which easily release HCN under slightly acidic environmental conditions; and (iii) total cyanide each potential source of HCN regardless of its originion (US EPA, 2004).

The term “cyanide” refers to all CN groups that can be determined analytically as cyanide ion (CN) via spectrophotometric or electrochemical measurements, usually following appropriate sample pre-treatment to release cyanide ion. The Environmental Protection Agencies have imposed maximum contaminant levels (MLC) for cyanide discharge into the environment. The MLC for WAD cyanide vary from 0.05 to 0.07 μg/L for drinking water and in the range between 200-500 μg/L for waste water. The MCL for total cyanide is much higher – 1 mg/L. The group of WAD cyanide has been a subject of special consideration as the assessment of environmental risk and efficiency of detoxification procedures depend on its analytical quantification.

The facts mentioned above highlight the main demands to cyanide determination methods in environmental objects: (i) high sensitivity to reach the low MLC; (ii) high selectivity to analyze a great variety of matrices; (iii) capability for speciation to quantify toxic cyanides; (iv) implementation in portable analytical devices to allow on-site analysis in real time. In the past few years, a variety of new cyanide sensors and improved cyanide determination methods have been reported. Nevertheless, it is not easy to respond to all of the requirements above. Recently, a review presenting the available methods for cyanide determination and assessing their flexibility to application in automated portable analyzers has been published (Surleva, 2009).

The potential of electrochemical detection is specially emphasized in view of its suitability for automation and miniaturization. In portable devices the amperomeric detection has been given preference regardless its low selectivity, which calls for cyanide separation and an on-line method by flow-injection, ligand exchange, and amperometric detection has been officially approved (US EPA, 2004). New flow-injection cyanide selective detectors obtained by thin-layer electrochemical deposition technique have been recently proposed (Neshkova et al., 2006).

The sensors are fully competitive with amperometric detection as far as the lower linear limit, sample throughput, and sensitivity are concerned. Moreover, the potentiometric detectors offer

additional advantages: selective response (so that the separation step could be omitted and thus the equipment simplified) and cyanide speciation. Due to the high sensitivity of UV-Vis spectroscopy a lot of research was done in attempt to improve selectivity, analysis time or to develop environmentally friendly procedures. A comparative study of some new and some established spectrophotometric assays for environmental cyanide was reported by (Drochioiu et al., 2008): (i) the Aldridge method and its variants with pyridine and pyrazolone; (ii) isonicotinate-barbiturate method that was useful to detect minute amounts of cyanide in vivo and in vitro; (iii) the reaction of cyanide ion with ninhydrin, which was proved to be fast, simple, highly selective, and free from most interference, but under reducing conditions; (iv) picric acid-based assay which was described to be highly selective, but yet less sensitive; (v) combined resorcinol-picric acid method which showed improved sensitivity. Although a lot of work has to be done to propose a robust method, these sensors show very low detection limit coupled with good selectivity, small sample volumes and rapid response. They work on "turn-off and–on" principle and are extremely suitable for portable signaling devises in dangerous environment. Analytical methods for cyanide determination in environmental samples are summarized in Table 1.

Table 1. Analytical methods for cyanide determination in environmental samples

Methods	Samples	LOD, µg/mL	Range, µg/mL	RSD, %	Recovery, %	Reference
Spectrophotometry	Tap, mineral and waste water	0.007	0.01-0.5	2-4	97-109	Abbasiet al., 2010
Spectrophotometry	Drinking water	0.11	0.26-6.5	2	-	Absalan et al., 2010
Spectrophotometry naked eye detection	Drinking water	0.03	4-8	-	95-105	Isaad et al., 2011
Voltammetry	Industrial waste	0.0002	0.001-3.9	1.4	98-104	Noroozifal et al., 2011
Spectrofluorimetry naked eye detection	Drinking water	0.008	0.5-4.7	2	99	Li et al., 2011

UV-Vis	Tap, bottle and ground water	16	50-2000	2.3	99-109	Ma and Dasgupta, 2010

5.2 Cyanide determination in biological samples

Human fluids contain cyanide due to different sources of cyanide exposure. Apart from sodium nitroprusside therapy (as a hypotensive agent) and ingestion of cyanide salt in the context of suicidal or homicidal attempts, the main sources of exposure are smoke from fires or cigarette smoking, accidental inhalation of hydrocyanic acid in the metal and plastic industries, and ingestion of various types of food such as cassava, cherry, or almond. Blood cyanide concentration is essential information in medicine and forensic science. Although the state of the objects for analysis is completely different, medical and forensic cyanide analyses have the same difficulties:

(i) First, sample storage and pretreatment significantly affect the results of the analysis. Prior to detection, cyanide needs separation from hemoglobin. This separation is most commonly performed by acidification using microdifusion in a Conway cell or nitrogen carrying into an alkaline trap solution. The acidification process is prone to errors due to incomplete releasing or artificial cyanide production. (ii) Second, standard methods for cyanide determination in blood are time consuming and cannot provide adequate data on real time basis. Many of the methods described in the literature are highly sensitive but do not have upper calibration limits high enough to be used in cyanide fatalities. Besides cyanide assay has to differentiate between bound and unbound cyanide to provide data for cyanide antidotes administration.

The postmortem specimens most frequently analyzed for cyanide in forensic toxicology are blood, spleen, liver, and brain. Blood cyanide concentrations lower than 0.25 μg⁄mL are considered normal, and those between 0.25 and 2–3 μg/mL as elevated, but not ordinarily causing death. Concentrations above 3 μg⁄mL are consistent with death in the absence of other relevant or toxicological findings (Gambaro et al., 2007). Animal tissues are other forensic targets for analyzing, especially when illegal use of cyanide compounds in the environment is

concerned (Mak et al., 2005). Therefore, cyanide determination in forensic analysis and cyanide monitoring at very low levels are of great importance (Meng et al., 2009).

The analytical techniques for cyanide detection in blood published before 2004 have been critically reviewed by (Lindsay et al., 2004). Here we present the latest achievements in cyanide determination in biological samples reported (Table 2). In attempt to improve efficiency and accuracy of the sample pre-treatment procedures a hollow fiber-protected headspace liquid-phase microextraction, a headspace single-drop microextraction or solid-supported liquid-liquid extraction combined either with capillary electrophoresis or chromatographic separation were proposed. Interesting approach for cyanide liberation without acidification is an enzymatic degradation of free and complexed cyanide (Mak et al., 2005).

Another research direction is aimed at the development of sensitive and selective detection systems. The lowest detection limit of 0.3 ng/mL was reported for capillary electrophorese with UV detection (Meng et al., 2009). The widest linear concentration range is reported for gas chromatography/mass spectrometry: 0.05–10 μg/mL (Frison et al., 2006) and 0.1–20 μg/mL (Liu et al., 2009). A high selective nafion-modified electrochemical sensor for cyanide determination at physiological pH without separation was described by (Lindsay and O'Hare, 2006), but additional validation in blood samples is needed. Cyanide instability in post-mortem blood samples was studied and sodium fluoride was proposed to be added to blood samples obtained from fire victims to reduce cyanide instability due to bacteriological activity (McAllister et al., 2011).

Chromatography, notably gas chromatography, has been particularly important in the measurement of cyanide in complex, especially biological samples. Uses with MS detectors have already been discussed in the foregoing; here we discuss use with two selective detectors, the nitrogen-phosphorus detector (NPD), and the ECD (Ma and Dasgupta, 2010).

Matrix isolation of analyte cyanide is typically achieved by acidification of the sample to produce HCN. The headspace can then be sampled either directly or via an SPME fiber. Further derivatization is not needed because the NPD responds sensitively to HCN. The GC-NPD approach has been widely used for cyanide determination in clinical and forensic needs. Many methods based on this principle and incremental improvements thereof have been published in the past. While during the period of this review no major novelties in GC-NPD based approaches were reported, the following are noteworthy. Compared results from a classical spectrophotometric method with those from an automated headspace GC-NPD approach and found them to be statistically equivalent. Headspace SPME sampling followed by GC-NPD analysis for the simultaneous determination of cyanide, acetonitrile, cis- and trans- crotononitrile, allylnitrile and butyronitrile at low µg/L concentration on rat and mice blood. The maximum RSD was less than 12% across the analytes and LOD less than 3 µg/L throughout (Ma and Dasgupta, 2010).

The ECD is more sensitive for appropriately derivatized analytes and tends to be more robust and stable than the NPD. HCN does not directly respond to the ECD and must be derivatized. While there was no dramatic new development, there were some important application papers. Applied headspace GC-ECD to measure cyanide and reported on cyanide distribution in various in human blood, kidney, brain, urine, and stomach content. Used chloramine-T to convert cyanide in cigarette smoke to cyanogen chloride, which is taken up in n-hexane, this was then analyzed by capillary GC coupled to a micro ECD. Ma and Dasgupta (2010) utilized the same derivatization principle to determine cyanide in blood except that the derivatization was conducted on a strip of filter paper in the sample vial headspace.

Table 2. Analytical methods for cyanide determination in biological samples

Methods	Samples	LOD, µg/mL	Range, µg/mL	RSD, %	Recovery, %	Reference
Gas chromatography	Whole blood	0.01	0.01-0.2	3-7	84-96	Felby, 2009
Gas chromatography/mass spectrometry	Plasma and urine	0.04	0.1-20	7	91-116	Liu et al., 2009

Electron captured electrophoresis	Urine and saliva	0.26	2.6-520	5.3-7	92-103	Ma and Dasgupta, 2010

5.3 Cyanide determination in plants

The human's health authorities pay special attention on cyanogens as toxic food constituents, as some cyanogenic plants are staple food in some countries and the population is exposed to high level risk of cyanide intoxication. The analysis of plant and the estimation of its cyanogen content have specific problems related to the need of: (i) hydrolysis and separation of cyanogens or produced cyanide from complex matrices, and (ii) sensitive detection systems. Most of the cyanide related diseases are reported in developing countries, so the availability of the analytical devises to small plants farms is of special importance.Some summaries of the methods for cyanogenic glycoside determination (although not exhaustive ones) can be found in (Herchi et al., 2012). Methods for determination of cyanogenic glycosides in plants and cyanide in foods are presented in Table 3.

Table 3. Methods for determination of cyanogenic glycosides in plants and cyanide in foods.

Methods	Samples	Cyanogenic compounds	Analyte	Range, µg/mL	Reference
Spectrophotometry enzymatic assay	Cassava roots	Amygdalin linumarin	Total cyanide	0.08-2.6	Tatsuma et al., 2000
Spectrophotometry picrate method	Cassava flour	Linumarin	Total cyanide	0.1-50	Bradury, 2009
GC spectrophotometry	Sorghum, sudan grass, forage	Dhurrin	Total cyanide	0.04-1.8	Goff, et al 2011

The main trends in the research on cyanogen determination could be summarized as: (i) development of sample pre-treatment procedure suitable for large range of matrices and a great number of cyanogens; (ii) development of efficient cyanide liberation and separation procedures; (iii) development of sensitive and selective detection systems suitable for analyzing small quantities of samples; (iv) development of low cost and easy to maintain equipment.

Cyanogenic glycosides can be determined directly by various chromatographic methods. An advantage of chromatographic method is the quantification of cyanogenic glycosides in their native form. Its wide application is limited for a lack of cyanogenic glycoside standards or their high cost. Indirect cyanogenic glycosides determination, also referred as determination of the plant cyanogenic potential, is based on quantification of HCN released after acidic or enzymatic hydrolysis of cyanogen glycosides (Table 3).

Efficient extraction and complete hydrolysis is the key for accurate determination of plant cyanogens. Spectrophotometric detection after different color formation reactions is the most widely used in total cyanogens determination: picrate paper assay (Bradbury and Denton, 2011), picrate based solid state detection (Abban et al., 2011); combined picrate/resorcinol method. Recently, the nynhidrin based method has specially modified for determination of total cyanogens in plants (Surleva and Drochioiu, 2012). A spontaneous enzymatic hydrolysis (at pH 6-8) was combined with extraction using bicarbonate solution or microdiffusion separation. The method is fast, cheap and environmentally friendly. Non-toxic reagents have been used. No special training or sophisticated instrumentation was needed.

6. Conclusions

This seminar paper provides a good example of how the demands of ecology, forensic science and medicine motivate the research and development of new analytical methods and instrumentation. Rapid cyanide analysis in blood or breath is ripe for new attractive approaches. There are fast acting antidotes for cyanide poisoning, whether from smoke inhalation or exposure to a weapon of terrorism. It is vital to determine blood or breathe cyanide levels fast and accurately so that an appropriate dose of the antidote can be readily determined. Physiological

half-life of free cyanide is short and concentration can be affected by storage conditions and many other factors. It is crucial to rapidly analyze such samples, if it possible in situ. The same demand is imposed also by ecology. Due to different toxicity of industrial cyanide containing pollutants, different detoxification procedures have to be applied so that the ecological equilibrium will not be disturbed at a large scale.

Quickly available and highly reliable information about cyanide contamination is required for this purpose. Because of the importance for clinical, forensic and very likely, security and antiterrorism applications, it has become urgent to establish rapid, sensitive, specific and robust ”point of care” cyanide analyzers.

The new colorimetric/fluorimetric probes working on “turn-of-and-on” principle have a lot of promise to be used in small alarm devices or spot tests. However, a lot of research is needed to validate them in real samples, e.g., air, natural waters, industrial wastewater, biological fluids like urine, blood, saliva, etc.

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8. References

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