Chapter 4

We saw in chapters 3 and 4 within the book that in order to solve an analytical problem, the sample type with identified analyte, the sample preparation step and the measurement technique are all interconnected. They can in some ways be considered as a "hub" within the 'analytical approach'. When the sample and the analyte(s) within it are defined as shown in chapter 3 and figure 3.1 (for identification and/or measurement purposes), the process of selection for this 'hub' is underway. However, before we go on to consider certain measurement problems and their solutions, it is worth considering a fundamental concept with regard to the "measurement" step itself. We have seen a number of measurement techniques presented already in chapter 4 and many rely on some form of instrument (if not all techniques, based upon the definition of how a measurement is actually achieved). If we reduce the process down to those measurements based upon an instrument that fundamentally involves an energetic process then, schematically, we are considering the following:

Stimulus: →	**Sample** Interacts with →	**Detector** sensitive to →	**Measurement**
Energy input based:	Stimulus:	: stimulus or emitted	and read-out
Upon:	Absorption / emission /	radiation from sample	
↓	Change of state =>	or physical / chemical	
	Physical or chemical	change of state	

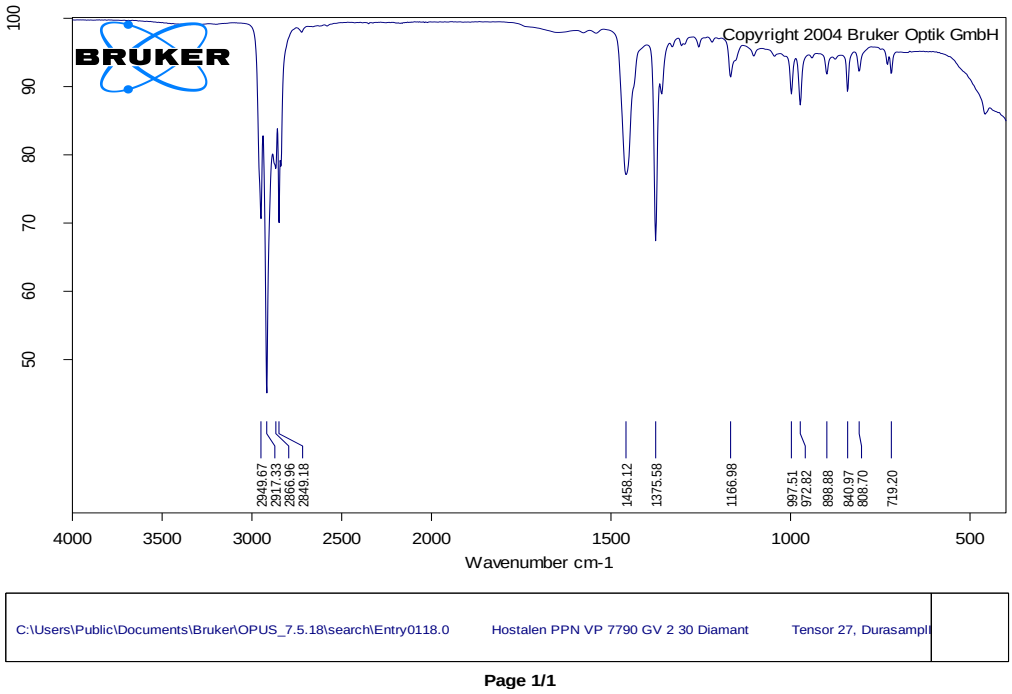
EMR
Electrical (Voltage or Current)
Heat, Chemical….etc.

How each of these parts within the schematic may be explained is best illustrated using an example technique we have already encountered, that of UV-visible spectrophotometry. Here the energetic input is from the visible and ultra-violet part of the electromagnetic (EM) spectrum. The **analyte** from the sample **interacts with this EM radiation** and **absorbs** the energetic radiation (**raising the analyte's energy** system accordingly). The **sensitive detector** system **responds to the intensity of the selected energy** both before and after its interaction with the analyte of interest and **presents a read-out**, allowing **a measurement** to be made – and thereby **allowing a comparison** to be made **between the presence and absence of the sample's analyte**. This process can allow both a qualitative and a quantitative measurement of the analyte if selection of the radiation to be measured and the detector to be used are carefully matched / controlled (e.g. wavelength of EMR, band-width of radiation, CCD, PDA, etc.).

Feedback to Problem 1

To help identify the "contaminant strips" found in the bread, it would be possible to use Infrared (IR) Spectroscopy of the solid strip, directly. This technique can be used in its attenuated total reflectance (ATR) mode on the solid to help answer some early questions on the contaminant's composition. The IR spectrum produced by a modern Fourier transform

instrument (FT-IR), usually covers the range 400 to 4000 cm^{-1} (wave numbers) and this "fingerprint" spectrum can be compared with a library database, found either with the instrument software or on-line, to confirm and identify the "plastic / polymer" present.



The above spectrum demonstrates a qualitative measurement (identification) performed in under a minute on a strip of 'plastic' (from problem 1) using FT-IR spectroscopy. It was found to be made of polypropylene.

It is also possible to use a simple energy dispersive (ED) or wavelength dispersive (WD) XRF Spectrometer directly on these solid contaminants to provide some supplementary qualitative / semi-quantitative elemental composition of the strips (~ carbon onwards; e.g. C, N, O, F, Cl, Br, filler materials, stabilisers, etc.).

The above techniques may be sufficient to confirm the identity (qualitative analysis) of the contamination strips and any potential health and safety (H & S) issues that may arise.

Feedback to Problem 2

You have been alerted to a possible issue because of the paint used for coating the child's toy. In many European countries and North America, there has been legislation in place since the late 20th century to restrict certain toxic metals, metalloids and organic compounds being incorporated in paint manufacture. This legislation is to be found on-line but the more obvious elements that come to mind are: lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), antimony (Sb), arsenic (As), etc. while there is also a range of organic-based dyes and pigments that have restrictions on their use. In the absence of any other information on the toy, the qualitative elemental content of its coating can be evaluated using the solids technique ED-XRF spectrometry. This may be undertaken directly on the toy's surface paint

layers (depending upon certain analytical conditions such as ‘critical depth’ and ‘substrate interference’) or on the flakes / scrapings resulting from the representative removal of the paint coating, etc.

For example, if the element ‘lead’ (Pb) was detected using ED-XRF directly, then a confirmatory analytical methodology would be adopted to determine its concentration above any legislative value. See http://www.who.int/ipcs/assessment/public_health/lead_paint.pdf

The presence of various organic compounds (binders, drying agents etc.) in paints, in addition to the colourant can cause the identification of any restricted organic dyes or pigments to be a more complex procedure. However, if direct evaluation of the colourant by IR spectroscopy is not possible then various simple but effective separation procedures can be adopted (liquid-liquid extraction, Soxhlet extraction, chromatographic techniques etc.) ending with an organic compound detection / identification system of suitable selectivity.

Feedback to Problem 3

The darker, slight brown tinted colouration and cloudiness may be linked and could present themselves due to a fine suspension of material that doesn’t readily settle. To test this, a phase separation process (in this case a possible solid suspension from an organic liquid) such as filtration or centrifugation (Chapter 3) could be employed. Use either a vacuum filtration system with a pre-cleaned < 0.22 µm filter (cellulose acetate) to retain any solid suspension material or a centrifugation process in a 50 mL tube at 4000+ rpm for 20 min to bring down the particulate material. If the supernatant liquid (that liquid left after processing) has the same appearance as the acceptable, comparison sunflower oil batch (clear, light straw yellow colour) then carefully transfer the clarified liquid to the fridge (< 5^{o}C, in the dark) for later analysis if required. The solid material collected from either processes can then be isolated and identified using a range of techniques offered in chapter 4 for organic / molecular or inorganic material. Initial exploratory investigations using the solid directly can centre around identification using elemental, molecular and structural approaches. Organic material using ATR-FT-IR spectroscopy, and even CHN(O,S) analysis for an elemental organic ratio breakdown; general elemental information using ED-XRF spectrometry to explore the range of elements outside the relatively limited organic series, for example halogens, phosphorus, sulphur, metalloids, metals etc. If the XRF spectrometric analysis shows a possible inorganic crystalline contribution, then X-ray diffraction (XRD) can be employed together with interrogation of the data base of powder diffraction patterns (international centre for diffraction data or ICDD; formerly JCPDS files) to help with identification of crystalline solids. Fine tuning of the identification, if required, is possible once the initial information is obtained.

It is noted from investigation of the constituents of sunflower oils, that the composition is based mainly around the triglyceride esters of Oleic and Linoleic acids (combined ~ 90%) with ~10 % from Palmitic and Stearic acids. However, this depends upon the processing, and other minor constituents can include carotenoids, waxes and tocopherols which have variable colour and solubility in the oil.

Because sunflower oil is primarily composed of the less-stable monounsaturated (e.g. Oleic) and polyunsaturated (e.g. Linoleic and Linolenic) fatty acids, it can be particularly susceptible to degradation by light, heat and by air; which is known to trigger and to accelerate oxidation effects. Refining by manufacturers of the basic sunflower oil through the use of solvent extraction, de-gumming, neutralization, and bleaching can make it more stable but also removes phospholipids, polyphenols and phytosterols which are more amber in colour. These materials are nutritionally acceptable but less stable, over time and when stored in above ambient temperatures.

It is noted that an analyst can, if they need to, identify in more detail the organic constituents of the sunflower oil (the glyceryl-fatty acid esters and the other components previously mentioned) using simple separation / derivatisation techniques. This includes saponification and / or solvent extraction and then methyl esterification to increase component volatility. This then allows identification of the oil components by separation techniques such as Gas Chromatography with a suitably selective detector (e.g. GC-MS, GC-FID, GC-NPD etc.; see table 4.4).

Feedback to Problem 4

Feedback to i)

i) The presence of (the NSAID) Phenylbutazone in meats sold for human consumption.

From the preparation step for this scenario (i) discussed in the on-line feedback for chapter 3, the analyte of interest, Phenylbutazone (PBZ) has been extracted from meat and cleaned-up by removing undesirable matrix components, and is in a suitable solvent ready for the measurement step. As was stated in chapter 3 and in the on-line problems for this chapter, it is actually noted that there is a close link between the analyte(s) or property to be measured, the sample preparation process and the final measurement technique. One would always have these three key points in mind when considering the specific analytical process. Looking at the analyte, an organic molecular compound which is not volatile, then the most obvious technique to consider first is high performance liquid chromatography with UV detection or possibly mass spectrometry (MS) detection (HPLC-UV or MS). The column type would be Reversed Phase (RP-HPLC) from the tables given in chapter 4.
It may be possible to produce a more volatile form of this molecule and so gas chromatography with a suitable detector, such as a nitrogen detector or mass spectrometer (GC-NPD or GC-MS) could be considered.

In all cases the low starting levels expected for this drug in the meat, might suggest the route to take would be that which provides some pre-concentration and / or use of the most sensitive detectors. The use of HPLC as a separating technique allows for the reduction of molecular interferences from matrix components extracted alongside the analyte of interest from the complex meat sample.

Brief feedback for all scenarios

Scenario →	i	ii	iii	iv	v	vi	vii	viii	ix	x
Measurement Technique step ↓	PBZ in Horse-Meat	PBA in Face Cream	Manuka in Honey	BPA in Printed Till and Card receipts	Radio-nuclides in plants and soils	TiO_2 in sun-screen	Scap-metals from waste sites	Elemental composition in mineral supplements	Fatty Acids in Soya-Beans	NH_4^+ ions in Waste water dis-charge
Possible direct measurement using	Some processing then ELISA				Gamma (γ) spectro-metry	X-ray fluoresc ence (WD); [XRD?]	X-ray fluore scence (ED)	X-ray fluorescence (ED)		ISE NH_4^+ selective
Measurement techniques after processing	HPLC with UV or MS detec-tion Or GC-FID / GC-MS [after deriv^n]	HPLC with UV or MS detec -tion Or GC-FID / GC-MS [after deriv^n	HPLC with UV or MS detec-tion Or GC-FID / GC-MS [after deriv^n]	HPLC with UV or MS detec-tion Or GC-FID / GC-MS [after deriv^n]	**Element al -** Gamma (γ) spectro-metry and ICP OES / MS	**Element al -** ICP-OES; X-ray fluoresc ence (WD);	**Eleme ntal -** ICP-OES	**Elemental -** ICP-OES and ICP-MS; X-ray fluorescence (WD);	GC-FID / GC-MS [after deriv^n Or HPLC with UV or MS detec -tion	Colouri-metric by Visible spectro metry

[After deriv^n = after derivatisation to a more volatile form; may be an option for GC analysis

Feedback for Problem 5

a) The type of plastic and possible range of plastics the mug is made from, can be investigated quite quickly using the direct solids analysis techniques of FT-IR spectroscopy, Raman spectroscopy, Differential scanning calorimetry, Thermo-gravimetric analysis and Scanning electron microscopy with EDS. Structural, molecular and elemental analyses can be performed using these techniques. Finger-printing of the polymers present is inherent in techniques such as FT-IR spectroscopy. See the measurement techniques for solids in the tables in chapter 4.

b) The above techniques will provide a basis upon which to plan the next step. The surface elemental scan using SEM-EDS together with freshly-cut surface analysis of the mug in various parts will provide the major elemental make-up for this primary evaluation. However as we saw before, secondary evaluation using various techniques of i) direct measurement, ii) decomposition and ii) extraction, on separate samples from the mug to get these into a suitable form for measurement, will be required if a suitable direct measurement technique indicates the selected (restricted) elements are present above particular levels / concentration (see feedback to Question 3 in chapter 3, on-line). This will as stated, allow measurement of their total elemental content and their leachable elemental and leachable compound content, to be compared with any regulatory or guidance values.

Suitable direct elemental measurement techniques of the plastic for their total content would include X-ray fluorescence spectrometry, when suitable calibration and matrix correction procedures are integrated into the determination.

Suitable elemental measurement in solution (resulting from a decomposition method for total content and from migration / leaching methods) would include the element specific techniques of ICP-OES, ICP-MS and solution-XRF for metals and metalloids, atomic fluorescence for elements like As, Sb and Hg, while the non-metallic elements such as halogens may be determined using solution-XRF, ISE, ion-chromatography etc. See Figure 4.12 and Table 4.6 in chapter 4 for guidance.