Removal of sodium dodecyl sulfate from protein samples

Andrius Žilionis

Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko St. 24, 03225 Vilnius, Lithuania

Sodium dodecyl sulfate (SDS) is a widely used detergent for protein denaturation and solubilization. However, application of SDS in the sample preparation for the liquid chromatographic-mass spectrometric analysis is limited because commonly used SDS concentrations interfere with reversed phase liquid chromatography and electrospray ionization mass spectrometry. In order to analyse SDS pretreated proteins by the above-mentioned methods SDS must be completely removed or its concentration must be lowered to less than 0.01%. In this work we present a comparison of different SDS removal strategies based on SDS ultrafiltration, protein precipitation and SDS precipitation methods. Every strategy was optimized so that the initial 4% SDS concentration was lowered to less than 0.01% and the initial sample volume remained unchanged. The modified Mukerjee's photometric method was used for the SDS quantitation in the presence of model protein bovine serum albumin and the recovery of model protein was evaluated using reversed phase ultra performance liquid chromatography. The main advantages and drawbacks of every strategy are discussed.

Keywords: SDS precipitation, SDS ultrafiltration, BSA precipitation, SDS quantitation, BSA RP-UPLC

INTRODUCTION

Sodium dodecyl sulfate is one of the most widely used detergents in protein chemistry. It has shown benefits for protein denaturation and solubilization. SDS is typically used at concentrations between 0.1 and 2%, but application of 4% SDS was also reported [1]. However, application of SDS in the protein sample preparation for the liquid chromatographic-mass spectrometric (LC-MS) analysis is limited because SDS is recognized to cause a significant chromatographic peak broadening in reversed phase liquid chromatography and signal suppression in electrospray ionization mass spectrometry. It was demonstrated that the threshold tolerance of SDS in LC-MS experiments was 0.01% [2]. Given the advantages of using SDS for protein sample preparation, procedures for detergent removal prior to LC-MS analysis are necessary.

Several different methods can be applied to reduce SDS concentration in the protein sample. The most popular of them are SDS precipitation with potassium ions, protein precipitation with cold acetone and chloroform-methanol-water protein precipitation. A direct comparison of these methods is complicated because the final sample volume, the protein recovery and the remaining SDS amount in the sample differ depending on a method used for a given protein. The final SDS concentration must be ≤0.01% and high protein recovery is preferable in the field of liquid chromatography and mass spectrometry. Since as high as 4% SDS concentrations are used in practice, either once applied SDS removal method could be not effective enough. Moreover, an additional protein concentration step is preferred if an SDS removal procedure results in an increased sample volume.

In this work we combined the above-mentioned SDS removal methods with an SDS ultrafiltration
procedure. Ultrafiltration with urea increases the protein concentration by lowering the sample volume and enables SDS removal at the same time. As a result, the SDS ultrafiltration with urea is a perfect additional step for all SDS removal methods. Since the initial sample volume remained unchanged in our experiments, the final SDS concentration in the protein sample and the recovery of model protein bovine serum albumin (BSA) were used for the evaluation of effectiveness of the suggested strategies. SDS quantitation in the presence of BSA was performed by the modified Mukerjee's photometric method and the recovery of BSA was evaluated using reversed phase ultra performance liquid chromatography. Every our suggested strategy was successfully applied to reduce the initial 4% SDS concentration to ≤0.01%.

**EXPERIMENTAL**

**Chemical reagents** were used as received without additional purification: BSA solution (2 mg/ml, for standard curve preparation with protein assays, Thermo Scientific, USA); SDS (≥98%, Merck, Germany); urea (≥99.5%, Carl Roth, Germany); acetone (≥99.8%,POCH S.A., Poland); methylene blue hydrate (≥97%, Sigma-Aldrich, USA); HCl (2 mol/l, Fisher Chemical, UK); chloroform (≥99.9%, Sigma-Aldrich, USA); trifluoroacetic acid (TFA, LC/MS grade, Fisher Chemical, USA); acetonitrile (LC/MS grade, Carl Roth, Germany); methanol (LC/MS grade, Carl Roth, Germany); KH₂PO₄ (≥99%, Sigma-Aldrich, USA); K₂HPO₄ × 3H₂O (for analysis, Sigma-Aldrich, USA); 1,3-dimethylimidazolium methylsulfate (for synthesis, Merck, Germany). All solutions were prepared by dissolving the required amounts of the reagents in bidistilled water unless otherwise noted.

The model protein sample was prepared by mixing 0.5 ml of the BSA solution (2 mg/ml), 0.4 ml of the SDS solution (10%, w/v) and 0.1 ml of water. The final concentrations of BSA and SDS were, respectively, 1 mg/ml and 4%. 150 µl of the model protein sample was used for investigation of every SDS removal strategy.

The ultrafiltration (UF) procedure was performed using Amicon Ultra 0.5 ml centrifugal filter units (Ultracel-3K, regenerated cellulose, MWCO 3 kDa, Merck Millipore, Ireland) and a Sigma 1-14 centrifuge with a fixed angle rotor (Germany). The protein sample containing SDS was placed in a filter unit and an 8 mol/l urea solution was added up to the 500 µl volume unless otherwise noted. The sample volume was reduced to approximately 130–150 µl by spinning the device at 14000 × g for 12 min. The ultrafiltration procedure was repeated several times depending on the strategy used (the exact numbers are given below). The filter device was placed upside down in a clean tube and spun for 2 min at 1000 × g in order to transfer the concentrated sample to the tube. The volume of the recovered sample was evaluated by a pipette and increased up to 150 µl by adding the required volume of the last collected filtrate.

The SDS quantitation in the presence of BSA was performed by the modified Mukerjee's photometric method as described elsewhere [3]. Briefly, one volume of the protein sample (100 µl) was mixed with four volumes of cold acetone (400 µl) and left at –20°C for 1 h, then centrifuged at 10000 × g for 10 min. The supernatant (125 µl) was mixed with 1 ml of a methylene blue solution (10 mg/l in 0.01 mol/l HCl), 200 µl of chloroform was added and the sample was mixed by thorough vortexing. The upper aqueous layer was transferred to a cuvette after spinning the sample at 1000 × g for 5 min and the absorbance was measured at 655 nm using a T60 U spectrophotometer (PG Instruments, UK). The same procedure was applied for all SDS standard solutions (0.025, 0.02, 0.015, 0.01, 0.005 and 0.002%, w/v) and the standard curve was used to determine the SDS concentration in the protein sample (Fig. [3]).

The ultra performance liquid chromatographic (UPLC) analysis of BSA was performed in the reversed phase mode using the Acquity Ultra Performance LC system (Waters, USA). BSA was separated on the Acquity UPLC BEH300 C4 column (1.7 µm, 2.1 × 100 mm) with the VanGuard pre-column (Waters, Ireland). Solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) were used for the gradient elution (0 min – 30% B, 5 min – 50% B, 10 min – 90% B). The column was equilibrated with the starting mobile phase for 10 min before the injection. The flow rate of the mobile phase was 0.25 ml/min, the column temperature was 25°C, the volume of injection was 10 µl and the detection wavelength
was 280 nm. The BSA standard solutions (0.03, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/ml) were prepared by mixing the required volumes of the 2 mg/ml BSA solution with the 8 mol/l urea solution, pH of which was firstly adjusted to neutral with TFA. The protein samples were diluted two times with the same 8 mol/l urea solution before the UPLC analysis and the standard curve was used for the evaluation of BSA recoveries (Fig. 2).

The cold acetone protein precipitation procedure for SDS removal was performed by mixing 150 µl of the model protein sample with 600 µl of cold acetone in a 1.5 ml polypropylene test tube. The mixture was left at –20°C overnight. The supernatant was discarded after centrifugation at 10000 × g for 10 min and additional 600 µl of cold acetone was added. The sample was kept at –20°C for 1 h and spun for 10 min at 10000 × g. The supernatant was discarded, 500 µl of the 8 mol/l urea solution was added and the precipitated protein was dissolved by vortexing. The ultrafiltration procedure was performed twice and the second time water was used instead of the 8 mol/l urea solution.

The chloroform-methanol-water (C/M/W) protein precipitation procedure was performed by mixing 150 µl of the model protein sample with 600 µl of methanol, 150 µl of chloroform and 450 µl of water in the 1.5 ml polypropylene test tube. The sample was mixed by vortexing and spun at 10000 × g for 10 min. The top layer (water and methanol phase) was carefully removed.
using a pipette, 450 µl of methanol was added and the sample was mixed by vortexing. The supernatant was discarded after centrifugation at 10000 × g for 10 min and the precipitated protein was dissolved in 500 µl of the 8 mol/l urea solution. The ultrafiltration procedure was performed twice and the second time water was used instead of the 8 mol/l urea solution.

The dodecyl sulfate precipitation with potassium ions (KDS precipitation) was performed using the 2 mol/l potassium phosphate buffer (pH ≈ 7.4), which was prepared by dissolving 0.5267 g of KH₂PO₄ and 1.3990 g of K₂HPO₄ × 3H₂O in 5 ml of water. The model protein sample (150 µl) was diluted with H₂O (150 µl) and 15 µl of the potassium phosphate buffer was added, the sample was mixed by pipetting and left in an ice bath for 30 min. The supernatant was placed in a centrifugal filter unit after centrifugation at 10000 × g for 10 min and the ultrafiltration procedure was applied twice, both times water was used instead of the 8 mol/l urea solution.

RESULTS AND DISCUSSION

An accurate quantitation of SDS and the evaluation of protein recovery are required to compare different SDS removal methods for a given protein sample. Mukerjee suggested a simple and accurate photometric SDS determination method based on methylene blue and dodecyl sulfate ion pair extraction. This strategy was reported being compatible with a number of biochemical reagents, nucleic acids and proteins. However, our previously reported results showed that a high concentration of protein prevented methylene blue extraction to the organic phase and thus interfered with SDS quantitation. In this study, the reliability of SDS quantitation in the presence of BSA was ensured by our previously presented modification of the Mukerjee's method, which enables an accurate SDS concentration measurement in the samples initially containing 1.8 mg/ml BSA (relative error <7% and relative standard deviation <5%, n = 8). The results of the SDS quantitation are presented in Fig. 3.

As SDS concentrations were firstly lowered to ≤0.01%, an accurate reversed phase liquid chromatographic evaluation of BSA recoveries became possible. The influence of SDS on the results of the UPLC analysis was evaluated by analysing 0.03 mg/ml BSA samples in water and in the 0.01% SDS solution, the relative error was 3.5% (n = 3, data not shown). Since all other BSA samples were diluted twice for the UPLC analysis, the SDS concentrations did not exceed 0.005% and the relative error was expected to be lower than 3.5%. BSA is

![Remaining SDS concentrations in BSA samples](image)

**Fig. 3.** The results of SDS quantitation: A, after UF with 8 mol/l urea; A*, after UF with water; B, after C/M/W precipitation + UF; C, after cold acetone precipitation with additional wash + UF; D, after cold acetone precipitation without additional wash + UF; E, after KDS precipitation; F, after sample dilution and KDS precipitation. The standard deviation is indicated by error bars (n = 3)
known to form aggregates, because of which multiple peaks are observed in reversed phase liquid chromatography [6]. In order to obtain single peaks BSA molecules were denatured by preparing standard solutions in the 8 mol/l urea solution. The model protein samples for SDS removal experiments contained 4% SDS. As a result, BSA was also denatured in those samples (only minor peaks of aggregates were observed) and all chromatograms were manually integrated for BSA quantitation (Fig. 4). The results of BSA recovery calculations are presented in Fig. 5.

**Fig. 4.** The chromatograms of BSA samples after SDS removal: A, after UF with 8 mol/l urea; B, after C/M/W precipitation + UF; C, after cold acetone precipitation with additional wash + UF; D, after cold acetone precipitation without additional wash + UF; E, after KDS precipitation; F, after sample dilution and KDS precipitation.

**Fig. 5.** The recoveries of BSA after SDS removal: A, after UF with 8 mol/l urea; B, after C/M/W precipitation + UF; C, after cold acetone precipitation with additional wash + UF; D, after cold acetone precipitation without additional wash + UF; E, after KDS precipitation; F, after sample dilution and KDS precipitation. The standard deviation is indicated by error bars (n = 3).
Andrius Žilionis

Ultrafiltration-based buffer exchange, also called desalting or diafiltration, is a widely used method for removing solvents and salts from the protein solution. The exchange of the buffer can be accomplished using a centrifugal filter unit by concentrating the sample, discarding the filtrate and reconstituting the concentrate to the original sample volume with the desired solvent. This process can be repeated several times until the concentration of the undesirable component is sufficiently reduced. In our experiments, once performed ultrafiltration procedure should reduce the concentration of the contaminating component approximately 3–4 times (see Experimental). Taking this into account, repeating the ultrafiltration procedure 5–6 times should reduce the initial 4% concentration of the contaminant to <0.01%. However, SDS removal by ultrafiltration is limited because dodecyl sulfate forms micelles at the concentrations above the critical micelle concentration (CMC). The CMC is very low (8.08 mmol/l in water) compared to the initial 4% SDS concentration. As a result, the major amount of dodecyl sulfate exists in a micellar form. Since the size of micelle is comparable with the size of protein, ultrafiltration is not able to separate them efficiently. Consequently, SDS micelles must be disrupted in order to effectively remove SDS by ultrafiltration.

It was reported that the CMC of detergents was increased by chaotropic solvents. We have tested the ultrafiltration with the 8 mol/l urea solution as the SDS removal step prior to the LC-MS analysis. Centrifugal filter units with regenerated cellulose membranes were used because these membranes were reported being less susceptible to fouling than polysulfone and polyamide ones in the SDS ultrafiltration. The smallest available pore size (3 kDa) was chosen in order to make the method useful for the maximum variety of proteins. The initial volume of the model protein sample was 150 µl and a 500 µl volume centrifugal filter unit was used. The ultrafiltration procedure was repeated until the concentration of SDS was lowered to ≤0.01% because this concentration was reported being the tolerance level of SDS in LC-MS experiments. In our hands, the 10 times repeated ultrafiltration procedure lowered the initial 4% SDS concentration to 0.0076% (Fig. A). The last tenth ultrafiltration procedure was performed using water instead of the 8 mol/l urea solution because high concentration of urea interferes with cold acetone protein precipitation, which is the first step of our SDS quantitation method. Ultrafiltration was not able to reduce the SDS concentration to ≤0.01% when the 8 mol/l urea solution was replaced with water for all 10 repeated procedures (Fig. A*). This result demonstrates that urea makes the ultrafiltration of SDS more efficient. However, the CMC most probably was not increased enough for disruption of all micelles in the model protein sample because the 5–6 times repeated ultrafiltration procedure did not reduce the SDS concentration to the level of tolerance in the LC-MS analysis. Since the disruption of micelles is not most probably complete in the 1.2% SDS solution (150 µl 4% SDS + 350 µl 8 mol/l urea), a significant reduction of the diluted sample volume should be avoided during ultrafiltration because of SDS precipitation risk. As a result, this method is impractical for larger initial volume samples unless larger volume centrifugal filters are used. On the other hand, only a negligible loss of BSA was caused by the SDS ultrafiltration with 8 mol/l urea using a 3 kDa regenerated cellulose membrane (Fig. A), and this method can be easily applied for low initial volume samples.

The CMC of detergents can be modulated by ionic liquids. It was reported that different ionic liquids can either increase or decrease the CMC of dodecyl sulfate. Beyaz et al. demonstrated that 30 mmol/l concentration of 1,3-dimethylimidazolium iodide increased the CMC of SDS to 170 mmol/l. It was also reported that the nature of the counterion had no noticeable effect on the observed CMC values. Taking this into account, the 30 mmol/l concentration of 1,3-dimethylimidazolium methylsulfate was expected to disrupt all micelles in the 4% SDS solution and thus enable an effective SDS removal by ultrafiltration. Unfortunately, our results demonstrated that ultrafiltration with 30 mmol/l 1,3-dimethylimidazolium methylsulfate was less effective than both the ultrafiltration with 8 mol/l urea and the ultrafiltration with pure water because the 10 times ultrafiltrated sample contained more than 0.025% SDS (the exact concentration was outside the quantitation range of our method and was not determined). The possible reasons for these results were not investigated because they are outside the scope of this study.
Several well-known SDS removal methods can be applied in the case of large initial volume protein samples. The most popular of them are protein precipitation with cold acetone, chloroform-methanol-water protein precipitation and SDS precipitation with potassium ions. Protein precipitation protocols enable both SDS removal and protein sample concentration because precipitated protein can be reconstituted in a lower volume of the desired solvent. The recovery of protein and the amount of remaining SDS depend on the extent to which the supernatant is pipetted from the remaining protein pellet. Removing as much solvent as possible maximizes the reduction of SDS, but it also maximizes the risk of aspirating a portion of the protein pellet. Moreover, the pellet becomes difficult to visualize at low protein levels (<5 µg) and the risk of protein loss increases [2]. Since our model protein sample contains 4% SDS, the concentration of SDS is also high in the supernatant and even a low remaining volume of the supernatant prevents a sufficient SDS removal for the LC-MS analysis. As a result, protein precipitation protocols are limited when the SDS concentration is high and the protein concentration is low. We combined protein precipitation protocols with the SDS ultrafiltration procedure in order to overcome the above-mentioned limitations. Firstly, protein precipitation with cold acetone or chloroform-methanol-water precipitation is performed to remove most of SDS. Instead of removing as much supernatant as possible, some supernatant is left to prevent aspirating a portion of the protein pellet when cold acetone precipitation is performed or to prevent disturbing the middle protein layer when chloroform-methanol-water precipitation is performed. This is in contrast to commonly used protein precipitation protocols, but a significant loss of protein is thus prevented. Secondly, the precipitated protein is reconstituted in the 8 mol/l urea solution and the ultrafiltration procedure is performed to reduce the concentration of remaining SDS to ≤0.01%. As a low volume of the 8 mol/l urea solution is used for the reconstitution (0.5 ml), the ultrafiltration using a 0.5 ml volume centrifugal filter unit can be performed independently on the initial sample volume. Our results demonstrate that either the above-mentioned protein precipitation protocol reduces the initial 4% SDS concentration below the tolerance level of the LC-MS analysis with a high BSA recovery (Fig. 5B, C) when followed by the twice repeated ultrafiltration procedure. The C/M/W protein precipitation protocol is more complicated to perform than cold acetone precipitation, but it requires less time to be performed and it should be considered when the time of sample preparation is a priority. It is worth noting that the cold acetone protein precipitation protocol without an additional precipitate washing step was also investigated and it was shown that an additional washing step does not reduce the recovery of protein (Fig. 5D) and enables a more complete removal of SDS (Fig. 5C, D). On the other hand, our results demonstrate that an additional washing step is not necessary for a successful sample preparation prior to the LC-MS analysis.

The last tested SDS removal method was dodecyl sulfate precipitation with potassium ions, which is based on the fact that solubility of the potassium salt of dodecyl sulfate is much lower than that of its sodium salt [1,3]. This method is fast and simple to perform. Potassium phosphate is recommended for general applications [14], but other potassium salts are also used in practice. Our results demonstrate that the initial 4% SDS concentration is sufficiently reduced for the LC-MS analysis by adding 15 µl of the 2 mol/l potassium phosphate buffer to 150 µl of the protein sample (Fig. 5E, F). Despite the fact that a low volume of the additional buffer precipitates a large amount of dodecyl sulfate and thus increases the initial sample volume only by 10%, the recovery of BSA was noticeably lower in comparison with the previously discussed SDS removal strategies (Fig. 5F). In order to obtain higher BSA recovery the initial sample was firstly diluted with water twice. The same dodecyl sulfate precipitation procedure was performed and a higher BSA recovery was obtained (Fig. 5F). On the other hand, the protein recovery was still significantly lower compared to ultrafiltration-based SDS removal strategies. Moreover, diluting the initial sample and/or using a lower concentration potassium phosphate buffer result in the increased sample volume and an additional sample concentration procedure may be required. It is worth noting that the ultrafiltration procedure was applied in order to reduce the concentration of potassium phosphate and to adjust the final sample volume to 150 µl after the KDS
precipitation. The removal of remaining SDS was not the purpose of ultrafiltration in this case. Since a significant dilution of the sample can be avoided and potassium phosphate does not interfere with reversed phase liquid chromatography, KDS precipitation can be applied as a fast single-step SDS removal procedure prior to the liquid chromatographic analysis of the protein sample unless the recovery of protein is of great importance. However, an additional removal of the potassium phosphate step is preferred in the case of bottom-up proteomics, because a high monovalent salt concentration may interfere with trypsin protease activity.

CONCLUSIONS

In this work several different SDS removal strategies were compared. The initial 4% SDS concentration in the BSA samples was successfully reduced below the tolerance level of the LC-MS analysis by SDS ultrafiltration with 8 mol/l urea, SDS precipitation with potassium ions or protein precipitation with organic solvents, followed by SDS ultrafiltration as a polishing step. In principal, the ultrafiltration-based SDS removal is simple to perform. Nevertheless, we recommend the SDS ultrafiltration procedure alone only for low initial volume samples because repeating ultrafiltration many times is a labour-intensive procedure and large volume centrifugal filter units are required for large volume samples. We also demonstrated that protein precipitation with cold acetone and chloroform-methanol-water precipitation resulted in both a nearly-complete SDS removal and a high protein recovery when combined with the ultrafiltration procedure. This strategy was tested using the initial sample volume of 150 µl, but larger volume samples are also acceptable because protein precipitation protocols enable both SDS removal and protein sample concentration while the following application of SDS ultrafiltration with urea ensures a high protein recovery and enables reduction of the remaining SDS concentration bellow the tolerance level of LC-MS experiments. Although dodecyl sulfate precipitation with potassium ions is much faster and less complicate to perform, this procedure was shown to cause a significant loss of BSA even when the model protein sample was firstly diluted twice. On the other hand, this simple method can be successfully applied when recovery of the protein is not of great importance. Moreover, ultrafiltration-based strategies cannot be used for very small proteins and peptides while dodecyl sulfate precipitation is not limited by the protein size. BSA was used as a model protein in our experiments and we demonstrated that the mentioned strategies differed in the BSA recovery, the variety of required chemical reagents, the sample preparation time and complexity. The main advantages and disadvantages of these strategies were discussed and the reader can choose the most appropriate method for his own applications.

ACKNOWLEDGEMENTS

The author thanks prof. Audrius Padarauskas for his critical review of the manuscript.

Received 23 August 2018
Accepted 11 September 2018

References

NATRIO DODECILSULFATO ŠALINIMAS IŠ BALTYMŲ MĖGINIŲ

Santrauka

Natrio dodecilsulfatas yra joninis detergentas, plačiai naudojamas denatūruoti ir soliubilizuoti baltymus. Tačiau jo naudojimas ruošiant mėginius skysčių chromatografinei ir masių spektrometrinei analizėms yra ribotas, nes įprastai naudojamos dodecilsulfato koncentracijos sukelia smailių išplatėjimų atvirkščių fazių skysčių chromatografijoje bei slopina elektropurkštuvinę analių jonizaciją. Norint minėtais metodais analizuoti dodecilsulfatu paveiktus baltymų mėginius, šis detergentas turi būti pašalintas arba jo koncentracija sumažinta bent iki 0,01 %. Šiame darbe buvo praktiškai išmėginti ir palyginti skirtingi dodecilsulfato šalinimo iš baltymų mėginių metodus, pagrįstę ultrafiltravimu, dodecilsulfato ir baltymo išsodinimu. Pradinis ir galutinis mėginio tūris visais atvejais siekė 150 µl, pradinės natrio dodecilsulfato ir modelinio baltymo jaučio serumo albumino koncentracijos buvo atitinkamai 4 % ir 1 mg/ml. Dodecilsulfato koncentracijos galutiniuose mėginiuose buvo įvertintos fotometriškai, o baltymo išgavos apskaičiuotos pagal atvirkščių fazių ultraefektyviosios skysčių chromatografijos analizės rezultatus. 4 % natrio dodecilsulfato koncentracija buvo sėkmingai sumažinta iki tinkamos mėginio analizei skysčių chromatografijos ir masių spektrometrijos metodais taikant dodecilsulfato ultrafiltravimą su 8 mol/l karbamido tirpalu, dodecilsulfato išsodinimą su kalio jonais bei sujungus baltymo išsodinimą organiniais tirpikliais su likutinio dodecilsulfato šalinimu ultrafiltravimo būdu. Pastaroji dodecilsulfato šalinimo strategija ne tik pasižymėjo didele baltymo išgava, tačiau taip pat gali būti lengvai pritaikyta didesnio pradinio tūrio mėginiams. Darbe aptarti skirtingų dodecilsulfato šalinimo metodų pagrindiniai privalumai ir trūkumai, leidžiantys konkrečioms eksperimentams lengviau pasirinkti tinkamiausią metodą.