

## ADSORPTION IN LIQUID SCINTILLATION COUNTING

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When a radioactive sample becomes adsorbed to the inner walls of a liquid scintillation counting vial instead of remaining in solution, the count rate obtained is no longer a simple reflection of its activity. Thus, the phenomenon of adsorption, although a subject of interest in many areas of physics and chemistry, is, in the area of practical liquid scintillation counting, an irritating and continuing problem. The following discussion is concerned with the practical aspects of recognizing and overcoming the problem.

### Nature of the Problem

As a result of the changed counting geometry of an adsorbed sample, and the back-scattering energy loss, counting losses in the channel of interest occur, coupled with the appearance of pulses in channels of lower energy. This overall effect is therefore identical to that caused by quenching — attenuation of the count rate and the pulse height shift to lower energy — with the following crucial difference: quenching is a property of the solution and so the count rate of an external standard is also controlled by it; adsorption, on the other hand, is a phenomenon concerning only the radioactive solute and not the solution as a whole, and thus the count rate of an external standard is essentially independent of the adsorptive state of the radioactive solute. Although this difference may be exploited (see below), the problem of adsorption is, therefore, a particularly insidious one in the sense that it gives rise to erroneous counting data in such a way that is not obvious in the normal operation of a liquid scintillation counter. As Litt and Carter have pointed out (1), the deceptive simplicity of operating modern counters makes the problem all the more acute.

In view of the pulse height shift caused by adsorption, the problem will clearly be more serious for situations in which narrow channels are employed.  $^{14}\text{C}$ — $^3\text{H}$  double-label counting, for example, could suffer from the double effect

of loss of counts in the  $^{14}\text{C}$  channel, and additional  $^{14}\text{C}$  spillover into the  $^3\text{H}$  channel.

### Detection of Adsorption

In our laboratory, the following three tests have been found useful to establish the question of adsorption or non-adsorption of a particular counting mixture.

1. Dilution with non-radioactive carrier. The idea of this test, extensively discussed by Litt and Carter (1), is to produce radioactive solute of such low specific activity that essentially all the surface sites are saturated with inactive carrier, with the result that the active material is in solution and is counted normally. Thus, for adsorbed compounds the apparent activity increases, while for non-adsorbed compounds the activity remains constant. Two disadvantages of the method are, firstly, that inactive material may not always be available, and secondly, that if the limit of solubility is exceeded, misleading results due to heterogeneous counting may be obtained. The possibility that the addition of inactive carrier may cause additional quenching is not, of course, of concern as this is corrected for by normal external standardization.
2. Vial emptying. Adsorption may be detected by emptying the suspected vial, refilling the vial with fresh scintillator solution, and recounting. In our experiments on  $^{14}\text{C}$ -labelled compounds in glass vials, there is a dramatic difference between non-adsorbed compounds (99.7% of activity removed from the vial) and adsorbed compounds (over 95% retained in the vial). It is worth noting that a small retention of activity (e.g. 16% for benzoic acid) does not appear sufficient to cause any counting anomalies (2).
3. Measurement of the Adsorption Shift. This method, developed in our laboratories three years ago (3), takes advantage of the fact that adsorption affects a properly chosen sample channels ratio (SCR) but leaves unaffected the external channels ratio (ESR), whereas quenching affects both SCR and ESR. Thus, the normal empirical relationship between SCR and ESR in the absence of adsorption may be derived, and the normal SCR calculated for any value of ESR. The Adsorption Shift is defined as the difference between the calculated and observed SCR. Adsorption gives rise to non-zero values of the Adsorption Shift.

### Prediction of Adsorption

Two prime requirements for adsorption problems to occur are firstly the counting of a compound with adsorptive properties, and secondly, a sufficiently high specific activity. An extensive survey of  $^{14}\text{C}$ -labelled organic radiochemicals in glass vials (Amersham-Searle low-level spectravials) was carried out in our laboratories in 1974 (5). It was found that the types of adsorptive radiochemical were, in fact, very few and essentially limited to compounds with a high oxygen content. The most prevalent group of adsorbed molecules were those with more than one carboxylic acid group, there being an interesting sharp distinction in this regard between mono- and dicarboxylic acids. In contrast, several other relatively polar compounds with high nitrogen content (e.g. barbituric acid, adenine) were not adsorbed. Litt and Carter (1) have found a number of amino acids to be adsorbed both on glass and, to a lesser extent, plastic vials. It is perhaps worth noting that organic chemists (reaction mechanism studies) are therefore unlikely to be troubled by adsorption problems, but that many biochemical studies (amino acids, compounds of intermediary metabolism) are liable to be plagued by the problem.

The second requirement for adsorption, that of sufficiently high specific activity, is clearly due to carrier saturation of adsorption sites mentioned above. In studies in our laboratory on glass vials (Amersham-Searle low-level spectravials) the critical concentration to saturate the sites was found to be approximately  $2 \times 10^{-6}$  M, corresponding to  $3 \times 10^{-5}$  mmoles/15 ml of counting solution and, if the counting activity is 50,000 dpm, to a critical specific activity of approximately 0.5 to 1.0 mCi/mmole, below which adsorption is likely to be of rapidly diminishing significance. It should be noted that  $3 \times 10^{-5}$  mmoles (above) corresponds to  $<1.0$  mg for compounds of molecular weight under 30,000 so that for compounds in this category, addition of 1 mg of carrier should eliminate the adsorption problem, provided that the material is sufficiently soluble.

### Overcoming the Adsorption Problem

Two approaches are possible. The first approach is to change the system in such a way as to eliminate adsorption of the radiochemical involved. The most obvious way of

achieving this objective is dilution with radioactive carrier to apparently constant activity, a method which is subject to the limitations mentioned above (detection of adsorption). Alternatively, a change in the type of vial or solution may give a non-adsorbed situation; recently, emulsion counting of sodium orthophosphate has been reported to eliminate the adsorption problems of solution counting (6).

The second approach is to make use of the erroneous data obtained from the adsorbed radiochemical. In a study in our laboratory in 1974 on  $^{14}\text{C}$ -labelled compounds, it was found that not only is the Adsorption Shift a good detector of adsorption, but also that the magnitude of the Adsorption Shift is reliably related to the extent of counting loss due to adsorption (7). Thus, by setting up an additional calibration curve, it becomes possible to correct for the adsorption counting losses (7). As is the case for external standardization, the calibration curve is applicable only for the instrument settings under which it was constructed.

#### References

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