Arch. Pharm. Res. Vol. 16, No. 1, pp. 57-61, 1993

A Turbidimetric Determination of Protein by Trichloroacetic Acid

Wahn Soo Choi[1], Kae Jong Chung[1], Man Sik Chang[1], Jae Kwang Chun[1], Hyang Woo Lee[2] and Sung Youl Hong[3]

[1]*Central Research & Development Laboratory, Yung Jin Pharmaceutical Co., Ltd., Kyunggi-Do 445-850 and* [2]*Department of Pharmacy and* [3]*Department of Genetic Engineering, Sungkyunkwan University, Suwon 440-746, Korea*

(Received February 12, 1993)

Based on the turbidimetric response of protein with 50%- trichloroacetic acid (TCA), this study aims to introduce an assay method for protein in solution. The standard procedure consists of mixing equal volume of sample solution (standard or unknown) with 50%-TCA solution and measuring the absorbance at 450 nm after 20 min. The absorbances of the solutions were almost stable over 120 min at room temperature. This assay method is simple, reproducible, and tolerant to many interfering substances. It can detect less amount than 10 μg/ml of bovine serum albumin. The assay method has low protein-to-protein variability over wide range of molecular weight.

Key words: Turbidimetry; Trichloroacetic acid; Protein determination

INTRODUCTION

A rapid and accurate method to quantify protein concentrations in a variety of biological samples is essential for the study in biochemical fields. The Lowry's protein assay method (Lowry *et al.*, 1951) has been widely used over about 40 years, although the method is susceptible to a wide range of nonprotein substances generally present in many biological extracts, and thus, we can not often estimate protein concentration accurately (Valiejo *et al.*, 1970; Kuno *et al.*, 1967; Lo *et al.*, 1972) Furthermore, instability of the Lowry's phenol reagent in alkaline solution and the two-step addition of the color-developing reagents are cumbersome and tedious. The other widely used Bradford procedure (Bradford, 1976) is relatively simpler and faster than the former method. However, it exhibits some variation depending on kinds of protein and the preparation of the Brilliant Blue G-200 solution is time-consuming.

Trichloroacetic acid (TCA) has been commonly used to precipitate proteins during many enzyme assays. Exploiting this property, a turbidimetric measurement of protein-TCA complex has been developed by several investigators (McElderry *et al.*, 1982; Marshall *et al.*, 1988) to estimate protein concentration. However, the method suffers from formation of insoluble protein complex which can be only alleviated by a large dilution of the reaction mixture and requires a large sample. In the present communication, we report a simple and quick one-step method to quantify protein concentration by using the TCA solution which overcomes most of the shortcomings described above.

Correspondence to: Wahn Soo Choi, Central Research & Development Laboratory, Yung Jin Pharmaceutical Co., Ltd., Kyunggi-Do 445-850, Korea

MATERIALS AND METHODS

Chemicals

TCA was obtained from Merck. Sodium azide, brij35, EDTA and urea were purchased from Sigma. Triton X-100 and sodium dodecyl sulfate were from Aldrich. All other reagents were best analytical grade available. Bovine serum albumin was obtained from Sigma. Bradykinin, ribonuclease A, chymotrypsinogen A, pepsin, ovalbumin, catalase and ferritin were purchased from Pharmacia Inc. Standard protein solutions were prepared in either isotonic saline or solutions containing the particular compounds under study. The Lowry reagent and protocol used in this study were as described (Lowry *et al.*, 1951) The Modified Bradford reagent and protocol used in this study were as described (Sedmak *et al.*, 1977).

Standard TCA procedure

A 50% (w/v) TCA solution is prepared in deionized water. The standard assay procedure is as follows: One

ml of protein solution to be determined (standard or unknown) is mixed with an equal volume of the 50% TCA solution in a test tube. Turbidity is developed immediately at room temperature and then slowly thereafter. After 20 min, further development of turbidity was not almost observed and so, the absorbances at 450 nm was taken against a blank prepared with deionized water in place of 50%-TCA solution.

Turbidity stability

The absorbances were recorded till 120 min in order to monitor any subsequent changes in absorbance values.

Interfering substance

A stock solution of BSA at a concentration of 100 μg/ml was prepared in isotonic saline. Stock solutions of each compounds listed in Table I. were also prepared in isotonic saline, but at twice the concentration shown. An automated pipetting device was used to dispense 0.5 ml of the BSA stock solution and 0.5 ml of the testing interfering compound stock solution into each of the tubes for the assays. The tubes marked "water blank" contained no BSA or potentially interfering compound while those marked "interference blank" (data not shown) contained no BSA but did contain the potentially interfering compound. All the tubes were then carried through the TCA method using the room temperature/20 min protocol. The amount of BSA found was then calculated from the net absorbances at the appropriate wavelength after substracting the "water blank" or "interference blank" (data not shown).

Table I. Protein-to-protein variation for the TCA assaymethod compared to the Bradford and Lowry methods for various proteins

Protein	M.W. (Dalton)	Conc. of protein (μg/ml)	μg/ml Protein found by		
			TCA[a]	Bradford[b]	Lowry[c]
Bradykinin	1,060	10	4.81	0.00	0.00
		20	5.18	0.00	0.00
		30	5.56	0.00	0.00
Ribonuclease A	13,700	10	10.22	4.89	15.34
		20	18.29	9.26	30.90
		30	28.70	12.95	44.79
Chymotrypsinogen A	25,000	10	8.51	4.83	11.45
		20	15.22	10.30	23.67
		30	22.50	16.59	35.34
Pepsin	32,700	10	8.89	0.00	17.56
		20	12.27	0.00	31.45
		30	15.22	0.00	45.90
Ovalbumin	43,000	10	11.14	6.47	11.45
		20	20.00	14.77	23.67
		30	31.29	21.38	35.34
B.S.A.	66,000	10	10.00	10.00	10.00
		20	20.00	20.00	20.00
		30	30.00	30.00	30.00
Catalase	232,000	10	4.44	4.23	5.90
		20	11.13	10.22	13.12
		30	19.09	17.42	19.78
Ferritin	440,000	10	11.59	6.13	7.01
		20	17.27	12.80	12.56
		30	29.03	15.60	25.90

[a,b,c] TCA, Bradford and Lowry assay were studied as described on the method section and BSA was used as the standard protein in all methods.

Protein-to-protein variability

The standard TCA room temperture/20 min protocol was used to conduct the protein-to-protein variation study. All of the proteins assayed were prepared at three concentrations of 10 μg/ml, 20 μg/ml and 30 μg/ml in isotonic saline. The same samples were used for the protein assay by the standard Lowry method (Lowry *et al.*, 1951) and the modified Bardford method (Sedmak *et al.*, 1977).

RESULTS AND DISCUSSION

General considerations

Most scientists in the life science fields often face to measurements of the amount of protein in solution. In 1951, Lowry *et al.* (Lowry *et al.*, 1951) reported the relatively exact and simple method. Since then, many methods that improved some defects of Lowry method have been reported (Hess *et al.*, 1978; Ohnishi *et al.*, 1978; Markwell *et al.*, 1978). Bradford reported a dye-binding method using coomassie brilliant blue G in 1976 (Bradford *et al.*, 1976). The method is more sensitive, simpler and faster than the Lowry method (Lowry *et al.*, 1951). But above two methods give some problems to scientists because of troublesome, tedious preparation of the solution and interfering characteristics against various non-protein substances.

TCA has been used for the precipitation of proteins in solution because of its chaotrophic property. Proteins in a solution are precipitated in the concentration of TCA more than specific concentration and the turbidity is in proportion to the amount of proteins in solution.

Spectra and TCA concentration

Fig. 1 shows spectral scans from 400 to 600 nm of solutions containing protein denatured by TCA. We can find out the fact that it has a tendency to increase near 400 nm. The wavelength (nm) to measure absorbances was set to 450 nm to avoid the interference of yellow color which has maximum absorbance at approx 380 nm and doesn't almost have any absorbance at 450 nm.

We studied to determine the concentration of TCA solution by which we could obtain reproducible results. As shown in Fig. 2, absorbances by each TCA solutions

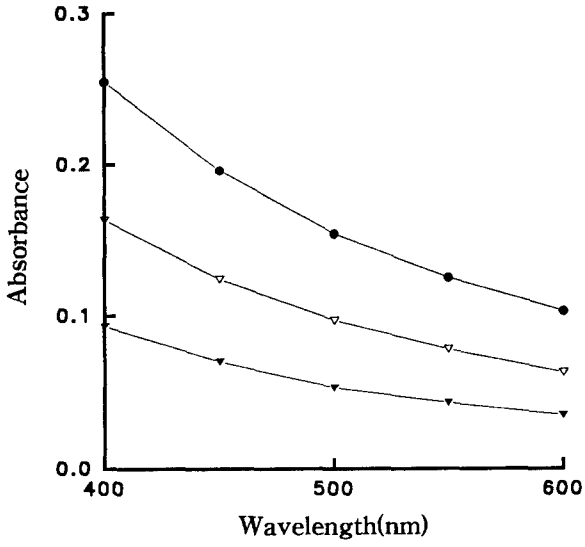

Fig. 1. Absorption spectra of the denatured BSA solutions on TCA assay. Mixture of 1 ml of 50%-TCA solution and 1 ml of protein solution at room temperature was scanned from 600 nm to 400 nm after 20 min. ●: 30 μg/ml, ▽: 20 μg/ml, ▼: 10 μg/ml.

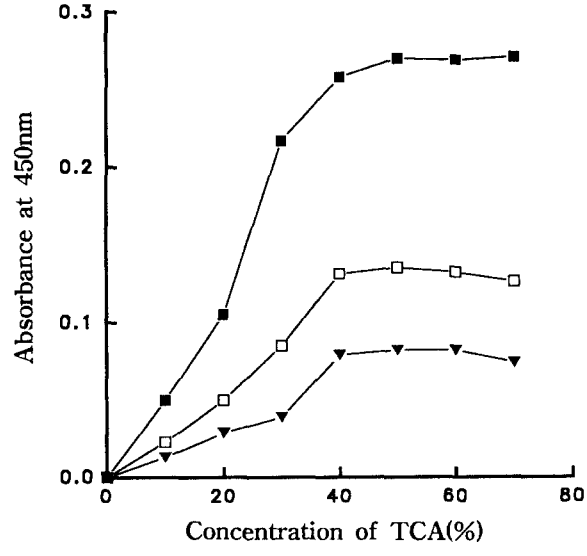

Fig. 2. Effect of trichloroacetic acid concentration. 1 ml of TCA solutions of each concentrations were added to 1 ml of BSA solution. After 20 min, absorbances were read a' 450 nm. All points represent the means of triplicates. ■: 40 μg/ml, □: 20 μg/ml, ▼: 10 μg/ml.

were reached to plateau over 50%-TCA solution. So the concentration of TCA solution was set to 50%-TCA solution. But if you keep the constant condition, you can quantify exactly protein in solution below 50%-TCA solution, too.

Reproducibility, sensitivity and linearity of the assay

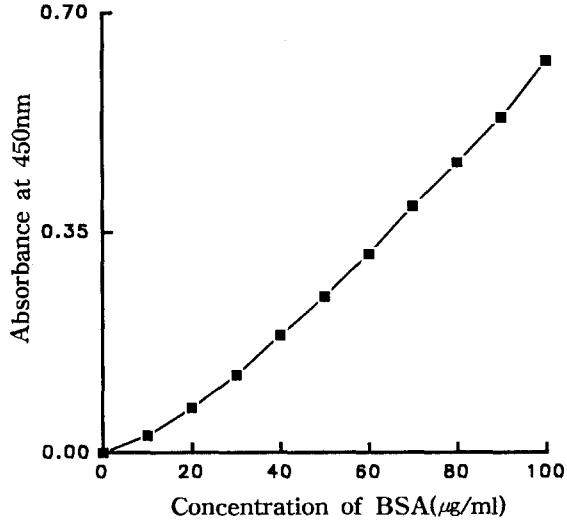

Fig. 3. Calibration curve for BSA by TCA assay. 1 ml of 50%-TCA was added to 1 ml of B.S.A. solution of various concentrations. After 20 min, absorbances were read at 450 nm. All points represent the means of four replicates.

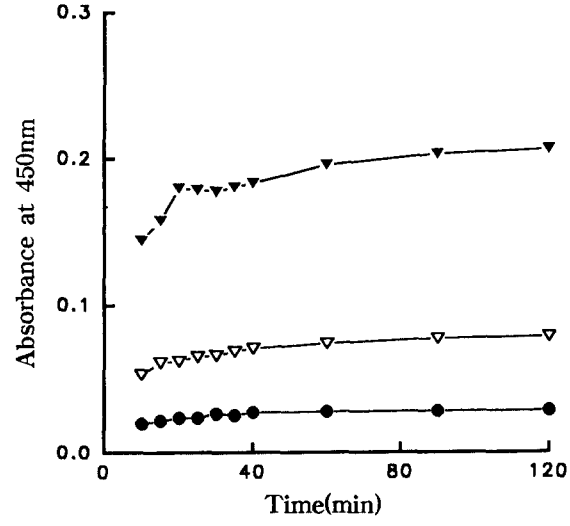

Fig. 4. Variation of absorbance with time after 1 ml of 50%-TCA solution was mixed with 1 ml of BSA solution. All points represent the means of triplicates. ▼: 40 μg/ml, ◀: 20 μg/ml, ●: 10 μg/ml.

As shown in Fig. 3, quadriplicate assays of bovine serum albumin at each concentrations were highly reproducible. As the result of statistical analysis, the standard deviation of the method was 0.0012. The assay was sensitive enough to estimate 25 μg/ml BSA solution which had the absorbance unit of 0.097 at 450 nm. We could quantify approx. 5 μg/ml BSA solution.

Stability of the turbidity

Fig. 4 shows the rate of the formation of turbidity on the assay system and its stability. The absorbances

Table II. Effect of various laboratory reagents on TCA, Bradford and Lowry assay methods

Sample (50 μg/ml BSA) in the following	μg/ml BSA found					
	TCA	mtd[a]	BRadford	mtd[b]	Lowry	mtd[c]
Water	50.00		50.00		50.00	
0.1 N HCl	55.00		37.92		40.90	
0.1 N NaOH	57.00		75.98		55.90	
0.2% Sodium azide	52.50		59.38		50.90	
1.0 M Sodium chloride	58.50		49.29		50.40	
50 mM EDTA (2Na)	53.50		70.32		486.50	
10 mM EDTA (2Na)	49.60		51.42		107.60	
3.0 M Urea	47.50		>100.00		52.60	
1.0% Triton X-100	ppt[d]		>100.00		ppt[d]	
1.0% SDS	68.20		39.03		54.80	
1.0% Brij 35	ppt[d]		>100.00		ppt[d]	
20.0% Sucrose	47.00		44.64		50.40	
10.0% Sucrose	50.00		49.10		48.70	
50 mM Glucose	51.20		52.86		95.90	
10 mM Glucose	52.00		54.44		73.10	
0.2% Sorbitol	50.90		50.63		135.90	
1.0 M-Glycine	45.00		>100.00		10.40	
0.25 M Tris	51.00		46.25		77.50	
0.125 M Tris	51.00		48.93		98.10	
10.0% Ammonium sulfate	53.00		>100.00		ppt[d]	
2.5% Ammonium sulfate	53.00		87.19		27.60	
5% Streptomycin sulfate	50.66		80.94		ppt[d]	
2.0 M Sodium acetate, pH 7.5	47.00		>100.00		47.10	
0.2 M Sodium acetate, pH 7.5	54.00		87.81		44.80	
1.0 M Sodium phosphate	48.00		>100.00		0.00	
0.1 M Sodium phosphate	51.00		63.26		37.00	
50 μM Guanidine	50.44		43.21		49.84	
50 μM Cytidine	50.51		52.22		51.23	
10 μg/ml DNA	63.70		55.08		47.36	
2 μg/mll DNA	51.39		54.76		48.18	
250 μg/ml RNA	51.76		69.79		66.99	

[a,b,c] TCA, Bradford and Lowry assay were studied as described in method section.

[d] The ppt means that we couldn't quantify proteins because the regeants used in the method were precipitated with the interering substance.

were monitored at 5 min intervals for 40 min and then at 30 min ntervals for 2 hrs. Most of turbidity was developed at 20 min after the addition of TCA solution and has stability until 120 min. Therefore, absorbances were read for this period. It has to be noted that absorbances has tendency to increase slightly with time after 40 min. We think that it is due to coagulation of denatured proteins in TCA solution. In order to get accurate determination, the absorbances should be read in between 20 min and 40 min after the addition of TCA solution.

Accuracy of the assay

Table I shows the results obtained from various proteins, such as bradykinin, ribonuclease A, chymotrypsinogen A, pepsin, ovalbumin, bovine serum albumin, catalase and ferritin, which were assayed by TCA-method, Modified Bradford procedure (Sedmak *et al.*, 1977) and Lowry's method (Lowry *et al.*, 1951) comparatively. Each of results from various proteins was analyzed by regression analysis. The results indicated that $Y=0.7424X+0.4373$ ($r=0.8551$) for TCA method; $Y=0.5077X-0.2506$ ($r=0.6895$) for Modified Bradford method; and $Y=1.0344X-0.3795$ ($r=0.7899$) for Lowry's method, respectively.

From these results, we can find out that the variability of the TCA method ($r=0.8551$) is much less than that of Modified Bradford method ($r=0.6895$) and that of Lowry's method ($r=0.7899$).

Table III. Measurement of the amount of protein from animal tissues and microorganism by TCA, Bradford and Lowry assay

Sample	μg/ml BSA found		
	TCA[a]	BRadford[b]	Lowry[c]
Mouse liver homogenated[d]	27.69	16.70	23.85
Mouse pancreas homogenated[d]	12.11	11.59	12.89
Mouse serum	10.19	45.83	56.39
Rabbit lung homogenate[e]	5.28	8.01	9.33
Streptomyces sp.−CDS[f]	1.23	1.42	3.17

[a,b,c] TCA, Bradford and Lowry methods were studied as described in method section.

[d] Each of the samples was prepared with 9-volume of 50 mM-phoslphate buffer to the weights of mouse liver and pancreas.

[e] Rabbit lung homogenate was prepared with 15-volume of 50 mM-phosphate buffer to the weight of acetone lung powder (L-0756, Sigma).

[f] Streptomyces sp. Y-183 cells were disrupted to 20%-cell (w/v) in 50 mM-phophate buffer (CDS: Cell Disrupted Suspension).

Interference by nonprotein components

Lowry's method (Lowry *et al.*, 1951) and Modified Bradford method (Sedmak *et al.*, 1977) are subject to interference by many biological substances and general reagents which are used in laboratories. So, we studied the interference effects of many substances to the three methods. Table II shows the results obtained by TCA assay, Modified Bradford assay and Lowry assay. With the TCA assay method, there is almost no interference effect except some detergents.

In the case of the solution containing more than 10 μg/ml DNA, TCA method was slightly subject to interference. But the Modified Bradford method (Sedmak *et al.*, 1977) is subject to interference by compounds such as strong alkaline solution, EDTA, urea, triton-X100, brij35, glycine, ammonium sulfate, sodium acetate buffer, sodium phosphate buffer, RNA and streptomycin sulfate etc. And we were difficult to quantify by Lowry's method (Lowry *et al.*, 1951) under the substances such as EDTA, triton-X100, brij35, glucose, sorbitol, glycine, tris buffer, ammonium sulfate, sodium phosphate buffer and streptomycin sulfate etc. The inter-

fering compounds selected in this study were chosen on the basis of being frequently encountered during the purification and isolation of proteins or because they were already known to interfere in the Lowry method (Ashni *et al.*, 1980). The data presented in Table II demonstrate that most of the compounds studied interfere significantly in the Modified Bradford and Lowry method, while TCA method is more tolerant of these compounds. This is a significant advantage of the TCA method. These results indicate that the TCA method can be used in many applications where the Bradford and Lowry method fails. But if a solution has more than 10 μg/ml DNA, you may have higher absorbance than the true one.

Measurement of the relative amount of proteins to that of BSA from various sources

Table III shows the results that protein solutions from various tissues and microorganism were quantified by TCA method, Modified Bradford method (Sedmak *et al.*, 1977) and Lowry's method (Lowry *et al.*, 1951). As shown in Table III the results from TCA method were similar with the results from the other two methods. But, in the case of serum from mouse, the amount of protein by TCA method was less amount than those measured by the other methods. From this result, we know that it is able to quantify the amount of protein in solution from various sources by TCA method. But If a solution contains some turbid material in itself, we have to read the absorbance of sample against the mixture of sample solution (1 ml) and water (1 ml) instead of the mixture of TCA solution (1 ml) and water (1 ml).

ACKNOWLEDGEMENTS

We would like to thank to Dr. J. K. Lee, Bioengineering Lab. in Yung Jin Pharm. Co. Ltd., for helpful hints and discussion and Miss E .K. Jin for skillful technical assistance.

LITERATURE CITED

Ashni, Y. and Catrvas, G. N., Highly reactive impurities in triton X-100 and brij 35: Partial characterization and removal. *Anal. Biochem.*, 109, 55-62 (1980).

Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72, 248-254 (1976).

Hess, H. H., Lees, M. B. and Derr, J. E., A linear Lowry-folin assay for both water-soluble and sodium dodecyl sulfate-solubilized proteins. *Anal. Biochem.*, 85, 295-300 (1978).

Kuno, H. and Kihara, H. K., Simple microassay of protein with membrane filter. *Nature (London)*, 215, 974-975 (1967).

Lo, C. and Stelson, H., Interferance by polysucrose in protein determination by the Lowry method. *Anal. Biochem.*, 45, 331-336 (1972).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

Markwell, M. A. K., Hass, S. M., Bieber, L. L. and Tolbery, N. E., A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.*, 87, 206-210 (1978).

Marshall, T. and Williams, K. M., Turbidimetric response of aqueous protein and detergent to trichloroacetic acid. *Biochem. Soc. Trans.*, 16, 640-741 (1988).

McElderry, L. A. Tarbit, I. F. and Cassells-Smith, A. F., Six methods for urinary protein compared. *Cli. Chem.*, 28, 356-360 (1982).

Ohnishi, S. T. and Barr, J. K., A simplified method of quantitating protein using the biuret and phenol reagents. *Anal. Biochem.*, 86, 193-200 (1978).

Sedmak, J. J. and Grossbery, S. E., A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G-250. *Anal. Biochem.*, 79, 544-552 (1977).

Valiejo, C. G. and Lagunas, R., Interferences by sulfhydryl, disulfide reagents and potassium Ions on protein determination by Lowry's method. *Anal. Biochem.*, 36, 207-212 (1970).