A Simple Technique for Eliminating Interference by Detergents in the Lowry Method of Protein Determination

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The presence of nonionic and cationic detergents interfered in the Lowry method of protein estimation by causing precipitate formation. The addition of 0.5% sodium dodecylsulphate in the alkali reagent prevented this precipitation without affecting colour development, and allowed the method to be used on detergent treated membrane preparations.

Nonionic detergents, such as Triton X-100, are frequently used to dissociate protein from membranes. However, both nonionic and cationic detergents interfere with the Lowry method of protein determination (1) by producing a yellow precipitate on addition of the Folin Ciocalteu reagent. This precipitate must be removed by centrifugation before the absorbance of the solution can be measured, and when the final detergent concentration is greater than about 0.15% this absorbance is markedly diminished (2).

The modification described here involves inclusion of an anionic detergent such as sodium dodecylsulphate in the assay mixture. This prevents the formation of any precipitate without affecting colour development.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin (BSA) was purchased from Calbiochem, sodium deoxycholate (DOC) from Mann Research Laboratories, and Triton X-155 from Sigma Chemical Company. British Drug Houses (Aust. Pty.) Ltd., Melbourne, supplied the Triton X-100, the Folin Ciocalteu reagent, and the sodium dodecylsulphate (SDS), specially pure grade. Tween 60 was obtained from Chemical Materials Ltd., Sydney, Teric 200 from Imperial Chemical Industries of Australia and New Zealand Ltd., Melbourne, and Hyamine 2389 from Robert Bryce and Co. Ltd., Sydney.
DETERGENTS AND PROTEIN ESTIMATION

Assay

For standard curve determinations, BSA, in amounts from 10 to 250 μg, was prepared in 0.5 ml of either water or varying concentrations of detergents (e.g., Triton X-100). Then either 0.5 ml water or 0.5 ml SDS or DOC was added at a concentration ten times that of the other detergent. The amount of BSA present was then determined by the standard Lowry method. In cases where a precipitate formed, samples were centrifuged after colour development and the absorbance of the supernatant measured as before at 750 nm on a single beam Unican SP 600. All assays were performed in triplicate and an average result taken.

RESULTS

Protein samples containing the nonionic detergents Triton X-100, Triton X-155, Tween 60, or Teric 200 gave a precipitate on addition of the Folin-Ciocalteu reagent. The same effect was observed with the cationic detergent Hyamine 2389. However, the anionic detergents DOC and SDS did not give any precipitate.

If a sufficient amount of one of these anionic detergents was added to the nonionic or cationic detergent before addition of the alkaline copper reagent, no precipitate formed.

The effectiveness of SDS and DOC in preventing precipitation with respect to these various detergents is shown in Table 1. With a range of nonionic and cationic detergents the addition of SDS at a concentration

<table>
<thead>
<tr>
<th>Detergents added</th>
<th>$A_{750}$ nm blank</th>
<th>$A_{750}$ nm 100 μg BSA</th>
<th>Increase in $A_{750}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>0.085</td>
<td>0.385</td>
<td>0.30</td>
</tr>
<tr>
<td>SDS</td>
<td>0.085</td>
<td>0.39</td>
<td>0.305</td>
</tr>
<tr>
<td>Triton X-100 + SDS</td>
<td>0.095</td>
<td>0.395</td>
<td>0.30</td>
</tr>
<tr>
<td>Triton X-155 + SDS</td>
<td>0.095</td>
<td>0.395</td>
<td>0.30</td>
</tr>
<tr>
<td>Tween 60 + SDS</td>
<td>0.085</td>
<td>0.395</td>
<td>0.31</td>
</tr>
<tr>
<td>Teric 200 + SDS</td>
<td>0.085</td>
<td>0.395</td>
<td>0.31</td>
</tr>
<tr>
<td>Hyamine 2389 + SDS</td>
<td>0.085</td>
<td>0.395</td>
<td>0.31</td>
</tr>
<tr>
<td>DOC</td>
<td>0.12</td>
<td>0.415</td>
<td>0.295</td>
</tr>
<tr>
<td>Triton X-100 + DOC</td>
<td>0.14</td>
<td>0.465</td>
<td>0.325</td>
</tr>
<tr>
<td>Triton X-155 + DOC</td>
<td>0.145</td>
<td>0.45</td>
<td>0.305</td>
</tr>
<tr>
<td>Tween 60 + DOC</td>
<td>0.14</td>
<td>0.46</td>
<td>0.32</td>
</tr>
<tr>
<td>Teric 200 + DOC</td>
<td>Yellow precipitate formed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyamine 2389 + DOC</td>
<td>Solution became yellow</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$a$ SDS and DOC concentrations were 2.5% in the 1 ml sample; all other detergent concentrations were 0.25% in the sample.
FIG. 1A. Effect of increasing Triton X-100 concentration in a 1.0 ml sample, with and without bovine serum albumin (BSA), on A750 nm in the presence of constant amounts of SDS or DOC. (●—●) 2.5% SDS in sample; (▲—▲) 2.5% DOC in sample. X—no BSA present in the sample. Y—100 μg BSA in the sample. Z—difference between A750 nm values for X and Y due to 100 μg BSA in the sample. Arrows indicate lowest percentage of Triton X-100 where precipitate was produced immediately on addition of Folin-Ciocalteu reagent. 1B. Effect of increasing anionic detergent (SDS or DOC) concentration in a 1.0 ml sample, with and without BSA, on A750 nm in the presence of a constant concentration of Triton X-100 (0.25%). (●—●) Varying percentage of SDS in the sample. (▲—▲) Varying percentage of DOC in the sample. X—no BSA present in the sample. Y—100 μg BSA added to the sample. Z—Difference between A750 nm values for X and Y due to the presence of 100 μg BSA in the sample. Arrows indicate highest percentage of SDS that did not prevent precipitation on addition of Folin-Ciocalteu reagent. Precipitates formed with all concentrations of DOC used.

ten times that of the detergent allowed protein estimations to be made without any precipitation occurring. DOC at the same concentration was effective in preventing precipitation in most cases but the absorbances were increased in its presence. In the case of Hyamine 2389, a yellow colour developed and a much higher absorbance was obtained, while with Teric 200, DOC did not prevent precipitation even at a concentration 40 times that of the Teric 200.

Figure 1A shows the effect of increasing the amount of Triton X-100 in the presence of a constant amount of either DOC or SDS with and without BSA present. Increasing the percentage of Triton X-100 caused
an increase in the absorbance of the solution, but this increased equally in the Triton X-100 blank and therefore did not affect the final result. The increased colour development appeared to be due to reaction of the Triton X-100 with the Folin–Ciocalteu reagent because the addition of Triton X-100 after the Folin–Ciocalteu reagent caused no precipitate to form even in the absence of anionic detergents. No increase in absorbance was observed under these conditions either. Any tubes containing a precipitate after full colour development were centrifuged before the absorbance was measured.

The presence of DOC caused the absorbance of the assay mixture to increase much more with increasing Triton X-100 concentration than with SDS present. Also the ratio of DOC to Triton X-100 necessary to prevent precipitation was about 7:1 which was considerably higher than

![Graph](https://example.com/graph.png)

**Fig. 2.** This illustrates the reduction in A750 nm caused by the removal, by centrifugation, of the precipitate formed in the presence of high Triton X-100 concentration. Both precipitate production and reduction in A750 nm were prevented by addition of SDS. (●—●) Increasing Triton X-100 concentration in a 1.0 ml sample containing 100 µg BSA. (▲—▲) Increasing Triton X-100 concentration in a 1.0 ml sample containing 100 µg BSA but with the addition of 3% SDS to the alkali reagent.
the 3:1 ratio of SDS to Triton X-100 required for the same purpose. If slightly less anionic detergent than this was used, a small amount of precipitate formed which redissolved before the 30 min allowed for colour development had elapsed. Even so, a higher absorbance than expected was usually observed under these circumstances.

Figure 1B shows the effect of increasing SDS and DOC concentrations at a constant Triton X-100 concentration with and without BSA present. Although increasing detergent concentration did not change the absorbance at 750 nm greatly, all solutions containing no anionic detergent or containing DOC had a higher absorbance than those containing SDS.

Over the range 0-250 µg BSA, there was no significant difference in absorbance at 750 nm whether Triton X-100 was present in the sample or not, provided either SDS or DOC was added. When the precipitate formed by Triton X-100 in the absence of SDS or DOC was removed by centrifugation, no decrease in absorbance compared to a normal protein determination was observed unless the Triton X-100 concentration was greater than 1% in the 1 ml protein sample. At higher Triton X-100 concentration however, a greatly lowered absorbance was obtained after centrifugation. This is illustrated by Fig. 2 which also shows that even at these high Triton X-100 concentrations precipitation was prevented by the addition of sufficient SDS, without affecting colour development.

**DISCUSSION**

These results show that the presence of anionic detergents in the reaction mixture prevented the precipitation of nonionic and cationic detergents in the Lowry protein determination.

DOC was suitable in most instances, but was ineffective in the cases of Hyamine 2389 and Teric 200. It also caused a greater increase in absorbance than did SDS. SDS was effective with all nonionic and cationic detergents tested and was required in lower concentration than DOC.

For these reasons it appeared that SDS was more universally suitable for the prevention of precipitate formation. To utilise this modification, the only alteration necessary to the Lowry method was the addition of 0.5% SDS to the alkali reagent. This reagent was then routinely stored at 25°C to prevent precipitation of the SDS. In extreme cases where very high concentrations of detergent were involved, the percentage of SDS could be increased at least up to 3% without having any detrimental effect on the original reagent or its stability. This simple modification enabled estimation of protein released from membranes by detergent treatment to be facilitated, without any reduction in the sensitivity of the method, provided reagent blanks were utilised.
ACKNOWLEDGMENT

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REFERENCES