blood offers the advantage of easy handling of samples without the need for immediate deproteination and centrifugation (4, 5). The test-strip method also provides rapid and precise measurements of lactate concentrations in whole blood (7). An amperometric assay of blood glucose with a ferrocene-mediated enzyme electrode has been developed and used to assay blood glucose with a single-use electrode strip (6, 8, 9). The conventional meters for glucose self-monitoring have glucose oxidase strips that change color. The disadvantages of these systems are that the user has to perform the test in a precisely timed cycle, and the blood-stained end of the strip that is inserted into the meter may contaminate it and cause inaccurate measurements. The assay system for measuring blood glucose with a single-use electrode strip resolves these issues.

Our assay system for measuring blood lactate also has a single-use electrode strip. The calibration curve for blood lactate was linear up to 20 mmol/L, which exceeded the maximum concentrations of blood lactate after exhaustive exercise. The required sample volume was 5 µL of whole blood, which seems particularly appropriate for use in obstetric and neonatal intensive care. In sports medicine, the use of a lancet for blood sampling makes it possible for coaches or athletes to measure blood lactate concentrations without the help of a doctor or a nurse. The new method is easy to operate, and the results are available in 60 s. The correlation between the lactate concentrations measured with the strip and the kit was high. In samples with a high lactate concentration, the lower the hematocrit, the higher the displayed lactate concentration. This result suggested that lactate concentrations in plasma samples could not be measured with this system. However, we observed a linear relation between the lactate concentrations in plasma and the magnitude of the electric current generated (Figure 2). The correct lactate concentrations in plasma were obtained when the concentrations were calculated with another calibration curve for plasma lactate measurements.

The new assay system is a rapid, convenient, and reliable method for measuring lactate concentrations in whole blood, and the meter itself is pocket-sized. These characteristics allow lactate to be measured at the bedside, in the laboratory, and even outdoors for application in clinical and sports medicine.

References

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Urine: Concentration of Urinary Proteins by Precipitation with Coomassie Blue

Thomas Marshall1,3 and Katherine M. Williams2

The precipitation of urinary proteins by Coomassie blue (Clin Chem 1992;38:1186–7) has been exploited for protein concentration prior to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Making the electrophoresed proteins visible by Coomassie blue staining confirms concentration and reveals ≤40 polypeptide bands in urine of normal protein content and distribution.

Making the electrophoresed proteins visible by silver staining confirms good protein recovery and suggests little modification of the electrophoretic profile. Excess dye can impair resolubilization of the protein and adversely affect electrophoresis, and should be removed by an acetone wash. The residual dye completely dissociates from the protein and can be used as a dye front during electrophoresis. The method combines protein assay and recovery and is particularly suited to high sample throughput. Concentration is simple, rapid (<30 min), and economic, but the choice of dye reagent is important because some commercial reagents are designed to enhance solubility of the protein–dye complex.

1 School of Health Sciences and 2 School of the Environment, University of Sunderland, Sunderland SR2 7EE, UK.
3 Address correspondence to this author at: School of Health Sciences, University of Sunderland, Fleming Bldg, Sunderland SR2 7EE, Tyne & Wear, UK. Fax 44 091 515 2557.
   Received January 18, 1993; accepted June 1, 1993.
Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) is a powerful tool for monitoring the presence of proteins indicating kidney malfunction and urinary tract infection (1). It has been favorably assessed relative to other separation methods as a technique likely to give urinary protein profiles consistent with a patient’s clinical status (2–6). However, analysis is complicated by low amounts of protein, which necessitates silver staining (7) or extensive concentration by means of membrane filters (e.g., Minicon; Amicon, Danvers, MA) (1). The former is technically demanding and the latter is known to induce protein loss (1, 4, 8–10) and to simultaneously concentrate of glycosaminoglycans, which can interfere with electrophoresis (11). SDS-PAGE of urinary proteins can be improved by preliminary treatment of the Minicon concentrate with ammonium sulfate (to precipitate proteins), EDTA (to solubilize complexes), and HCl (to hydrolyze glycosaminoglycans). However, the method is complex and unsuitable to high sample throughput. We have recently reported formation of insoluble protein–dye complexes (12) and the precipitation of urinary proteins (13) after the Bradford protein assay (14). The assay is specific for protein and can be exploited for the concentration of urinary proteins prior to SDS-PAGE. This combines protein assay and recovery, and is ideal for high sample throughput. It is particularly useful when making visible the electrophoresed proteins by Coomassie blue staining.

Materials and Methods

Reagents. Acrylamide, N,N′-methylenebisacrylamide, N,N,N′,N′-tetramethylethylenediamine, Tris, SDS, glycine, ammonium persulfate, glycerol, methanol, and acetic acid were purchased from BDH Chemicals, Poole, Dorset, UK. Bio-Rad protein assay dye reagent concentrate was purchased from Bio-Rad Labs., Hemel Hempstead, Herts, UK. Pierce Coomassie protein assay reagent and Pierce Coomassie Plus protein assay reagent were gifts of the Pierce Chemical Co., Rockford, IL.

Samples. Urine samples (n = 24, predominantly early-morning midstream) were collected from healthy individuals and various patients. The samples were stored at −20 °C, thawed, and clarified by centrifugation (1500 × g, 5 min). The samples were randomly mixed to generate six pools for Coomassie blue precipitation and SDS-PAGE. Ten samples were also randomly pooled to generate a low-protein urine pool, and five additional samples pooled to generate a high-protein urine pool, for evaluation of different dye reagents.

Concentration of urinary proteins. A stable dye reagent concentrate (5×) was prepared by dissolving 0.1 g of Serva Blue G (Serva Fine Biochemicals, Garden City Park, NY) in 50 mL of ethanol, adding 100 mL of phosphoric acid, and diluting with 50 mL of water. Just before use, the concentrate was diluted fivefold with water. For precipitation, 600 μL of urine was mixed with 2.4 mL of dye reagent and then centrifuged (13 400 × g, 5 min; M.S.E. Micro Centaur Centrifuge, Loughborough, UK). The supernate was decanted and discarded, and the protein–dye complex was resuspended in 1 mL of acetone to remove excess dye prior to centrifugation (13 400 × g, 5 min). The proteins were resolubilized in 30 μL of a “dilute” sample-denaturing solution (per liter, 20 g of SDS and 200 g of glycerol in 62.5 mmol/L Tris·HCl buffer, pH 6.8). The sample mixtures were heated at 95 °C for 10 min.

SDS-PAGE. The unconcentrated urines were prepared for electrophoresis by mixing 900 μL of urine with 200 μL of glycerol and 100 μL of a “concentrated” sample-denaturing solution (per liter, 10 g of SDS in 625 mmol/L Tris·HCl buffer, pH 6.8) (7). The samples were heated at 95 °C for 10 min and, for direct comparison, coelectrophoresed alongside their respective protein concentrates with a Micrograd electrophoresis unit and Consort E455 power supply unit (Flowgen Instruments, Sittingbourne, UK). Briefly, the denatured samples (15 μL) were loaded in agarose wells on 60 to 200 g/L polyacrylamide gradient gels and electrophoresed in pre-cooled (4 °C) SDS buffer (per liter, 1 g of SDS, 25 mmol of Tris, and 200 mmol of glycerol) at 50 mA/gel until the dye front reached the bottom of the gel (7, 15). The gels were fixed overnight in 200 g/L trichloroacetic acid and the protein bands were then made visible by staining with Coomassie blue (15).

To assess protein recovery after the dye precipitation, we diluted 5-μL aliquots of the protein concentrates with 95 μL of the “dilute” sample denaturing solution and coelectrophoresed these alongside the respective unconcentrated urines at an equivalent protein load (7.5 and 10 μL, respectively, assuming 100% recovery of protein in the concentrate). The gels were fixed in 500 mL/L methanol/100 mL/L acetic acid and the protein bands were made visible by silver staining (16) prior to densitometry (UltraScan XL Laser Densitometer, LKB, Milton Keynes, UK).

Evaluation of different dye reagents. To assess the precipitation properties of different dye reagents, we mixed increasing volumes (25–200 μL) of either the low-protein or high-protein urine pools with 1 mL of the respective dye reagent: laboratory preparation (diluted), Bio-Rad reagent (diluted 1:5), Pierce reagent, and Pierce Plus reagent. In each case, the absorbance (A495) was measured against a reagent blank in a Jenway (Dunmow, UK) Model 6100 spectrophotometer, and the assay mixtures were centrifuged (13 400 × g, 5 min) prior to absorbance measurement of the respective supernates. Assay of appropriate volumes of either low-protein or high-protein urine pools indicated protein concentrations of 0.06 and 0.30 g/L, respectively, with good agreement between the values obtained with the different dye reagents.

Results and Discussion

SDS-PAGE of equal volumes of the unconcentrated urines and their respective resolubilized Coomassie blue precipitates clearly demonstrated recovery and concentration of the urinary proteins in the dye precipitates (Figure 1). Although only albumin and Tamm–Horsfall mucoprotein were readily detected in the nonconcen-
trated urines (Figure 1, tracks 2–7), the urinary protein profiles of the respective dye concentrates were characterized by the detection of ≤40 polypeptide bands (Figure 1, tracks 8–13). SDS-PAGE of equal amounts of the nonconcentrated urines and their respective resolubilized Coomassie blue precipitates (diluted back to starting volume) followed by silver staining (Figure 2) and densitometry (Figure 3) confirmed good protein recovery and little change in the urinary protein profiles. There may be some loss of Tamm–Horsfall mucoprotein and occasional changes in the distribution of low-molecular-mass proteins (Mr < 20,000), but otherwise the protein patterns of the nonconcentrated urines and their respective concentrates were very similar (Figures 2 and 3).

Reproducibility can be a problem with laboratory preparation of Coomassie blue dye reagent; thus, commercial reagents are available for protein assay. We have evaluated the protein precipitation properties of these reagents with both low-protein and high-protein urines (Figures 4 and 5). Although our laboratory-prepared reagent proved optimal (Figures 4A and 5A), the Bio-Rad reagent gave a similar response (Figures 4B and 5B). The Pierce Coomassie and Pierce Coomassie Plus reagents were unsuitable (Figures 4C, 5C, and 4D, 5D, respectively), presumably because their composition has been designed to enhance solubility of the protein–dye complex. In this respect, they may be optimal for protein assay (13) but are unsuitable for protein concentration.

It is evident that Coomassie blue precipitation only works within a limited range of urinary protein amounts, since a transition from an insoluble to a soluble protein–dye complex occurs at higher protein concentrations: >30 mg/L dye (Figure 5). Similar studies with purified proteins indicate that this transition is characteristic of an individual protein and varies in inverse proportion to the relative response of proteins to the Bradford assay (17). This presumably reflects the dye binding/solvation properties of an individual protein but is also influenced by the concentration of dye and the presence of additives (e.g., SDS, phenol, or sodium hydroxide) in the reagent (17). Thus, if a reagent is designed to enhance shelf life or solubility, its modified composition may influence the transition in solubility [e.g., as with the Pierce reagents (Figures 4C, 4D, 5C, and 5D)]. Occasionally, urine may show residual absorbance following Coomassie blue precipitation at low protein concentration. This can be readily distinguished from the intense blue supernatant characteristic of the transition in solubility at high protein concentra-

---

**Fig. 1.** Coomassie blue-stained SDS-PAGE patterns of urinary proteins before and after concentration
The urine pools (tracks 2–7) were precipitated with Coomassie blue and the respective protein concentrates (tracks 8–13) were resolubilized in 1/20 of the volume. A = albumin, TH = Tamm–Horsfall mucoprotein, M = Pharmacia–LKB low-molecular-mass calibration proteins.

---

**Fig. 2.** Silver-stained SDS-PAGE patterns of urinary proteins before and after concentration
The urine pools (tracks 2–7) were precipitated with Coomassie blue and the respective protein concentrates (tracks 8–13) were diluted and coelectrophoresed at an equivalent load. Abbreviations as in Fig. 1.

---

**Fig. 3.** Densitometric scans of silver-stained SDS-PAGE patterns of urinary proteins before and after concentration
The urine pools (scans 2 and 4) were precipitated with Coomassie blue and the respective protein concentrates were and coelectrophoresed at an equivalent load (assuming 100% protein recovery) (scans 3 and 5). The M, calibration proteins (scan 1) were phosphorylase b (arrow), albumin, ovalbumin, carbonic anhydrase, triphenyltropolone, and ovalbumin. Quantitative recovery of protein was consistently >90% for albumin (A) and Tamm–Horsfall mucoprotein (TH). The recovery of other proteins (small arrow) was quantitatively >100%. The reason for this is unclear—it may reflect increased dissociation of high-molecular-mass complexes or, alternatively, protein degradation. The pattern alterations were reproducible for an individual urine and consistent between urines, although variable in degree.
terference, A). Absorbance protein (Ag), because SDS-PAGE of the resolubilized protein appears to be due to nonprotein interference, because SDS-PAGE of the resolubilized protein was measured at 595 nm before and after (C) centrifugation.

**Fig. 4.** Comparison of dye reagents for precipitation of the low-protein urine pool. Increasing volumes of urine were mixed with 1 mL of laboratory-prepared reagent (A), Bio-Rad (B), Pierce Coomassie (C), or Pierce Coomassie Plus (D) reagents. Absorbance was measured at 595 nm before (B) and after (C) centrifugation.

**Fig. 5.** Comparison of dye reagents for precipitation of the high-protein urine pool. Increasing volumes of urine were mixed with 1 mL of laboratory-prepared reagent (A), Bio-Rad (B), Pierce Coomassie (C), or Pierce Coomassie Plus (D) reagents. Absorbance was measured at 595 nm before (B) and after (C) centrifugation.

Coomassie blue precipitate confirms good recovery of protein.

The precision of the method was good (CV <5%), as evaluated by precipitation of multiple aliquots of the same sample on the same day (Figure 6) and on different days with different batches of reagent (results not shown).

Concentration of urinary proteins by precipitation with Coomassie blue combines protein assay and recovery and is ideal for electrophoretic comparison of equal amounts of protein by Coomassie blue staining; i.e., the individual protein response to dye-binding on protein assay is likely to reflect its response to staining. Upon resolubilization, the residual dye completely dissociates from the protein, and the electrophoretic mobility of the protein appears to be unaffected.

**Fig. 6.** Denitometric scans of Coomassie blue-stained SDS-PAGE patterns of urinary proteins precipitated from multiple aliquots of the same sample.

Quantitative recovery of protein in the major peaks of seven sample aliquots indicated a CV <5%. The scans correspond to four aliquots (1–4) of the same urine precipitated with the same batch of dye on the same day. Abbreviations as in Fig. 1.

References
Solid-Phase Extraction Protocol for Isolating Retinol-d₄ and Retinol from Plasma for Parallel Processing for Epidemiological Studies

Steven R. Dueker,¹ Jennine M. Lunetta,¹ A. Daniel Jones,² and Andrew J. Clifford¹³

Solid-phase extraction permits the parallel processing of samples in large numbers. We have applied this technique to the isolation of retinol isotopomers from plasma of humans participating in a study of vitamin A stable isotope dilution. The isotopomers were analyzed by gas chromatography/mass spectrometry. The extraction involves the separation of retinol from its aqueous matrix with a C₁₈ silica-based sorbent followed by removal of lipid contaminants with an aminopropyl silica-based sorbent. Overall recovery of retinol from plasma was 47.2% ± 1.8%. Purity of the retinol isolated from plasma is comparable with that obtained with a single HPLC method. This method permits the preparation of 32 samples per day by one analyst. Elimination of the need for HPLC permits sample preparation in the field with a minimum of equipment and technical skill.

Indexing Terms: vitamin A · gas chromatography/mass spectrometry · epidemiological application

Vitamin A (retinol) is an essential nutrient that has been linked to cancer prevention by recent epidemiological evidence (I, 2). Serum retinol concentrations are maintained nearly constant over a wide range of vitamin A intakes and do not accurately reflect an individual's total body stores of vitamin A (3). In well-nourished individuals, 90–95% of the total body vitamin A is stored in liver, making vitamin A analysis of liver biopsies the most direct approach for determining vitamin A status. However, liver biopsies for the purpose of assessing vitamin A status cannot be justified in epidemiological studies.

Recently, isotope dilution methods have been developed for estimating the total body stores of vitamin A (4, 5). The ratio of labeled to unlabeled retinol is determined in total retinol isolated from plasma drawn 10 or more days after ingesting labeled retinyl acetate. Current methods in our laboratory involve precipitation of plasma proteins, extraction of retinol with hexane, purification by HPLC, and derivatization to retinol tert-butylidimethylsilyl ether (t-BDMS-retinol). The ratio of labeled to unlabeled ethers is determined with gas chromatography (GC) coupled with selected ion monitoring mass spectrometry (SIM/MS) (6). Sample preparation is time-consuming (nine plasma specimens/day), making the method unsuitable for the rapid analysis of large sample sets.

We have developed a solid-phase extraction (SPE) procedure that utilizes C₁₈ and aminopropyl (NH₂) silica-based sorbents and allows for the parallel processing of 32 specimens/day by an individual analyst without the need for HPLC. The two-column SPE extraction replaces the hexane extraction and HPLC steps of our previous method. The C₁₈ sorbent was chosen for its

¹ Department of Nutrition and ³ Facility for Advanced Instrumentation, University of California, Davis, CA 95616-8869.

³ To whom correspondence should be addressed. Fax 916-752-8966; E-mail AJCLIFFORD@HAMLET.UDAVIS.EDU.

Received March 2, 1993; accepted June 4, 1993.

4 Nonstandard abbreviations: SPE, solid-phase extraction; GC/MS, gas chromatography/mass spectrometry; t-BDMS-retinol, retinol tert-butylidimethylsilyl ether; SIM/MS, selected ion monitoring mass spectrometry; NH₂, aminopropyl; MTBSTFA, N-methyl-N-tert-butylidimethylsilyl trifluoroacetamide; retinyl-d₄ acetate, trans-retinyl-10,19,19-d₄ acetate; and BHT, butylated hydroxyltoluene.