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Review

Liquid–gel partitioning and enrichment in the analysis of organochlorine contaminants

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ABSTRACT

A review is given of methods for analysis of organochlorine compounds in which the lipophilic gel Lipidex is used for extraction and purification. Some of the compounds analysed are DDT, DDE, hexachlorobenzene (HCB), hexachlorocyclohexane (α-, β-, γ-HCH), dieldrin, oxychlordane, *trans*-nonachlor, pentachlorophenol, polychlorinated biphenyls (PCBs), including the non-*ortho* planar congeners, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and metabolites of chlorinated paraffins. Applications to analyses of human milk, cod liver oil, bile, urine, water and indoor air are discussed.

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1. INTRODUCTION

Organochlorine compounds are widely spread in the environment, and many of them constitute a danger to the environment and risks for our health. Such well-known contaminants include pesticides (*e.g.*, DDT, hexachlorobenzene, dieldrin, chlordane), industrially used products [*e.g.*, polychlorinated biphenyls (PCBs), naphthalenes (PCNs) and paraffins (CPs)] and unintentionally produced contaminants such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). These compounds possess several common properties; they are persistent, lipophilic and accumulate in mammals. The most toxic of the compounds, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been extensively studied, and a structure–toxicity relationship with this compound has been confirmed for many of the organochlorine aromatics [1,2]. Analysis of the organochlorine compounds in different compartments of the environment and in humans is of great interest in order to establish the present condition and time-related trends and to evaluate the effects of the contaminants. The multiplicity of compounds and the low levels to be analysed from complex matrices make the analytical work complicated. Generally, the analyses include the following steps: extraction of lipids and lipid-soluble compounds, isolation of organochlorine compounds from the bulk of the co-extracted material, purification and separation into groups for identification and determination of concentrations by electron-capture gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

A variety of methods have been used for the extraction of biological materials, and some reviews of the common methods have been published [3–5]. The classical partitioning between solvents of different polarity [6,7] has been modified and applied in many cases [8–10]. The drawback with this method is that several extractions must be made and emulsions may be troublesome. These problems can in some cases be eliminated by addition of sodium sulphate. The sample is mixed with sodium sulphate to a dry matter which is extracted by maceration [11] or shaking with a non-polar solvent [12,13], or it can be filtered or eluted [14,15]. The dry sample can also be transferred to a Soxhlet apparatus and refluxed with a non-polar solvent. This method is reported to give lower levels of PCBs than the method of saponification and subsequent liquid–liquid extraction [16]. In recent years, successful attempts to use supercritical extraction of biological [17–19] and environmental samples [20] have been described. However, problems with interfering contaminants from carbon dioxide have been reported.

After the preliminary isolation of the compounds, co-extracted material must be removed. Usually several purification steps are required to get the sample clean enough for GC or GC–MS analysis. In the case of biological samples, the first step is to remove lipids and other co-extracted components without losing the lipid-soluble organochlorine compounds. For this purpose saponification and treatment with sulphuric acid have been used. However, strong alkali degrades PCDDs and PCDFs [21,22] and concentrated sulphuric acid destroys certain pesticides, *e.g.*, dieldrin [23]. Column chromatography on aluminium oxide, silica gel, Florisil and activated charcoal is most frequently used for further purification and separation of the compounds. The activities of the adsorbents have been varied and the silica gel is sometimes impregnated with silver nitrate or sulphuric acid or modified to strong basic silicates [3–5,24]. Several techniques have been applied in order to improve the efficiency of the chromatographic systems. In this respect, favourable fractionation of organochlorine compounds from butterfat has been demonstrated using high-performance liquid chromatography (HPLC) with silica columns [5]. Gel-permeation chromatography with cross-linked polystyrene resins (Bio-Beads) has also been used for effective removal of lipids [26–28] and for separation of chlorinated paraffins from other halogenated compounds [29].

The identification of analytes and the determination of their levels are difficult even with clean extracts. There are a large number of chlorinated compounds with similar chemical and physical properties, and not all the compounds are separated by any available single GC column. Furthermore, the concentrations of the compounds to be analysed may differ in the range of ppm to ppt (10^{-6} to 10^{-12}, w/w), making the quantitation of the lower concentrations in the mixture impossible. Therefore, the compounds are separated into groups suitable for further analysis. Such group separations are achiev-

ed by column chromatography using the above-mentioned adsorbents in different combinations. Activated charcoal has generally been used for separation of planar (PCDDs, PCDFs and certain PCBs) from non-planar compounds [24,29–32]. Recently, Haglund *et al.* [33] managed to separate PCBs and PCNs into groups, characterized by the planarity of the substances, using HPLC on 2-(1-pyrenyl)ethyldimethylsilylated silica.

Usually pesticides and PCBs are analysed in the same samples and separate analyses are performed for *e.g.* PCDDs and PCDFs. With the increasing number of pollutants and the possibility of interaction between compounds in their toxic activity, there is a need for multicomponent methods for analysis of a range of analytes in the same sample. For this purpose methods have been developed for simultaneous analysis of a large number of halogenated compounds [29,31,34].

Simple and effective methods are needed for extraction and purification of biological samples for multicomponent trace analysis by GC and GC–MS. In order to replace the laborious liquid–liquid extractions in separatory funnels, other techniques for partitioning have been developed. A liquid–liquid partition method in which one phase is supported on macroporous diatomaceous earth has been used for extraction of milk with light petroleum saturated with acetonitrile [35] and for extraction of oil and fat samples with hexane–acetonitrile mixtures [36]. Solid phase extraction using octadecylsilane-bonded (ODS) silica has been used in the analysis of, for example, water [37] and serum [38].

In our laboratory liquid–gel partition using the lipophilic gels Lipidex 1000 and 5000 is employed for extraction and preliminary purification of biological samples. These gels have been successfully used in the analysis of steroids and bile acids and have also been found to be efficient for other lipid-soluble compounds. Depending on the sample matrix, different procedures have been developed for the transfer of analytes into the gel. The present paper reviews procedures for enrichment on Lipidex in the analyses of organochlorine contaminants.

2. LIPIDEX GELS

2.1. Structure

Lipophilic gels were originally synthesized by methylation [39] or hydroxypropylation of the hydroxyl groups of the cross-linked dextran matrix of Sephadex [40,41]. The hydroxypropylated product, Sephadex LH-20 (Pharmacia, Uppsala, Sweden), has been additionally substituted in order to obtain gels with different functional groups and properties. The Lipidex gels are derivatives of Sephadex LH-20 in which the hydroxypropyl groups have been reacted with long-chain epoxides [41,42] to introduce non-polar alkyl substituents. Two neutral lipophilic gels, Lipidex 1000 and Lipidex 5000, are commercially available from Packard Instruments, Downers Grove, IL, USA. Similar gels are also available from Sigma, St. Louis, MO, USA, as hydroxyalkoxypropyl dextrans. Lipidex 1000 contains 10% and Lipidex 5000 contains 50% (w/w) hydroxyalkyl groups. The higher degree of substitution makes Lipidex 5000 hydrophobic and less polar than Lipidex 1000. The gels are delivered in methanol. Before use they are washed with aqueous ethanol at 70°C and ethanol to remove contaminants from the synthesis [43]. The gels can also be re-used after appropriate washing.

2.2. Properties and mechanisms

The properties of Lipidex and the mechanisms for distribution of compounds in liquid–gel chromatography have been discussed and reviewed [39,41,44]. The Lipidex gel consists of a three-dimensional network. The hydrophobic substituents make the gel repel water and contract in polar solvents while swelling in less polar organic solvents. The bed volume varies with the polarity of the solvent and can be modified by mixing different solvents. The distribution of analytes between the mobile (solvent) and gel phases depends on the polarities of the analytes, the solvent and the gel. Extraction of aqueous solutions with Lipidex gels closely resembles extraction with a solvent of medium polarity and differs markedly from extractions with ODS silica and Amberlite XAD-2, in which adsorption occurs to both polar and non-polar sites. In aqueous media, Lipidex 1000 and 5000 have a higher selectiv-

ity for non-polar compounds than, for example, ODS silica and other solid sorbents [44]. This is an advantage, since cleaner extracts are obtained when analysing non-polar compounds. This is also seen from the yield of organic compounds from urine: the two solid sorbents extracted 2–3% of the total dry weight, while Lipidex 1000 extracted less than 0.1% [45]. The size-exclusion properties of the gel matrix are important for exclusion of macromolecules from the extract but are of little discriminatory importance for the extraction of compounds of medium molecular size.

3. APPLICATIONS

Owing to their selectivity and high capacity, Lipidex gels have been applied in several areas of analysis. In our laboratory, Lipidex 1000 has been successfully used for extraction and purification of steroids of low and medium polarity from different biological samples, *e.g.*, urine [45], tissues [46] and human milk [47]. Bile acids have been extracted from aqueous media as ion pairs [48]. The more hydrophobic Lipidex 5000 has been used for extraction of tetrahydrocannabinol from human fat [49]. An automated method using lipophilic ion-exchanging gels has been developed for enrichment of bile acids and metabolites of mono(2-ethylhexyl) phthalate from urine [50]. This indicates future possibilities for development of simpler procedures for sample treatment using these gels for extraction and purification.

The Lipidex gels have also found several applications in the analysis of organochlorine contaminants. Lipidex 1000 has been used for separation of the more polar conjugated metabolites from the non-polar unconjugated products of chlorinated paraffins [51]. In the early studies, Lipidex 1000 was shown to be an efficient sorbent for fat and *p,p'*-DDT from cow's milk [52]. In this experiment *p,p'*-[^{14}C]DDT was added to the milk and the pesticide could be eluted and separated from 90% of the lipids. In subsequent studies, the more hydrophobic Lipidex 5000 has been used for extraction and purification of organochlorine pesticides, PCBs, PCDDs and PCDFs [31,32,34,53–55].

The gels can be used for chromatography both with normal- and reversed-phase systems [41]. The extraction of compounds with Lipidex is performed in a reversed-phase mode, while the purification and separation can be performed in a normal-phase system. The distribution of organochlorine compounds in a normal-phase system from Lipidex 5000 is shown in Table 1. A mixture of compounds was applied on top of a column of 5 g of Lipidex 5000 prepared in hexane. The gel was eluted with hexane and then with dichloromethane–hexane (1:1). The

TABLE 1

ELUTION OF ORGANOCHLORINE COMPOUNDS FROM LIPIDEX 5000

Recoveries in 5-ml fractions of hexane (fractions 1–20) and dichloromethane–hexane (1:1) (fractions 21–24). + = Compound is present but was not quantified.

Compound	Recovery (%) in fractions											
	1	2	3	4	5	6	7	8–20	21	22	23	24
Hexachlorobenzene		16	84									
α-HCH					86							
β-HCH											56	44
γ-HCH						54	46					
trans-Nonachlor				90								
Heptachloroepoxide				97								
Dieldrin				100								
p,p'-DDT			+	71								
p,p'-DDE			90									
p,p'-DDD						84						
PCBs (Clophen A50)			+	+								
Camphechlor			+	+	+							

separation of dieldrin and β-HCH from other compounds has been used as a purification step in the GC analysis of breast milk [34].

The partitioning of compounds from a sample solution into the Lipidex gel is dependent on the combined effect of the nature of the gel, the solvent associated with the network of the gel and the analyte. Thus, the efficiency of transfer can be modified by rinsing the gel before use with an appropriate solvent. In the analysis of organochlorine contaminants, the Lipidex was washed with methanol [34,53] or 2-propanol [32] and the solvent was removed by suction. The "dry" gel still having some enclosed polar solvent in the matrix was used in the analysis. The need for 2-propanol in Lipidex for quantitative yields of non-polar lipids was first noted in studies on the extraction of sterols and sterol esters in plasma [56].

4. EXTRACTION PROCEDURES

Because of the widespread occurrence of organochlorine contaminants, the nature of the samples to be analysed varies greatly. It is an advantage if analytical strategies can be used which are independent of the nature of the sample. One way to achieve this is to bring all samples into a common form before the start of the purification and separation steps. The aim of our studies is to design methods to transfer the contaminants from different types of samples into Lipidex gels from which column beds can be prepared for subsequent washing and elution.

4.1. Filtration of fluids

4.1.1. Bile

The simplest method for enrichment and purification is to filter a solution through a column bed of the gel [44,45,48,52]. This method was applied in a study of metabolites of chlorinated paraffins in bile [51]. A column bed of Lipidex 1000 in methanol was washed with water. The bile was diluted with 0.6 *M* acetic acid and filtered through the column. The metabolites conjugated with N-acetylcysteine and glutathione were retained and could be eluted with methanol. The unconjugated material was mainly eluted with methanol–chloroform. The more polar conjugated bile acids, which are present in high concentrations in bile, are not retained. Thus, they are removed and will not interfere in subsequent analytical steps.

4.1.2. Water

The somewhat less polar Lipidex 5000 was used in the analysis of pentachlorophenol (PCP) in water and urine [53]. The gel was washed with methanol and the solvent was removed by suction. The gel (1 g) was transferred to a chromatographic column and washed with 20% methanol in water. The sample of water (100 ml) was mixed with 2 ml of formic acid and passed through the gel. The column was then eluted with 10 ml of 20% and 5 ml of 50% methanol in water. This gives a gradual change of the gel character with subsequent swelling of the gel and enables elution with a non-polar solvent. The aqueous solvent in the void volume was expelled with nitrogen and the retained DDT and TCDD were eluted with 35 ml of hexane. When 1 ml of hexane had passed through the column, the flow was stopped for 10 min to permit maximum swelling of the gel in this solvent. Finally, PCP was eluted with 10 ml of acetone. The PCP was derivatized and analysed by GC.

4.1.3. Urine

Non-hydrolysed and hydrolysed urine samples (2 ml) have been analysed for PCP [53]. The hydrolysis was performed with hydrochloric acid or enzymatically with *Helix pomatia* digestive juice. The samples were then treated in the same way. Formic acid (0.5 ml) was added to the hydrolysate and the mixture was passed through a bed of 2 g of Lipidex 5000 followed by 50 ml of water, 10 ml of 50% and 25 ml of 70% methanol in water. Nitrogen was blown through the column to expel the remaining polar solvent and the elution was then performed with 35 ml of hexane and 15 ml of acetone. The elution with 70% methanol and hexane efficiently removed the bulk of different endogenous compounds and interfering substances to give an extract which was sufficiently clean for GC analysis of PCP after derivatization. This method exemplifies the combination of a reversed-phase mode for partitioning of the compounds into the gel and normal-phase elution for purification of the sample on the same Lipidex bed.

4.2. Filtration of gases

4.2.1. Indoor air

Pentachlorophenol is very strongly sorbed to Lipidex and was shown to be directly transferred from laboratory air to Lipidex [53]. A flow of air through a column (2 cm I.D.) containing 5 g of Lipidex 5000 was obtained by suction. The outlet of the column was connected to a flask containing isooctane and ended in the solvent. At different time intervals the sampling was stopped and the column was eluted with 50 ml of hexane to eliminate most of the non-polar compounds and then PCP was eluted with 50 ml of methanol. However, this fraction was not clean enough for GC analysis and purification was achieved by treatment with sulphuric acid. The linear increase of PCP with time of sampling clearly indicated a constant level of PCP in the laboratory air [53].

4.3. Batch extraction

4.3.1. Human milk

Milk has a high fat content and the non-polar lipids are present in chylomicrons surrounded by a layer of phospholipids and proteins. This layer has to be disrupted for the lipids to be extracted, and conditions for quantitative extraction by a fast filtration method have not yet been established. Instead, the fat and fat-soluble compounds can be partitioned into the gel by shaking a mixture of milk and Lipidex under suitable conditions [34]. The extraction was performed in a flask (Erlenmeyer shape) with a PTFE-lined screw cap. A 10-ml aliquot of milk in the flask was mixed with 10 ml of formic acid and 5.0 g of Lipidex 5000 (washed with methanol and dried by suction) was added. The flask was placed at 35°C and shaken for 2.5 h. The mixture was then poured into a glass column (2 cm I.D.) and the solvent was drained. The resulting gel bed was washed with 40 ml of 30% methanol followed by 50 ml of 50% methanol in water. The organochlorine compounds and part of the lipids were eluted with 75 ml of acetonitrile. Remaining lipids were eluted with 60 ml of chloroform–methanol–hexane (1:1:1, v/v/v). The acetonitrile fraction was submitted to further purification and separation steps (chromatography on aluminium oxide, silica gel, activated charcoal) before analysis by GC and GC–MS. Details of the method are described in refs. 31 and 34. The addition of formic acid to the milk was essential for complete transfer of organochlorine compounds and lipids into the gel. Formic acid decreases the binding of chlorinated compounds to proteins [57]. Probably it disrupts the chylomicrons and facilitates the extraction of organochlorine compounds in the lipid core. The slightly elevated temperature keeps the lipids dissolved and the sample mixture uniform. The method has been used in trend studies of organochlorine contaminants, including DDT, DDE, hexachlorobenzene (HCB), isomers of hexachlorocyclohexane (HCH), dieldrin, oxychlordane, *trans*-nonachlor, pentachlorophenol and congeners of PCBs, PCDDs and PCDFs, in milk from individual and pooled samples from Swedish mothers, collected from 1972 to 1989 [31,54,55], and in studies of the correlation of levels with the fat content of milk [54]. Milk from the early sampling periods, 1972–1980, was previously analysed by a method using liquid–liquid partitioning [9]. The agreement between the two methods was established by analysing the same milk by both methods [34]. By the retrospective analysis of stored milk it was possible to calculate the time-related changes in the levels of compounds (PCDDs, PCDFs, planar PCBs) for which there were no techniques available at the time of sample collection [54,55] (Tables 2–4).

4.3.2. Cod liver oil

An oil or solution of oil cannot be filtered through Lipidex or treated in the same way as milk. Because of the non-polar character of both the sample and the gel, there will be no transfer of non-polar compounds to the gel phase. However, the polarity of an oil solution in hexane can be changed by addition of 2-propanol and water, which causes a partitioning of the organochlorine compounds into the Lipidex. This is demonstrated in the analysis of PCBs in cod liver oil [32]. The oil (10 g) was dissolved in hexane (100 ml) and a 2-ml aliquot was subjected to analysis. The sample was mixed with 15 ml of 2-propanol in an Erlenmeyer flask and 5.0 g of Lipidex 5000 (washed with 2-propanol and dried by suction) were added. During the extraction procedure, water (40 ml) was added from an attached dropping funnel equipped with a pressure equalizer at a rate of 0.5 ml/min. The extraction was per-

TABLE 2

MEAN LEVELS OF PCDDs AND PCDFs IN HUMAN MILK FROM SWEDISH MOTHERS

All values are pg per g of fat.

Year	1972	1976	1980	1985	1989
Number of mothers	227	245	340	102	100
2,3,7,8-Tetra-CDD	5	5	3	1	3
1,2,3,7,8-Penta-CDD	9	7	6	5	7
1,2,3,6,7,8-Hexa-CDD	45	40	31	30	38
1,2,3,4,6,7,8-Hepta-CDD	119	96	70	69	57
Octa-CDD	458	371	338	244	268
2,3,7,8-Tetra-CDF	4	3	3	2	2
2,3,4,7,8-Penta-CDF	32	29	17	14	17
1,2,3,6,7,8-Hexa-CDF	14	14	8	8	7
1,2,3,4,6,7,8-Hepta-CDF	24	21	7	8	8
Octa-CDF	6	4	5	5	2

formed in a water bath at 35°C for 2.5 h. The mixture was then transferred to a glass column and subsequently eluted as described for milk. This procedure separated about 60% of the lipids from the PCB-containing fraction (eluted with acetonitrile). Further purification and separations were made prior to analysis by GC and GC–MS [32]. The method was successfully used in an international intercalibration of methods for analysis of planar PCBs.

TABLE 3

MEAN LEVELS OF TOXIC NON-*ORTHO* PCBs IN HUMAN MILK FROM SWEDISH MOTHERS

All values are pg per g of fat.

Year	1972	1976	1980	1985	1989
Number of mothers	195	204	431	102	140
3,3′,4,4′-Tetra-CB (PCB 77)	76	41	29	35	27
3,3′,4,4′,5-Penta-CB (PCB 126)	298	253	166	102	98
3,3′,4,4′,5,5′-Hexa-CB (PCB 169)	67	74	65	43	47

TABLE 4

MEAN LEVELS OF TOXIC MONO-*ORTHO* PCBs IN HUMAN MILK FROM SWEDISH MOTHERS

All values are pg per g of fat.

Year	1972	1976	1980	1985	1989
Number of mothers	135	153	431	102	140
2,3,3′,4,4′-Penta-CB (PCB 105)	15	16	8	6	7
2,3′,4,4′,5-Penta-CB (PCB 118)	60	46	31	20	25
2,3,3′,4,4′,5,-Hexa-CB (PCB 156)	20	19	13	11	13

5. CONCLUSIONS

The liquid–gel partitioning technique is advantageous compared with liquid–liquid partitioning as no emulsions are formed and the passage of sample through a column bed replaces repeated extractions and centrifugations. By suitable modifications of the liquid–gel partitioning procedure organochlorine contaminants from different sample matrices can be transferred into the gels. In this way a common starting point is obtained for subsequent purification, and analytical strategies can be similar for many types of samples. The structure and polarity of the gels permit sequential use of solvents in the reversed- and normal-phase modes, so that the extraction–transfer step can be combined with separation and purification. Finally, the methods have been developed for small sample sizes permitting the use of small column systems and solvent volumes.

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