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Systematic comparisons of various spectrophotometric and colorimetric methods to measure concentrations of protein, peptide and amino acid: Detectable limits, linear dynamic ranges, interferences, practicality and unit costs

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ABSTRACT

There is limited and inconclusive information regarding detectable limits and linear dynamic ranges of various quantitative protein assays. We thus performed systematic comparisons of seven commonly used methods, including direct spectrophotometric quantitation at λ205 and λ280 nm (A205 and A280, respectively), bicinchoninic acid (BCA), Biuret, Bradford, Lowry and Ninhydrin methods. Purified BSA, porcine kidney extract, tryptic digested peptides derived from purified BSA, and glycine, were used as representative purified protein, complex protein mixture, peptide and amino acid, respectively. Bradford method was the most sensitive assay (LOD=0.006 mg/ml) and had the widest range of detectability (LOD–UOD=0.006–100 mg/ml) for purified protein and complex protein mixture. For peptide, A205, A280, Lowry and Ninhydrin methods had a comparable LOD (0.006 mg/ml), but Ninhydrin method had the widest detectability range (LOD–UOD=0.006–100 mg/ml). For amino acid, A205 and Ninhydrin methods had a comparable LOD (0.006 mg/ml), but A205 had a wider detectability range (LOD–UOD=0.006–6.250 mg/ml). Biuret method offered the widest linear dynamic range for purified protein and complex protein mixture (0.391–100 mg/ml), A280 offered the widest linear dynamic range for peptide (0.024–6.250 mg/ml), and Ninhydrin method offered the widest linear dynamic range for amino acid (0.024–0.195 mg/ml). Both Laemmli's and 2-D lysis buffers had dramatic interfering effects on all assays. Concerning the practicality and unit costs, A205 and A280 were the most favorable. Among the colorimetric methods, Bradford method consumed the least amount of samples and shortest analytical time with the lowest unit cost. These are the most extensive comparative data of commonly used quantitative protein assays that will be useful for selecting the most suitable method for each study.

1. Introduction

Quantitative protein assay is one of the essential methodologies in protein research and life sciences [1]. It is used in a prerequisite step prior to subsequent protein analysis, e.g. gel electrophoresis, proteomic analysis, Western blot analysis, ELISA, enzyme kinetic study, etc. Many assays have been established and are widely used in protein research and life sciences. Commonly used assays to measure protein concentrations in the samples include direct spectrophotometric quantitation at λ205 and λ280 nm (A205 and A280, respectively), bicinchoninic acid (BCA), Biuret, Bradford, Lowry and Ninhydrin methods [2–4]. Selection of these quantitative protein assays is usually based on compatibility of these methods with the samples to be analyzed [1,3]. One of the most important factors for such selection is the estimated range of concentrations and interfering substances contained in the samples. As such, Biuret assay is the method of choice for the samples with high protein concentrations (5–160 mg/ml), while BCA, Bradford and Lowry methods are more suitable for the samples with low protein concentrations (1–2000 μg/ml) [3]. The samples containing reducing agents or copper chelating reagents are frequently measured by Bradford method, whereas those containing detergents are better measured by BCA assay [3]. Occasionally, additional steps

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to dilute or pretreat the samples (e.g. dialysis, precipitation) to reduce effects of interfering substances prior to measuring protein concentrations may be required [5].

Previously, there were some attempts to compare various quantitative protein assays aiming to define the most suitable one for various applications [6–13]. Due to the disparate results among these previous studies [6–13], it is inconclusive that which one is the most suitable quantitative protein assay for general applications. It seems that each assay has some advantages for certain applications but, on the other hand, exhibits some disadvantages for other applications [6–13]. Moreover, there is limited information on the detectable limits and linear dynamic ranges of the individual methods. Therefore, extensive comparisons of the commonly used quantitative protein assays in a systematic manner are crucially required to address this issue.

The present study aimed to systematically compare detectable limits and linear dynamic ranges of seven commonly used quantitative protein assays, including A205, A280, BCA, Biuret, Bradford, Lowry and Ninhydrin methods. Purified bovine serum albumin (BSA), porcine kidney extract, tryptic digested peptides derived from purified BSA, and glycine with 2-fold serial concentrations (ranging from 0.006 to 100 mg/ml) were used as representative purified protein, complex protein mixture, peptide, and amino acid, respectively. The examined parameters included lower limit of detection (LOD), upper limit of detection (UOD), range of linearity and % interference by Laemmli's and 2-D lysis buffers, which are the most commonly used lysis buffers in biochemical and proteomic studies, respectively. In addition, practicality (concerning the sample consumption or the amount of sample that must be discarded and could not be recovered after the assay, number of pipetting/reaction, and estimated time consumed) and approximated unit costs were also compared.

2. Materials and methods

2.1. Sample preparation

BSA (Sigma-Aldrich Corp.; St. Louis, MO), porcine kidney protein extract, tryptic digested peptides derived from purified BSA, and purified L-glycine (Sigma-Aldrich Corp.) were used as the representatives of purified protein, complex protein mixture, peptide, and amino acid, respectively.

For complex protein mixture, dry powder of porcine kidney extract was prepared from a porcine kidney bought from a local fresh poultry market. Briefly, a cube of porcine kidney tissue (approximately 1 g) was chopped to several small pieces and washed several times with ice-cold phosphate buffered saline (PBS). The sample was then brisky frozen by liquid nitrogen and ground into powder using pre-chilled mortar and pestle. Proteins were extracted from the tissue powder using a lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS (3[(3cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate), 120 mM DTT, and 40 mM Tris-base. The extracted protein solution was dialyzed against deionized (dI) (18.2 MΩ cm) water (1000 ml × 4 changes for 24 h) through cellulose dialysis tubing membranes with a molecular weight cut off of 6000–8000 Da (Spectrum Laboratories; Rancho Dominguez, CA) to remove compositions of the lysis buffer and to retain only porcine kidney proteins in the solution. Kidney proteins were then dried using a lyophilizer (ModulyoD-230, Thermo Fisher Scientific; Waltham, MA).

For peptide, 1 g purified BSA (Sigma-Aldrich Corp.) was dissolved in 100 ml Tris–HCl buffer (pH 8.0) and incubated with 5 ml of 200 mM DTT at room temperature (RT) for 1 h. The sample was subsequently incubated with 20 ml of 200 mM iodoacetamide at RT for 1 h and then with 20 mg trypsin (Invitrogen; Grand Island, NY) at 37 °C for 16 h. The tryptic digested peptides were then dried using the ModulyoD-230 lyophilizer (Thermo Fisher Scientific).

For all samples, dry weight, one of the gold standard methods for measuring protein amount [14], was used for preparing the sample solutions at a concentration of 100 mg/ml in dI water. These samples were then serially (2-fold) diluted in dI water, resulting to 15 sample concentrations ranging from 0.006 to 100 mg/ml.

3. Quantitative protein assays

All following measurements were performed in triplicate and dI water was used as the blank control.

3.1. Direct spectrophotometric quantitation at λ205 and λ280 nm (A205 and A280)

A205 and A280 methods were the simple direct spectrophotometric measurements of absorbance of peptide bonds at λ205 nm and aromatic amino acids, cystine and disulfide bonds of cysteine residues at λ280 nm, respectively [2]. Briefly, 1 ml of each sample was added into a 1 ml quartz cuvette, which was then placed into a UV–visible spectrophotometer (Shimadzu; Kyoto, Japan). Absorbance of the sample was then read at λ205 and λ280 nm, respectively.

3.2. BCA method

BCA assay combines the reduction of Cu^{2+} to Cu^{1+} by peptide bonds of the protein in an alkaline solution with the selective colorimetric reaction of BCA–Cu^{1+} to form purple complex, which is strongly absorbed at λ562 nm [15]. BCA assay was performed according to the manufacturer's protocol. Briefly, 100 μl of samples was added into 2 ml of BCA working reagent (catalog #PI23227, Pierce Biotechnology; Rockford, IL), which contains BCA reagents A and B (50:1). The reaction mixture was incubated at 37 °C for 30 min in a water bath. Subsequently, the reaction mixture was cooled at RT and the absorbance was measured at λ562 nm using a UV–visible spectrophotometer (Shimadzu).

3.3. Biuret method

Biuret assay is based on the reaction of cupric ions to peptide bonds of protein under alkaline condition. This reaction changes blue color of the solution to purple [16]. Biuret assay was performed according to the manufacturer's protocol. Briefly, 20 μl of each sample was mixed with 1 ml of Biuret reagent (catalog #T1949, Sigma-Aldrich Corp.) and further incubated at RT for 10 min. The absorbance of the mixture was measured at λ540 nm using a UV–visible spectrophotometer (Shimadzu).

3.4. Bradford method

Bradford assay measures proteins by their binding capacities to Coomassie Brilliant Blue (CBB) to form a protein-dye complex. This interaction shifts the peak absorbance of the dye from λ465 nm to λ595 nm, resulting to change of the solution color from red-brown to blue [17]. The procedure was performed as described previously [17] using Bio-Rad protein microassay (catalog #500-0006, Bio-Rad Laboratories; Hercules, CA). Briefly, 2 μl of each sample was mixed with 798 μl of dI water. The solution was then incubated with 200 μl of Bradford reagent at RT for 5 min. The absorbance of the mixture was measured at λ595 nm using a UV–visible spectrophotometer (Shimadzu).

3.5. Lowry method

The principle of Lowry assay is similar to that of Biuret assay, but Lowry assay includes folin-phenol reagent into the reaction to enhance the sensitivity of the Biuret reaction [18]. Modified Lowry assay (catalog #TP0200, Sigma-Aldrich Corp.) was employed in this study. The procedure was performed according to the manufacturer's protocol. Briefly, 200 μl of each sample was added into 2.2 ml of reagent A (Biuret reagent) and further incubated for 10 min at RT. Thereafter, 100 μl of reagent B (folin and Ciocalteau's phenol reagent) was added into the reaction mixture and further incubated at RT for 30 min. The absorbance of the mixture was measured at λ550 nm using a UV–visible spectrophotometer (Shimadzu).

3.6. Ninhydrin method

Ninhydrin assay measures protein concentration based on the amount of amino acid residues. This principle involves the reaction between free amino group with 1,2,3-indantoin monohydrate (ninhydrin), resulting to a complex product with Ruhemann's purple color, which has the peak absorbance at λ570 nm. Briefly, the samples were hydrolyzed at 100 °C overnight using 6 N HCl to completely breakdown the intact proteins into amino acid residues. Thereafter, 2 ml of each hydrolyzed sample was mixed with 2 ml of ninhydrin working reagent containing 0.5 g of ninhydrin (ACS grade) (catalog #151173-10G, Sigma-Aldrich Corp.) in 30 ml of isopropanol and 20 ml of 0.1 M acetate buffer (pH 5.5). The reaction solution was incubated in boiling water bath for 15 min, cooled at RT and then added with 3 ml of 50% ethanol. The absorbance of the mixture was measured at λ570 nm using a UV–visible spectrophotometer (Shimadzu).

4. Lower limit of detection (LOD) and upper limit of detection (UOD)

The absorbance values obtained from each method were plotted against the concentrations (0.006–100 mg/ml) of each sample to determine LOD and UOD of each method for individual samples. LOD was defined as the lowest concentration of which absorbance could be distinguished from that of the blank control (dI water) with significant p value (<0.05), whereas UOD was defined as the highest concentration that still had significant difference in its absorbance as compared to the adjacent lower concentration. As such, UOD was the first concentration that formed the plateau phase of the dose-response curve. All LOD and UOD values reported in the "Results" section were derived from average values of triplicate sets of data obtained from three independent sets of experiments.

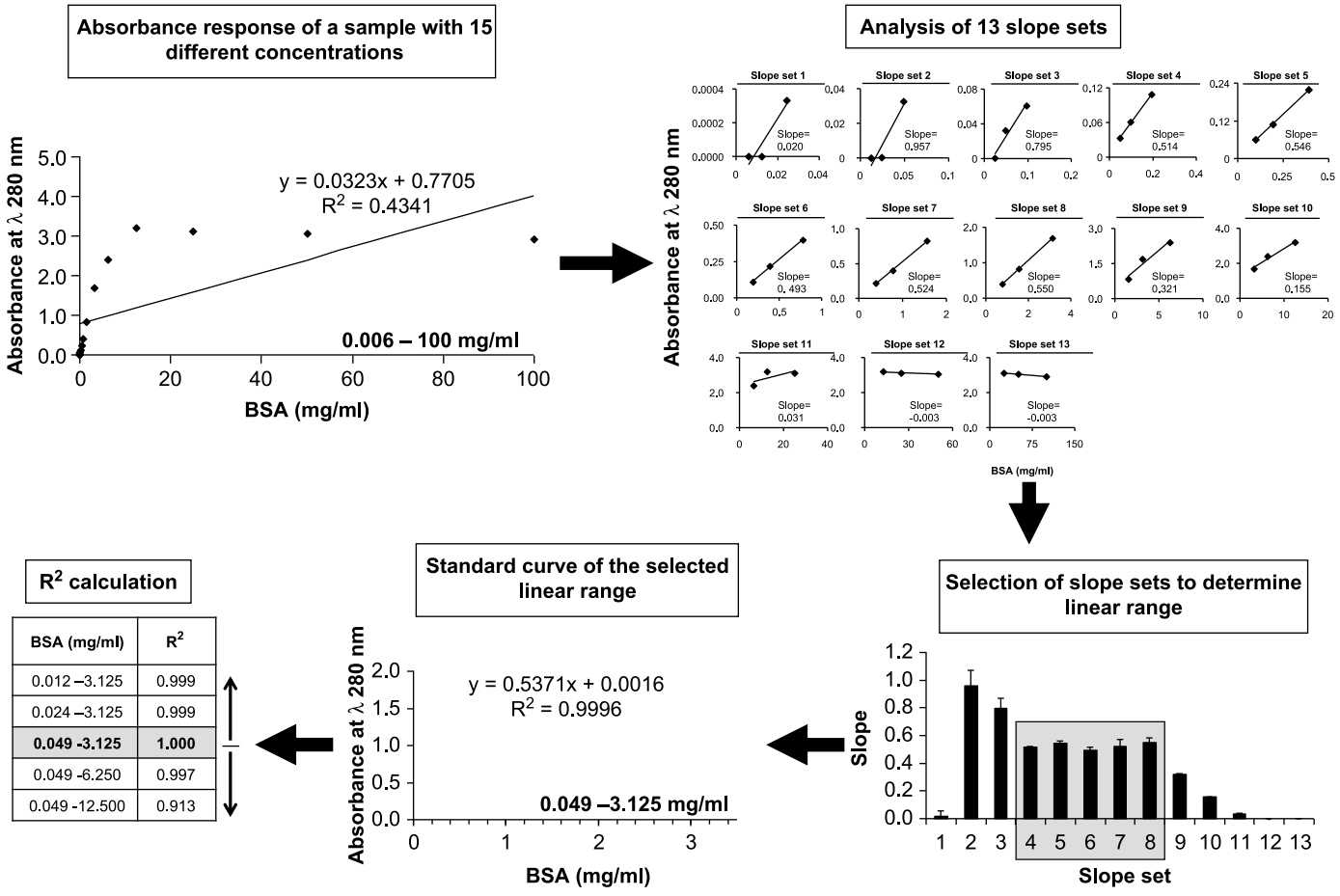

Fig. 1. Schematic diagram to summarize workflow to determine the linear dynamic ranges of quantitative assays used for measurement of protein, peptide and amino acid concentrations in the samples (BSA was used as an example). Initially, screening of the linear dynamic range of each assay for individual samples was performed using 15 of 2-fold concentrations (from 0.006 to 100 mg/ml) of individual samples. A total of 13 slope sets were generated using 3-consecutive concentrations for each slope set. Multiple comparisons among all these 13 slope sets were performed and the two or more consecutive slope sets, which had no statistically significant differences, were highlighted and selected. All the concentrations within the selected consecutive slope sets were then used to establish a standard curve. To validate the linearity of the selected consecutive slope sets, coefficient of determination (R^2) of this range was compared to those of the other extended ranges of concentrations (which extended to include 2 higher or 2 lower concentrations from the selected linear range). It would be expected that R^2 of the selected linear range was greatest (and closed to 1.000).

5. Linear dynamic range

Initially, screening of the linear dynamic range of each assay for individual samples was performed using all 15 (2-fold) concentrations (from 0.006 to 100 mg/ml) of individual samples. Three consecutive concentrations were used for determining a slope of linear curve. This resulted to a total of 13 slope sets generated from all 15 concentrations (Fig. 1). Multiple comparisons among all these 13 slope sets were performed (as detailed in "Statistical Analysis") and the two or more consecutive slope sets, which had no statistically significant differences, were highlighted and selected. All concentrations within the selected consecutive slope sets were then used to establish a standard curve. To validate the linearity of the selected consecutive slope sets, coefficient of determination (R^2) of this range was compared to those of the other extended ranges of concentrations (which extended to include 2 higher or 2 lower concentrations from the selected linear range) (Fig. 1). It would be expected that R^2 of the selected linear range was greatest (and closed to 1.000).

6. Interference by Laemmli's and 2-D lysis buffers

Interfering effects of Laemmli's and 2-D lysis buffers on each quantitative protein assay for individual samples were determined using the average concentrations of these samples within the linear dynamic range of each assay (as detailed above). In this set of experiments, dry BSA, porcine kidney protein extract (prepared as aforementioned), tryptic digested peptides derived from purified BSA (prepared as aforementioned), and purified L-glycine were resuspended in Laemmli's buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.01% bromophenol blue, 60 mM Tris–HCl; pH 6.8) or 2-D lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% (v/v) ampholytes (pH 3–10), 120 mM DTT, and 40 mM Tris-base). The samples dissolved in dI water served as the controls. The interfering effects of Laemmli's and 2-D lysis buffers were quantitated and are reported as %Interference, which was calculated using the following formula:

$$\%\text{Interference} = \frac{(\boldsymbol{C}_{\text{Buffer}} - \boldsymbol{C}_{\text{dI}}) \times 100}{\boldsymbol{C}_{\text{dI}}}$$

where $\boldsymbol{C}_{\textbf{Buffer}}$ was the concentration measured from the sample in Laemmli's or 2-D lysis buffer, whereas $\boldsymbol{C}_{\textbf{dI}}$ was the concentration measured from the same sample in dI water. The positive value of %Interference reflected over-estimation (the obtained concentration was significantly greater than that obtained from the sample in dI water), whereas the negative value of %Interference reflected under-estimation (the obtained concentration was significantly lower than that obtained from the sample in dI water). All data of %Interference reported in the "Results" section were derived from average values of triplicate sets of data obtained from three independent sets of experiments.

7. Practicality and unit cost

Assay-related parameters including practicality (concerning the sample consumption or the amount of sample that must be discarded and could not be recovered after the assay, number of pipetting/reaction, and estimated time consumed) and approximated unit costs were also compared among different quantitative protein assays. The details for amount of samples used, number of pipetting/reaction, and estimated time consumed were noted. Unit cost was calculated using the following formula:

$$\text{Unit cost (US\$/reaction)} = \frac{\text{Total price of an assay kit}}{\text{Total number of reaction by an assay kit}}$$

Note that only consumables (not including instrumentations and accessories, which are hard to compare) were used for this calculation.

8. Statistical analysis

Multiple comparisons were performed using ANOVA with Tukey's post-hoc test (SPSS, version 13.0). Linear regression analysis was performed to define the correlation between the two parameters. P values < 0.05 were considered statistically significant.

9. Results and discussion

Previously, evaluation of quantitative protein assays was based mainly on analysis of the purified proteins (particularly BSA), because the complex protein mixture was more difficult to interpret due to protein-to-protein variability [3]. In our present study, purified BSA, porcine kidney extract, tryptic digested peptides derived from purified BSA, and purified glycine were used as the representative samples for purified protein, complex protein mixture, peptide, and amino acid, respectively. The main reasons to employ these four samples in this study are: (1) They are common types of samples submitted to quantitative protein assays; (2) While there are several methods available for measurements of purified proteins and complex protein mixtures, fewer are available for measurements of peptides and amino acids of which the demands have been increasing, particularly in the proteomics/peptidomics era [19]; and (3) This study intended to evaluate effects of different sample types on each quantitative protein assay, i.e., limits of detection, ranges of linearity, and interferences by sample buffers. To compare individual assays in a systematic manner, we used a wide range of concentrations of these samples (from 0.006 to 100 mg/ml) prepared from their dried weights with serial 2-fold dilutions. Thereafter, a total of 15 concentrations of purified BSA, porcine kidney extract, tryptic digested peptides derived from purified BSA, and purified glycine were measured by seven commonly used quantitative protein assays.

We first evaluated the detectable limits of individual quantitative protein assays for each sample type (Table 1). The data showed that Bradford method was the most sensitive assay (LOD = 0.006 mg/ml) and had the widest detectable limits (LOD to UOD = 0.006–100 mg/ml) for purified protein (purified BSA) and complex protein mixture (porcine kidney extract). These data were consistent to those reported in previous studies indicating that Bradford assay was very sensitive and suitable for the sample with low protein concentrations such as the urine [6]. Ninhydrin assay and A205 had a comparable sensitivity (LOD = 0.006 mg/ml) as of Bradford method for detection of purified BSA and porcine kidney extract, respectively. Nevertheless, UOD of Ninhydrin assay and A205 (UOD = 0.781 mg/ml for both) for detection of purified BSA and porcine kidney extract, respectively, was much lower than that of Bradford method (UOD = 100 mg/ml). This result strengthened that Bradford method provided the widest detectable range for purified proteins and complex protein mixtures. On the other hand, the least sensitive method for detection of purified BSA and porcine kidney extract was Biuret assay (LOD = 0.391 mg/ml), consistent to the results obtained from previous studies demonstrating that Biuret was insensitive and was not appropriate for detecting proteins in the sample with low protein concentrations [3,6].

For peptide, A205, A280, Lowry and Ninhydrin methods had a comparable LOD (0.006 mg/ml), but Ninhydrin method had the

Table 1
Comparative analysis of detectable limits and linear dynamic ranges of various quantitative protein assays.

Parameters	Direct spectrophotometric assay		Colorimetric assay				
	A205	A280	BCA	Biuret	Bradford	Lowry	Ninhydrin
Lower limit of detection (LOD)							
Purified BSA (mg/ml)	0.012	0.049	0.024	0.391	0.006	0.012	0.006
Porcine kidney extract (mg/ml)	0.006	0.049	0.024	0.391	0.006	0.012	0.049
Peptide (mg/ml)	0.006	0.006	0.012	NA	NA	0.006	0.006
Purified glycine (mg/ml)	0.006	NA	NA	NA	NA	NA	0.006
Upper limit of detection (UOD)							
Purified BSA (mg/ml)	0.195	12.500	3.125	100	100	3.125	0.781
Porcine kidney extract (mg/ml)	0.781	3.125	6.250	100	100	6.250	1.563
Peptide (mg/ml)	25.000	25.000	6.250	NA	NA	12.500	100
Purified glycine (mg/ml)	6.250	NA	NA	NA	NA	NA	0.195
Range of linearity							
Purified BSA (mg/ml)	0.012–0.195	0.049–3.125	0.024–1.563	0.391–100	0.781–12.500	0.012–1.563	0.024–0.391
R^2	0.999	1.000	1.000	1.000	0.999	0.998	0.995
Porcine kidney extract (mg/ml)	0.024–0.195	0.098–1.563	0.049–1.563	0.391–100	0.781–6.250	0.098–1.563	0.195–1.563
R^2	0.998	0.999	0.998	0.998	0.998	1.000	0.997
Peptide (mg/ml)	0.006–0.098	0.024–6.250	0.024–1.563	NA	NA	0.024–0.391	0.012–0.098
R^2	1.000	1.000	0.996	NA	NA	0.999	0.999
Purified glycine (mg/ml)	0.006–0.098	NA	NA	NA	NA	NA	0.024–0.195
R^2	1.000	NA	NA	NA	NA	NA	0.999

Abbreviations used: A205, absorbance at λ205 nm; A280, absorbance at λ280 nm; BCA, bicinchoninic acid; BSA, bovine serum albumin; NA, not applicable; R^2, coefficient of determination.

widest detectability range (LOD to UOD=0.006–100 mg/ml) (Table 1). The data also showed that Biuret and Bradford methods are not applicable for peptide measurement. For amino acid, both A205 and Ninhydrin method had a comparable LOD of 0.006 mg/ml, but A205 had a wider range of the detectability (LOD to UOD=0.006–6.250 mg/ml) (Table 1). It should be noted that only these two methods are theoretically and practically applicable for detection and measurement of amino acid residues. Our data confirmed that glycine is not detectable or measurable by other five methods (Table 1).

The accuracy of a quantitative protein assay relies on precise correlation between actual concentrations and measured values (mostly absorbance derived from UV–visible spectrophotometry). Linear dynamic ranges of individual protein assays for each sample were screened systematically using 15 of 2-fold concentrations (from 0.006 to 100 mg/ml) of individual samples (Fig. 1). Briefly, each slope set was derived from 3-consecutive concentrations and a total of 13 slope sets were obtained. Multiple comparisons were performed among all these 13 slope sets and the two or more consecutive slope sets, which had no statistical differences, were highlighted and selected. All the concentrations within the selected consecutive slope sets were then used to establish a standard curve (Supplementary Figs. S1–S7). To validate the linearity of the selected consecutive slope sets, coefficient of determination (R^2) of this range was compared to those of the other extended ranges of concentrations (which extended to include 2 higher or 2 lower concentrations from the selected linear range). Our data demonstrated that R^2 values of the selected linear ranges of individual protein assays for each sample were greatest (and closed to 1.000) as compared to the extended ranges of concentrations (Supplementary Figs. S1–S7).

For purified BSA and porcine kidney extract, the broadest range of linearity was obtained from Biuret assay (linear dynamic range=0.391–100 mg/ml, R^2=1.000 and 0.998 for purified BSA and porcine kidney extract, respectively) (Table 1; Supplementary Figs. S4A and B). This result was consistent to previous reports [3,6], in which the major advantage of Biuret assay over the others was its wide linear range. On the other hand, A205 had the narrowest range of linearity for purified BSA and porcine kidney extract (0.012–0.195 and 0.024–0.195 mg/ml, respectively) (Table 1; Supplementary Figs. S1A and B). Thus, A205 is useful to quantitate samples with diluted protein concentrations ($<$200 μg/ml) [2]. For peptide, the widest range of linearity was obtained from A280 (linear dynamic range=0.024–6.250 mg/ml; R^2=0.999) (Table 1; Supplementary Fig. S2C), whereas Ninhydrin method offered the narrowest linear dynamic range (0.012–0.098 mg/ml) (Table 1; Supplementary Fig. S7C). For glycine, which was detectable only by A205 and Ninhydrin methods, Ninhydrin assay offered a slightly wider linear dynamic range (Table 1; Supplementary Fig. S7C).

The precision of quantitation is also determined by effects of interfering substances, if any, in the samples. Unfortunately, most of chemicals in sample buffers (lysis/extraction buffers) have some degrees of such interference [3], which may lead to over- or under-estimation of protein, peptide, and amino acid concentrations. However, effects of these commonly used sample buffers on protein, peptide, and amino acid quantitation were not thoroughly examined in the past. Among these sample buffers, Laemmli's and 2-D lysis buffers are most widely used in current protein research and life sciences. We therefore evaluated the interfering effects of these two commonly used sample buffers on protein, peptide, and amino acid quantitation in our present study.

To evaluate such effects in a systematic way, we used the average concentrations of proteins, peptide, and amino acid within their linear dynamic ranges of each assay as described with details above to ensure that the samples were in the measurable ranges and errors of quantitation was affected by interfering compositions in the sample buffers, not from the limited ranges of detectability of each assay. Dry purified BSA, porcine kidney extract, tryptic digested peptide, and purified glycine were dissolved in dI water, Laemmli's buffer or 2-D lysis buffer. Concentrations of proteins, peptide or glycine derived from the samples in Laemmli's or 2-D lysis buffer were compared to those obtained from the samples dissolved in dI water (Supplementary Figs. S8–S14). The results illustrated that both Laemmli's and 2-D lysis buffers had dramatic effects on all seven commonly used assays (Table 2). For measuring purified BSA and porcine kidney extract, Biuret assay had the greatest interfering effects from both Laemmli's (418% and 1049% over-estimation, respectively) and 2-D lysis buffers (768% and 1499% over-estimation, respectively) (Table 2; Supplementary Fig. S11). In contrast, Bradford

Table 2
Comparative analysis of %Interference by Laemmli's and 2-D lysis buffers in various quantitative protein assays.

Interference by	Direct spectrophotometric assay		Colorimetric assay				
	A205	A280	BCA	Biuret	Bradford	Lowry	Ninhydrin
Laemmli's buffer							
Purified BSA (%)	37	176	−86	418	−56	203	259
Porcine kidney extract (%)	932	147	−79	1049	3	470	96
Peptide (%)	259	140	−123	NA	NA	1663	27,825
Purified glycine (%)	3687	NA	NA	NA	NA	NA	259
2-D lysis buffer							
Purified BSA (%)	35	176	110	768	32	203	−66
Porcine kidney extract (%)	896	146	61	1499	139	470	−40
Peptide (%)	264	404	327	NA	NA	1665	2782
Purified glycine (%)	3607	NA	NA	NA	NA	NA	−77

Abbreviations used: A205, absorbance at λ205 nm; A280, absorbance at λ280 nm, BCA, bicinchoninic acid; BSA, bovine serum albumin; NA, not applicable.

Table 3
Comparative analysis of practicality and unit costs of various quantitative protein assays.

Parameters	Direct spectrophotometric assay		Colorimetric assay				
	A205	A280	BCA	Biuret	Bradford	Lowry	Ninhydrin
Sample consumption or the amount of sample that must be discarded after the assay (µl/reaction)	0	0	100	20	2	200	2000
Number of pipetting/reaction	1	1	4	3	4	4	4
Estimated time consumed (min/reaction)	5	5	50	15	10	35	1465
Approximated unit cost (US$/reaction)	0	0	0.392	0.144	0.045	0.320	0.059

Abbreviations used: A205, absorbance at λ205 nm; A280, absorbance at λ280 nm; BCA, bicinchoninic acid.

assay had the least interfering effects from Laemmli's and 2-D lysis buffers for measuring purified BSA and porcine kidney extract (Table 2; Supplementary Fig. S12). For measuring peptide, Ninhydrin method had the greatest interfering effects from both Laemmli's (27,825% over-estimation) and 2-D lysis buffers (2782% over-estimation) (Table 2; Supplementary Fig. S14C). However, BCA method had the least interfering effects from Laemmli's buffer, whereas A205 had the least interfering effects from 2-D lysis buffer for measuring peptide (Table 2; Supplementary Figs. S10C and S8C, respectively). For measuring purified glycine, A205 had greater interfering effects from both Laemmli's (3687%) and 2-D lysis buffers (3607%) (Table 2; Supplementary Fig. S8D), as compared to Ninhydrin assay.

The marked interferences from Laemmli's and 2-D lysis buffers alerted us for attention before submitting protein, peptide and amino acid samples recovered in these sample buffers for quantitation. It is advisable to strictly use protein, peptide and amino acid standards dissolved in the same buffer as of the samples, not dI water, to create a standard curve. Moreover, the same sample buffer should be used as the blank control for any quantitative protein assays. This is the simplest and probably the best way to avoid interfering effects from sample buffers. However, the investigators have to create several protein, peptide, or amino acid standards for individual sample buffers to ensure the precise quantitation in each type of samples.

In addition to detectable limits, linear dynamic ranges and interferences by sample buffers, selection of quantitative protein assay may also rely on the practicality of each assay and its unit cost. In our present study, the sample consumption (or the amount of sample that could not be recovered after the assay), number of pipetting/reaction, estimated time consumed, and unit cost were compared among individual assays (Table 3). The data demonstrated that both direct spectrophotometric methods (A205 and A280) were the simplest (required only one pipetting step) and consumed least amount of the samples. A205 and A280 determined protein/peptide concentrations based on light absorbance, which did not require any chemical reagents, additional manipulations and colorimetric reactions. Therefore, proteins and peptides could be fully recovered for subsequent analyses [2]. Moreover, both A205 and A280 consumed the shortest analytical time and had the lowest unit cost (indeed, there were no consumables used in these methods thereby the unit cost was zero) (Table 3). There is no doubt that A205 and A280 methods can be performed in any laboratory with an available UV–visible spectrophotometer. Comparing among five colorimetric assays, Bradford method consumed only a minimal amount of sample (2 µl/reaction), which was 50-fold, 10-fold, 100-fold and 1000-fold less than those consumed in BCA, Biuret, Lowry and Ninhydrin assays, respectively. In addition, Bradford method required the shortest analytical time (10 min/reaction) and had the lowest unit cost (0.045 US$/reaction) as compared to the other colorimetric assays (Table 3).

In summary, we present herein the most comprehensive data obtained from systematic comparative analyses of commonly used quantitative protein assays in term of their detectable limits, linear dynamic ranges, interferences, practicality and unit costs. Each method has both advantages and disadvantages that must be considered when ones select an appropriate method for measuring concentrations of proteins, peptides or amino acids in the samples. Our data will be useful for selecting the most suitable quantitative assay for each study.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.06.058.

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