Application Note # 162

Concentration analysis of nutritionally important proteins from milk

Concentrations of proteins were determined from milk and milk-based infant formula using Multi-Parametric Surface Plasmon Resonance (MP-SPR) instrument. α-Lactalbumin (ALAB) is a nutritionally important protein for infants, and immunoglobulin G (IgG) is an important part of the secondary immune response. Concentrations of unknown samples were determined based on calibration curves. The assay was a direct binding assay using an anti-ALAB antibody or anti-IgG immobilized on the surface. The same assay format can be used to detect other similar proteins of interest such as antibiotic residues, toxins, allergens or nutrients.

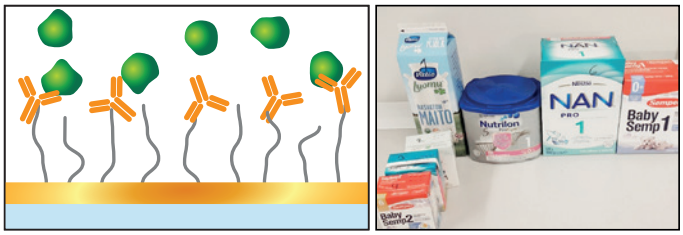

Figure 1. Concentration of α-Lactalbumin or immunoglobulin G determined from different milk and baby formula products using direct binding assay.

Introduction

α-Lactalbumin (ALAB) is the most abundant whey protein in human milk and it is nutritionally important for suckling infants. ALAB is regularly added to infant formulas derived from bovine milk as a part of the humanization process, and it can also be used as a marker for heat-hydrolyzation in hypoallergenic infant formulas. This makes accurate concentration determination of the intact protein from milk of prime interest. [1]

IgG in milk exhibits specific activity against bacteria that are pathogenic in humans. Concentration of anti-microbial factors in cow's milk has an impact on the shelf life of raw milk and it may provide additional health benefits.

Multi-Parametric Surface Plasmon Resonance (MP-SPR) is an excellent platform for building immunological concentration assays for complex matrices, such as milk. The proven SPR detection method is widely used in different immunological studies, such as kinetic, affinity and concentration determinations in antibody production and biopharmaceutical drug development. The robust fluidic setup and unique optical configuration of MP-SPR technology enable assays from crude samples such as milk, sea water, plasma, serum and saliva.

Materials and methods

The direct detection assay for qualitative concentration determination of ALAB and IgG from milk was adapted from H. Indyk (2009). The measurements were performed using a fully automated four channel MP-SPR Navi™ 420A ILVES instrument and BioNavis CMD-3D sensor slides. The immobilization method was standard amino-coupling including NHS and EDC activation, protein coupling and ethanolamine deactivation of the CMD surface. For ALAB detection, a polyclonal anti-ALAB antibody was used (Bethyl Labs A10-128A) and for IgG detection a polyclonal anti-IgG antibody was chosen (Jackson ImmunoResearch 301-005-003). Calibration standards were ALAB (Sigma-Aldrich L6010, 14 kDa) and bovine IgG (Jackson ImmunoResearch 001-000-003, 150 kDa). The running buffer was HBS+T (Hepes buffered saline with 0.05% Tween 20) for ALAB and PBS+T (Phosphate buffered saline with 0.05% Tween 20) for IgG. The regeneration solution for analytes was 10 mM Glycine-HCl pH 2. Data was analyzed using TraceDrawer™ for BioNavis v. 1.8.

The samples were different milk products bought from a Finnish supermarket (Fig 1). Powder samples were prepared according to the instructions on the package into liquid form and further diluted by a factor of 1000 or 50 (normal milk) and 5000 (infant products). The measurements were performed using a 90 second sample injection (calibration or milk sample), a 120 second wait time and a 60 second regeneration injection for ALAB. With IgG, a longer injection time was used. The calibration was performed between 0 - 1000 ng/mL range for ALAB and 0 – 75 000 ng/mL for IgG. The calibration curve was fitted with the 4-parameter Low-High equation, which was also used to determine the protein concentrations in the samples. In the ALAB measurement a quality control sample was included in the series, having a concentration of 100 ng/mL.

Results and discussion

The immobilization of anti-ALAB and anti-IgG antibodies was successful on CMD sensor surface. The reference-corrected sensograms of the protein detection are typical antibody-antigen interaction curves (Fig. 2). The calibration concentrations formed a typical calibration plot. The ALAB samples are positioned relatively evenly on the calibration curve, whereas the IgG concentration does not show as large differences in milk products studied. The calculated results of ALAB and IgG concentrations are displayed in the Table 1 and 2.

There was practically no ALAB protein detected in hypoallergenic formulations of milk (S3 and S5). This was expected, as proteins have been heat-hydrolyzed, and therefore it is presumed that no intact ALAB remains. The normal milk (S1) had the lowest concentration of the ALAB in it, which was also an expected result, as ALAB is routinely added only to the infant formulas for nutritional reasons.

AN_162.810.a

Excellence in Surface Plasmon Resonance
BioNavis

e-mail : info@bionavis.com
www.bionavis.com

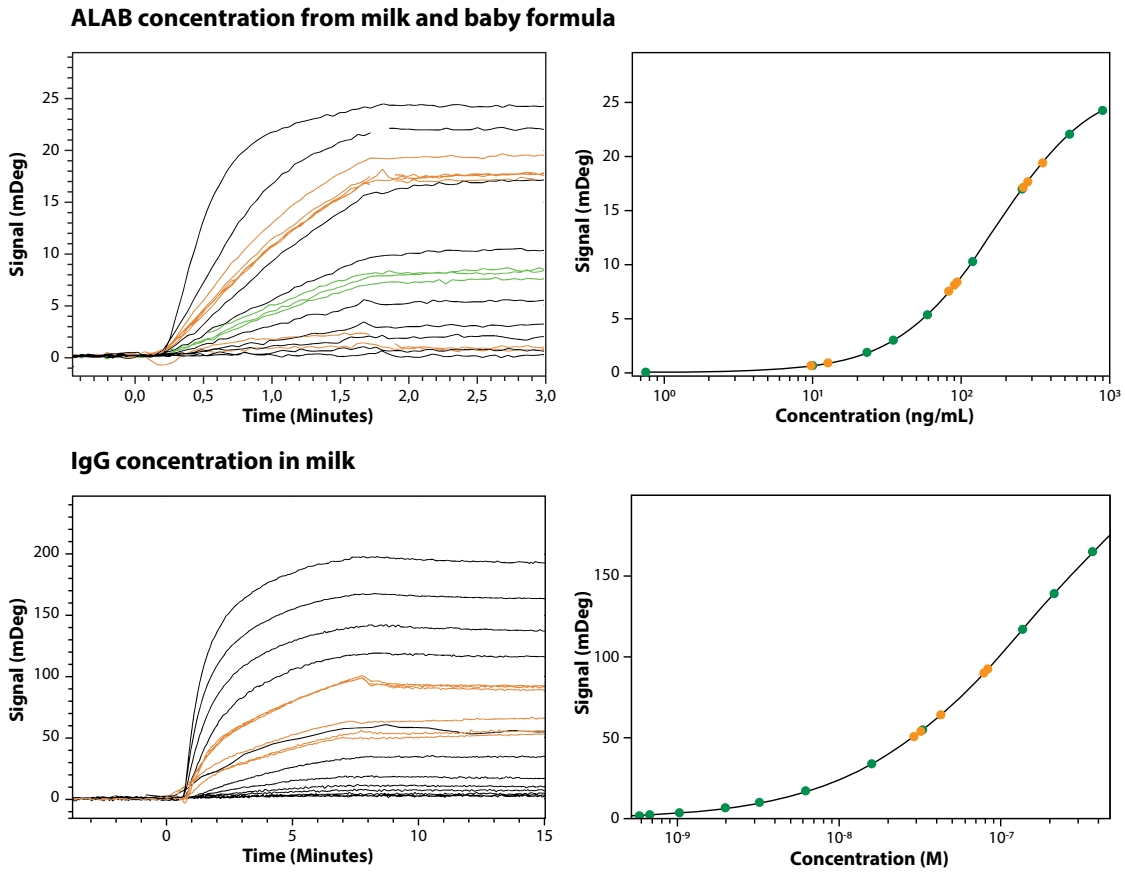

Figure 2. Sensograms and concentration vs. signal plots of ALAB and IgG concentration analysis.
Left: sensograms – calibration samples, – quality control samples, – milk samples
Right: concentrations vs. signal plot – calibration injections, – milk and quality control samples.

Conclusions

The MP-SPR is an excellent platform for concentration determination of proteins with immunoassays. The method can easily reach ng/mL sensitivity when high-affinity antibodies are used. The method presented here can also be used with other milk proteins, such as β-Lactoglobulin. MP-SPR is not limited only to milk as the measurement matrix but can be used also with other food-related matrices, such as juice and eluted wheat dust, or in other crude samples, such as 100% serum, urea and sea water.

References:

[1] Harvey Indyk, International Dairy Journal 19 (2009) 36–42

Table 1. ALAB concentration in milk products

Sample	Sample name	Type of original product	ng/mL	Dilution factor	Original conc. (mg/mL)
S1	Valio organic fat-free milk	Liquid	**1070***	1000	**1,07**
S2	Arla Little baby organic 1 (0-6m)	Liquid	**263,0**	5000	**1,32**
S3	Nestle NAN 1 H.A (0-6m) Hypoallergenic	Liquid	**12,8**	5000	**0,06**
S4	Semper Baby Semp 2 (6+m)	Liquid	**280,0**	5000	**1,40**
S5	Nutricia Nutrilon Pro Expert 1 HA (0-6m) Hypoallergenic	Powder	**9,8**	5000	**0,05**
S6	Nestle NAN pro 1 (0-6m)	Powder	**278,0**	5000	**1,39**
S7	Semper Baby Semp 1 (0-6)	Powder	**280,0**	5000	**1,40**
	* out of calibration curve				

Table 2. IgG concentration in milk products

Sample	Sample name	Type of original product	ng/mL	Dilution factor	Original conc. (mg/mL)
S8	Arla light milk (1% fat)	Liquid	**1180** **1160**	50	**0,62** **0,61**
S9	Arla fat-free milk	Liquid	**263,0** **472**	50	**0,33** **0,25**

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 420A ILVES and 220A NAALI
Sensor surfaces: Carboxymethyl dextran (CMD) or regenerable avidin kit (SPR102-AVI-2)
Software: MP-SPR Navi™ Control, DataViewer, and TraceDrawer for MP-SPR Navi™.

AN_162.810.a

Excellence in Surface Plasmon Resonance
BioNavis
e-mail : info@bionavis.com
www.bionavis.com