Trace Solvent Contamination as Determined by Surface Pressure Measurement

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Abstract □ Amphipathic contaminants, present in organic solvents, were detected by spreading the solvent on water and measuring its surface pressure. The method is quite sensitive and, in many cases, is superior to conventional techniques.

Keyphrases □ Solvent contamination, trace—determination □ Contaminants in solvents—surface pressure effect □ Surface pressure measurements—solvent contamination determination

A number of instances have been reported in which the presence of small quantities of impurities caused a significant change in the properties of the system. For example, the stability of suspensions of channel black in toluene was increased considerably by the presence of traces of moisture in the solvent (1). Contact angle hysteresis in experiments on wetting of solids by liquids has been ascribed to the presence of impurities (2). In studies of adsorption by solids from the liquid phase a high order of solvent purity is necessary. Traces of contaminants may be preferentially adsorbed, leading to spurious results (3). Similar considerations apply to work on monolayers. Surface-active impurities will be concentrated at the interface resulting in a considerable error. Grease from the fingers may represent an important source of contamination (4).

Spectroscopy and chromatography are often used to check the purity of solvents. Both of these methods are capable of detecting small concentrations of foreign substances, under favorable conditions. However, spectroscopy is useful only at wavelengths at which the pure solvent does not itself strongly absorb. Of the various chromatographic techniques, gas chromatography is probably the most sensitive. Contaminants of very low volatility (polymers for example) may be sorbed so strongly by the column that they are not eluted for many hours.

In this communication, another method for detecting solvent contamination is described. A small quantity of the liquid to be tested is spread on an aqueous substrate. Water-insoluble molecules and a number of water-soluble amphipathic molecules and polymers will be concentrated at the air-water interface, causing changes in surface tension which are easily detectable.

EXPERIMENTAL

Water employed as the substrate was deionized and then distilled in an all-glass still. All organic liquids were reagent or spectro grade. Glassware was cleaned in chromic acid cleaning solution prior to use. Near IR spectra were obtained using the Beckman DK-2 spectrophotometer. All gas chromatography was performed using a Perkin-Elmer vapor fractometer, model 154. A column of propylene glycol was employed; helium was the carrier gas.

The Teflon surface balance has been previously described (5). Surface tension was determined by the Wilhelmy plate method (6), using a roughened platinum plate. The apparatus employed had a sensitivity of 0.1 dynes/cm. The water surface was swept with the Teflon barrier. Then 1 ml. of the organic liquid to be tested was placed dropwise on the water surface and allowed to spread. After a waiting time of 10 to 15 min. the barrier of the surface balance was moved so as to reduce the available surface area to 80 cm.^{2} and the surface tension was determined.

Table I—Surface Pressure of Pure Organic Liquids Spread on Water

Liquid	Surface Pressure, dynes/cm.
Hexane	0.2
Benzene	0.2
Isopropanol	1.2
Acetone	0.7

RESULTS AND DISCUSSION

The spreading experiments are summarized in Table I. Results are expressed in terms of surface pressure, which is the decrease in surface tension using a clean water surface as reference. All of the liquids tested had positive spreading coefficients (7), and spreading was observed visually in all cases. Volatile, poorly soluble liquids, such as benzene and hexane, would be expected to disappear very rapidly from the surface, producing a negligible surface pressure. However, even liquids as water-insoluble as these may diffuse through the surface layers of water and require some time for back diffusion to the surface and subsequent evaporation (8). For this reason, it is necessary to wait for about 10 min. after spreading before measuring surface pressure. Pure, insoluble liquids will then indeed yield no significant surface pressure (Table I).

Molecules of water-soluble substances, such as isopropanol and acetone, are distributed between the surface and bulk regions, resulting in a low surface pressure (Table I). The surface pressure values obtained for these solvents were independent of surface area and persisted after the surface was swept clean. The observed surface pressures were therefore due to the presence of solvent molecules in the interface and not to impurities. Regardless of the solubility of the solvent, impurities concentrated at the surface cause large changes in surface pressure, which indicate their presence.

The sensitivity of the spreading test was recently demonstrated in this laboratory. A sample of benzene spectrograde, taken from an old bottle which had been opened many times, gave a surface pressure of 11.3 dynes/cm. when spread on water. Near IR spectra of the contaminated benzene and of benzene known to be pure were identical. Gas chromatography at low attenuation of the impure sample yielded a large peak and a much smaller one (whose area was about $^1/_{2000}$ that of the large peak). The pure sample of benzene produced the same two peaks of the same relative size.

The impure sample of benzene was passed through a silica gel-alumina column. The purified material, spread on water, gave a surface pressure of 0.2 dynes, showing that the contaminant had been removed by the column.

Assume, for purposes of calculation, that contamination occurred as a result of careless handling and that the contaminant was a skin lipid. Skin phospholipids spread on water occupy an area of about 80 to 100 Å.^{2} per molecule at moderate surface pressures (9). Under the conditions of the author's spreading test one could therefore be capable of detecting less than 10^{16} molecules, or about 10^{-8} moles of contaminant.

The spreading test may be extended to nonspreading liquids which are volatile or water-soluble. Such liquids may be deposited on the surface by first dissolving them in a solvent (*e.g.*, hexane)

which is capable of spreading. The spreading test may also be applied in certain cases to the detection of solid contaminants, such as detergents which are leached by solvents. The recent report of silicones present in disposable syringes (10) suggests another possible application. Silicones are surface active; if spread on water, as little as 0.01 mg. can easily be determined (11).

Although the spreading method is very sensitive, it is limited to those impurities which are not very volatile and which have some affinity for the surface. Included in this category are surfactants, lipids, most polymers, and some other materials such as steroids and antibiotics. The method is incapable of detecting, for example, the presence of low boiling homologs or isomers in hexane. Therefore it should not be considered a substitute for other methods of checking purity. Nevertheless, if it is necessary to determine trace quantities of surface-active impurities, the spreading method represents a convenient and efficient tool.

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Anticholinesterase Activity of Some Degradation Products of Physostigmine

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Abstract ☐ The anticholinesterase activities of four degradation products of physostigmine have been determined *in vitro* using biochemical and biological assay techniques and *in vivo* by means of the rat chromodacryorrhea response. The degradation products are at least 100 times less active than the parent compound as anticholinesterase agents. Eseroline, rubreserine, and eserine blue are effective in high concentrations against the cholinesterase of horse and rabbit plasma but are much less active against the red blood cell cholinesterase; they exert no inhibitory action on the two human cholinesterase enzymes. *In vivo*, the degradation products are at least 1000 times less active than physostigmine. Lack of coloration of solutions of physostigmine does not necessarily indicate full anticholinesterase activity because a colorless product of hydrolysis, eseroline, possesses little or no anticholinesterase activity.

Keyphrases ☐ Physostigmine—degradation products ☐ Anticholinesterase activity—physostigmine degradation products ☐ Biological assay—physostigmine degradation products ☐ *In vitro* assay—physostigmine degradation products activity

When physostigmine in solution undergoes decomposition, a red-colored compound is formed which turns blue on further decomposition (1). Hydrolysis which initially removes the urethane grouping and produces eseroline, a colorless compound (Scheme 1), is followed by oxidation to yield rubreserine, a red material, which is converted into eserine blue or eserine brown. The molecular weight of eserine blue exceeds that of rubreserine and it has been suggested that condensation of rubreserine with other physostigmine degradation products occurs at this stage in the reaction. The end product of the degradation by heat or by exposure to oxygen and alkali is eserine brown.

The anticholinesterase activity of these degradation products has been investigated. Their biological activity has particular relevance as solutions of physostigmine are used in ophthalmology. Furthermore, in the preparation of eye drops sterilization is effected by filtration or by autoclaving the solution at 98 to 100° for 30 min. (BPC, Supplement, 1966) or by steam sterilization at 121°. (USP XVII, 1965). This heating process may result in decomposition of the physostigmine, with possible loss of activity. A preliminary note concerning this work has been published (2).

METHODS

Warburg Manometric Technique—The anticholinesterase activity of the different compounds was compared by this technique using horse serum (0.5 ml.) as the source of cholinesterase and acetylcholine (0.5 ml.) at a final concentration of 0.0138 *M* (3) as the substrate. The pH value of the incubation mixture was controlled by adding 1.5 ml. of 0.04 *M* sodium bicarbonate (adjusted to pH 7.6 by the addition of hydrochloric acid) and each compound under test was added to 0.5 ml. of this bicarbonate solution before addition to the incubation flask. The total volume of fluid in each flask was 3 ml. After gassing with a mixture of 95% nitrogen and 5% carbon dioxide for 10 min., the flasks were incubated at 37° and the manometers read at 10-min. intervals for 30 min. Values in the text are the mean of three experiments. Differences in percentage inhibition greater than 15% are significant ($p = 0.05$).

Biological Assay Technique—Horse serum (0.1 ml.) was used as the source of cholinesterase, with acetylcholine as the substrate (2 ml. of 5 mcg./ml.). Each compound under test was dissolved in 2