



Fig. 1. Silicate-salinity relationship, March 1961–February 1962, at the Koko Head monitoring station, Oahu, Hawaii

changes in standing crops of phytoplankton. Consequently, it seemed of interest to investigate the possibility that variations in trace metals and other chemical species might reflect changes in water type.

Analysis of dissolved silicate and particulate iron was carried out on a weekly basis on surface water samples collected at the Koko Head station from March 1961 through April 1962. The monthly mean concentration of silicate-Si ranged from 0.49 to 1.81 µg-atom/l. and that of particulate iron from 0.50 to 3.44 µg-atom/l.

Although seasonal variations of both silicate and particulate iron were discernible, it was not possible to relate these to known local biological activity in the waters. A plot, however, of the monthly mean values of silicate versus the corresponding monthly salinities reveals three distinct distribution groups (Fig. 1). With the studies of Seckel and others<sup>1</sup> on the surface water types of the Hawaiian islands in mind, it can be assumed that the water types moving in and out of the island chain have varying silicon and iron content and that the groups in Fig. 1 indeed represent these different water types. The biological history of each water type is undoubtedly different and hence is responsible for the variation in silicon and particulate iron content. Similar characteristic distribution groups were also observed with particulate iron. Inorganic phosphate and nitrate did not show such distinct groups but rather indicated annual cycles<sup>2</sup>. It is not known whether data on other trace metals will reveal information similar to that obtained from silicate and particulate iron. However, if such proves to be the case, trace element content could be used advantageously under the proper conditions as an additional tracer for distinguishing water types.

We are indebted to the Bureau of Commercial Fisheries (Honolulu) for making available salinity data obtained at the Koko Head monitoring station.

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<sup>1</sup> Seckel, G. R., *Fish. Bull.*, **61**, 371 (1962).

<sup>2</sup> Zeitlin, H., and Higaki, S. (unpublished).

### Thin-layer Chromatography in S-tanks of Mixtures containing Free Fatty Acids

FREE fatty acids may be readily separated from other lipids by chromatography on thin layers of silica gel. The solvents used generally contain a small amount of acetic acid<sup>1-4</sup> the purpose of which is to suppress dissociation of the fatty acids being chromatographed and thereby prevent serious 'tailing' of the spots.

Stahl<sup>5</sup> has described the separation of phenolic acids with an acid-free developing solvent on silica gel containing a small amount of oxalic acid. Experiments in this laboratory have shown that mixtures containing free fatty

Table 1. *R<sub>F</sub>* VALUES OF VARIOUS FREE FATTY ACIDS AND GLYCERIDES IN AN S-TANK

Oleic acid	0.68
9 and 10-Hydroxy stearic acids	0.13–0.18
9 and 10-Keto-stearic acids	0.40–0.50
1-Monostearin	0.03
1,3-Distearin	0.43
Tristearin	0.93

Adsorbent: Silica gel G (Merck) + 2% w/w oxalic acid. Humidity: circa 55%. 20° C. Solvent: 1 : 1 cyclohexane : di-iso-propyl ether (purified over alumina, grade 1). 15 cm development. Detection by charring was not affected by the oxalic acid.

acids may also be chromatographed with an acid-free solvent in ordinary rectangular tanks, provided that there is some acetic vapour in the atmosphere of the tank. Presumably this vapour is rapidly absorbed by the dry layer and behaves in much the same way as the oxalic acid in Stahl's experiments.

These observations may usefully be applied to the chromatography of fatty acids in S-tanks. S-tanks<sup>6</sup> have several advantages over normal tanks; but when they are used for chromatographing fatty acids in solvent systems containing a little acetic acid, they have the disadvantage that the fatty acids often move as an irregular spot immediately ahead of a sharp secondary solvent front, some distance below the true solvent front. This secondary front arises from the frontal analysis or demixing of the solvent mixture; the effect is particularly pronounced in S-tanks, in which absorption of solvent vapour by the dry part of the layer is normally at a minimum<sup>7</sup>.

To overcome this problem we have found one of two alternatives necessary. Either the plate may be exposed, just before development, to acetic acid vapour for up to one minute, or about 2 per cent (based on the weight of silica gel) of oxalic acid may be incorporated into the slurry when preparing the chromatoplates. Either course ensures that free acid is dispersed over the whole adsorbent before development begins.

Of the two methods, the addition of oxalic acid is the more reliable and has given satisfactory results with oleic acid (up to at least 50 µg per spot). The oxalic acid has not been observed to lead to isomerization of 1,3 distearin on a chromatoplate or to poor binding of adsorbent.

A suitable system for the separation of free fatty acids and glycerides is given in Table 1.

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<sup>6</sup> Stahl, E., *Dünnschicht-Chromatographie* (Springer-Verlag, Berlin, 1962).

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### Inhibition of Hyaluronic Acid Degradation by Dimethyl Sulphoxide

THE presence of ascorbic acid in vitreous humour<sup>1</sup> is of great importance in the process of post-mortem degradation, since hyaluronic acid, the major polysaccharide component of vitreous humour<sup>2</sup>, has been shown<sup>3</sup> to be degraded by reducing agents such as ascorbic acid and hydroquinone, in the presence of oxygen (the so-called ORD—oxidative reductive depolymerization—reaction). It is generally held that this depolymerization is brought about by free radical action, since radical scavengers such as sodium diethyldithiocarbamate have been shown<sup>4</sup> to inhibit the reaction, and in an analogous investigation of the degradation of alginate, Smidsrod, Haug and Larsen<sup>5</sup> have postulated a free radical reaction with peroxide intermediates. In our examination of the preservation of human vitreous humour a major consideration has been