Work Package 9: Heavy Metals

Introduction

Heavy metals (HM) are highly toxic to both plants and animals, and are known to have well-documented neurotoxic (lead, mercury), hematotoxic (lead) and nephrotoxic (lead, cadmium, mercury) effects on humans.

The current standard techniques employed for trace heavy-metal analysis include Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma-Mass Spectrometry (ICPMS). However, these methods require expensive equipment, involve time-consuming sample treatment, and pre-concentration steps that can only be carried out by trained professionals.

Given this situation, there has been an effective barrier with regards to screening for heavy metals at various stages of food production. In order to achieve one of the most important practical objectives of food safety, i.e. that of preventing heavy metal contamination into the food production chain as early as possible, the electrochemical method developed is a step to overcoming this problem. Electrochemical methods are envisioned as a complement to the aforementioned standard techniques. They are especially attractive as they employ inexpensive and portable instrumentation that can be used by personnel with limited training in various environments.

Figure 1. The basic instrumentation needed for lead detection.

Work Package Progress and Results

In the context of the BioCop project, systems for both lead and mercury detection have been developed and evaluated for their practical application in milk and baby foods.

Lead Detection

The overall approach was based on a classical electrochemical technique (anodic stripping) that has been coupled with novel disposable sensors. This approach is easy to produce (using thick film technology) and can be used with portable, cost-effective, and user-friendly instrumentation.

Screen-printed electrodes (SPEs) have been designed and constructed after devising and testing a combination of chemical layers. Further to this, the selection and testing of different inks and silver pastes for screen-printing were performed.
Finally, after the evaluation of electrochemical behaviour and performance with real samples using various combinations, the choice of method allowed the analytical performance for detecting lead in milk samples to be at its best.

While the final objective was to have a system applicable for use within different settings, and also for screening. The method was still required to deliver the requisite sensitivity for regulation and to meet additional criteria.

Additional criteria was to replace the highly toxic Hg in the classic method. For this purpose the SPEs have been modified by deposition of a Bi-film on the surface of the working electrode. The presence of lead is then monitored by i) an accumulation step in which the lead forms an alloy with bismuth, and ii) a subsequent stripping step in which a specific lead peak is observed with an area that is proportional to the lead concentration.

In order to enhance the sensitivity of the sensor, different polymers were used to modify the sensor before the Bi-film deposition. Nafion® was ultimately chosen, giving a 2.5-fold enhancement in sensitivity and achieving a detection limit of 1.5 parts per billion (ppb), considerably below the legal limit (1.5ppb) for lead in milk. Finally, the system developed was validated by measuring lead in standard solutions. This validation was carried out at the leading technical and scientific body of the Italian National Health Service.

Another focus of the BioCop project is to link improved sample preparation procedures with the new detection technologies. For milk, a new pre-treatment method involving acid precipitation of proteins (using HCl and HClO4) was devised. The pre-treatment protocol also involves sonication and the addition of other agents (H2O2 and ferrocyanide) to enhance recovery by releasing lead from proteins and also to avoid interference from copper. With these procedures, the method meets all the analytical requirements to accurately determine whether the lead content in the milk is lower than the EU legal limit of 20 ppb.

In a parallel process, dedicated software for lead detection was developed in collaboration with the BioCop partner, Palmsens. Taking into consideration the final procedure developed for the measurement in treated milk samples, the software was designed not only to control the analytical processes but also to guide the operator through each step. Using the instrument and software as developed; the operator can provide the input data indicated by the software and view the concentration of lead in milk on the digital screen at the end of the procedure.

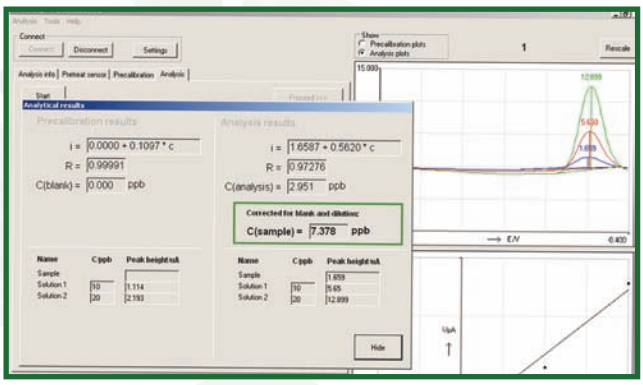

Figure 2. Software designed to control analytical processes but also to guide the operator through the procedure of lead detection.

Mercury Detection

The experimental approach used involved the synthesis of novel mercury chelating groups that could be cross-linked to magnetic microbeads. These were then tested in a system using enzymes for signal amplification. Although a reasonable detection limit was achieved for inorganic mercury, there were insuperable problems related to sample preparation and the liberation of Hg to enable its detection in milk and baby foods. for detection in milk and babyfoods Thus further development of this approach was not undertaken.

Benefits of the BioCop Project

Consumer

Faster and more cost-efficient detection can ultimately lead to enhanced levels of food safety for consumers, by detecting heavy metals that end up in numerous products that consumers then consume. The relative simplicity of the method developed means that it could be used in many of the small-scale businesses involved in the processing of milk.

Food Industry

This new method provides numerous potential advantages for the food industry. It would significantly lower costs and provide faster testing to assure that milk and baby food products are in conformity with the regulatory limits. Moreover, the method can be carried out by unskilled personnel in "normal" laboratory conditions equipped with "usual" instrumentation

(water bath, centrifuge, portable potentiostat) making it possible to monitor at the various stages of production. Thereby avoiding economic loss from contaminated lots and, as a result, improving the overall safety of the final product.

Scientist

Several technical advances have been achieved which provide numerous benefits to the scientist. These include the development of a complex sensor based on screen-printed electrodes and bismuth/Nafion films. These have demonstrated that:

- SPEs are useful and practical substrates for sensor development.
- Bismuth can replace the highly toxic mercury for lead detection by anodic stripping.

The treatment of milk with concentrated acid, coupled with H2O2, ferricyanide and sonication steps, allows sufficient recovery of lead. This is so that it can be determined at ppb levels, (relevant for meeting regulatory standards) within 2 hours and without the use of sophisticated instruments.

Training/Workshops

WP9 showcased a video at the DG SANCO Conference on ''Delivering for Tomorrow's European Consumers'' Technology exhibition in Brussels, Belgium on the 29th October 2008.

A workshop "Screening and confirmatory methods for the detection of heavy metals in food" at the Università Degli Studi di Roma Tor Vergata (URTV) took place on the 2nd - 3rd July 2009. At this event, a demonstration of the developed BioCop methods to the network of European heavy metal testing laboratories were performed.
For further information, please visit:
www.workshop.wp9.biocop2009.uniroma2.it

Other events WP9 have participated in include, the International conference, marketplace and matchmaking event for Food and Feed Safety and Quality, ''Rapid methods Europe 2008'' and the BIOCOP/CASCADE Joint Spring School, Nantes, France (26th-30th March 2007)

Future Activity

Collaboration with the new BioCop partner ''VPHL'' in providing training opportunities for stakeholders in electrochemical assays – due to take place in Asia in 2010.