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Public Analysts in their modern role as Scientific Advisers provide analytical and consultative support for Local Authorities for the many services required by the Ratepayers. The Public Analyst has, therefore, an interest in a very wide range of local authority functions in addition to his traditional statutory responsibility for the safety and quality of food. This Annual Report seeks to illustrate the extent to which those responsibilities have expanded.

In spite of the reduced resources currently available, new legislation and proposals continue to be generated, influencing the day to day work of the Public Analyst.

The most significant and serious impact on the work of the profession in 1980 has undoubtedly been the reduction in resources and the consequent lowering of levels of protection afforded to the general public. There is no doubt that it is not the generation of legislation which protects the consumer, but the effective enforcement of it. It is ironic that these levels of public protection should be lowered at a time when the attractions to circumvent the law are at their strongest, and the quality of products and systems for their control are most at risk.

In order to make enforcement more cost-effective, Public Analysts have played a leading role in the co-ordination of some of the enforcement effort between Food and Drugs Authorities. Over 30 joint projects had been completed in collaboration with Trading Standards Officers and London Environmental Health Officers to prove the feasibility of the system before recommendations were made to the Local Authorities Committee on Trading Standards (LACOTS) that a measure of national co-ordination of enforcement could be adopted.

The Association is conscious of its responsibility to make a professional contribution to Europe and in a specifically scientific area has identified equivalent enforcement laboratories in Europe. The Council of the Association has undertaken the publication and distribution of a quarterly European Bulletin to provide a forum for discussion between scientists within the Community. The ready exchange between scientists which the Bulletin is designed to promote could, it is believed, be invaluable in helping to establish satisfactory methods of analysis for incorporation into Community legislation.

In a similar connection, the Association, together with the Environmental Health Officers' Association, the Institute of Food Science and Technology and the Institute of Trading Standards Administration, has sponsored the establishment of the United Kingdom section of the European Food Law Association. The main conference of EFLA was held in October, this year in Madrid, when

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members of the Association of Public Analysts took an active part. This new link will provide a sound basis upon which a positive contribution can be made in the development of future legislation in the Community.

A feature of the generation of legislation in the United Kingdom is the consultation between the Ministry of Agriculture, Fisheries and Food, the organisations involved in enforcement and other interested bodies. The Association has provided professional comment on all relevant proposals throughout 1980, including the new Food Labelling Regulations which embody many aspects of European philosophy, without sacrificing the consumer's right to be informed, in order that a value-for-money judgement can be made at the time of purchase.

Official methods of analysis are a feature of the EEC legislation, and it is vital that these official methods are accurate and reliable. Detailed negotiation and trials of the methods are essential parts of legal surveillance and the Association is pleased that the Ministry of Agriculture, Fisheries and Food is making use of the experience and expertise of Public Analyst laboratories in funded collaborative studies. Recent experiences with a method for the determination of a food preservative, 2-hydroxy-biphenyl, illustrated the difficulties, which in this case had resulted from translation errors. The Association is gratified to note that this particular problem has at last been resolved as a consequence of its representations.

Most significant changes in U.K. legislation are usually preceded by published reports of expert committees which make recommendations to Ministers on any changes that are required. Such a Report was produced by the Food Standards Committee of the Ministry of Agriculture, Fisheries and Food, in 1980, following several years' deliberation on the very vexed question of the composition of meat products. As early as 1975, the Association made representations to the Ministry expressing its concern at the unsatisfactory nature of the legislation and giving evidence for the deterioration in quality of meat products in general. The incorporation of water and other non-meat materials, and new methods of processing meat products, are the cause of considerable concern to this Association and it notes with satisfaction that these matters are largely covered by the main recommendations of the FSC Report which, if adopted, will substantially improve the situation. The Association will continue to do all in its power to ensure that the consumer's right to make an informed choice is upheld and preserved.

Changes in legislation produce a steady demand for the development of new analytical methods by Public Analysts, if the law is to be adequately enforced and the consumer properly protected. During 1980, such developments have been necessary in a variety of areas. In food for human consumption, new general regulations halved the maximum permitted levels of lead as a contaminant, and collaborative trials for the determination of trace metallic contaminants have highlighted many problems, which are being resolved, as regards the accurate determination of these elements at very low concentrations. Public Analysts are also having to turn their attention to other contaminants which may arise from food packaging materials, particularly residues from PVC plastic, under the Materials and Articles in Contact with Food Regulations.

An aspect of bacterial contamination which again came to light in 1980 was the detection of histamine-like toxins produced in Scombroid fish due to pre-processing spoilage. Such fish, including canned sardines, canned tuna and smoked mackerel, were identified as containing excess levels of toxin, as a consequence of which they caused sudden outbreaks of food poisoning among susceptible consumers.

The Association has been represented on committees with the Ministry of Agriculture, Fisheries and Food, along with other interested parties concerned with new legislation and particularly with special methods of analysis for fertilisers, and feeding stuffs in the area of medicinal additives in animal feeds. Work on unsolved problems in Vitamin A determination is continuing. The presence of mycotoxins in feedingstuffs has been highlighted by the findings of Public Analysts and also by the "Survey of Mycotoxins in the United Kingdom" (4th Report by the Steering Group on Food Surveillance: Working Party on Mycotoxins, available from Her Majesty's Stationery Office). As a result, the importation of certain materials containing the mycotoxin aflatoxin, has now been banned. The great concern over the occurrence of the carcinogen aflatoxin in animal feeds and animal feed ingredients, where parts per billion of the compound have to be detected, illustrates the current demand for very high levels of analytical expertise. It is anticipated that work on mycotoxins will be of increasing importance.

For nearly a century, the control of the quality of drugs on retail sale was covered by the same Act of Parliament as the sale of food. The Medicines Act separated the control of Drugs from that of Food, but it failed to implement any continuation of the control of drugs on retail sale. Hence after 6 years there is still no provision for the sampling and analysis of drugs on the "General Sales List"—that is, sales "over the counter". The public has therefore lost the protection, which it has a right to expect, against poor quality or out-dated or deteriorated stock. The Association is firmly of the opinion that this should be a Local Authority function which is completely independent of pharmaceutical interests and is making appropriate representations.

Waste disposal is just one of the many aspects of pollution control in which members of the Association have an important contribution to make. Most of the waste generated in the country is put back into the land under controlled conditions. Highly polluted leachates which may come from these landfill sites must be monitored regularly, and methods of treatment devised and put into operation. Even before a site is designated for this purpose, scientific investigational work involving tracer tests, ground and surface water drainage, and other pollution factors is carried out in order to assess its suitability for the intended purpose, and subsequently for the re-development of the completed landfill sites.

Under the Deposit of Poisonous Wastes Act, 1972, the objective has been to ensure that toxic and dangerous wastes have been disposed of in a manner which will not give rise to any environmental hazard, including poisoning and pollution. Public Analysts provide a service which includes analytical checks on materials either deposited or to be deposited, as well as liaison with Industry and the gathering of information regarding toxic wastes.

In November 1980, the Control of Pollution (Special Wastes) Regulations received royal assent, and will make it even more essential for scientific control of the highest calibre. The questions whether a particular waste is "special" within the meaning of the Regulations, or its constituents are in sufficient concentration to be dangerous to life, require much analytical work followed by a sound interpretation of toxicity data. Within some Local Authorities, Public Analysts are the only persons qualified in these subjects. Many of our members are already involved in this work and in more general matters of environmental pollution, and provide services for monitoring of air pollution, the re-use of land, and the quality of water supplies. In this latter context, it is anticipated that Public Analysts must necessarily be involved with the new water quality Directive emanating from the European Community and which in due time must be enforced in the United Kingdom.

The realisation of the hazards arising from asbestos dust, particularly during the stripping of lagging from boilers, etc., and problems with other insulation materials, has led to the need for examination of large numbers of specimens of insulating material to safeguard the health of operatives. Hazards can also arise in schools, offices and other places of work as well as in domestic premises where asbestos may have been used. This has involved members of the Association in an additional heavy burden. During the Year, the Association, through the Royal Society of Chemistry, has contributed comment on a Consultative Document which was issued by the Home Office on changes in the law relating to "breathalising" motorists suspected of driving with excess alcohol in the blood. A great deal of work has gone into the development of instruments which will give "instant" readings of the alcohol in the breath of the suspect as an indication of blood alcohol level. The Transport Bill at present before Parliament seeks to legalise their use. The basic objections on which representations were made by the Association of Public Analysts in association with the RSC are that (a) the suspect is robbed of the safeguard against wrongful conviction afforded by the present law, in that there can be no independent analysis of the specimen, (b) no instrument is consistently infallible, and (c) the correlation between the concentrations of alcohol found in the breath and that present in the blood is poor, and successive breath readings can vary by as much as 20 per cent.

Public Analysts are constantly aware that they were originally appointed with the specific and unfettered duty to protect the consumer in all scientific matters of determination and interpretation in relation to the food we eat. This Annual Report illustrates and emphasises the extent to which those responsibilities have necessarily expanded, and it spotlights those matters of current concern to the profession on behalf of the public at large, if those terms of reference are to be met effectively across that wider field of responsibility.

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Headspace Gas Chromatography for the Analysis of Vinyl Chloride and Other Monomers in Plastic Packaging and in Foods*

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A generalised review is presented covering the types of plastics used for various food packaging applications in the United Kingdom, and the typical levels of residual monomers (vinyl chloride, acrylonitrile, vinylidene chloride and styrene) to be found in the packaging and in the foods themselves. The analytical method of choice for monomers is undoubtedly headspace gas chromatography and for this technique, both the practical experimental difficulties and likely sources of error are discussed, with particular reference to the European Economic Community (EEC) method of analysis for Vinyl Chloride in PVC and in Foods. Examples to illustrate the sensitivity, specificity and precision of headspace monomer analysis have been drawn both from the authors' own experiences and from recent participation in UK and EEC collaborative trials.

The contamination of foods and beverages by trace amounts of materials which have migrated from plastics used in their packaging is of concern both on the grounds of adversely affecting product quality and of food safety. A large number of substances represent potential sources of migration ranging from unpolymerised monomers[1–4] or oligomers[5] to auxiliary agents incorporated in the plastics formulation to facilitate stability, production and processing of the material; these might include antioxidants[6], U.V. stabilisers[7], plasticisers[8], lubricants[9] and heat stabilisers[10] depending on the type of plastic and its application.

However, the detection in beverages of vinyl chloride in 1974[11], associated with earlier findings on its carcinogenicity[12], has tended to centre attention on the migration of monomers. Vinylidene chloride[13], acrylonitrile[14] and styrene[15] have by analogy evoked a similar interest but the main developments in terms of both methods of analysis and of monitoring plastics and foodstuffs have been for vinyl chloride. This impetus has been further sustained in the European Economic Community (EEC) by the introduction of statutory limits for residual vinyl chloride in plastics and foods, and the therefore necessary development of a legislative method of analysis capable of the required sensitivity and precision. The EEC Directive[16] stipulates a maximum permitted limit of 1 mg/kg of residual vinyl chloride in materials and articles intended for food

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contact, and a limit of 0·01 mg/kg of vinyl chloride released from packaging into foods. Allied to the Directive is a method of analysis for plastics and foods[16] using headspace gas chromatography and this method has been the subject of many collaborative trials both within the EEC and amongst Public Analysts within the United Kingdom.

It has only been since the adoption of this Directive[16] and of the earlier more general Materials and Articles in Contact with Food Regulations[17] that the Public Analyst has generally been involved in any consideration of plastics migration or, therefore, with any of the associated problems of plastics analysis and the headspace analysis of these contaminants in foods. Hence the object of this review is to provide something of an introduction to this subject by giving some generalised background information on the usage of plastics for food packaging, to provide data on the likely typical levels of monomers in plastics and foods and to discuss the experimental difficulties and sources of error of this required methodology. The comments are made particularly with respect to vinyl chloride although the individual problems associated with the analysis of different monomers are outlined as these may become the future subject of legislative control.

Types of Plastics used for Food Packaging

It is notoriously difficult to obtain information on which plastics are used with what foods, because of commerical secrecy, because of the constantly changing pattern of usage for economic reasons, and also because of the fragmented nature of the industries involved. However recent publications from The Ministry of Agriculture, Fisheries and Food's Working Parties on vinyl chloride[18] and vinylidene chloride[19] have provided some valuable data on the food packaging applications of these plastics and it is possible to make some generalised statements in particular with respect to plastics containing monomers of interest (for this reason polyolefins have been excluded). A simplified picture is shown in Table I which, although by no means exhaustive, does give some of the major uses of plastics for food packaging. For flexible films, however, the situation is further complicated by their use in combination with one another, for example as coatings, as co-polymers and as laminates where complex systems are frequently necessary to achieve desired physical properties for specific applications. In addition to retail food packaging one should also be aware of the potential for contamination of foods arising from contact with plastics during food processing (e.g. plastic piping, processing equipment), during bulk storage and at the other extreme, from contact with plastics in the consumer's home during food preparation in the kitchen.

Headspace Gas Chromatography for Monomer Analysis

The monomers of primary concern to date have been vinyl chloride (VC), vinylidene chloride (VDC), acrylonitrile (AN) and styrene, and in each case the preferred method is that of headspace gas chromatography, using, where possible, a specific detector either to eliminate interferences or to increase sensitivity, or both. The analysis of styrene in foods can however require an

alternative approach because of factors which will be more fully discussed later.

Headspace gas chromatography consists, essentially, of placing the sample for analysis in a closed vessel, allowing the compound of interest to equilibrate between the sample and the surrounding vapour usually with heating in order to obtain rapid equilibration) and finally to inject a sample of this equilibrated "headspace" into a gas chromatograph. (The term "equilibration" has been emphasised because this is an essential condition for quantitative analysis.) It is possible to perform the necessary operations manually, but automated chromatographs are available dedicated to headspace chromatography, which combine the advantages of automated sample handling with enhanced reproducibility[20,21].

Two major advantages of headspace chromatography make this technique especially suitable for the analysis of monomers. Firstly, in contrast to direct injection techniques[22, 23] (where, for example, a 10 per cent. solution of a plastic in a suitable solvent is injected directly onto the column) only volatile species enter the chromatograph, so there is no danger of contamination of the front of the GC column with polymer or involatile constituents of foods. Secondly, a much increased sensitivity is possible because, depending on the distribution coefficient of the monomer between food/plastic and headspace, considerably larger amounts of monomer may be loaded onto the column using normal injection volumes of 0·25 to 1·0 millilitres of vapour. Naturally, the less volatile the monomer, the less this advantage becomes. Furthermore, if a fat or other lipophilic phase is present in the sample/headspace system, then the partition coefficient is likely to become less favourable and thus the sensitivity of the method would again be reduced. Consequently, of the four monomers mentioned above, styrene is the least amenable to headspace analysis and where only very small amounts are present, such as is normally the case for foods, an alternative method of analysis (involving concentration by steam distillation prior to gas chromatography) may become necessary.

Foods also present problems in terms of interfering volatile components and it is correspondingly important to verify the assignment of any gas chromatographic peak as being due to the monomer in question. Normally this would be done by employing columns with different GC stationary phases but where a mass spectrometer is available, the technique of single—or multiple—ion monitoring of the chromatogram offers both a powerful confirmatory test and the added advantage of increasing the sensitivity of the analysis.

DIFFICULTIES AND SOURCES OF ERROR IN MONOMER ANALYSIS

Although headspace GC is basically simple there are a number of seemingly minor but nevertheless vital precautions to be taken to obtain reproducible and accurate data. The initial sampling strategy, for example, is more critical than for alternative methods. With plastics it is usual to take 200-mg samples but it must be appreciated that the distribution of residual monomer will vary in the article being assayed, depending upon the varying thickness and/or processing temperatures of the package. Thus, for bottles, the neck and shoulder is usually considerably thicker than the walls. Hence, although the concentration of

monomer might be expected to be reasonably constant in the polymer resin used for manufacture of the item, processing will cause monomer to be lost in differing degrees from the various parts of the article.

With liquid foods (5-gramme samples frequently used) there is little problem but it should be recognised that most monomers can readily be lost from the sample while it is being dispensed. In addition, any sample being stored before analysis must be held in a completely full container, otherwise much of the monomer will be lost into the headspace of that vessel, prematurely. This also applies to standard solutions. With solid foods there is again the question of obtaining a representative sample. Particulate samples can be broken into small pieces but bulk foods such as margarine must be well homogenised, preferably under liquid nitrogen to avoid monomer losses during this process. Without homogenisation it would be expected that food close to the edge of the container might show higher levels of monomer.

It is usually necessary to dissolve plastic samples in some suitable solvent in order that headspace equilibrium may be attained in a reasonable space of time. This too can cause problems as some plastics are only partially soluble and all normally require several hours swelling in the solvent (frequently overnight) before solution can be assured. For some plastics e.g. VDC coatings on a cellulose substrate, dissolution is not possible and a "hot-jar" technique is used where the film is directly heated. Here it is necessary to obtain a balance between promoting rapid equilibration of the monomer and avoiding polymer degradation leading to artefact formation.

The major difficulty with manual headspace analysis lies with the actual injection of headspace vapours into the GC column. Great care is essential in order to obtain a representative sample of the headspace gas. Firstly, it is necessary to ensure that the gas syringe used is at or above the temperature of the vapours in order to avoid condensation of the volatiles on the barrel of the syringe. If this cannot be arranged because of limitations due to syringe construction, then the syringe should be as hot as possible. In all cases the plunger of the syringe should be "pumped" several times both on sampling from the headspace vessel to ensure a representative sample, and between injections with clean air to minimise "ghosting" from any adsorption effects on the glass walls of the syringe.

It is preferable to use a syringe designed to enable pre-pressurising of the vapour sample to that of the GC carrier gas before injection. Sharper GC peaks are obtained with this technique and the risks of sample loss through "blow-back" through the inlet septum are reduced. However, all these problems can be avoided, of course, by the use of an automated headspace chromatograph if the expense can be justified.

Other difficulties that might be experienced are interference peaks from a number of sources. Perhaps the least obvious of these arises from contamination of the laboratory atmosphere. When a sample vial is closed, a substantial volume of potentially contaminated atmosphere could be included with the sample in this manner. It is particularly important not to seal the vials in a laboratory where quantities of neat monomer have been handled. Vials should stand in a clean environment at least 10 minutes before being stoppered or,

preferably, the samples dispensed in an area free of monomers and other potential interferences. Another source of interference peaks is the septum used to seal the vials. When heated these may liberate volatiles and in some cases it may be necessary to use PTFE-faced septa. For the plastics analysis, care should also be taken over the purity of the solvents and, for example for the N,N-dimethylacetamide used for PVC analysis it may be necessary to purge the solvent with nitrogen gas for some time to remove volatile impurities which could co-chromatograph and interfere with the vinyl chloride determination.

One other practical point concerning the chromatography is that some samples, particularly foods and normally all the solvents used for dissolving the plastics, may give rise to headspace components which elute only very slowly from a GC column and which are liable to interfere with subsequent injections. Two remedies are available, "baking-out" the column or using a back-flush technique. The latter is usually preferable, especially with automated systems when highly reproducible retention times are required for computer data processing.

CHOICE OF GC DETECTOR FOR MONOMER ANALYSIS

For VC analysis, flame ionisation has been the detector of choice[24,25] the ECD (the normal detector for halogenated compounds) being relatively insensitive to vinyl chloride unless operated in a chemically sensitized mode[26] (incorporating nitrous oxide in the carrier gas). With an FID a detection limit of 0·1 mg/kg VC in PVC and between 0·002 and 0·005 mg/kg VC in foods is easily attainable. Figure 1 shows a typical chromatogram illustrating the determination of VC in a fruit drink (data from this laboratory's participation in the EEC 3rd collaborative trial on vinyl chloride in foods). These chromatograms, obtained using an automated headspace gas analyser (Perkin Elmer F42) show characteristic negative peaks after injection due to a pressure pulse in the chromatographic system. The VC peak is eluted rapidly, and from a comparison with the unspiked food can be seen to elute in a clear early region of the chromatogram. For aqueous systems, the distribution coefficient of VC between food and headspace is highly favourable to the latter and a limit of detection of 0·002 mg/kg is easily achieved. The internal standard shown in Figure 1 was diethyl ether the use of which with an automated method of analysis was however found to offer little advantage in terms of improved reproducibility, but for manual techniques its use would be regarded by the authors as essential. In Figure 2, from the same EEC collaborative trial, the standard addition curve is shown for VC in salad oil from which the oil was estimated to contain 0·018 mg/kg VC (spiked by the trial organiser with 0·02 mg/kg). Good straight line standard addition curves are readily obtained with the EEC method of analysis, replicate additions of VC to the oil giving almost identical VC peak heights. The less favourable partition coefficient for VC in oil gives a less favourable limit of detection compared with aqueous foods of around 0·005 mg/kg. For the determination of acrylonitrile in ABS plastics and in foods the nitrogen specific alkali flame ionisation detector (AFID) has been universally employed[14, 27, 28] offering both improved specificity and increased sensitivity over the FID. In

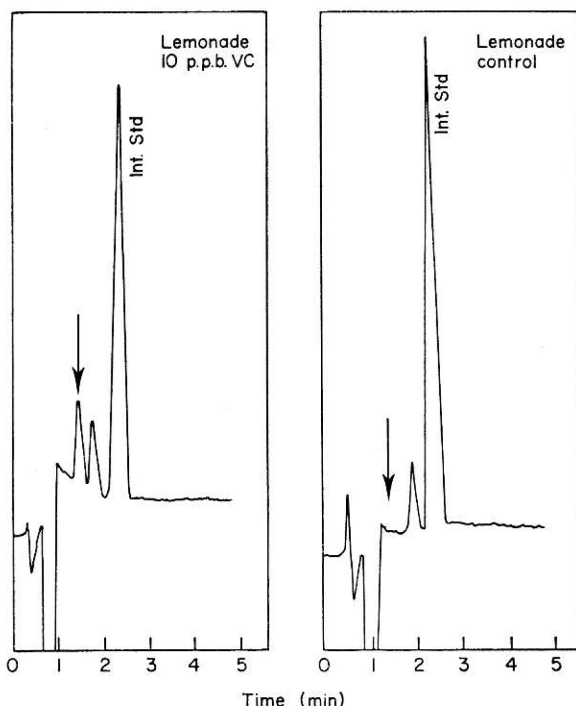

Fig. 1. FID chromatogram showing vinyl chloride in lemonade (from the 3rd EEC Collaborative trial on methods of analysis for VC in foods). Perkin Elmer F42 automatic headspace analyser operated under the following conditions: column 3 m × 2 mm id stainless steel packed with 25 per cent. di-*iso* decylphthalate + 0·5 per cent. Atpet 80 on Diatomite CAW (60 – 70 mesh) with nitrogen carrier at 35 millilitre per minute operated isothermally at 85°C. The sample was equilibrated at 60°C for >2 h prior to analysis. (Injection time =9 s, Analysis=2·2 min, Backflush=2·2 min) GC attenuation 1 × 4. Internal standard – diethyl ether.

Figure 3 typical chromatograms are shown for AN determined in soft margarine and in an ABS tub, both results obtained during the course of a recent survey of retail foods packaged in ABS. An internal standard, proprionitrile, has been used in this instance to compensate for any slight change in the response of the AFID which might occur over long periods – which would be the case for unattended operation of the automatic headspace analyser.

For VDC the detector of choice has been the ECD[3, 13, 29], where for analysis of film VDC quantification was possible at 0·001 mg/m^2; this was equivalent to between 0·04 and 0·06 mg/kg depending on the film grammage and for foods the detection limit was 0·005 mg/kg[13]. Foods could be analysed in the preferred manner by automatic headspace equipment, but for films the small vial capacity did not allow sufficiently large film areas to be sampled to be fully representative of the packaging material and a manual technique was therefore employed.

As mentioned earlier, styrene is not an ideal candidate for analysis by headspace chromatography, for with a boiling point of 145°C its volatility is low by comparison with VC, VDC or even AN (boiling points—17°C, 37°C and 78°C respectively). However, as concentrations of styrene in polystyrenes and

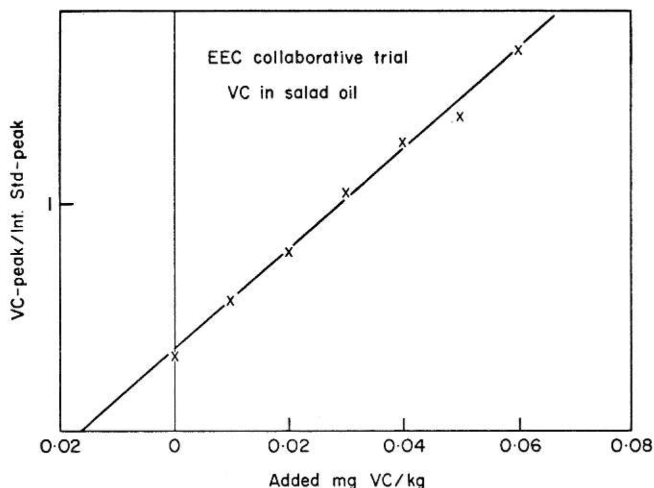

Fig. 2. Standard addition curve for the determination of vinyl chloride in salad oil (from the 3rd EEC Collaborative trial on methods of analysis for VC in foods). GC conditions identical to Fig. 1. Extrapolated level of vinyl chloride present = 0·018 mg/kg compared with a spiking level of 0·02 mg/kg.

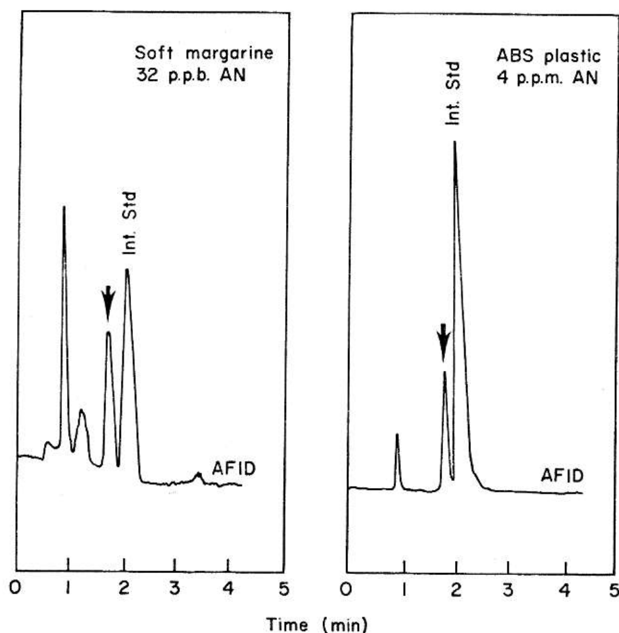

Fig. 3. AFID chromatograms of acrylnitrile: soft margarine and an ABS plastic tub (unpublished results from Ministry of Agriculture, Fisheries and Food Working Party on Acrylonitrile and Methylmethacrylate survey of retail foods 1979). Perkin Elmer F42 automatic headspace analyser operated with an AFID under the following conditions: column – 2 m × 2 mm id stainless steel packed with 0·2 per cent. Carbowax 1500 on Carbopack C (80 – 100 mesh) with nitrogen carrier gas at 40 millilitre per minute operated isothermally at 90°C. The sample was equilibrated at 70°C for 1 h prior to analysis. (I=9 s, Analysis=2·5 min, BF=2·5 min) GC attenuation: × 16 for margarine; × 128 for ABS. Internal standard – proprionitrile.

copolymers are relatively high, the limit of detection of 1 mg/kg styrene which can be achieved by a modified solution headspace approach[30] is quite adequate. This modified technique involves dissolving the polymer in an organic solvent (N,N-dimethylacetamide) in the normal manner and then altering the composition of the solvent phase to decrease styrene solubility and thereby increase its equilibrium concentration in the headspace. For this purpose water was found to be the most effective solvent[30], normally being free of interfering organic contaminants and not being sensed by the GC—FID. Linear calibration curves are obtained despite the consequent precipitation of polystyrene. The modified headspace approach has also been applied to 2-ethylhexylacrylate (b.p 214°) in polymers[30] and to the determination of 1,1,1-trichloroethane (b.p. 74°C) in PVC resins and bottles[31].

For the determination of styrene in foods, the limits of detection of headspace GC are generally inadequate for most practical purposes ranging from the most favourable case of yoghurt 0·02 mg/kg[32], to the most unfavourable of a high fat content food, say soft margarine with a limit of 0·350 mg/kg[32].

This problem has been overcome by the use of distillation techniques[33,34] as a preconcentration prior to either conventional GC[33,34] or LC[15] analysis, but these methods tend to be time consuming, involve large sample sizes and are generally inconvenient for food survey purposes. The mass spectrometer, however, is now routinely used in many laboratories as a highly selective chromatographic detector for quantitative measurements in either single- or multiple-ion monitoring modes, offering the added advantage of much increased sensitivity over the FID.

Using these mass spectrometric techniques, identification and quantification of monomers in plastics and in foods have been previously reported,[35,36,37] but used primarily as a confirmatory method. The increased sensitivity of detection attainable by this method over an FID is, however, such that for styrene in foods, the headspace approach can then be employed achieving limits ranging from 0·001 to 0·015 mg/kg[32]. This work was carried out with an automatic headspace analyser coupled direct to the mass spectrometer, using m/z = 104 (the molecular ion of styrene) for detection[32], which is the preferred method for its combination of rapid throughput of samples and precision of results.

Typical Monomer Levels in Retail Packaging and Foods

It is obviously difficult to generalise about levels of monomers likely to be found in retail packaging and foods, but as there have been many surveys of the retail outlets in the United Kingdom, it is possible to summarise the situation in broad terms as shown in Table II. There will of course, be exceptions to these ranges but nevertheless the general indication of likely levels is a useful starting point for the analyst new to this type of problem. For vinyl chloride in a 1978 survey (Ministry of Agriculture, Fisheries and Food—unpublished survey results) of 24 retail bottles analysed for VC, 23 contained less than 0·1 mg/kg and 22 contained less than 0·4 mg/kg. Ten out of 11 PVC tubs examined contained less than 0·6 mg/kg. These results contrast sharply with the situation in 1974 when a typical level in a PVC bottle was 50 mg/kg[18], although this level

TABLE I
EXAMPLES OF PLASTIC PACKAGING AND FOODS PACKAGED

Polymer	Form	Examples of food applications
PVC*	Bottle	Orange drink, cooking oil, mineral water, draught beer
	Rigid film (nesting)	Butter, potato salad, cakes, biscuits and chocolates
ABS	Tub	Soft margarine
Polystyrene	Tubs	Diary products—(yoghurt, cream, cottage cheese) honey, chocolate spread
	Trays	Fresh meat, fish, fruit
Poly (vinylidene chloride)*	Film coating	Biscuits, confectionery, snack foods, cheese, dried foods, cooked meats
	Bag or chub (co-polymer)	Pâté, black pudding, natural and processed cheese, gammon, ham, cooked meats
Poly (ethyleneterephthalate)	Bottle	Carbonated beverages (lemonade, cola, etc.)

*For more detailed information see references 18 and 19.

had fallen sharply by 1975 to 3 mg/kg through the introduction of improved manufacturing practices at the polymer resin processing stage[18]. For foods in the 1978 survey VC was not detectable in 34 of the 35 samples analysed, and only just measurable at 0·003 mg/kg in one sample of a concentrated orange drink.

For acrylonitrile in ABS tubs, levels of AN are in the range indicated in Table II, the majority being at the lower end of the scale and similarly for the contained soft margarines. Concentrated butter for cooking and lard, also packaged in ABS, likewise showed levels of AN within these ranges. For VDC in films, the levels measured in a number of surveys have been very low and for PVDC coating on cellulose normally below the limit of detection ($<0{\cdot}001$ mg/m^2). For the foods packaged in films, VDC has never been detectable at a limit of 0·001 mg/kg, but has been just measurable in chub pack products (e.g. black pudding, liver pâté, etc.) at between 0·005 and 0·01 mg/kg. Only very limited data is currently available for styrene levels in retail packaging and in foods packaged in polystyrene. It is generally recognised that styrene levels (as indicated in Table II) in plastics are significantly higher than those found for the other monomers, partly because of polymer solubility in the monomer and because of facile depolymerisation which occurs on heating polystyrene. The limited data available for retail foods however shows that styrene levels are not

TABLE II
TYPICAL MONOMER LEVELS IN RETAIL PACKAGING AND FOODS

Monomer	Application	Typical range in polymer *p.p.m.*	Foodstuff	Typical range in food *p.p.m.*
Vinyl chloride†	PVC bottles	0·1 – 0·6	Orange drink	<0·002 – 0·005
Acrylonitrile	ABS tubs	1·5 – 10·0	Soft margarine	<0·01 – 0·04
Vinylidene chloride‡	PVDC/PCV copolymer*	0·02 – 0·3	Liver pâté	0·005 – 0·01
	PVDC/PP film*	0·2 – 1·0	Biscuits	<0·005
Styrene	ABS tubs	100 – 300		
	Polystyrene tubs	350 – 1200	Single cream	0·005 – 0·04

*PVC Poly (vinylidene chloride); PP Polypropylene.
†Reference 18.
‡Reference 13 and 19.

similarly proportionally higher than for other monomers, and for example levels ranging from 0·005 to 0·04 mg/kg have been found in single cream which confirm survey data produced elsewhere[4,34].

Concluding Remarks

In conclusion, it can be said that if a few precautionary measures are taken, headspace gas chromatography offers a combination of simplicity and sensitivity which ensures that it will be the method of choice for most monomer analyses in both plastics and foods. In particular, the EEC method of analysis for vinyl chloride has evolved through a number of collaborative trials, and the methodology requirements for reproducibility and detection limits are based on practical analysis of foods and plastics, although it must be said that analyst performance with these methods does show significant improvement with increasing experience. The usefulness of headspace techniques is not, however, limited to monomer analysis[38] and as experience is gained in the methodology, and its use becomes more widespread, no doubt further applications for trace analysis of volatile organics in food will become apparent.

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Uses of Enzymes in Analytical Chemistry*

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The paper reviews the basic features of enzyme-catalysed reactions, and distinguishes between the optimal conditions required for monitoring enzyme concentrations (and, therefore, those of inhibitors and activators) and substrates. Recent advances are emphasised, including insolubilised enzyme and bacterial electrodes, enzyme-based sample decomposition, drug and pesticide detection, and apo-enzyme activation, as well as more common applications.

Enzymes are naturally-occurring protein-based macromolecules which catalyse reactions of great selectivity and sensitivity. There are several general categories of enzyme such as those which catalyse particular redox reactions, or cause hydrolysis of certain molecules. Individual enzymes include lactate dehydrogenase, which catalyses the oxidation of lactate to pyruvate, and glucose oxidase, which catalyses the oxidation of glucose by oxygen to gluconic acid. Well over 1000 enzymes are known.

The glucose oxidation (which occurs in aqueous solution with molecular oxygen at room temperature and forms hydrogen peroxide) is a good illustrative example of how very selective enzyme reactions are. Any normal chemical oxidation of glucose would produce a range of products or even gross degradation of the molecule, and would also convert other, similar molecules. The enzyme reacts only with glucose and produces only one product.

One of the earliest enzymes to have its structure worked out was lysosyme, a small enzyme with a molecular weight of only just above 14 000 (some enzymes have much larger molecular weights). The very first was ribonuclease, in 1963, which has 124 amino acid residues in its chain, lysozyme has 129. A spatial model of the enzyme shows a cleft in the molecule (the active site) into which a polysaccharide molecule fits neatly.

Some enzymes are more complicated and have a metal ion as part of the structure. The metal usually is found within the active site so that it participates as a redox partner or by co-ordination in the reaction with the reactive species. Alkaline phosphatase (which catalyses the hydrolysis of phosphate esters in alkaline solution) contains a zinc ion bound to three different nitrogen atoms in the protein chain. Zinc is also present in alcohol dehydrogenase. The other metals which commonly occur in enzymes are the metals which are usually referred to as essential trace elements (Cu, Mn, Mo, etc). Metals which usually have a redox function occur in enzymes which have a redox function. Zinc

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ions, which normally have no redox properties, often occur in enzymes which cause hydrolysis.

The methods used for monitoring enzyme catalysed reactions can involve many of the standard instrumental techniques of analytical chemistry[1,2]. Spectrophotometry, spectrofluorimetry, electrochemical techniques and thermometric measurements are all widely used. For example, for simple measurement of an alkaline phosphatase-catalysed reaction, colourless *p*-nitrophenylphosphate may be hydrolysed to the yellow *p*-nitrophenolate ion, which enables the reaction to be followed by spectrophotometric measurement of the yellow colour. Oxidations involving oxidases (for example, of amino acids) produce peroxide. This can be measured by reaction with reagents which are oxidised to coloured products (e.g. *o*-dianisidine) or with homovanillic acid to give a fluorescent product. It is essential that the monitoring reaction is much faster than the enzyme-catalysed reaction.

There is a group of oxidative enzymes called dehydrogenases which differ from oxidases inasmuch as they cause oxidation by the participation of a co-enzyme (often nicotinamide adenine dinucleotide, NAD^+). The co-enzyme is an essential reagent in the enzyme system. It takes up a position on part of the active site. The substrate (reacting compound) reacts with the co-enzyme in the presence of the enzyme to form the product and simultaneously forming reduced co-enzyme, NADH. An example is the metabolism of alcohol, where it is decomposed under the influence of alcohol dehydrogenase and NAD^+:

$$C_2H_5OH + NAD^+ \rightleftharpoons CH_3CHO + NADH + H^+$$

Such reactions can be monitored by measuring the NADH formed. The spectrum of the NAD^+–NADH system shows a peak at 240 nm for NAD^+ and NADH and another 340 nm for NADH only. Thus NADH can be measured spectrophotometrically at 340 nm. NADH is also fluorescent so can be measured using a spectrofluorimeter, with a gain of two orders of magnitude in sensitivity compared to spectrophotometry.

Enzyme activity is defined by the Internation Unit, which is the amount of enzyme which catalyses the conversion of 1 μmol of substrate per minute under optional conditions.

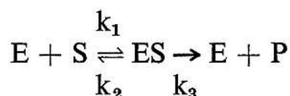
Michaelis–Menten Equation

The mathematical equation which for many years has been used to describe the kinetics of enzyme-catalysed reactions is the Michaelis–Menten equation:

$$v = \frac{V_{max}\,[S]}{K_m + [S]}$$

where v is the rate of reaction (e.g. rate of formation of product), when the substrate concentration is [S], K_m is a constant (known as the Michaelis constant) and V_{max} is the maximum rate attainable, which can be achieved when there is a large excess of substrate, and is proportional to the initial enzyme concentration $[E]_0$. This treatment assumes that a substrates first of all interacts with an enzyme E to form an addition compound ES. The compound in the

active site then reacts to form a product P and release the enzyme, so that it continues to function:



$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

When the above equation of rate against substrate concentration is plotted, as [S] increases, v increases until it becomes constant at V_{max} (Figure 1). K_m is the substrate concentration which gives a rate equal to 0·5 $V_{max.}$

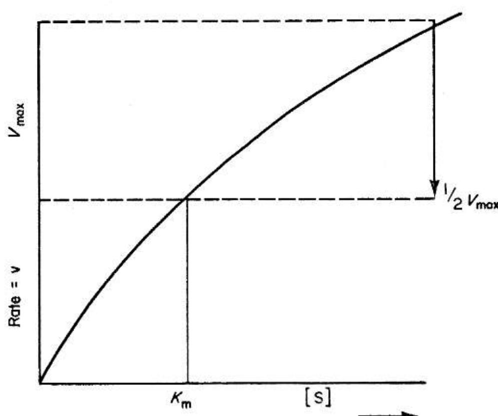


Fig. 1. Effect of substrate concentration on rate of a simple enzyme-catalysed reaction.

Analytical Applications

For analytical applications in general, it may be necessary to measure the concentration or activity of an enzyme, or to use an enzyme-catalysed reaction to measure the concentration of a substrate. The reaction conditions will differ depending on whether substrate or enzyme is to be determined.

Monitoring Substrate Concentrations

If an enzyme-catalysed reaction is used to monitor substrate concentrations, the kinetics are simplest if only small concentrations of substrate are present, so that $K_m \gg [S]$. Then $v = V_{max} [S]/K_m$, i.e. the rate is directly proportional to the substrate concentration. A typical spectrophotometric response for different initial substrate concentrations is shown in Figure 2.

The change in absorbance over a fixed time interval, e.g. $t_o - t_1$, is a direct measure of the reaction rate. Kinetic measurements are faster to obtain than those where the reaction is allowed to go to completion, and the requirement for small substrate concentrations implies a trace determination.

Although single enzyme-catalysed reactions are useful for determining a wide range of compounds, for example, urea, via hydrolysis to ammonia in the presence of urease, or ethanol, using alcohol dehydrogenase and NAD^+,

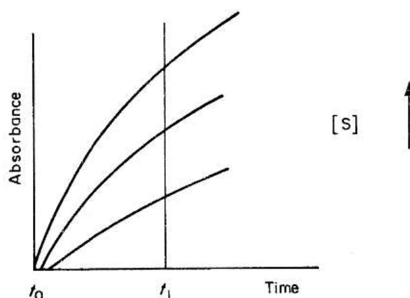
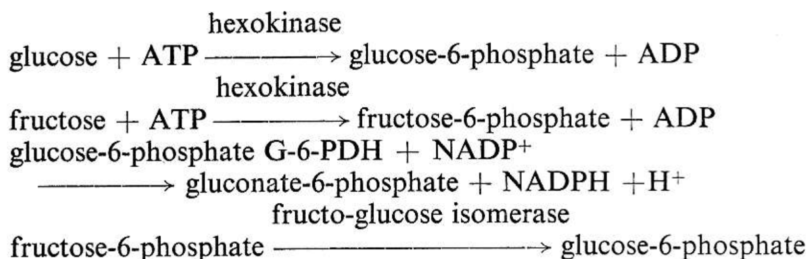


Fig. 2. Change of absorbance with time for different substrate concentrations in a simple enzyme-catalysed reaction.

much greater scope is achieved by using sequences of enzyme-catalysed reactions.

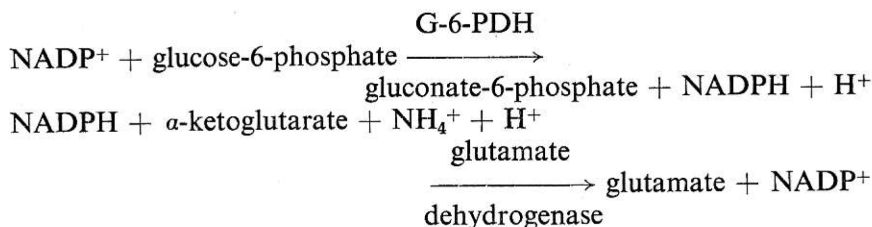
For example, an analysis applicable to honey (a mixture mostly of glucose and fructose) may be carried out by letting both sugars react with adenosine triphosphate (ATP) in the presence of hexokinase. The mixture of sugar-6-phosphates formed is treated with glucose-6-phosphate dihydrogenase (G-6-PDH) and the coenzyme NAD phosphate, $NADP^+$, which only react with the glucose phosphate. NADPH is formed and can readily be measured spectrophotometrically. Next fructo-glucose isomerase is added to isomerise the fructose-6-phosphate to glucose-6-phosphate. The G-6-PDH reaction produces more NADPH to give a measure of the fructose.



Enzyme methods exist for the estimation of a host of other organic and inorganic compounds[1,2].

Enzyme Cycling

The concept of enzyme cycling is interesting[3]. It is a very sensitive technique. Traces of $NADP^+$, for example, when present with reacting species such as glucose-6-phosphatase and G-6-PDH, produce the gluconate and an equally small amount of NADPH. If α-ketoglutarate and glutamate dehydrogenase are also present, a further reaction occurs, with release of glutamate and $NADP^+$. The released $NADP^+$ reacts with more substrate and the cycle repeats, at up to 20 000 times per hour. Gradually, glutamate and gluconate accumulate, and can be measured enzymatically after an appropriate time. The method is so sensitive that it has been used to determine the quantity of the co-enzyme in a single cell.



Monitoring Enzyme Concentrations

In the presence of a large amount of substrate, $[S] \gg K_m$. Thus the reaction rate becomes constant and independent of substrate concentration, i.e. $v = V_{max}$ (zero order reaction). Therefore the slope of an absorbance — time plot, representing the rate of reaction, is linear, and increases with increasing initial concentration of enzyme, because $V_{max} \propto [E]_o$.

Clinical chemists are concerned to measure levels of enzymes as a means of diagnosing various clinical conditions and differentiating between conditions like chronic hepatitis and cyrrhosis of the liver[4]. Glutamate oxaloacetate transaminase activity is high in both conditions. Cholinesterase levels are slightly reduced in hepatitis patients but greatly reduced in cyrrhosis patients.

Inhibitors and Activators

The behaviour of enzymes can be influenced adversely or favourably by various additives. Thus beryllium adversely affects alkaline phosphatase and the more beryllium one adds the more the enzyme is inhibited. This effect can be used to measure ng amounts of beryllium[5,6]. The inhibiting affect on enzymes is the reason for the toxicity of beryllium in the body. Alcohol dehydrogenase is inhibited by pg amounts of silver, and an analytical method based on this would be equivalent to having a spectrophotometric reaction with a molecular absorptivity of 10^{10} l mol^{-1} cm^{-1} [6]. The inhibiting effect of fluoride on some enzymes has been used to determine fluoride at ng levels. Inhibition of esterase systems is normally used[7–9]. Pesticides inhibit enzyme systems, and in particular they are potent inhibitors of cholinesterase. Some pesticides are determined by measuring the extent to which they inhibit this enzyme[1]. The inhibition of cholinesterase obtained from insect sources is far greater than the inhibition of the enzyme from mammals.

The inhibition effect can be used to monitor separated pesticides on a thin layer chromatography plate[10]. If one sprays with the enzyme, where there is a pesticide the enzyme will be inhibited. The enzymatic reaction is the hydrolysis of acetyl choline, which liberates acetic acid. So an acid/base indicator in the spray will reveal as acidic those areas where there is no inhibition of the enzyme. Where the enzyme is inhibited there is no acid released and hence no colour change.

The class of drugs known as monoamine oxidase inhibitors has been determined, based on this property, at the Home Office Central Research Laboratory[11]. The technique has since been extended to other classes of drugs[12]. Tetrahydrocannabinol, for example, inhibits glutamate dehydrogenase, and this

provides a means of detecting it at the 100 ng level. For many drugs cholinesterase is the most susceptible enzyme and by using that, tetrahydrocannabinol may be detected down to 8 ng ml^{-1}. Even ascorbic acid has an inhibiting effect on cholinesterase[13].

On the enzyme activation side, one finds that in extracting enzymes from cells, essential metals are sometimes lost, forming apo-enzymes. If the metal ion is returned to such apo-enzyme extracts, their activity is restored. For example, magnesium activates *iso*citrate dehydrogenase and can be determined by measuring the degree of activation[14]. Likewise, metals can be deliberately removed from enzymes to provide a sensitive and highly selective means of determining such metals based on their activation effect. Zinc can be removed from alkaline phosphatase by EDTA, for example, and the apo-enzyme used to determine traces of zinc[15].

Applications in Sample Decomposition

Enzymes find use in laboratories for purposes other than for quantitative assays. The Laboratory of the Government Chemist has used enzymes to assist in the extraction of various colouring matters from foods[16]. Dyes which bind to protein (such as Brown FK in kippers) are easier to liberate if the protein is destroyed with an enzyme such as papain. Some drugs become bound to protein and the protein is sometimes precipitated with reagents such as tungsten before drug assay. Much better recoveries of such drugs are obtained if the protein is destroyed by enzymes such as *Subtilisin Carlsberg*[16].

When teeth are examined for lead, a more realistic recovery of lead is obtained if the teeth are first cleaned by digesting off blood and soft tissue with the aid of papain[17]. In museum work, parchments and old documents may be stuck together with paste or glue. Sometimes they can be separated by digesting away the adhesive by means of enzymes[18].

Insolubilised Enzymes

Enzymes may be made insoluble by binding them by means of various functional groups in their molecules to chemically activated surfaces such as glass or polymer[19]. Such bound enzymes may be used in a number of ways. When held in columns, for example, solutions can be made to pass over and react with particles with enzyme coatings, thus retaining the enzyme for use over and over again, in, for example, flow analysis systems.

Another way in which an immobilised enzyme has been used is when cholinesterase is bound to a porous sinter through which water samples possibly containing a pesticide are continuously passed. After sufficient cycling, the bound enzyme becomes inhibited to a greater or smaller degree. The sample solution is replaced by a solution containing acetyl choline passing through the same sinter. If the enzyme has been greatly inhibited this can be detected by the low rate of conversion. This system has been adopted as an automatic water monitoring device for pesticides where an audible warning sounds when contaminants exceed a pre-set level. Air monitoring devices of a similar kind have also been devised[20].

Enzyme Electrodes

Immobilised enzymes find use when bound to films covering the surface of electrodes[21,22]. Species approaching such electrodes can become changed as they pass through the membrane bearing the enzyme. Thus, a layer of glucose oxidase over an oxygen electrode allows glucose to be measured by the indirect measurement of the depletion of oxygen at the electrode as a result of the catalysed oxidation of glucose. As glucose oxidase is very selective, if the electrode is dipped into a solution of a mixture of sugars, it will measure only the glucose. Glass electrodes, or ammonium ion sensitive electrodes, can similarly be combined with amino acid oxidase to measure amino acids or with urease to monitor urea. The speed of response is now 5–10 seconds for a glucose electrode. A universal detector for enzyme reactions might be the thermistor detector, measuring heat changes as the substrate passes over immobilised enzyme in a specially designed cell[23].

A few investigators have recently extended the immobilisation concept, by attaching living bacteria and other microorganisms to polymers, and keeping them alive whilst covering suitable electrodes. Thus yeast has been chemically bound to a porous polymer and placed over an oxygen electrode. If this is dipped into a solution containing an antibiotic (nysastin) some of the yeast dies, and less oxygen is consumed. So with increasing amount of antibiotic present, increasing amounts of oxygen are available to the electrode[24]. The opposite effect can be turned into a means of measuring acetate, because acetate will stimulate yeast growth, more oxygen is consumed, and oxygen at the electrode is decreased[25].

Such electrodes have a slow response, but if one can wait the necessary 10 minutes or so, such electrodes extend significantly the range of compounds which may be determined by electrode response. Thin slices of pigs kidney have been incorporated into an electrode, making use of their glutamine oxidase content[26]. If this electrode is dipped into glutamine solution, ammonia is liberated, so an ammonia-sensitive electrode is used as detector. That electrode responds only to glutamine in the presence of other similar compounds, and it is more reliable than if the enzyme had been extracted and immobilised on to a membrane. The kidney slices are said to remain effective for a month. The bacteria which cause tooth decay (by converting dietary carbohydrate to acid) have been immobilised and used in conjunction with a glass electrode to measure carbohydrates in solution by means of the response of a glass electrode to the generated acid.

Conclusions

Enzymes are playing an increasingly important and widespread role in solving analytical problems. As well as providing sensitive and selective methods of determination for compounds in foods and beverages, clinical, biological and environmental samples, recent and current developments are greatly expanding the mode and fields of application of enzymes. This is leading to greater availability of enzymes and substrates as well as to improved economics of use. There is no reason why this trend should not continue during the next decade.

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Estimation of Coffee, Chicory and Glucose Solids Contents of Instant Coffee Products

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Simple methods for the determination of caffeine, total ketoses after hydrolysis and total aldoses after hydrolysis as measures of the coffee, chicory and glucose solids contents respectively are described. These methods are in use in the Government Analyst's Laboratory in Zimbabwe, and may be of use in other laboratories lacking high pressure liquid chromatography apparatus.

The Zimbabwe, Food and Food Standards (Coffee) Regulations, 1981 allow instant coffee products to be manufactured from the soluble solids of coffee, chicory and from glucose solids. The designations of the various products and the proportions of solids which they may contain are laid down in the Regulations. The methods have been developed to monitor the products to ensure compliance with the Regulations.

Discussion of Methods

COFFEE CONTENT

Caffeine content is determined as a measure of coffee content. The method for determination of caffeine incorporates features from previously published methods[1,2]. Substances (particularly 5-hydroxymethylfurfural) present in roasted chicory interfere in the measurement of caffeine by ultraviolet spectroscopy, unless they are first reacted with bisulphite and the effect of any remaining interfering substances obviated by careful choice of wavelengths for readings.

The caffeine is extracted with chloroform, the chloroform evaporated and the caffeine determined in 50 per cent. alcoholic solution. Determination of caffeine in chloroform is possible, but less accurate because the u.v. maxima of caffeine and residual interfering substances are much closer together in chloroform than in 50 per cent. alcohol.

Caffeine may also be determined by gas chromatography using a flame ionisation detector, a 100 cm 2·5 per cent. OVI column and triphenyl methane as an internal standard. Good agreement was obtained on comparison of the methods (see Table II).

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The u.v. method is quicker than the gas chromatography method, but should not be used for any mixture containing ingredients other than coffee, chicory and glucose solids without careful checking.

CHICORY CONTENT

Chicory yields about 50 per cent. of ketose sugars on hydrolysis whereas coffee and glucose solids do not yield significant amounts of ketose sugars. It is therefore possible to estimate the chicory solids content of instant coffee products from the ketose sugars determination, provided that it is established (by thin layer chromatography or other means) that the product does not contain sucrose. Certain liquid instant coffee products contain added sucrose, and in this event, estimation of chicory content cannot so readily be made by determination of ketose content after hydrolysis. The method used for determination of ketoses is adapted from the published method of Tsutsui *et al.*[3]

GLUCOSE SOLIDS CONTENT

The Regulations for Zimbabwe permit instant coffee and chicory mixtures to contain glucose solids. The proportion of glucose solids must not exceed the combined mass of ground coffee and chicory used in manufacture. Glucose solids consist of dextrose, maltose and dextrins in varying proportions. Using this method, the maltose and dextrin constituents of glucose solids are largely hydrolysed to dextrose and the total dextrose is determined as reducing sugars. At the same time, the inulin and fructose constituents of chicory are broken down into products which do not have reducing ability. Dextrose and any other remaining reducing sugars are reacted with mixed Fehling's reagents and the residual dissolved copper determined by atomic absorption. One of the official methods for determination of reducing sugars could be used as an alternative to atomic absorption.

Experimental

APPARATUS

Varian Superscan 3, u.v. – visible recording spectrophotometer (or similar).
AA5 atomic absorption spectrophotometer (or equivalent).
pH meter.
Laboratory flask shaker.

REAGENTS

1. *pH 6·5 Buffer*. Dissolve 8 g of sodium hydroxide in 70 ml of water, add 10 ml of glacial acetic acid, adjust pH with dilute acetic acid, using a pH meter, and dilute to 100 ml with water.
2. *Sodium metabisulphite.*
3. *Chloroform, redistilled.*
4. *Zinc acetate solution.* Dissolve 21·9 g of crystallised zinc acetate and 3 ml of glacial acetic acid in water and dilute to 100 ml.

5. *Potassium hexacyanoferrate (II) solution.* A 10·6 per cent. m/v aqueous solution.
6. *Perchloric acid – urea solution.* To 26 ml of 50 per cent. aqueous urea solution add 24 ml of 60 per cent. perchloric acid.
7. *Concentrated hydrochloric acid,* 33–34 per cent. m/v.
8. 5 *M sodium hydroxide.*
9. *Fehling's reagent No. 1.* Dissolve 69·28 g of copper sulphate ($CuSO_4 5H_2O$) in water and make up to 1 litre.
10. *Fehling's reagent No. 2.* Dissolve 100 g of sodium hydroxide and 346 g of sodium potassium tartrate in water and make up to 1 litre.
11. *Diluted mixed Fehling's reagents.* Take 25 ml of Fehling's reagent No. 1, add 25 ml of Fehling's reagent No. 2 and dilute to 500 ml.
12. Standard solution of 0·002 per cent. w/v caffeine in 50 per cent. alcohol.

Methods

METHOD FOR DETERMINATION OF CAFFEINE

To 0·5 g of instant coffee powder add 0·5 g of sodium metabisulphite and 5 ml of pH 6·5 buffer and mix. Add 100 ml of chloroform and cool in ice water. Shake mechanically for 30 min and then cool in ice water again. Transfer to a separating funnel and filter the chloroform layer through a rapid filter paper. Evaporate 10 ml of the chloroform extract in a tube immersed in warm water using an air jet, and dissolve the residue in 10 ml of 50 per cent. alcohol.

Record absorbance readings at 272·5 nm and 291 nm and determine the difference. Compare with 0·002 per cent. caffeine in 50 per cent. alcohol and then calculate the caffeine content of the instant coffee.

Similarly treat 0·2 g portions of ground coffee samples and determine the caffeine content. From the caffeine contents of the instant coffee and the ground coffee, estimate the quantity of ground coffee used in the manufacture of the instant coffee. The chloroform extract remaining after the determination of caffeine by u.v. may be used for the determination of caffeine by gas chromatography.

Recoveries of caffeine from spiked chicory by the u.v. method ranged from 97 to 103 per cent.

METHOD FOR DETERMINATION OF KETOSES

Weigh out 1·0 g of instant coffee powder into a flask and add 80 ml of water. Warm on water bath for 30 min. Cool, add 2 ml of zinc acetate solution and mix. Add 2 ml of potassium hexacyanoferrate solution and mix again. Dilute to 100 ml and filter. Into a 10-ml stoppered tube pipette 1·0 ml of the extract, and 5·0 ml of perchloric acid – urea solution, mix. Similarly treat 1·0 ml each of standard solutions of fructose containing 1 mg and 2 mg respectively. Place tubes into a vigorously boiling water bath (95°C in Salisbury) for 25 min, making sure that the contents of the tubes are submerged. Remove, cool and stand for 45 min to 1 h. Read absorbance at 508 nm, 604 nm and 700 nm.

Absorbance due to ketose may be found from the expression:
E604 – 0·5 (E508 + E700).

Calculate the ketose content of the instant coffee in terms of fructose. Similarly treat 0·25-g portions of ground roasted chicory root samples and determine the ketose content. From the ketose contents of the instant coffee and the ground chicory, estimate the quantity of ground chicory used in the manufacture of the instant coffee product.

METHOD FOR THE DETERMINATION OF GLUCOSE SOLIDS

Use the same extract of the coffee product as used for the determination of ketoses. Pipette 0·8 ml of the extract into a 10-ml stoppered tube and add 3·0 ml of concentrated hydrochloric acid. Dilute to 6·0 ml with water and mix. Similarly treat 3·0-ml aliquots of 1 per cent. extracts of ground coffee and ground chicory, a 1·5-ml aliquot of 0·4 per cent. glucose solids and 0, 0·5, 1·0, 1·5 and 2·0-ml aliquots of a 0·4 per cent. solution of dextrose (containing respectively, 0, 2, 4, 6 and 8 mg of dextrose). The 0·4 per cent. solution of glucose solids may be made from glucose syrup after deriving the solids content from the refractive index. Duplicate determinations for the instant coffee product and glucose solids solution are advisable.

Place tubes (without stoppers) into a boiling water bath for 20 min making sure contents are submerged. Remove, cool, and transfer to 100-ml beakers and make alkaline with 8 ml of 5 M NaOH. Without delay, add 15 ml of diluted mixed Fehling's reagents and bring to the boil for 1 min. Cool, dilute to 500 ml and mix. Centrifuge aliquots and spray the supernatant liquid on an atomic absorption spectrophotometer using a copper lamp at 327·4 nm, and an air-acetylene flame.

Plot a graph of readings against dextrose (mg) and then with the aid of the graph calculate the aldose contents of the instant coffee product, ground coffee, ground chicory and glucose solids. Finally after making corrections for the aldose contents of the coffee and chicory constituents, determine the glucose solids content of the instant coffee product.

Discussion of Estimation of Coffee Chicory and Glucose Solids Contents

The Zimbabwe, Food and Food Standards (Coffee) Regulations, 1981 define the constituents of coffee products in terms of the ingredients used in manufacture. These ingredients are in effect, ground roasted coffee berries, ground roasted chicory root and glucose solids (the water free material in glucose syrup). The estimation of these ingredients from the determination of caffeine, ketose and aldose depends on the values for these constituents assigned to the original ingredients, and on the extraction rate assumed for coffee solids and chicory solids from ground coffee and ground chicory. Typical analyses by the given methods of the ingredients used in manufacture in Zimbabwe and of extracted solids are given in Table I. An extraction rate of 37 per cent. for coffee and 80 per cent. for chicory is assumed.

It must be stressed that analytical results and extraction rates are based on

TABLE I
CONSTITUENTS OF COFFEE, CHICORY AND LIQUID GLUCOSE

Ingredients	Caffeine *per cent.*	Ketose *per cent.*	Aldose *per cent.*
Ground coffee	1·1	0	2
Extracted coffee solids	3·0	0	5
Ground chicory	0	46·0	8
Extracted chicory solids	0	57·5	10
Glucose syrup (82 per cent. solids)	0	0	74·4
Glucose solids	0	0	90·7

limited data obtained from raw materials produced in Zimbabwe and may not be applicable to raw materials from other countries.

ANALYSIS OF FOUR INSTANT COFFEE PRODUCTS

Four instant coffee products manufactured in Zimbabwe, designated A, B, C, and D, were analysed and the results, which for ketose and aldose are the means of duplicate determinations, are given in Table II. From the results in

TABLE II
ANALYSIS OF FOUR INSTANT COFFEE PRODUCTS

Instant coffee	Caffeine *per cent.* (by u.v.)	Caffeine *per cent.* (by GLC)	Ketose *per cent.*	Aldose *per cent.*
A	0·15	0·16	15·8	63·4
B	0·38	0·38	12·0	64·6
C	0·46	0·47	13·7	57·3
D	0·57	0·59	12·8	56·1

Table II, estimations of the three constituents were made and these figures are given in Table III. Finally from the values obtained for the three constituents of instant coffee products, and assuming there are no other constituents, estimations were made in Table IV of the ingredients used in manufacture as required by the Regulations.

TABLE III
CONSTITUENTS OF FOUR INSTANT COFFEE PRODUCTS

Instant coffee	Coffee solids *per cent.*	Chicory solids *per cent.*	Glucose solids *per cent.*	Total *per cent.*
A	5·2	27·5	66·6	99·3
B	12·7	20·9	68·2	101·8
C	15·5	23·8	59·7	99·0
D	19·3	22·3	58·3	99·9

Bearing in mind the variability of natural products; the results in Table IV would in practice be rounded off in favour of the manufacturers.

TABLE IV
INGREDIENTS USED IN MANUFACTURE OF FOUR PRODUCTS

Instant coffee	Ground coffee *per cent.*	Ground chicory *per cent.*	Glucose solids *per cent.*
A	12·2	29·9	57·9
B	26·7	20·3	53·0
C	31·9	22·6	45·5
D	37·7	20·2	42·1

Conclusion

Caffeine, ketose after hydrolysis, and aldose after hydrolysis can be determined by the methods presented. The three constituents used in the manufacture of instant coffee powders, i.e. ground coffee, ground chicory and glucose solids can be estimated from the values obtained for the determination of caffeine, ketose and aldose. The determination of chicory content of liquid coffees containing sucrose would require a different approach.

The methods have however, also been used in the analysis of ground coffee and chicory mixtures, and the glucose solids method can be adapted to determine the starch content of mixtures containing roasted cereal.

The author is grateful to the Government Analyst for helpful discussions during the development of these methods, and to the Secretary for Health for permission to publish this paper.

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The Determination of Extraneous Water in Frozen Chickens

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Comparative determinations of extraneous water in frozen chickens by different methods of analysis are reported and critically examined. It is shown that the prescribed EEC methods are of little value for enforcement purposes. Of the official methods the Annex III procedure is shown to give the most consistent results, but the calculation is shown to be too generous, since it permits a substantial amount of extraneous water to be undetected. The Stubbs and More procedure is reliable and has some advantage.

A Council Regulation (EEC)[1] laying down common standards for the water content of frozen and deep-frozen chickens, hens and cocks has amongst its aims the establishment of reliable methods for the precise determination of the water content added during processing.

The Regulation requires that regular checks on water uptake in processing shall be made as described in Annex I, describes a rapid check method in Annex II and two referee methods in Annexes III and IV as amended.[2] It will be demonstrated that the prescribed methods of analysis are neither reliable nor precise. The Regulation does not at the present time apply to poultry treated with polyphosphates.

The check prescribed in Annex I consists of measuring the increase in weight of 20 carcases from after defeathering and evisceration to the end of the process. It should, therefore, provide a reliable estimate of the water uptake in that part of the process. Great difficulty was found at a local packing station in complying with the maximum tolerance of 5 per cent. prescribed in the Annex. Samples which were drawn for chemical analysis by the procedure in Annex III were found invariably to satisfy the prescribed tolerance and indeed to show little or no apparent extraneous water.

A number of further experiments were carried out in which individual carcases were each examined following the procedures described in Annexes I, II, III and IV. None of the carcases contained added polyphosphate.

The rapid detection method in Annex II consists simply of measuring the fluid loss obtained on thawing 20 carcases. It is stated that if the average loss exceeds 5·2 per cent. it is highly probable that the amount of water absorbed during processing exceeds the limit figure. The Regulation provides that if the loss does not exceed the prescribed level the poultry shall be deemed to comply. If the loss exceeds the limit, or if it is suspected that polyphosphates have been

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added, one of the methods of chemical analysis described in Annexes III and IV is to be applied.

Annexe III requires the determination of moisture and nitrogen on seven whole carcases after maceration. The method assumes a technically unavoidable water absorption during preparation of 7·4 per cent. Allowing for this, and including a confidence interval, the highest permissible level for the average weight of water is stated to be given by the following formula for chickens.

$$W_9 = 3{\cdot}82 \times RP_A + 59$$

where RP_A is the average weight of protein in the seven carcases.

In Annexe IV analysis for water and fat is carried out on the flesh separated from each of seven carcases. The theoretical physiological water content in grammes, including confidence interval is given as

$$(M_5 - M_8 - M_9) \times 3{\cdot}54 + 27$$

M_5 = weight of flesh
M_8 = weight of water
M_9 = weight of fat

From this and the determined water the extraneous water content is calculated. An average tolerance of 6 per cent. is allowed.

Prior to the publication of these Regulations, meat content was almost invariably determined in the United Kingdom by the so called Stubbs and More[3] procedure. A further figure was therefore obtained by this method according to the following formula:

$$\text{Extraneous water} = \left\{100 - \frac{N \times 100}{3{\cdot}70} - F\right\}\frac{\text{Wt flesh}}{\text{Wt carcase}}$$

where N = per cent. nitrogen in flesh
F = per cent. fat in flesh
(using a nitrogen factor[4] (namely 3·70) recommended by the Analytical Methods Committee of the Society for Analytical Chemistry).

Procedure

Clearly it is not possible to follow precisely the preliminary preparation laid down in each Annex on the same carcase. Hence the following procedure was adopted.

(1) The weight of water uptake was determined in the production establishment following the procedure described in Annex I.

(2) From the 20 test carcases, seven were taken at random and submitted to the laboratory in a frozen condition.

(3) The weight of drained liquor was obtained following the procedure laid down in Annex II. The liquor being retained.

(4) The drained carcase was then dissected following the procedure laid down in Annex IV.

(5) The separated flesh was combined with the drained liquor from (3) macerated and subjected to a chemical analysis for moisture, fat and protein.

TABLE I

ESTIMATION OF APPARENT EXTRANEOUS WATER IN CHICKEN CARCASES BY VARIOUS METHODS

Sample No.	Weight *grammes*	Annex I Weight Gain (A) *per cent.*	Annex II Drip (B) *per cent.*	Annex II error (C)	Annex III *per cent.*	Annex III error	Annex IV *per cent.*	Annex IV error	Stubbs and More *per cent.*	Stubbs and More error
4–1	1568	5·0	3·3	−1·7	0·7	−4·3	−0·8	−5·8	5·8	+0·8
4–2	1742	5·9	2·0	−3·9	2·1	−3·8	1·1	−4·8	4·9	−1·0
4–7	1619	3·5	2·3	−1·2	−2·6	−6·1	−0·7	−4·2	2·5	−1·0
4–9	1437	6·6	3·6	−3·0	5·0	−1·6	2·3	−4·3	9·1	+2·5
4–12	1731	6·1	3·5	−2·6	4·1	−2·0	2·9	−3·2	7·8	+1·7
4–15	1356	6·0	3·2	−2·8	−1·7	−7·7	1·2	−4·8	6·8	+0·8
4–25	1497	5·8	3·8	−3·0	−0·8	−6·6	−5·9	−11·7	6·1	+0·3
5–3	1392	9·6	4·5	−5·1	0·3	−9·3	0·6	−9·0	6·5	−3·1
5–8	1819	5·0	2·5	−2·5	0·8	−4·2	−3·7	−8·7	5·9	+0·9
5–11	1385	6·2	3·2	−3·0	2·1	−4·1	−1·5	−7·7	8·2	+2·0
5–17	1320	7·7	4·6	−3·1	0·8	−6·9	−2·9	−10·6	7·1	−0·6
5–20	1280	6·1	3·1	−3·0	−2·3	−8·4	−7·3	−13·4	4·8	−1·3
5–23	1534	5·0	2·0	−3·0	−1·7	−6·7	−10·4	−15·4	5·9	+0·9
5–25	1543	9·4	5·3	−4·1	2·1	−7·3	−3·1	−12·5	6·3	−3·1
6–1	1349	3·9	3·0	−0·9	2·9	−1·0	2·3	−1·6	6·8	+2·9
6–4	1536	9·1	5·1	−4·0	5·4	−3·7	3·5	−5·6	10·5	+1·4
6–5	1049	6·5	5·2	−1·3	3·6	−2·9	4·3	−2·2	9·6	+3·1
6–15	1066	5·8	4·0	−1·8	1·9	−3·9	4·1	−1·7	7·6	+1·8
6–20	1512	4·6	3·5	−1·1	−1·0	−5·6	−6·7	−11·3	3·9	−0·7
6–23	1511	3·9	3·0	−0·9	2·9	−1·0	2·2	−1·7	6·7	+2·8
6–24	1720	5·4	3·1	−2·3	−0·3	−5·7	−6·7	−12·1	5·2	−0·2
7–4	1578	5·0	2·2	−2·8	2·1	−2·9	−0·6	−5·6	7·3	+2·3
7–7	1795	6·8	2·3	−4·5	−2·9	−9·7	−5·8	−12·6	3·5	−3·3
7–10	1941	6·3	2·8	−3·5	1·6	−4·7	−1·5	−4·8	6·0	−0·3
7–13	1269	8·3	4·6	−3·7	1·1	−7·2	4·5	−3·8	6·7	−1·6
7–16	1740	14·5	5·5	−9·0	7·6	−6·9	6·1	−8·4	11·1	−3·4
7–20	2076	14·0	5·2	−8·8	9·7	−4·3	3·5	−10·5	13·8	−0·2
7–24	1438	3·7	2·1	−1·6	0·4	−3·3	−0·3	−4·0	6·9	+3·2
8–2	1325	8·2	4·6	−3·6	1·8	−6·4	−5·4	−13·6	9·4	+1·2
8–3	1637	8·5	3·6	−4·9	1·5	−7·0	3·3	−5·2	6·2	−2·3
8–11	1197	7·0	4·2	−2·8	8·3	+1·3	5·9	−1·1	12·8	+5·8
8–12	1384	5·9	3·9	−2·0	6·1	+0·2	−4·6	−10·5	13·3	+7·4
8–17	1497	7·3	4·7	−2·6	2·6	−4·7	3·2	−4·1	8·3	+1·0
8–18	1790	4·9	2·1	−2·8	2·1	−2·8	1·4	−3·5	6·5	+1·6
8–25	1196	11·0	6·4	−4·6	5·8	−5·2	5·9	−5·1	12·7	+1·7
Mean				−3·2		−4·8		−7·0		0·7
Standard deviation				1·8		2·6		4·1		2·4
Standard error (sample of seven carcases)				0·7		1·0		1·5		0·9

Column C is derived by deducting B from A.

The separated skeleton was ground and also subjected to analysis for moisture, fat and protein.

(6) The results of analysis on the combined flesh and drained liquor were calculated to give the extraneous water content using the formula provided in Annex IV. From the same analysis the total meat content was calculated using the Stubbs and More procedure and the extraneous water obtained according to the formula given above.

(7) The results of analysis of the flesh and drained liquor mixture and the bones were combined together in proportion to the weights of flesh and bones to give the analysis of the whole carcase. The extraneous water was calculated from the figures so derived by the formula in Annex III. In order, however, to make this strictly comparable with the results obtained in the other procedures the figure so obtained was expressed as a percentage and the tolerance of 7·4 per cent., which is allowed for in the calculation, was added to the figures so obtained.

Results

The results of all these analyses given in terms of percentage of extraneous water are shown in Table I. For the purpose of this table it is assumed that the weight gain obtained by the Annex I procedure is a true estimate of the extraneous water. It is clearly an underestimate as will be discussed later. The column against each procedure shown as error is the simple difference between the result obtained and that found in Annex I.

Table II illustrates the effect of taking the mean results for the seven carcases comprising the sample taken on each occasion. It demonstrates that whilst the Annex I check procedure indicates a failure to meet the required standard, the results of chemical analysis by Annex III or IV easily satisfy the standards prescribed in those Annexes. Indeed, it is our experience that it is most unlikely that even the most unsatisfactory output from the processing plant would be condemned by either Annex III or IV.

TABLE II

PERCENTAGE OF EXTRANEOUS WATER IN CHICKEN CARCASES (MEANS OF SEVEN DETERMINATIONS BY VARIOUS METHODS)

Sample No.	Annex I	Annex II	Annex III	Annex IV	Stubbs and More
4	5·6	3·1	1·0	0	6·1
5	7·0	3·6	0·3	−4·0	6·4
6	5·6	3·8	2·2	0·4	7·2
7	8·4	3·5	2·8	0·8	7·9
8	7·5	4·2	4·0	1·4	9·9

Units are per cent. extraneous water.

Discussion

Clearly no useful purpose can be served by enforcement Authorities using the present procedures in Annex III or IV for the detection of extraneous water. A study of the results reported in Table I may indicate whether some improved

procedure can be devised, or whether the natural variation in the parameters determined by chemical analysis is such that no better system is possible.

It is suggested that the standard deviations of the error figures in Table I are a measure of how consistent the various methods may be. The simple Annex II procedure shows the lowest standard deviation. Annex IV shows a significantly higher figure than both Annex III and Stubbs and More which are not significantly different. It is interesting to note that in Annex IV the physiological water is obtained as a function of the dry non-fat solids of the flesh and that this figure is derived by difference, an unsatisfactory procedure increasing the possibility of error. This method is clearly less reliable and must be considered unsatisfactory. Annex III, based on analysis of the whole carcase, and Stubbs and More on the analysis of the flesh alone show substantially the same range of error indicating that the separation of the flesh is not a factor.

In considering the figures for mean error it must be remembered that the Annex I results do not take account of water absorbed in the defeathering process. There appears to be little reliable information on the amount of water uptake in this stage. However, the maximum water absorption for dry chilled poultry is given as 2 per cent. in Annex II, 2·9 per cent. in Annex III and 2·3 per cent. in Annex IV. Assuming that this occurs in defeathering it is suggested that the mean water uptake in this process might be assessed as approximately 1·5 per cent. If this figure is accepted the overall mean errors for the four methods of analysis are as follows:

Annex II	Annex III	Annex IV	Stubbs and More
−4·7	−6·3	−8·5	−0·8

The reason for the failure to confirm excess extraneous water by chemical analysis is apparent. Clearly the calculations and tolerances in the official methods are too generous. It must be remembered that the calculations in Annexes III and IV incorporate a confidence interval.

Consideration of the data obtained from these experiments leads to the following conclusions.

Annex II

The mean error for this procedure of nearly 5 per cent. means that any carcase showing measurable drip must be suspect. This test seriously underestimates the extraneous water. It is misleading and should be discarded.

Annex III

The overall mean error is minus 6·3 per cent. and with a standard error for seven carcases of only 1·0, this could clearly be reduced. The data on which the formulae are based should be checked and re-examined. Nevertheless, even after revision it appears likely that true extraneous water contents of over 10 per cent. may not always be detected.

Annex IV

The wide range of error obtained by this method makes it unsatisfactory and it should be rejected.

Stubbs and More

This method provides results as consistent as those obtained by Annex III. The low figure for mean error gives excellent confirmation of the nitrogen factor recommended by the Society for Analytical Chemistry. It has the additional advantage that since the flesh is separated from the bone the determination of added polyphosphates is simplified. The presence of ground bone is a complication if this test is applied to a carcase prepared under Annex III. The Regulation does not at present apply to polyphosphate treated poultry, but it is nevertheless necessary to establish its absence.

The method gives a similar confidence level to Annex III. It is unlikely that this can be improved upon in any method employing proximate analysis due to the natural variations in the parameters on which the calculations are based.

This paper illustrates the pitfalls which may arise in prescribing methods of analysis in legislation. A more satisfactory procedure is to allow the expert analyst freedom of choice remembering that he must justify his results in the Courts.

From the results reported here the Annex III and Stubbs and More procedures show the most consistent results. Clearly the factors and confidence interval in the Annex III calculation are much too generous, but, bearing in mind the limited number of results reported and the assumption regarding the water uptake in defeathering, it would be unwise to suggest amended factors. No confidence interval has been incorporated in the Stubbs and More results and yet no injustice would occur from their use.

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Book Review

THE MEASUREMENT OF BREATH ALCOHOL By V. J. Emerson, R. Holleyhead, M. D. J. Isaacs, N. A. Fuller and D. J. Hunt. Forensic Science Society and Scottish Academic Press, 1980, Pp. 70, £10.00.

This is a hard backed book and is an account of experimental work that was done to evaluate breath alcohol testing instruments following the Blennerhasset report on Drinking and Driving in 1976.

The instruments are designed to analyse a sample of breath for its alcohol concentration and present the results as an equivalent blood alcohol concentration. This is based on a statement made in 1972 by U.S. specialists in alcohol research that a physiological relationship exists between the alcohol concentration in 2100 millitres of expired alveolar air and in 1 millilitre of blood. A temperature of 34°C is taken since this is the one at which breath leaves the mouth. The use of rigid temperature control is important as the results may vary by as much as 6 per cent. with 1°C temperature variation.

Three different types of instruments were used in 13 different locations for periods of 2 months each and the results carefully examined and recorded together with the (printable) comments of the operators who had all received a minimum of training.

On broad lines the results were in good agreement with blood alcohol test results, adjusted for the time elapsing between tests during which metabolism occured. A second breath test taken a few minutes after the first one confirmed the results. It is essential to have 2 litres of alveolar air for each test hence the subject will need time to recuperate after the first test. Likewise asthmatic sufferers may require special exemption for the test as could people subject to spontaneous pneumothorax.

At present haemophiliacs are known to be at risk with blood alcohol tests but it is essential to make the point plain to the "man on the beat". Mouth alcohol is likely to vitiate the breath test hence the need to wait for its disappearance—as at present and also "burps" from people consuming alcohol when on low calorie diet may affect it. The low calorie diet may also produce volatile compounds which behave similarly to alcohol in the breath but the effect is small, although even the small effect mentioned in the book could be critical in the "80" mg region.

The book is basically just an account of the work done on these instruments and as such is free from criticism except that in my own opinion 991 valid tests may be insufficient on which to base such an important step as to alter the method of test which may condemn many people unfairly.

No mention is made of the number of tests done in places like Northern Ireland, Canada, Australia, New Zealand and much of the U.S.A. I understand in this country that about 120,000–130,000 samples are taken under the Road

Traffic Act each year and about 10 per cent. of these are sent for private "check ups".

In Northern Ireland and New South Wales (Australia) where the new type breath tests have already been in operation for some time I understand that about 10 per cent. of the persons stopped, exercised their option to have blood or urine samples taken to confirm the results obtained from breath tests. If this be so, then it is likely that there will be an increase in the number of tests in the areas of greatest risk, i.e. "80", "150" and possibly 200 mg of alcohol per 100 millilitres of blood.

The book reviewed was singularly free from typographical errors—only one small one could be found and the text is excellent and well written. The tables and figures of results are impressive. The price of the book is high except for specialists to whom it can be thoroughly recommended. It is well bound and legible.

Possibly any developments should be investigated on a wider basis than the one on which this experiment was based and thoughts go back to original experiments during the 1950s when Public Analysts, the then Royal Institute of Chemistry and the then Society for Analytical Chemistry were also involved.

G. V. JAMES

J. Assoc. Publ. Analysts, 1981, **19**, 73

Graham Sherratt

It was with great regret that we heard the news of the death of one of our Senior Past Presidents, Graham Sherratt.

Some of Graham's university colleagues viewed his decision to join Ruddock in private practice early in his career with considerable scepticism. In due course he proved his critics to be wrong and built up a thriving practice becoming virtually the established leader of the part time or consultant Public Analysts in the North of England.

His first appointment was as Public Analyst to the County Borough of Warrington in 1930 and at the time of his retirement he served in this capacity 18 different authorities mainly in the North of England and Wales.

Water analysis was one of Graham's great interests and he was consultant analyst to the Warrington Water Department, Cheshire River Board and the Gwynedd River Board. He was also one of the consultant analysts to the Anglers Co-operation Association and took part in a number of important legal cases aimed at reducing the pollution of fishable rivers. After his retirement, he was the joint author with George Newsom QC of a book entitled "Water Pollution (a guide to those who are concerned with grappling with river pollution)"; published in 1972.

Graham had a great regard for our Association and was one of the Senior Public Analyst members of the old Society of Public Analysts who initially supported the formation of the new Association. This was at a time when some of his contemporaries viewed the formation of a new Association with many misgivings. Sherratt served on the Council of the Society for Analytical Chemistry for three different periods, was a Vice-President for two periods and Chairman of the North of England Section in 1960. He became our President in 1959 and until his retirement in 1970 he took a very active part in all the work of Public Analysts and in the development of the Association. The gavel which our Presidents now use sometimes with such vigour was presented to the Association by Graham in memory of Herbert Evans.

One of Sherratt's major contributions to Public Analyst work was his chairmanship of the newly formed Board of Examiners for the Branch E Examination of the Royal Institute of Chemistry (now the Royal Society of Chemistry). At the time of his appointment, this examination was at its lowest ebb and attracted very few candidates with the result that the country might well have been faced with insufficient adequately qualified analysts to fill Public Analyst appointments as they became vacant.

The writer was one of the first examiners appointed by the new Board and was, therefore, fully aware of the problems the new chairman had to face. The support that the chairman gave to the examiners in their efforts to improve the general standard of the Examination was greatly appreciated and will always be remembered.

Graham devoted many hours to his practice but, nevertheless, he found time for other interests such as photography, shooting, fishing, sailing and the countryside in general.

J. H. Hamence