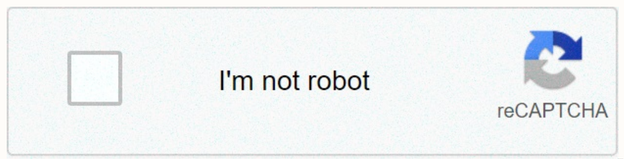

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Extinction coefficient calculator protein concentration

Volume 182, issue 2, 1 November 1989, pages 319-326View full text Author information copyright and license information Denialo-fighting Malaria Immunology and Vaccineinology, National Institute of Allergy and Infectious Diseases, National Institute of Health, Rockville, MD 20852, USA*Correspondence: Dam Zhu, Laboratory of Malaria and Vaccineology, National Institute of Allergy and Infectious Diseases, National Institute of Health, Rockville, MD 20852, USA. Phone: (301) 402-7957; Fax: (301) 480-1962; vog.hin.diain@uhzd; Alan Saul, Novartis Vaccines Global Health Vaccine Institute (NVGH), Via Fiorentina 1, 53100 Siena, Italy, Telephone (39 0577 245129); FAX (39 0577 243540); moc.sitravon@luas.nalla1Prevulent address: Department of Rheumatology and Immunology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, PR China, 2Presented address: Novartis Vaccines Institute of Global Health S.r.l. (NVGH), Via Fiorentina 1, 53100 Siena, Italy This study reports a method for determining the total concentration of protein or protein concentration of interest in protein-protein conjugate using ultraviolet absorption, after determining the moland ratio of proteins in conjugates from which extination can be calculated. A Microsoft Excel template has been developed to solve the problem of using amino acid analysis data to determine the molar ratio. The percentage mass of each protein in the conjugate is calculated from the amino acid composition data using the smallest squares method in the Microsoft Excel solving function, and the percentage mass is converted into a molar part of each protein using the corresponding molecular weight. The molar ratio is obtained by dividing the molar part protein 1 of the molar part protein 2. A weighted extination coefficient is calculated using the molar ratio and the total protein concentration is determined using ultraviolet absorption at 280 nm. The accuracy of the method is checked using mixtures of known proteins. This study provides a quick, simple and accurate method for determining the concentration of protein in protein-protein conjugates. Keywords: Concentration of protein-protein conjugate, molar ratio, Microsoft Excel solver, Amino acid analysis, least square analysisLawing a poor immunogenic compound with a high immunogenic protein carrier protein to increase immunogenicity is a common practice in the biological science field and has widespread application. Our previous studies have shown that significantly higher antibody titres can be achieved by when the leading antigens of the malaria vaccine blocking the transmission of the virus are conjugated with the complex of external membrane proteins (OMPC) of Neisseria meningitidis serogroup B (Wu et al., 2006) or recombinant notoxic Pseudomonas aeruginosa ExoProtein A (rEPA) (Qian et al., 2007 and An accurate assessment of the concentration of conjugated protein is essential for downstream research. The most critical Most Critical (c) the molar ratio of protein-protein conjugates, which can be used to calculate the extination coefficient of the conjugate, shall be assessed. A number of methods were developed to assess the molar ratio of protein-protein conjugates, including radioactively labelled proteins (Green et al., 1982), sodium dodecyl sulphate electrophoresis (SDS) (Jones et., 1989), spectrophotometric method (and Jones al., 1989; Sahidhar, etc., 1994), matrix-assisted laser desorption/ionization of light (MALDI-TOF) mass spectrometry (Pakarinen et al., 2002) and capillary electrophoresis (Safi et al., 2007). However, the ratios determined by these methods are estimates and may not be suitable for accurately measuring the concentration of protein of the conjugate. It turns out that analysis of amino acids is the most accurate method for determining the molar ratio of protein in protein-protein conjugates. In 1989, Antoni and Presentini reported on a DOS method and with at least squares to determine the molar ratio of two different proteins in conjugates using the results of the amino acid analysis. Schuller and colleagues presented a comprehensive Microsoft Excel- and smallest squares method for determining the ratios of small peptides to keyhole limocyanin (KLH) using amino acid analysis (Shuler et al., 1992). As the technology rapidly evolves, the program written in BASIC language for vax 750 computer, described in Anthony and presentini paper is no longer suitable for today's applications; and the method developed by Schuller depends on the method used to calculate the protein composition of amino acid analysis data, which requires thorough verification. In this message, we present a simple and accurate method by which the molar ratio of protein-protein conjugates can be determined by a Microsoft Excel solver-based template using amino acid analysis data. The total protein concentration in the conjugate and the concentration of the individual protein components can be accessed using calculated extination coefficients (Pace et al., 1995). The accuracy of this method is verified by calculating molar ratios in known protein mixtures. This method must have general uses in which the protein concentration of protein-protein conjugates must be determined. Recombinant Pichia pastoris expresses Pvs25 (MacDonald and Narum, not published), Pfs28 (MacDonald and Narum, not published) and AMA1-FVO (Kenedy et al 2002) proteins, as well as Escherichia coli expressed ExoProtein A (rEPA) (Qian et al 2007) protein have been produced with methods developed in the Laboratory of Malaria Immunology and Vaccination (LMIV), national institute of allergy and infectious diseases, National Institutes of Health, protein concentrations are determined by ultraviolet absorption at 280 nm. BSA was purchased by Thermo Fisher Scientific.The known molar ratio of the protein mixture prepared according to Table 1. The mass % protein 1 and 2, experimentally prepared molar ratio protein 1 / protein 2 2cPfs25BSA0.7700.75237.512.575%25%1.111252551%49%3.33212.537.525%75%9.995 AMA1-FVOEPA0.4370.84151515%85%0.187101034%66%0.56215561%39%1.685 Pvs 25EPA0.6901.265151045%55%2.650Pvs28EPA1.3081.225101052%48%3.396The amino acid composition was determined by the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University (New Haven, CT). Hydrolyse the samples in vacuo for 16 hours at 115°C in 100 µl 6N HCl/0,2 % phenol (with 1 nmole norvaline/100 µl as an internal standard) to grind the protein into free amino acids. After hydrolysis, HCl is dried in a vacuum centrifuge and the amino acids obtained dissolved in 100 µl of 0,02N HCl (with 2 nmole taurine/100 µl as the second internal standard). The analysis of amino acids is carried out on Hitachi L-8900 PH Amino acid analyser, which uses ion exchange column with pH and temperature gradients to separate amino acids and postcolonal conductor with ninhydrin for detection at 570 nm and 440 nm. The EZChrome Elite software (for Hitachi) is used to work with the analyzer and collect and analyze the data. Fourteen amino acids were used in our calculation. During HCl hydrolysis, asparagine is converted into aspartic acid and glutamine glutamine glutamic acid; therefore these amino acids are reported as total values of ASX and Glx. Cysteine, Tryptophan, thrionin and serine recovery are usually low, methionine can be partially oxidized, and proline quantitatively is often inaccurate due to intervention by cysteine, so these amino acids have been excluded from the calculation. Table 2 is a Microsoft Excel template for calculating the molar ratio. The percentage of composition of each amino acid in each protein (or conjugate) is obtained by dividing the experimental nmol of the amino acid into protein 1 (X1), protein 2 (X2), or conjugate (Y) with a total nmol (Σ) of X1, X2, or Y to produce normalized X1 (protein 1), X2 (protein 2) and Y (conjugate), respectively. Y is the experimentally determined percentage composition of an amino acid in a conjugate. The theoretical percentage of composition (Σl) of an amino acid is calculated by formula: Σi = (X1i × Z1) + (X2i × (1−Z1)), where I is an amino acid and Z1 represents the percentage mass of protein 1 that is produced when Σ (Y-Σ) 2 is the smallest as determined by the microsoft decider (see Appendix). The percentage by mass of protein 1 (Z1) is then converted into a molar part in the conjugate part by dividing the percentage by mass of its molecular weight. The molecular weight may be either intact or modified molecular weight, i.e. molecular weight, amino acid residues used. Molar Molar protein 2 is calculated by dividing the percentage mass (100 % - percentage mass of protein 1) by the molecular weight (intact or modified molecular weight). The molar ratio of protein 1 to protein 2 is obtained by dividing the molar part protein 1 with the molar part protein 2. This template can be easily used to determine the molar ratio of proteins in the conjugate. Template in Microsoft Excel format for molar ratio calculation*ABCDEFGHI Experimental data (nmole)Normalized data (%)ˉY(Y−ˉY)2 X1X2YX1X2Y1Asx2.58861.54702.22390.19990.11880.15820.15797.2703 × 10−82Glx2.08692.44612.46030.16120.18790.17500.17503.5468 × 10−93Gly1.20520.55890.91850.09310.04290.06530.06713.1088 × 10−64Ala0.37281.29230.93630.02880.09930.06660.06531.5886 × 10−65Val1.62321.00571.42440.12540.07720.10130.10047.6615 × 10−76Ileu0.88710.38550.67890.06850.02960.04830.04834.3462 × 10−97Leu0.86761.77251.44880.06700.13610.10300.10293.2973 × 10−88Tyr0.35340.52990.49750.02730.04070.03540.03421.2879 × 10−69Phe0.23590.78420.53610.01820.06020.03810.04003.5186 × 10−610His0.52070.37940.48730.04020.02910.03470.03453.2240 × 10−811Lys2.00961.69242.01240.15520.13000.14310.14219.5056 × 10−712Arg0.19570.62540.43690.01510.04800.03110.03221.2362 × 10−6 13Σ12.946713.019314.06131.001.001.001.2603 × 10−5 14Modified molecular weight of protein 1b15551.20Molar portion of protein 1c3.0967 × 10−5 15Modified molecular weight of protein 2b53361.28Molar portion of protein 2d9.7156 × 10−6 16% mass of protein 1a0.4816Molar ratio of Protein 1/Protein 2e3.1873After determining the molar ratio of a conjugate, the molar absorption coefficient at 280 nm for the protein conjugate was calculated by the equation described by Pace et al. (1995), i.e. ε280 (M−1 cm−1) = (no. trp) × (5500) + (no. × (1490) + (no. cys pair) × (125). The weighted extination coefficient (ml mg-1) is calculated by dividing ε280 by the molecular weight of the protein conjugate. derived from ExPASY. Weighted conjugate extination coefficient = [(extination protein antigen coefficient × milligram protein antigen) + protein carrier protein coefficient extination coefficient] ÷ (1 + milligram protein antigen). Nominal molan ratios (known molane ratios of protein mixtures) and calculated molan ratios by method, the mean percentage accuracy is 94,55 ± 3,36 % (intact molecular weight) or 96,39 ± 1,70 % (modified molecular weight) when calculated using this method, it shows that the method developed in this study , is able to accurately determine the molar ratio of protein conjugate.Comparison of the nominal molar ratio and calculated mollandationProtein 1/ Protein 2Molar ratio protein 2 /h)aMolar ratio protein 1/ protein 2 (calculated) b % accuracy from intact MWdV modified MWe from modified непокътнат MWe MWe AMA1-FVO/EPA0.1870.2010.19792.6394.690.5620.5840.573 95.9697.951.6851.6461.61497.6595.77 Pvs 25/EPA2,6 6 502.4222.62891.4199.18Pvs28/EPA3.3963.3703.19599.2 2 494.07 Average accuracy rate94.5596.39 Standard deviation3.361.70 The protein sequence ratio is easily accessible from the ExPASY location. The weighted extination coefficient of the conjugate can be calculated using the extination factors of the two components after their ratio has been determination. It can be calculated directly using the Pace equation, once the composition of the amino acid of the conjugate is known. The weighted extination coefficients of 8 conjugates are calculated using these two methods. As expected, the results are almost identical to the average deviations ± the standard deviation of 0,065 % ± 0,070 % (data not shown). These small variations can be merged by the rounding of the extinction factor of each monomer protein and suggest that the direct use of the Tempway equation is a better approach to calculating the protein conjugate extinction factor. The concentration of the total protein in a conjugate can be obtained by dividing ultraviolet absorption at 280 nm (UV280) by the extination coefficient or directly from an analysis of amino acids with the correction of missing residues. The concentration of protein of interest can be calculated by the following equation: concentration of proteinofintered ={(molarratio×molecular weightrecounted)÷[(molarratio×mmuld weightprotein)+weight from the protein slide)}×concentrationpotener. Although previously reported method is able to calculate the ratio of peptide to protein molar in conjugate, the accuracy of the calculation depends on the method used to calculate the composition of the protein from data for the analysis of amino acids. The theoretical amino acid composition of peptide is used for calculation in the original paper (Shuler et al., 1992). However, the results were not satisfactory when the theoretical amino acid composition of proteins were used to calculate some ratio of protein mixtures in our study. Accuracy is improved when amino acid compositions are experimentally determined. However, there are several ways to calculate the experimental composition of amino acids and they require extensive verification. This study uses the primary data in the amino acid nmole obtained directly from the amino acid analysis, eliminating the error that may occur when nmol is converted into a number of residues, as was done in the previous method. We also found that Microsoft Excel Solver, a uch. In addition, the model for calculating the ratio (Table 2) calculates the ratios in a short and reproducible manner. Post-translating modifications are common in proteins. During the analysis of amino acids, most of the post-translysesm modifications are removed from the therefore not quantified by the analysis. Modifications cannot be removed (or partially removed) by hydrolysis, a more accurate result may be obtained if the theoretical amino acid sequence or composition is known together with a molecular weight that includes all modifications. In fact, with the exception of glycosilation, many post-translational modifications represent only a small percentage of the total protein molecular mass and thus do not have a significant effect on amino acid analysis data. Fourteen amino acids were used in our calculation. However, an amino acid is eliminated from the calculation if it has undergone a chemical modification or is used as a stabilizer in the solution. In many cases, protein conjugate consists of a mixture of conjugates with a wide range of protein to protein ratios. The method described in this study is able to determine the average ratio of protein conjugate. Additional purification may be necessary if a more accurate ratio of a particular conjugate product is to be determined. Analyzing amino acids can take time and costly. A quick and easy way to assess the molar ratio of the protein in the conjugate can be achieved using spectrophotometry. According to beer-lambert law, A280nm = €lC; where € is the coefficient of molar absorption (M−1 cm-1), l is the length of the path (cm), and C is the protein concentration (M). Molar absorption coefficient € = A280nm/lC = A280nm/C when l is 1 cm. In routine practice, researchers may choose to use a weighted extinction coefficient. i.e. absorption (E) for 0,1 % solutions (= 1 g/l), E = A280nm/C (protein concentration in mg/ml) when using 1 cm thick cuvette. The concentration of protein (C) of the conjugate can be determined by a number of methods and therefore, E can be experimentally determined. If the composition of each monomer protein is known, the weighted extination factor at different protein-to-protein ratios can be obtained from ExPASY or directly from the Pace equation and a standard extination coefficient curve for the conjugate to the mole can be generated. The molar ratio of protein 1 to protein 2 can be calculated from the equation of the standard curve. As expected, the accuracy of this assessment is lower than that determined by the method developed in this study. In summary, a quick, simple and accurate method was developed to determine the molar ratio of proteins in protein-protein conjugates using data for the analysis of amino acids. This allows the calculation of the protein concentration using the calculated extination factors. This method should have common applications to determine the protein concentration in each peptide-peptide, peptide-protein-protein and protein-protein-protein conjugate.*This worksheet is designed to analyze as many residues as possible, the user select residues for use and/or delete residues that may result in low accuracy. Thank you to Dr. Myron Crawford, Kek. Kek. Resource Lab, Yale School of Medicine for analysis of amino acids. This work was supported by part of the Department of Intramur Research, NIAID, NIH. Install a Microsoft Excel solving tool as specified by the manufacturer. Target cell: target cell for calculating the sum of (Y-ˉl)2, as in Table 2, is $I $13Equat to: check min optionFrom changing cells as % table protein 1, as in Table 2, is $D $16C for Restrictions: leave blankChoose a method to solve: GRG NonlinearOnce configured, click the Solve button on the Date tab to run the Spreadsheet Solving program and message Solver find a solution. All limitations and optimal condition are met. Click on the Ok button, the molar ratio of protein 1 to protein 2 appears automatically. Publisher Disclaimer: This is a PDF file of a non-employed copy that has been accepted for publication. As a service to our customers, we provide this early version of the manuscript. The manuscript will be subject to copying, printing and review of the evidence received before it is published in its final citation request. Please note that errors that may affect the content can be detected during the production process, as well as any legal waivers that apply to the magazine. 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