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New Approaches in the Quantitation of Total Proteins from Latex Gloves

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Aspects of protein purification/concentration and detection were modified to enhance sensitivity, rapidity and practicality in assaying total proteins from latex gloves. In purifying the eluted proteins by acid precipitation, a combination of 5% trichloroacetic acid and 0.2% phosphotungstic acid was found to precipitate proteins most effectively. Maximal colour development in the Lowry micro-assay for proteins was attained when the protein precipitate was re-dissolved in 0.25M sodium hydroxide and when 6% Folin reagent was utilised in the reaction. As a simpler alternative to the colorimetric assay, precipitated proteins were quantitated by UV absorption at 280 nm. The UV readings correlated well with results obtained by the Lowry assay. In another approach towards rapid assessment, proteins present in situ *on the latex glove was approximated by staining with Naphthalene Black.*

The occurrence of Type I hypersensitivity in a small proportion of people using latex products has been attributed to allergenic proteins that are present in the products[1,2]. To assess the amount of residual proteins that might diffuse out of latex products (specifically latex gloves), methods have been described to purify and concentrate the proteins obtained in glove eluates and to assay the proteins using a modified Lowry micro-method[3]. While these procedures and their adaptations are currently being routinely employed, some aspects of the methodologies are re-examined with the view to enhance sensitivity, rapidity and practicality in assaying total proteins from latex gloves.

PROTEIN PURIFICATION AND CONCENTRATION

Assays to quantitate latex glove proteins are susceptible to interference by non-proteinaceous substances present in the test sample. To minimise interference to the assay, a convenient purification procedure is to precipitate the proteins using trichloroacetic acid (TCA) and phosphotungstic acid (PTA)[3]. By this approach, the precipitated proteins are recovered by centrifugation while most of the soluble non-proteins that might interfere with the assay are discarded with the supernatant. Acid precipitation of proteins offers another advantage where protein concentrations are very low and thus difficult to quantitate accurately. By re-dissolving the precipitated proteins in a smaller volume of solvent (commonly sodium hydroxide) than that of the original sample, a concentration effect is achieved. While the combination of 5% TCA and 5% PTA to precipitate latex glove proteins has been reported[3], no systematic study has been made to determine the most effective concentrations of the acids.

In an investigation, a latex glove eluate was precipitated by various combinations of TCA and PTA, with each reagent ranging from 0 to 10% (w/v) final concentration. It was observed that 1 to 10% TCA combined with 0.2% PTA

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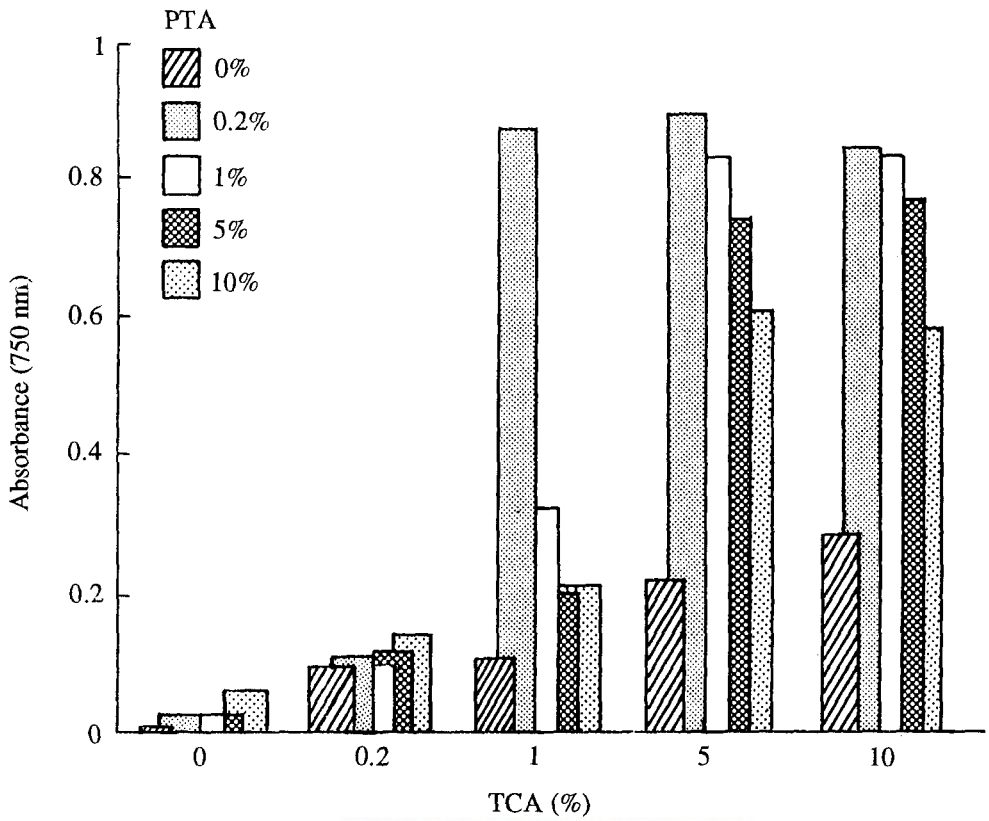

Figure 1. Effectiveness of various combinations of TCA and PTA in precipitating proteins from latex glove eluate. TCA was added to the test sample first in all cases. Results are the means of two replicates.

was most effective in precipitating proteins from 6 ml of latex glove eluate containing 46 g/ml *(Figure 1)*. It is recommended that the glove eluate be precipitated with 5% TCA first, followed by 0.2% PTA *(Appendix 1)*.

PROTEIN ASSAY

Modified Lowry Micro-assay

To quantitate the low levels of proteins eluted from latex gloves, a modified micro-Lowry procedure following the general approach of Faridah and Yeang[3] was adopted. In their report, 0.1*M* sodium hydroxide was used to dissolve the precipitated proteins while the amount of Folin reagent employed in the Lowry micro-assay was 6.25%. Lowry *et al.*[4] noted that the pH of the reaction mixture was critical to its sensitivity and that this was controlled by the relative contents of sodium carbonate/sodium hydroxide that was alkaline and the Folin reagent that was acidic. Their recommendation, that has since been widely adopted, was to have about 0.1*M* sodium hydroxide in the reaction mixture prior to addition of the Folin reagent and to use a final concentration of 2% Folin reagent even though they reported that higher sensitivity could be achieved with 0.2*M* sodium hydroxide coupled with 6 or 7% Folin reagent.

In a study to determine the optimal sodium hydroxide - Folin reagent combination, latex glove proteins dissolved in various concentrations of sodium hydroxide (0 – 0.3*M*) were assayed by the Lowry reaction where the final Folin reagent concentration ranged from 2 to 7%. As shown in *Figure 2*, 0.2 – 0.25*M* sodium hydroxide in the test sample (0.18*M* after addition of *Reagent C*) coupled with 6% Folin reagent (final concentration) gave optimal colour production in the Lowry micro-assay.

UV Absorbance at 280 nm

Two commonly used procedures to assay for proteins are the method of Lowry *et al.*[4] and the method described by Bradford[5]. Both

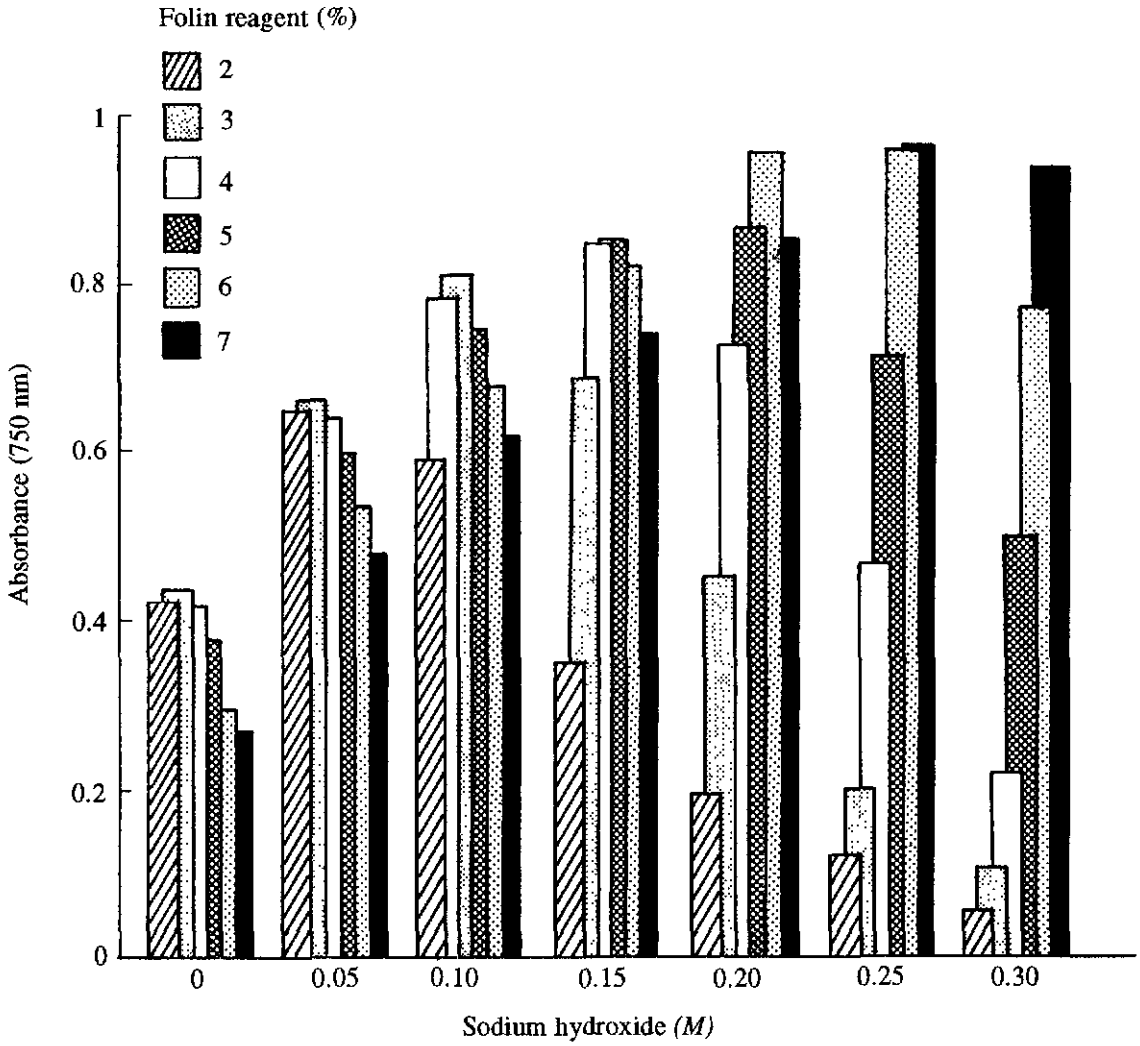

Figure 2. Effect of sodium hydroxide concentration in the test sample and Folin reagent concentration on the sensitivity of the Lowry micro-assay. Results are the means of two replicates.

are colorimetric assays that involve the reaction of proteins with specific reagents. While not particularly complex, these assays – more so the Lowry assay – require a number of steps to complete before reading the colour developed in the reaction mixture. Another common method to assay for proteins in a solution is by directly measuring the UV absorbance of the test sample at 280 nm. The procedure is rapid and does not require the use of reagents, but it is not preferred because of its low sensitivity (ten to twenty times less sensitive than the Lowry assay[4]) and also because of its wider variation in response to different proteins depending on their amino acid composition[4]. Recently, Yip[6] assayed proteins from latex products by their absorption at 280 nm following separation of the proteins by HPLC. The total proteins, calculated from the integrated area under the UV absorbance curve, was found to be well correlated to the Lowry assay.

In the present study, samples from six brands of gloves were unwashed or washed in water for 5 s, 10 s, 30 s, 5 min or 1 h to simulate protein removal by dry film washing in the factory. The samples were air-dried and then subjected to extraction for 3 h with water. The glove eluates (6 ml) were precipitated with 5% TCA and 0.2% PTA and the precipitates re-dissolved in 0.8 ml 0.25*M* sodium hydroxide to attain a 7.5-fold increase in concentration. Samples were drawn for the Lowry micro-assay while similar samples were also read for absorbance at 280 to quantitate proteins. Preliminary experiments had earlier shown that a second reading at 260 nm to correct for the presence of nucleic acids was unnecessary. Nucleic acids were also not detected by electrophorectic separation of the eluate followed by ethidium bromide staining.

The results obtained from the Lowry assay for proteins eluted from the six brands of gloves were found to be well correlated with their UV absorbance, with correlation coefficients above 0.9 in all cases *(Table 1)*. The relationship between UV absorbance and the Lowry values were sustained even where the eluted proteins were very low (as in *Glove N*, ranging from 16.0 to 20.5 g/ml). The low sensitivity normally associated with protein detection by UV was mitigated by concentration of the sample following TCA/PTA precipitation (as mentioned above) and by the fact that UV absorbance by proteins from latex glove eluate was relatively high (more than three times that of bovine serum albumin). When the data from all the six brands of gloves were combined, a correlation coefficient of 0.870 was obtained *(Figure 3)* indicating that quantitation of latex glove proteins by UV absorption was only slightly influenced by the different latex sources and different latex formulations used by the six manufacturers. Hence, comparisons of protein levels across brands was generally possible. In using UV absorbance to assay proteins, a calibration should be carried out with the same samples being assayed both by UV absorbance and by Lowry assay.

Staining with Naphthalene Black

While quantitating proteins in an acid-precipitated sample by UV absorbance is uncomplicated, there is a need for an even simpler method to estimate proteins with a minimal requirement of laboratory equipment. Such a method would be useful for in-factory monitoring where the need for rapidity of protein assessment might take precedence over the need for very high precision. With this objective in mind, a method was developed for a rapid approximation of proteins present *in situ* on the latex glove without the need to elute the proteins out. In this method, the protein present was visually approximated by the uptake of a protein stain, Naphthalene Black.

TABLE 1. CORRELATION BETWEEN PROTEIN CONCENTRATION AND UV ABSORBANCE IN ELUATES OF GLOVES THAT WERE WASHED FOR VARYING PERIODS

Glove	Range of protein concentrations[a] (μg/ml)	Correlation coefficient between protein concentration[a] and absorbance at 280 nm
P	6.4 – 126.9	0.904
J	10.0 – 104.0	0.995
C	6.3 – 87.1	0.960
F	9.0 – 62.0	0.904
G	7.0 – 59.2	0.914
N	16.0 – 20.5	0.984

[a]Based on Lowry micro-assay. Samples have been concentrated 7.5× after TCA/PTA precipitation

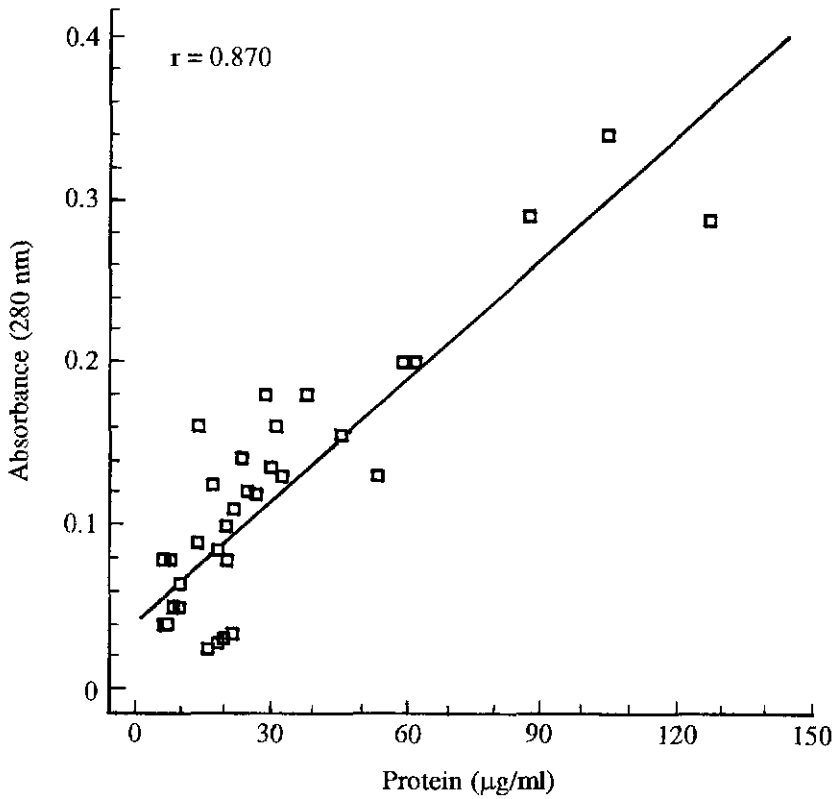

Figure 3. Correlation between protein concentration in latex glove eluates and absorbance at 280 nm. Results combined from six brands of gloves.

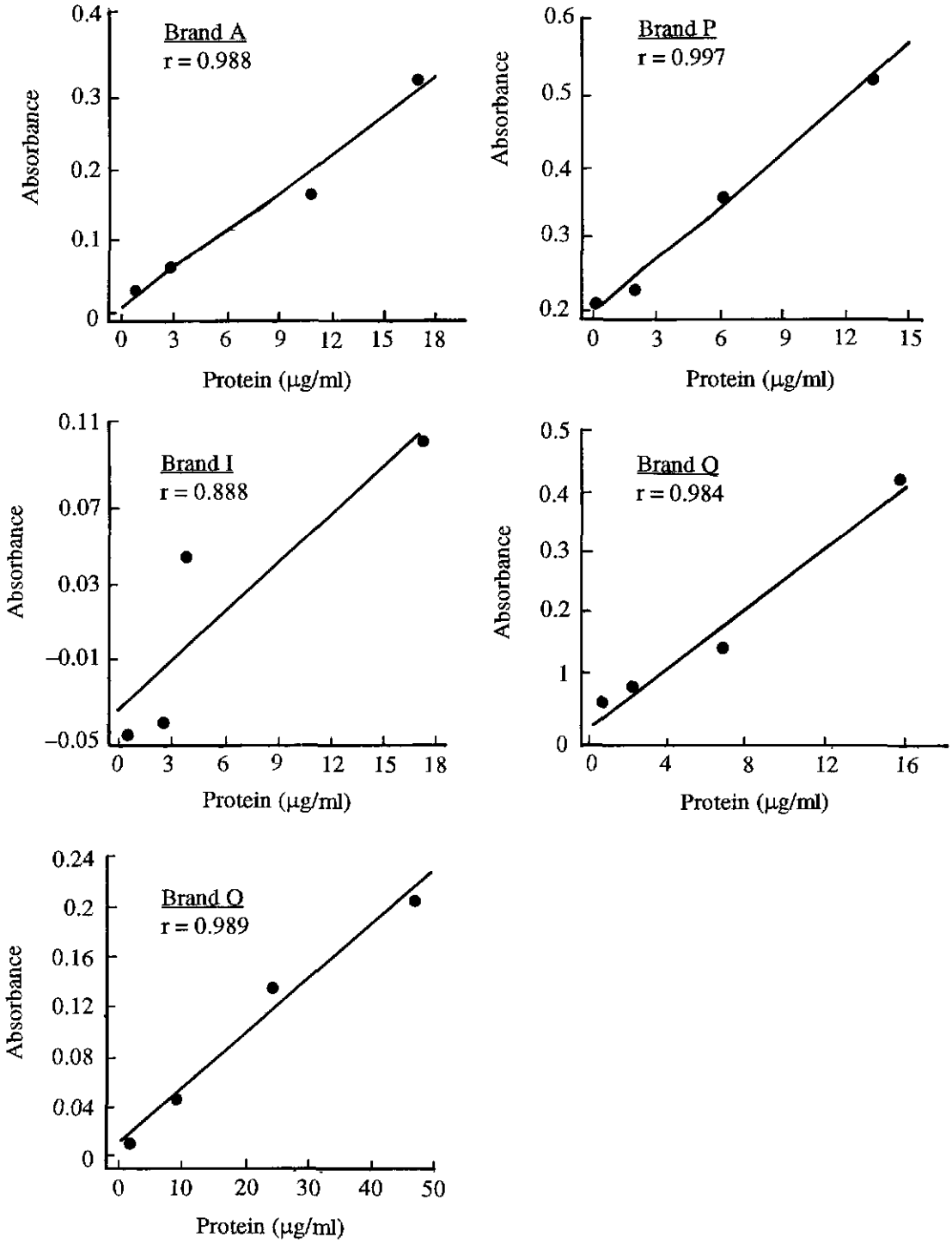

Figure 4. Correlation between protein concentration in latex glove eluates and absorbance at 665 nm.

It was previously reported that soluble proteins of latex films could be slectively stained *in situ* with Naphthalene Black[7] whereas insoluble proteins were poorly stained after 3 h. As soluble proteins were located at the inner surface of the glove[7], it was possible to shorten the time of staining since there was no necessity for a time lapse to allow the stain to diffuse into the depth of the glove. Satisfactory colouration of the inner glove surface due to protein could be attained with 5 min staining using 0.1% Naphthalene Black in 50% ethanol and 7% acetic acid followed by de-staining for 5 min with a mixture of 10% ethanol and 7% acetic acid.

In a study, samples from five brands of gloves were unwashed or washed in water for 10 s, 30 s, 5 min or 1 h to simulate protein removal by dry film washing in the factory. A portion of each glove sample was cut off and the inner surface was eluted[8], then assayed for protein by the Lowry micro-assay. Another portion of each sample was affixed on to a glass sheet (with the surface to be stained exposed) using double adhesive tape so that only one surface was stained/destained. Although it was intended in practice to assess the stain intensity by eye, a photometric assessment was carried out for experimental purposes in this instance. The stained glove films were photographed using colour slide film and these were cut to size and placed into the sample cuvette of the spectrophotometer. A spectrophotometric scan showed maximal absorbance at 665 nm and this wavelength was used to read the photometric absorbance of the slides of the glove samples. Usually, only the inner glove surface was significantly stained as most of the soluble proteins were located there[7,8]. The slide of the stained outer glove surface that had earlier been washed 1 h (so that there would be very little proteins remaining) was used as a blank for other readings.

A comparison of the photometric readings with the Lowry protein readings showed a good correlation between the two variables. Of the five brands of gloves evaluated, the correlation coefficient in four of the brands exceeded 0.98 while the fifth brand had a correlation coefficient of 0.888 *(Figure 4)*. Whereas the protein assay by UV absorbance assessed protein levels across brands, stain intensity correlated well with extractable proteins for the same brand (specifically the batch) of gloves only. Hence, it would be necessary to re-calibrate stain intensity with protein if the latex source or formulation is changed.

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APPENDIX I

Protocol for TCA/PTA Protein Precipitation and Modified Lowry Micro-protein Assay

PROTEIN PURIFICATION AND CONCENTRATION

1. Add 1 ml 35% (w/v) TCA to a 6 ml of test sample. (The test sample should not be too alkaline as to neutralise the acid precipitant.) Mix and stand about 5 min. Then add 1 ml 1.6% PTA (w/v), mix and allow to stand 20 min.

2. Centrifuge the mixture for 30 min at about 1500 g preferably in a refrigerated centrifuge. Decant and discard the supernatant. (Keep the centrifuge tube inverted for several minutes to ensure thorough removal of the supernatant).

3. Re-dissolve the precipitate (which is a very thin film where protein concentration is low) in 0.8 ml 0.25*M* NaOH for at least 20 min[a].

PROTEIN QUANTITATION

4. The test reaction may be carried out directly in the centrifuge tube. Add 0.3 ml *Reagent C* to the 0.8 ml test sample already present in the centrifuge tube from *Steps 2* and *3*. Mix well and stand for 10 min.

5. Add 0.1 ml *Reagent D*. Mix well the contents of each individual test tube *immediately* upon adding *Reagent D* (use a vortex mixer). Stand the mixture at room temperature for 30 min and read the absorbance on the spectrophotometer at 750 nm.

6. The concentration of protein is read against a standard protein used for calibration (*e.g.* bovine serum albumin, 0 to 100 μg/ml).

The assay reagents are prepared as follows:

Reagent A: 6% sodium carbonate in distilled water

Reagent B: 1.5% copper sulphate in 3% sodium citrate

Reagent C: (Working reagent prepared on the day of assay) 50 ml *Reagent A* mixed with 1 ml *Reagent B*

Reagent D: Folin Reagent diluted to 72% with distilled water.

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[a]In the event that the precipitate is abundant, the volume of NaOH may be increased so that the photometric reading does not fall outside the range of the calibration standards. Pipette 0.8 ml of the test solution into another centrifuge tube or a test tube and proceed with *Step 4*. An adjustment on the protein concentration is made to correct for this dilution.