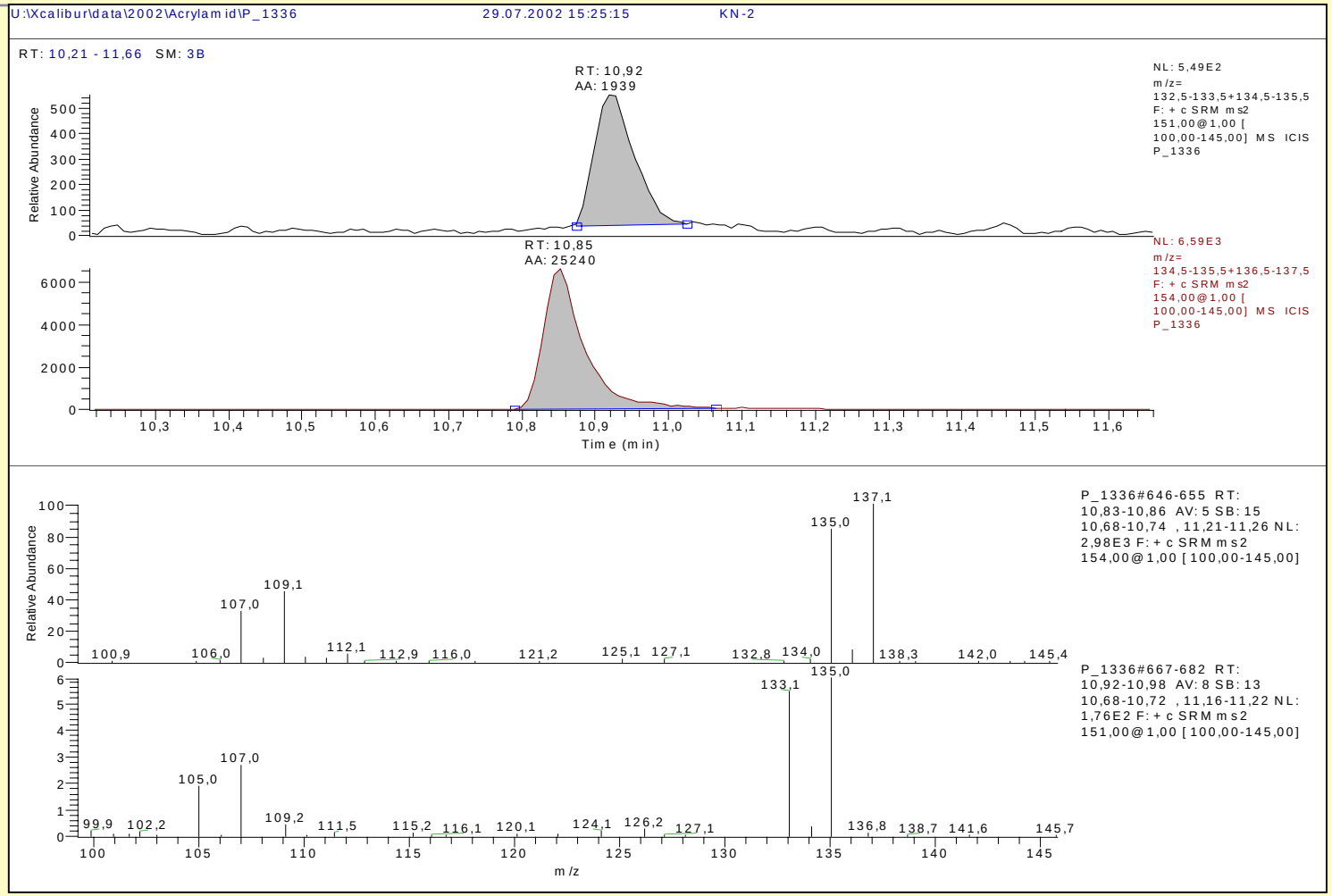
GC-MS-method with bromination (2)

- Extraction with water (40°C –80°C, Ultra Turrax, Ultrasonic bath, mechanical stirring)
- Treatment with diastase (if necessary)
- *Alternatively: Extraction with organic solvents and reextraction with water*
- Clearing procedure with Carrez solutions I and II

GC-MS-method with bromination (3)

- Bromination with Br_2 or with KBr + $KBrO_3$
- Removing of excess bromine with sulfite
- Extraction of dibrompropionylamide with ethylacetate
- Evaporation of solvent = concentration step

GC-MS-method with bromination (4)



GC-MS-method with bromination (5)

- GC-MS equipment is available in much more laboratories than LC-MS-MS equipment (and it is much cheaper)
- Higher sensitivity and lower detection limits because of the concentration step
- Characteristic mass-spectrum with dibromine-pattern (m/z 133/135 and m/z 105/107)
- High separation power of capillary-GC

GC-MS-method with bromination (6)

- Less problems with interferences in complex matrices like coffee or cocoa
- Complex and time consuming clean-up procedure
- HBr-elimination can take place on active surfaces of injector and capillary
- During the bromination step an exchange ^{2}D . ^{1}H is possible, if D_3-acrylamide is used as internal standard

Clean-up procedure for LC-MS-MS (1)

- *Recommendable for fatty matrices (e.g. potato chips): Partial defatting procedure by soaking the dry sample with hexane /butylmethylether*
- Extraction with water (e.g. ultrasonic bath at 40°C)
- Addition of internal standard ($^2D_3$-acrylamide)
- Clearing procedure with Carrez solutions I and II
- Filtration oder centrifugation

Clean-up procedure for LC-MS-MS (2)

- Removing of interfering compounds by passing a solid phase extraction (SPE) cartridge
- „Easy“ matrices (potato chips, french fries, crisp bread, butter cookies): RP-18-phase
- „Difficult matrices“ (coffee powder, cacao): multi functional phases (RP-18 + anion exchanger phase + cation exchanger phase

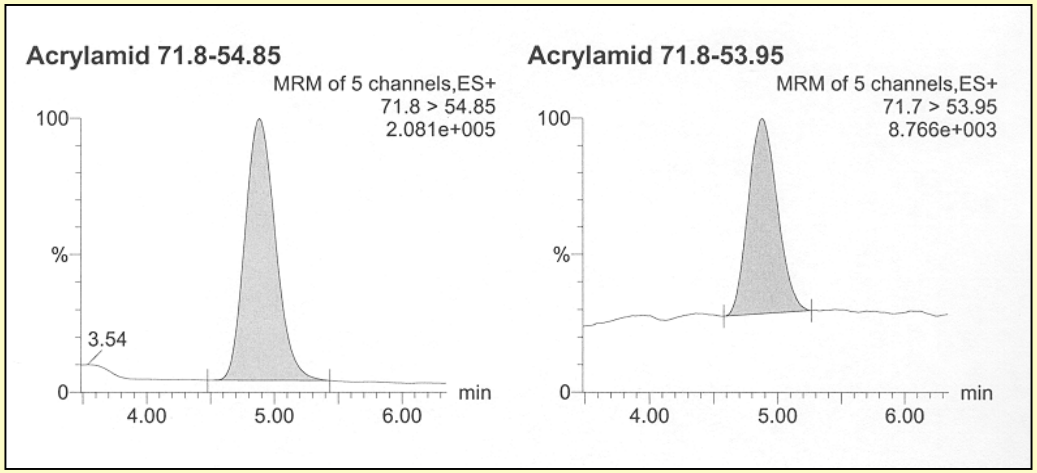
Clean-up procedure for LC-MS-MS (3)

- Many interfering substances are retended on the cartridge, but not acrylamide → no concentration effect for acrylamide
- Enrichment of acrylamid is possible by retention on special phases (Oasis HLB, active charcoal, cation exchanger at pH < 2,5) but this methods are not robust.
- Enrichment of acrylamide also is possible by salting out into organic solvents followed by reextraction into water.

Recommended parameters of LC-MS-MS

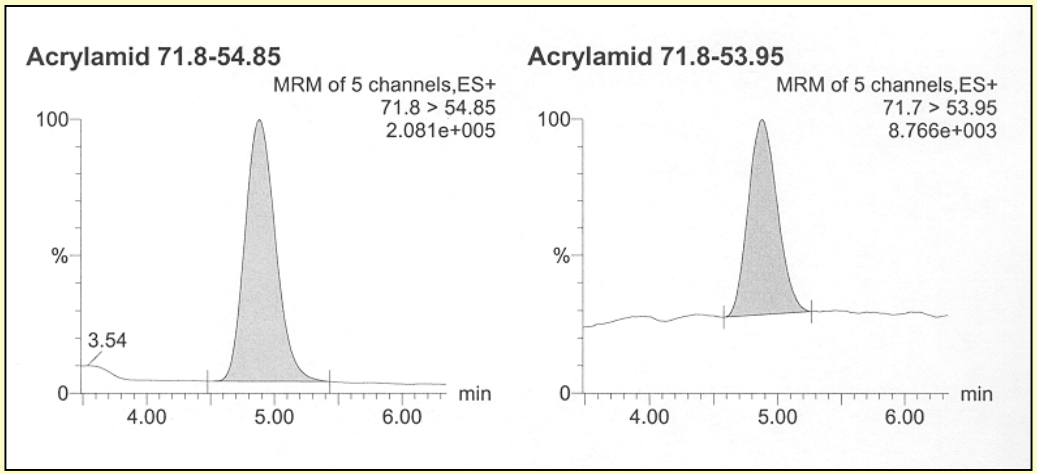
- Column: Hypercarb (graphite) or suitable RP-18-phases
- Solvent water/acetonitrile/formic acid (99:1:0,05)
- Ionisation: Elektrospray positive (ESI)
- Quantifier-ion: m/z = 72 → 55 (ISTD: 75 → 58)
- Qualifier-ions: m/z = 72 → 54; 72 → 44
- Qualifier ions: very low intensity !

LC-MS-MS chromatograms



acrylamide-standard

44 ng/ml



Potato chips

(796 ng/kg)

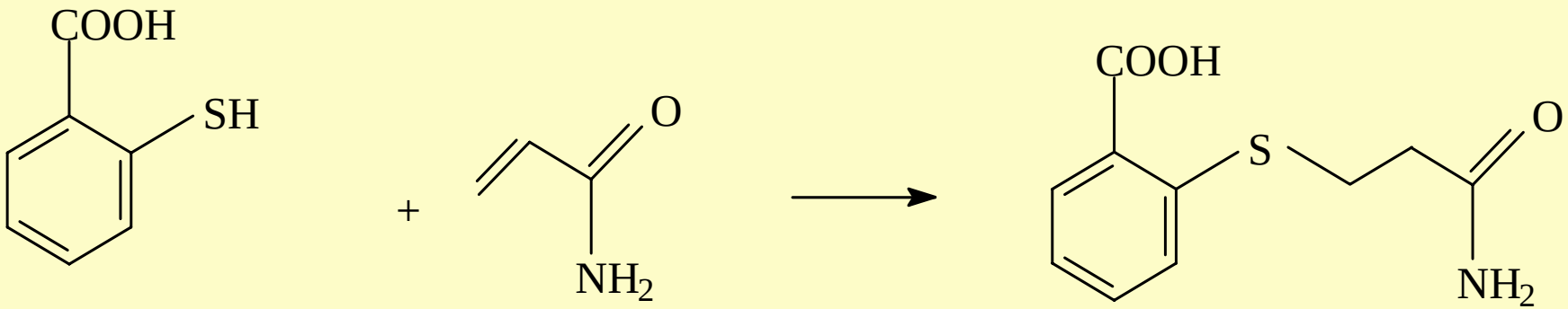
Aspects of LC-MS-MS

- Easy to handle, high troughput is possible
- No concentation step, but dilution of the sample
- No characteristic mass spectrum, low intensity of qualifier ions, verificaton problem at low levels
- In „difficult matrices“ like coffee powder and cacao, extraction problems and interferences are frequent
- Matrix induced quenching effects are frequent, the use of an isotope-labelled internal standard is obligatory

GC-MS without derivatisation

- For GC-MS without derivatisation a very effective clean-up procedure is required.
- **Problem**: Formation of acrylamide in the injector, if traces of asparagine and sugars are present in the extract.
- Formation of acrylamide often very high in PTV-injectors (programmable temperature vaporizser)
- Check clean-up procedure and injection with a solution of asparagine and fructose

LC-MS / derivatisation with mercaptobenzoic acid



LC-MS / derivatisation with mercaptobenzoic acid

- Higher retention on RP-phases, because of the lower polarity of the derivate leads to better separation from interfering substances.
- Less interferences, higher mass range and higher sensitivity in MS
- No LC-MS-MS is required, a benchtop LC-MS-system is satisfactory
- **^{13}C-labelled internal stand is required**

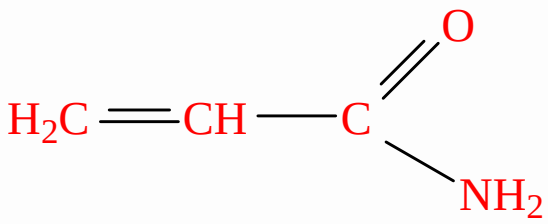
Limits of detection (simple matrices)

- GC-MS (with bromination): 2 – 20 µg/kg
- LC-MS-MS: 10 – 50 µg/kg
- GC-MS without derivatisation: 10 –100 µg/kg
- LC-MS with derivatisation: 25 µg/kg
- (high price) LC-MS-MS with optimum sensitivity in the low mass-range : < 1 µg/kg

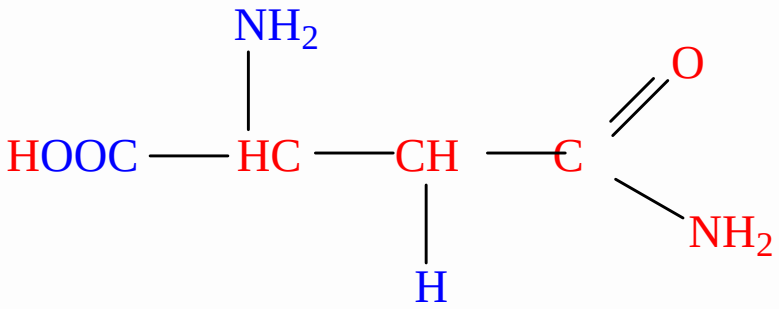
Presuppositions for acrylamide formation

- Free asparagine
- Free reducing sugar (glucose, fructose)
- Low water activity
- Product temperature > 100°C

Asparagine delivers the backbone of acrylamide

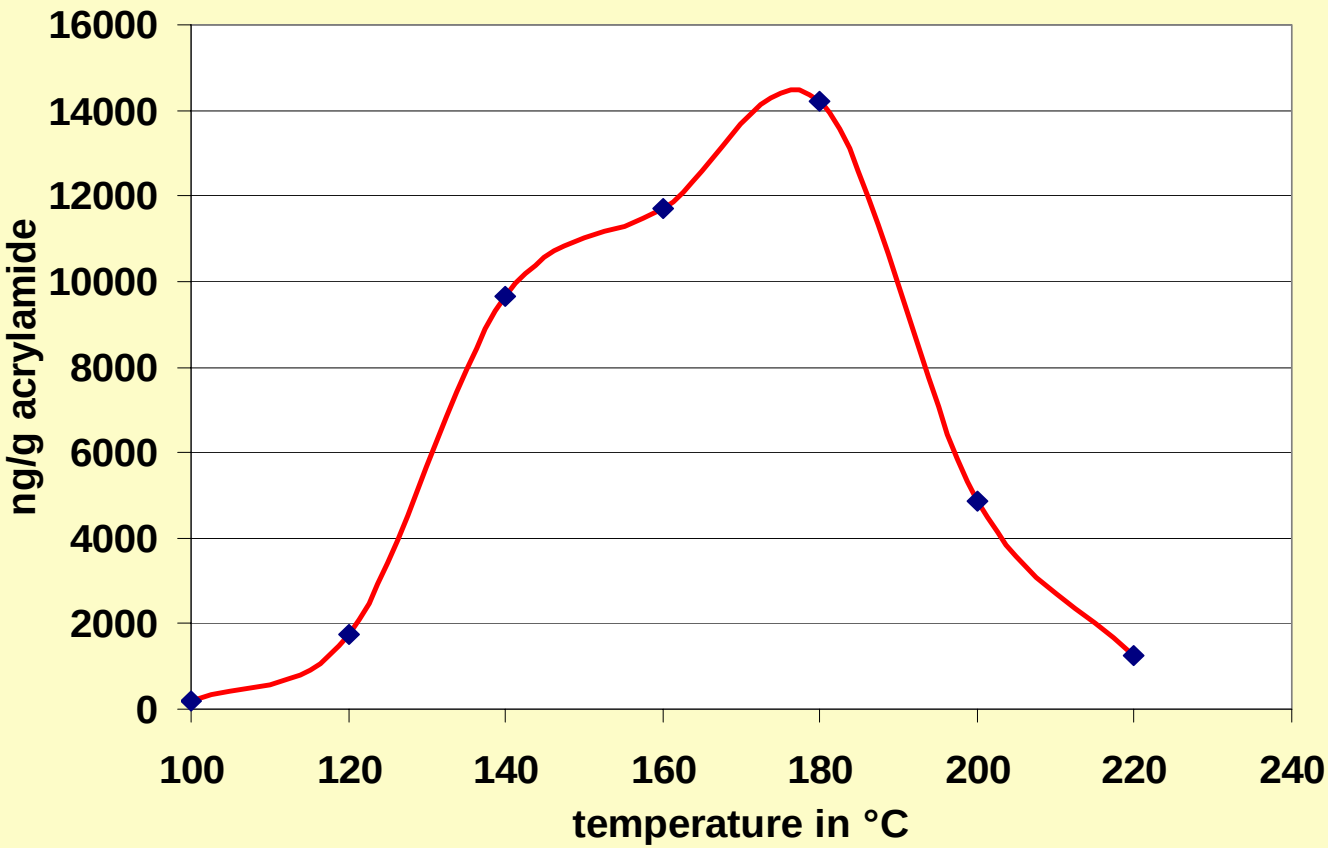


Acrylamide



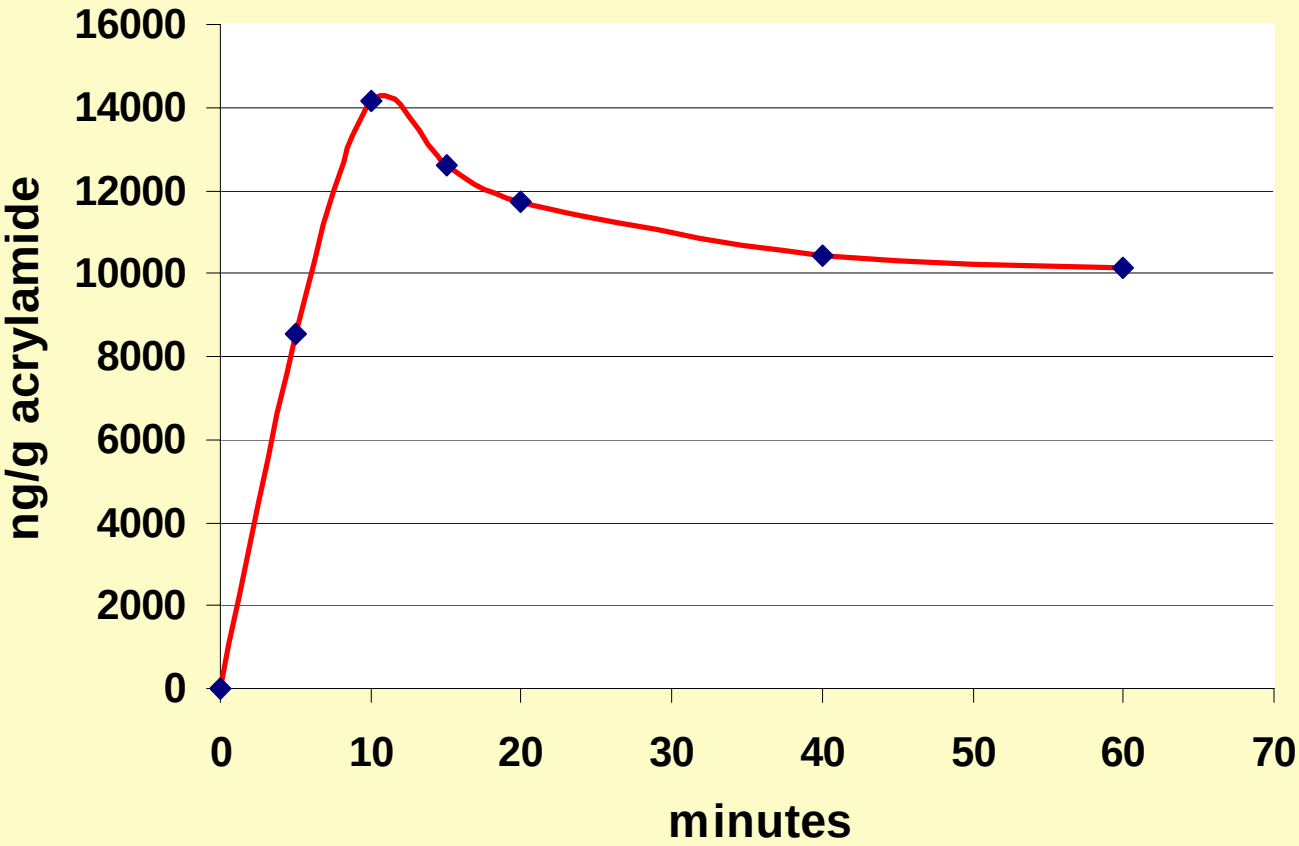
Asparagine

Acrylamide formation in instant potato powder (1)



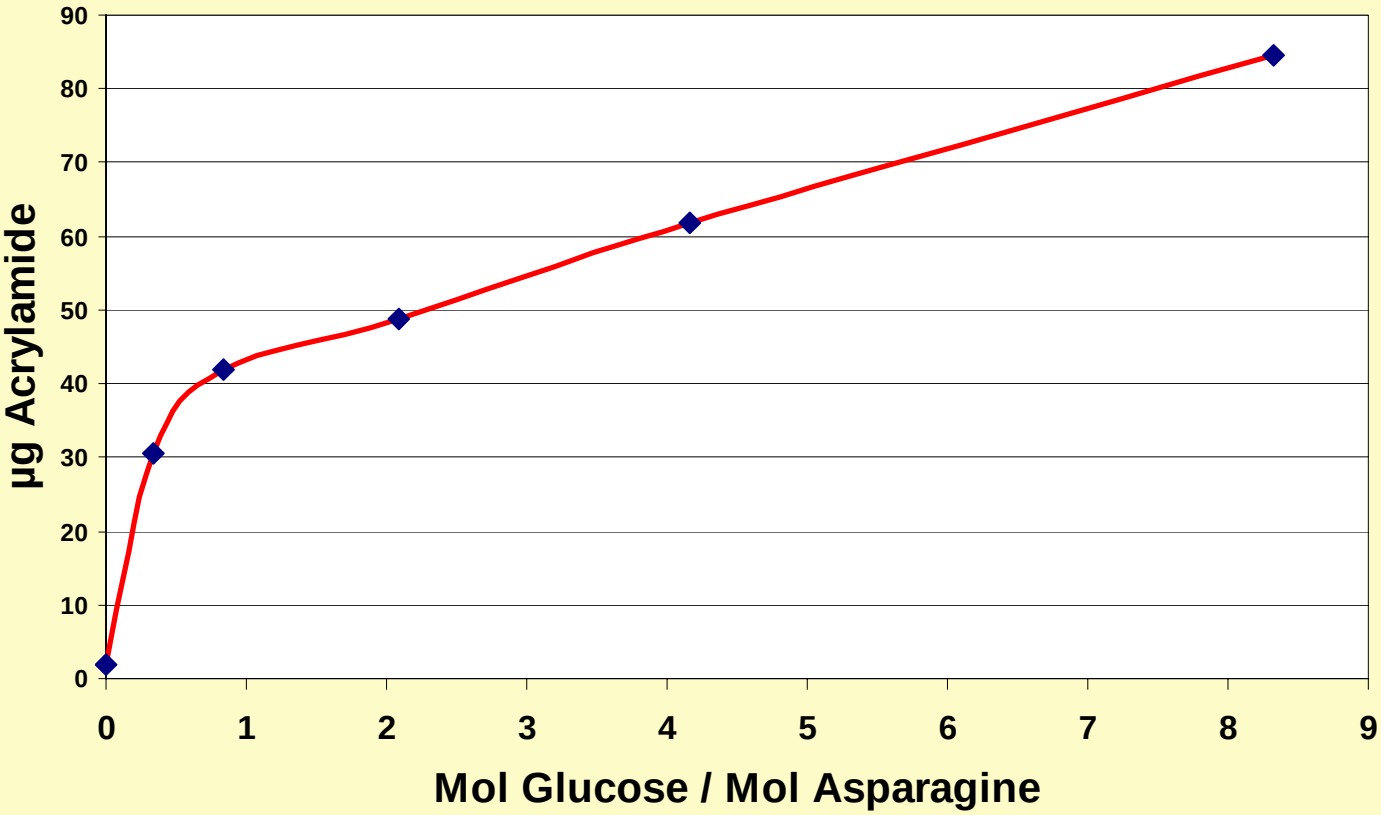
Acrylamide formation in instant potato powder (2)

Heating at constant temperature (160°C)



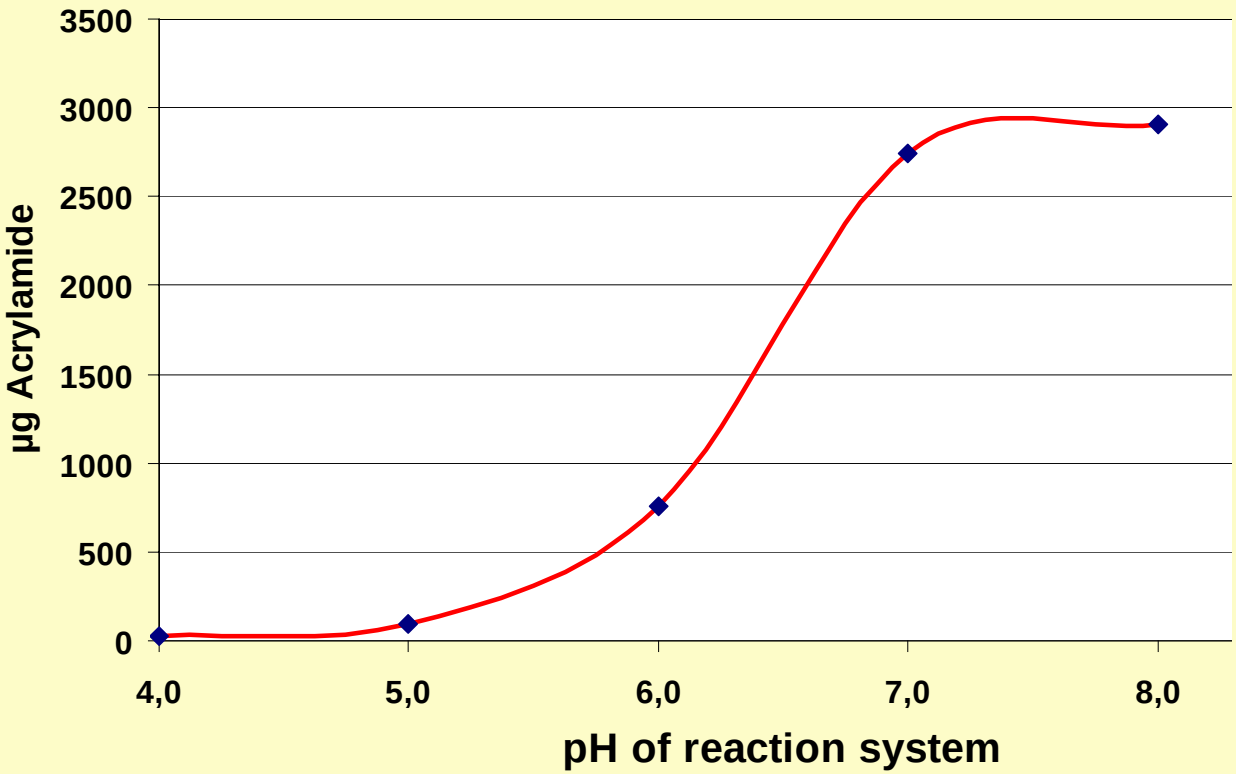
Acrylamide formation – reducing sugars

Model experiments with different glucose/asparagine ratios

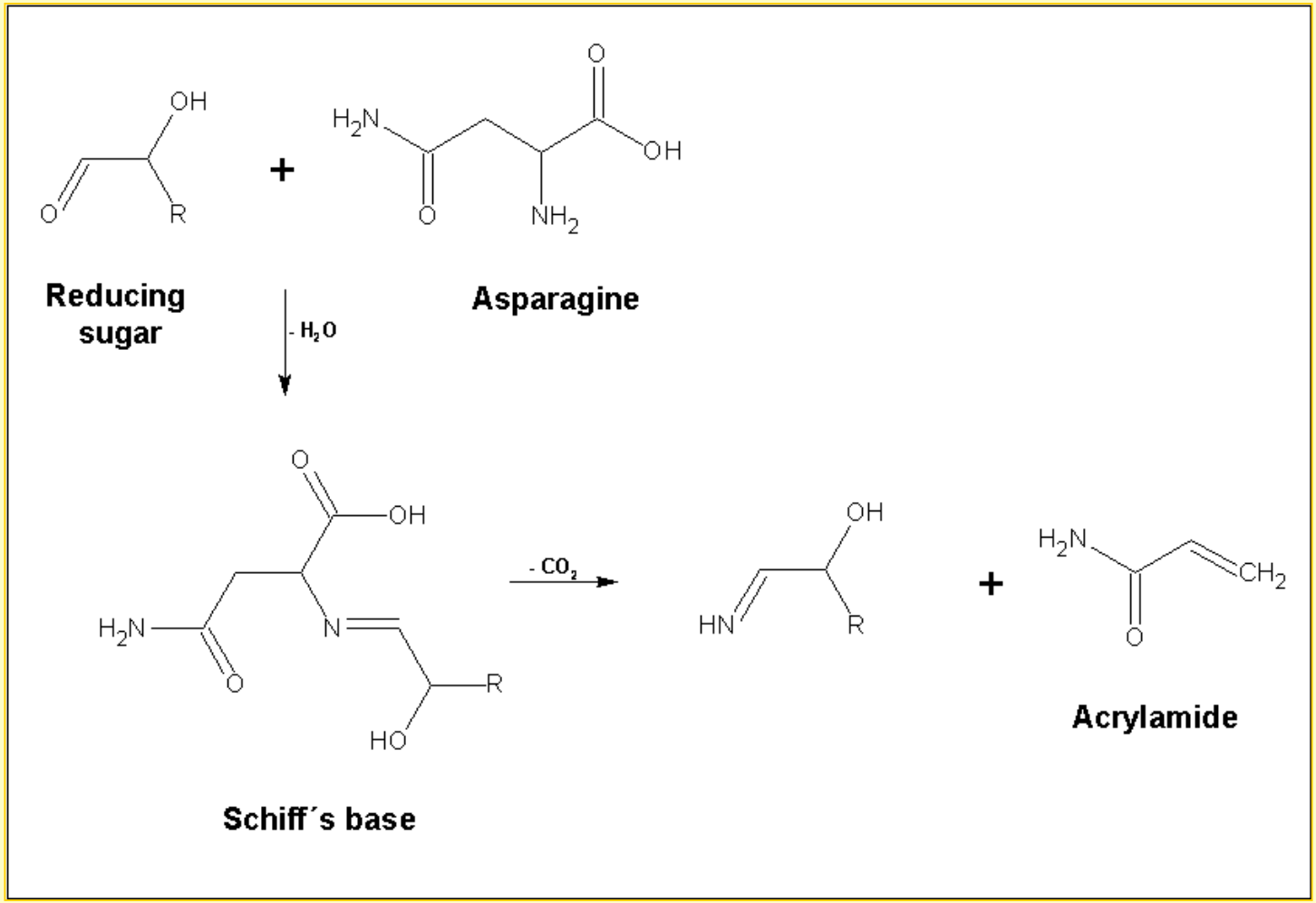


Acrylamide formation – pH value

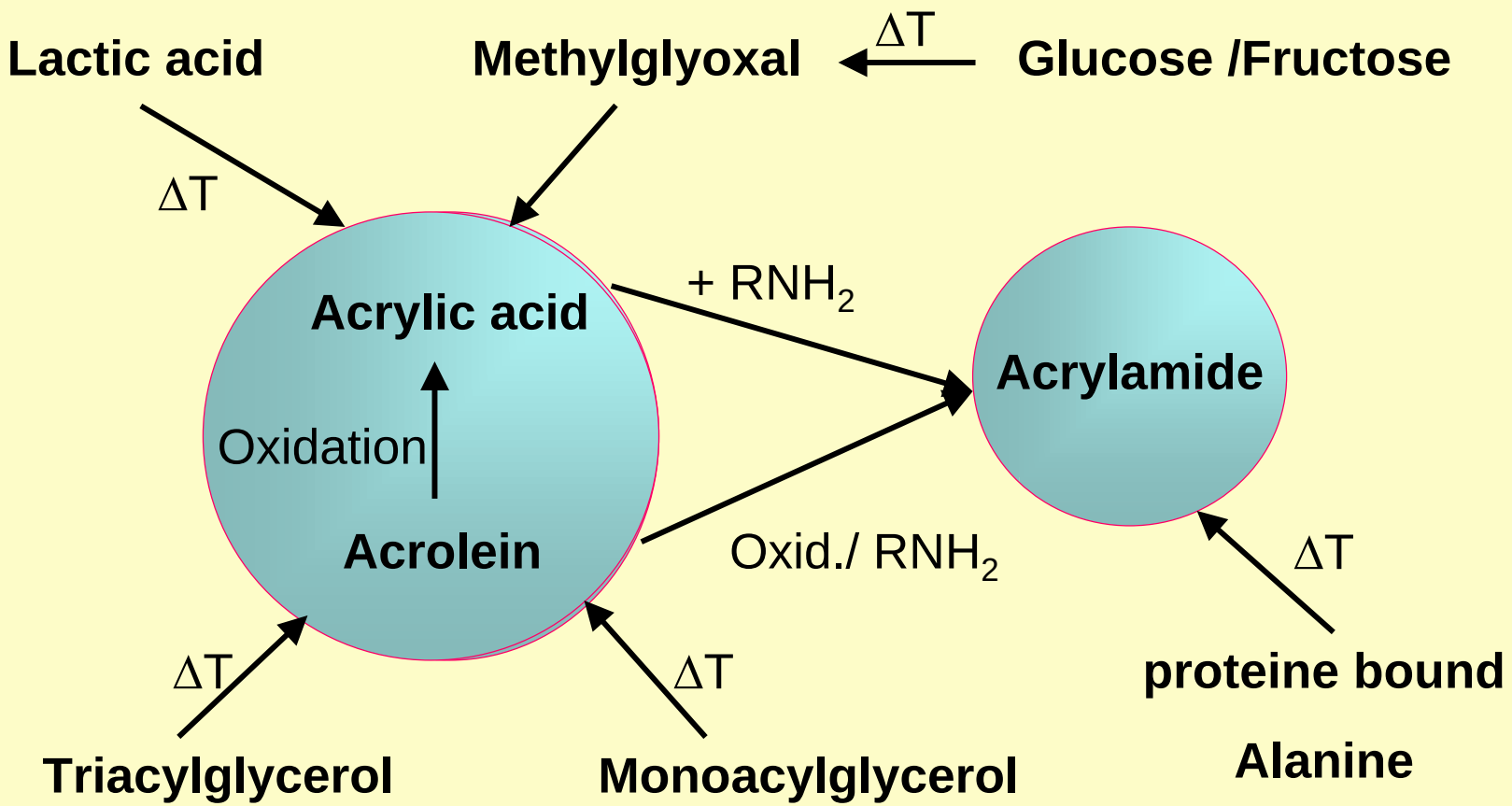
Model experiments with glucose+asparagine at different pH



Mechanism of acrylamide formation



Alternative (less important) formation routes



... Thank you very much for your friendly attention