ABSTRACT: Protein solubility is very important for protein crystallization, bioprocess development, and protein application. In this study, a method based on the stability of a protein dispersion system is proposed for fast assessment of protein solubility, which mainly involves ultrasonic dispersion, differential centrifugation, and spectral measurement (UDDCS) and curvature estimation. The appropriate ultrasonic time and centrifugal time were experimentally determined at first. The results show that the relationship between the standard deviation and the protein concentrations originally added accords with the modified exponential equation, and the corresponding concentration of the maximum curvature point is defined as the solubility of the protein. Lysozyme solubility data in NaCl aqueous solutions and zein solubility data in ethanol aqueous solutions are selected to verify the UDDCS method by comparing the data obtained by the UDDCS method and the results from references, and the results indicate that the UDDCS method is reliable, universal, and time-saving. Finally, measurements of zein solubility in NaOH solution and casein solubility in urea aqueous solution were conducted as test cases by the UDDCS method.

HIGHLIGHTS
- A method based on the stability of a protein dispersion system for a general evaluation of protein solubility is proposed.
- Ultrasonic dispersion and differential centrifugation technologies are utilized in the method.
- A modified exponential equation, its curvature equation, and the maximum curvature are selected to analyze the experimental data and determine the protein solubility.
- The method is verified by data from the literature and tested by case studies.

1. INTRODUCTION
Protein solubility, defined as the concentration of soluble protein, is very significant in protein crystallization, bioprocess development, and protein application. In protein crystallization, the bottleneck of the three-dimensional structural analysis of protein molecules is the crystallization of suitable high-quality crystals. Protein solubility is one of the most important parameters to study the crystallization process, and it is particularly important to determine the driving force for nucleation and growth processes. In bioprocess development, the knowledge of protein solubility under various conditions is one of the most essential data needed for a directed process development. The process steps in the biotechnological purification sector where knowledge of protein solubility is absolutely crucial comprise controlled protein precipitation, chromatography, aqueous two-phase systems (ATPS), and protein formulation. In protein applications, protein solubility is a major issue in many fields,
including high-throughput structural genomics, human diseases, and industrial applications.

When determining solubility data for the development of the processes mentioned above, one has to distinguish between the requirement of thermodynamically sound data and “rough” estimates on regions where spontaneous precipitation/aggregation might occur. Therefore, the methods for measuring protein solubility presented in the literature can be divided into those aiming at determining the thermodynamic solubility and the kinetically controlled precipitation. The thermodynamic solubility curve can be determined very precisely nowadays and is crucial for a scientific thermodynamic approach. Nevertheless, it is also characterized by a need for protein crystals before starting the experiment, sophisticated instrumentation, and relatively long experimental timelines. Although, for most processes, the biotechnology industry only requires data on spontaneous precipitation and kinetic effects in the time frame of hours to days, the protein solubility curve based on the kinetically controlled precipitation still lacks precise solubility determination. A considerable amount of methods describing ways to determine protein solubility can be found in the literature. The methods based on the thermodynamic solubility include equilibrium methods, microcolumn techniques, the scintillation method, several interferometry methods, the spin filter method, the artificial neural network method, and so on. Usually, establishing a solubility curve of protein is time-consuming mainly because the precipitation or the growth rate of protein crystals is much slower than that of small molecules.

This study focuses on a method based on ultrasonic dispersion, differential centrifugation, and spectral technology (namely, UDDCS), which aims at fast assessment of protein solubility with reasonable reliability. A modified exponential equation, its curvature equation, and the maximum curvature are selected to analyze the experimental data and determine the protein solubility. Previously published methods and their results are selected to verify the UDDCS method, and zein and casein are selected as the model proteins to test the UDDCS method.

2. FUNDAMENTAL OF THE UDDCS METHOD

The UDDCS method is proposed based on the principle of protein dissolution, which is introduced first as follows.

2.1. Base Principle of Protein Dissolution. The principle of protein dissolution is explained by the protein dissolution two-step theory and a protein/precipitant phase diagram.

According to the theory of dry protein dissolved in solvents of Van Oss, most dried proteins are not immediately soluble in solvents, but they do become solvent-soluble if they are capable of being strongly solvated. In other words, solubility of dry proteins is a two-step procedure, that is, step 1: solvation and step 2: solubilization. It must be assumed that in step 1, the tertiary configuration of the peptide chains, upon solvation, reasumes its native position in which the more solvophilic moieties become oriented outward. Once a sufficient degree of solvation is achieved, the solvated proteins become completely soluble in solvents (step 2), which was proved by Li et al. in that the primary reason could be explained as solvent protonation.

Based on the theories mentioned above, Figure 1 shows the protein/precipitant phase diagram, which indicates that the protein/precipitant phase diagram is divided into four zones by three curves with the concentration increase of protein

Figure 1. Schematic illustration of the concentrations for an arbitrary system via solvent evaporation or protein addition in a protein/precipitant phase diagram.

dispersions. The three curves are the solubility curve, the ML curve between the metastable and labile zone, and the LP curve between the labile and precipitation zone, respectively. The four zones are the undersaturated zone, metastable zone, labile zone, and precipitation zone, and the latter three zones belong to the supersaturated zone.

When solvent evaporation has occurred or more protein is added to an arbitrary protein dispersion system located in the undersaturated zone, as it is at point N in Figure 1, the protein concentration increases and the thermodynamic solubility curve is crossed (point B). In the undersaturated zone, according to the two-step theory, the protein dispersion system is completely solvated and dissolved where the system is stable. However, in the metastable zone, the solvation degree of protein molecules decreases and so does the stability of the dispersed system. Nevertheless, unless the solution is seeded by a crystal or crystallization is initiated by other means, no crystals will form in the metastable zone. With a continuously concentrating sample concentration, the labile zone is reached at point C. At point C, the system stability deteriorates dramatically from the metastable zone to the labile zone because concentrating the solution further means that spontaneous nucleation can occur. Thus, in the labile zone, the solvation degree of protein molecules further decreases, and so does the stability of the system. In the precipitation zone, the protein will form amorphous aggregates where the stability of the system is the worst. Meanwhile, the reports on the time needed to establish equilibrium in the supersaturated region suggest that the rate of solvent evaporation is too rapid to determine the “thermodynamic” solubility. Thus, it can be assumed that the phase transition curve measured represents the transition to a region where spontaneous precipitation or aggregation occurs.

In short, protein dissolution includes dispersion, solvation, and solubilization. The stability of an arbitrary protein dispersion system deteriorates along with increasing concentrations of the protein dispersion. At the ML curve containing point C, the rate of stability deterioration of the dispersion is the fastest because, in the metastable zone, no crystals form; however, in the labile zone, spontaneous nucleation occurs.

2.2. Proposal for the UDDCS Method. When developing the UDDCS method, we focus on the stability of the protein dispersion or solution, and the “solubility curve” obtained is the
ML curve in Figure 1. To obtain the “solubility curve”, three important problems should be taken into account: (1) how to make protein disperse and dissolve fast and completely, (2) how to evaluate the stability of the protein dispersion, and (3) based on analysis of the stability of the protein dispersion, how to evaluate the solubility of the protein.

To solve the three problems mentioned above, first, ultrasonic and stirring dispersion are utilized to obtain the protein dispersion system, which is desirably dispersed in a short time. When the protein is added to a solvent and dispersed using ultrasonic and stirring, it will be quickly dispersed into small particles, and a colloid dispersion system will soon be established. The inner solvophilic moieties of protein molecules will be outstretched and soon solvated. Thus, the system can be dispersed completely and approaches the dissolution equilibrium in a short time.

Second, the stability of a protein dispersion system can be evaluated by the variation of supernatant concentrations at different centrifugal speeds because the dispersed but not dissolved protein particles should be separated at a certain centrifugal speed. The greater the variation, the less stable the protein dispersion system is, and the extent of variation can be expressed by calculating the standard deviation ($s_i$) of supernatant concentrations at different centrifugal speeds. Thus, in the UDDCS method, differential centrifugation technology is utilized to evaluate the stability of the protein dispersion.

Third, according to the principle of protein dissolution mentioned above, the stability of the protein dispersion deteriorates along with the addition of the protein in a certain solvent. At the ML curve in Figure 1, the stability of the protein dispersion shows the fastest deterioration. Therefore, if the $s_i$ of a series of protein concentrations originally added ($c^{add}_i$) is calculated in terms of the second step, the relationship between $s_i$ and $c^{add}_i$ can be correlated, and the curvature equation of the fitted curve should have a maximum curvature value. The $c^{add}$ corresponding to the maximum curvature value, that is, the ML curve shown the in Figure 1, is defined as the “protein solubility” in the UDDCS method. Experimental results show that the relationship between $s_i$ and $c^{add}_i$ accords with the modified exponential equation.

3. MATERIALS AND METHODS

3.1. Materials. Zein was purchased from Sigma-Aldrich Shanghai Trading Co. Ltd. (food grade quality, China). Lysozyme (Ly) and casein (Cas) were purchased from the Shanghai Yuanye Bio-Technology Co., Ltd. (biological reagent, China). NaCl, NaOH, tetramethylsilane (TMS), ethanol (Et), dimethylsulfoxide (DMSO), and urea were purchased from the Guangdong Guanghua Sci-Tech Co., Ltd. (AR, China). Water used in the experiment was deionized water.

3.2. Experimental Procedure, Apparatus, and Data Analysis. The apparatus and experimental procedure of the UDDCS method is illustrated in Figure 2, which contains three procedures, that is, ultrasonic dispersion and stirring, differential centrifugation, and measurement by a UV—vis spectrometer. Procedure a of Figure 2 shows that, before measuring the protein solubility, pre-dispersion is required to be carried out for evaluating the approximate range of protein solubility. At room temperature, protein is gradually added to a certain solvent and ultrasonically dispersed by stirring thoroughly until it forms a protein dispersion with obvious precipitation. Therefore, the approximate protein solubility is obtained. As shown in procedure b of Figure 2, according to the pre-dispersion result, proteins with equal quality intervals ($m_i$) are added to a certain number of beakers ($i$) containing the same volume of solvent ($v$) and ultrasonically dispersed with stirring thoroughly until forming a protein dispersion with a certain $c^{add}_i$, where $c^{add}_i$ is calculated by eq S1. Procedures c and d of Figure 2 illustrate that each protein dispersion mentioned above is evenly decanted into several centrifugal tubes ($n$) and centrifuged at different speeds of $j_n$ (rpm). The centrifugal tubes with different $c^{add}_i$ values are centrifuged together at the same centrifugal speed. After centrifugation, the centrifugate supernatant ($c^{vis}_i$) is removed (procedure e of Figure 2), diluted (procedure f of Figure 2), and measured by use of a UV—vis spectrometer (procedure g of Figure 2). Triple tests were run in parallel. To directly indicate the relationship of the parameters mentioned above, the arrangement for data analysis of the UDDCS method is listed in Table 1.

3.2.1. Appropriate Ultrasonic Time. All samples were dispersed at a constant ultrasonic strength of 40 kHz and 200 W, and the ultrasonic time can be determined by testing the supernatant concentration of protein dispersions as a function of ultrasonic time. For example, at ambient temperature, eight

![Figure 2. Schematic overview of the experimental procedure: (a, b) ultrasonic dispersion and stirring, (c, d) differential centrifugation, and (e—g) measurement by UV—vis.](https://doi.org/10.1021/acsomega.2c03666)
protein dispersions were prepared, and for each dispersion, 1 g of zein weighed by an analytical balance (AL104, Mettler Toledo, Switzerland) was added to 50 mL of 8 g/L NaOH aqueous solutions and dispersed by stirring at a speed of 300 rpm by a stirrer (MS-M-S10, Dragon-Lab, China) for 10 min until without visible aggregations then treated by ultrasonic (KQ5200B, Kunshan Ultrasonic Instrument Co. Ltd., China) and diluted with solvent. Then, \( c^3 \) is tested by a UV−vis spectrometer.

As shown in Figure 2, procedures a and b are used to obtain the approximate protein solubility, and c and d are used to get the supernatant of the protein solution through differential centrifugation, and the concentration is measured by UV−vis (procedures e, f, and g). The \( s_i \) of the protein solution at different centrifugal speeds with same \( c^3 \) values is calculated, and when the protein is added, a series of \( s_i \) values can be obtained. Therefore, the equation of \( s_i \) as a function of \( c^3 \) can be fitted as an exponential equation, and the \( c^3 \) corresponding to the maximum curvature value of the equation is defined as the “protein solubility” in the UDDCS method.

### 3.2.4. Data Analysis.

Table 1 shows the arrangement for data analysis of the UDDCS method, \( c^3 \), \( j_i \) and \( c^3 \), are listed and the standard deviation of each row is calculated. \( s_i \) is calculated by eq S6, where \( \bar{c_i} \) is the mean value of \( c^3 \),

\[
\bar{c_i} = \frac{\sum_{n=1}^{n} c^3_i}{n}
\]

According to the principle of the UDDCS method mentioned above, the tendency of \( s_i \) from top to bottom should increase, that is, \( s_1 < s_2 < s_3 \ldots < s_8 < s_9 \ldots < s_5 \) which follows the modified exponential equation; therefore, eq 1 is adopted to correlate \( s_i \) and \( c^3 \),

\[
s_i = a \exp(c_i^3/b)
\]

where \( a \) and \( b \) are the amplitude and decay constants of the modified exponential function, respectively.

The maximum curvature point of eq 1 is calculated as follows. The curvature of equation of eq 1 can be expressed as eq S7,

\[
K = \frac{1}{s_i^2} \frac{d^2}{ds_i^2} \ln S_i
\]

where \( K \) is the curvature, \( s_i^2 \) and \( s_i^4 \) are the first and second derivatives of eq S7, respectively. Since eq S7 has a maximum curvature point, there is a numeric to make the first derivative of eq S8 equal to zero. The first derivative of eq S8 is obtained from eq S9. If \( K = 0 \), eq S10 is obtained.

Therefore, the corresponding concentration at the maximum curvature point, where the rate of stability deterioration is fastest, can be obtained by eq 2 and determined as the solubility of the protein (S).

\[
S = \frac{b}{\sqrt{2a}}
\]

### 3.3. Verification and Test Studies.

#### 3.3.1. Verification.

Ly solubility in NaCl aqueous solutions were measured by Berg et al.\(^{33}\) in which a high-throughput method was utilized and by Forsythe et al.\(^{30}\) where a microcolumn technology was used. The zein solubility in Et aqueous solutions was measured by Evans et al.\(^{31}\) based on measuring the zein “critical precipitation temperature”. The UDDCS method was verified by comparing the solubility data of Ly and zein measured by the UDDCS method at 18 °C and pH 6.5. Measurement of each \( c_i^3 \) was repeated in triplicate.

The zein solubility in Et aqueous solutions with different Et: H\(_2\)O (w/w) ratios, namely, 11.4:1, 10.76:1, 10.1:1, 9.46:1, 8.8:1, 8.15:1, and 7.5:1, was measured using the UDDCS method at 20 °C, where the mass of H\(_2\)O was 3 g for each experiment.

#### 3.3.2. Test Studies.

For further illustrating the feasibility of the UDDCS method, the solubility of zein in NaOH aqueous solutions and Cas in urea aqueous solutions was evaluated by the
UDDCS method. Measurement of each $i j n$ was repeated in triplicate.

To determine the zein solubility in NaOH aqueous solutions at 20 °C in 50 mL of NaOH aqueous solution, experiments were carried out and $c_{NaOH}$ was set as 2, 4, 6, 8, 10, and 12 g/L. $^1$H NMR spectra were recorded on a Bruker spectrometer (Bruker AV600, Bruker Co., Switzerland) to investigate the effect of $c_{NaOH}$ on the zein solubility too where zein was dissolved in deuterated DMSO, and all chemical shifts were reported in the parts per million downfield from TMS.

For the effect of temperature on the zein solubility in NaOH aqueous solutions, experiments were carried out using 50 mL of 6 g/L NaOH aqueous solution at 10, 20, 30, 40, 50, and 60 °C.

While measuring the Cas solubility in urea solution, 0, 2, 4, 6, 8, and 10 g of urea were added to 50 mL of $H_2O$ for increasing the solubility, 0.1 g of NaOH was also added to each solution.

4. RESULTS AND DISCUSSION

4.1. Determination of Ultrasonic Time, Centrifugal Time, and Protein Standard Curves. Figure S1 shows the relationship between the supernatant concentration of the protein dispersion and ultrasonic time, and the error bars represent the standard deviation. Figure S1 indicates that the supernatant concentration of protein dispersion reached a balance after ultrasonics for 40 s. Thus, 40 s of ultrasonic time is selected as the feasible ultrasonic time in this study.

Figure S2 indicates that, despite the protein concentration, the supernatant concentration become stable when the centrifugal time is approximately 10 min, and if the centrifugal speed increases, the centrifugal time will shorten. Thus 10 min is sufficient and selected as the proper centrifugal time.

In this study, Ly, zein, and Cas were selected as the model proteins, and the results of standard curve equations of Ly in NaCl aqueous solution, zein in Et/H$_2$O and NaOH aqueous solution, and Cas in urea aqueous solution are shown in eqs S2−S5, respectively.

4.2. Verification of the UDDCS Method. 4.2.1. Ly Solubility in NaCl Aqueous Solutions. Figure 3 demonstrates that $s_i$ rises with the increase of $c_{Ly}$, and all the fitted curves correlated with eq 1 accord well with the exponential growth. The fitting parameters of $a$, $b$, and $R^2$ of the fitting equations in Figure 3 are listed in Table S1, and the value of $R^2$ suggests that eq 1 can be successfully used to correlate $s_i$ and $c_{Ly}$. The fitting parameters of equations in Table S1 are substituted into eq 2, and the Ly solubility data in different $c_{NaCl}$ values are obtained and shown in Figure 4. It indicates that $S_{Ly}$ decreases along with the increase of $c_{NaCl}$ and reaches a low limit. Compared with Ly solubility data measured by Berg et al.$^{43}$ and Forsythe et al.$^{50}$ under the same conditions, Figure 4 indicates that the solubility curve measured by the UDDCS method is located in the middle of the other two curves.

The differences among the three solubility curves of Ly under the same conditions can be explained by the principles of the three methods. The principle of measuring the Ly solubility by a high-throughput method in Berg et al.’s work$^{43}$ is to observe whether the precipitation occurs. The principle of microcolumn
technology in Forsythe et al.’s work\textsuperscript{50} is to make the protein solution reach the thermodynamic equilibrium as far as possible.

According to the principle of the UDDCS method, the protein solubility curve is measured based on the stability of protein dispersions. Therefore, the Ly solubility curve of Berg et al.\textsuperscript{43} in Figure 4 basically is the LP curve containing point D in Figure 1, the solubility curve of Forsythe et al.\textsuperscript{50} basically is the solubility curve containing point B in Figure 1, and the “solubility curve” measured by the UDDCS method is basically the ML curve containing point C in Figure 1. The result completely accords with the original design of the UDDCS method.

4.2.2. Zein Solubility in Et Aqueous Solution. Figure 5 shows the relationship between $s_i$ and $c_{\text{zein}}^{\text{add}}$ in different Et:H$_2$O (w/w) solutions at 20 °C, and the fitting parameters of $a$, $b$, and $R^2$ of the fitting equations are listed in Table S2, which indicates that eq 1 can be successfully used to correlate $s_i$ and $c_{\text{zein}}^{\text{add}}$.

The fitting parameters are substituted into eq 2, and zein solubility data in different Et:H$_2$O (w/w) solutions are obtained.

\begin{align*}
\text{Figure 4}. \text{Comparison of Ly solubility data measured by different methods.} \\
\text{Figure 5}. \text{Relationship between } s_i \text{ and } c_{\text{zein}}^{\text{add}} \text{ in different Et:H}_2\text{O (w/w) solutions at 20 °C. (A) 11.4:1, (B) 10.76:1, (C) 10.1:1, (D) 9.46:1, (E) 8.8:1, (F) 8.15:1, and (G) 7.5:1.}
\end{align*}
and shown in Figure 6. It shows that $S_{\text{zein}}$ decreases along with the increase of Et:H$_2$O ratios (w/w). Figure 6 also demonstrates the comparison of zein solubility data measured by Evans et al.$^{51}$ and the UDDCS method under the same conditions; it indicates that, at low $S_{\text{zein}}$, the results of the two methods are consistent, but when $S_{\text{zein}}$ increases, the data measured by the UDDCS method is smaller than that of Evans.$^{51}$

The differences between the two solubility curves of zein under the same conditions can be explained by the principles of protein dissolution and the two methods. The principle of measuring the zein solubility in Evans et al.'s work$^{51}$ is to measure its “critical peptization temperature”, that is, the temperature above which zein is soluble in all proportions and below which it is soluble only to the extent of 2% or 3%. At a low $S_{\text{zein}}$, if sufficient zein is added to a certain solvent system, except the dissolved zein, the undissolved part will precipitate directly rather than suspend. The solubility data measured by the two methods is the mass of the dissolved zein, and thus the solubility data are consistent. At a high $S_{\text{zein}}$, when sufficient zein is added to a certain solvent system, except the dissolved zein, the suspension part of zein will increase. If the zein solubility is established by measuring its “critical peptization temperature”, the small crystals or aggregations in the suspension will exist steadily in the protein dispersion. However, the small crystals or aggregations in the suspension will be separated when using the UDDCS method. Therefore, at a high $S_{\text{zein}}$, the solubility data measured by the UDDCS method is smaller than that of Evans et al.$^{51}$

Thus, the results of verification suggest that the UDDCS method is by no means able or meant to determine the thermodynamic solubility of a protein but determines the process-relevant stability data where the time frame allows when the metastable zone or the labile zone is reached.$^{48}$ According to the principle of protein dissolution shown in Figure 1, the protein solubility data measured by the UDDCS method would be sufficient for many applications.

In brief, it is suggested that the UDDCS method has three advantages, that is, it is reliable, universal, and time-saving. The reliable property of the UDDCS method has been verified by different methods mentioned above. The UDDCS method is a universal method based on the principle of protein dissolution because it is designed for statistics and analysis of a number of experimental results by using the constructed mathematics model. Meanwhile, the UDDCS method is a dynamic method in essence, and it does not need preparation of protein crystals and have protein solutions reach the thermodynamic equilibrium. The time consumed in each part is approximately 30 min for dispersion, 20 min for differential centrifugation, and 60 min for UV−vis measurement. The total time needed in the UDDCS method is approximately 110 min to obtain a solubility point, and thus it can evaluate the solubility curve of a certain protein within 12 h. Therefore, it is time-saving compared with other thermodynamic and dynamic methods, which need several months or weeks to obtain a solubility curve.

4.3. Results of Test Studies. 4.3.1. Effect of $c_{\text{NaOH}}$ on Zein Solubility. Fitted curves of $S_2$ as a function of $c_{\text{zein}}$ in different $c_{\text{NaOH}}$ values at 30 °C are shown in Figure S3, and fitting parameters of $a$, $b$, and $R^2$ of the fitting equations are listed in Table S3. The fitting parameters are substituted into eq 2, and zein solubility data are obtained, as shown in Figure 7. It can be seen that $S_{\text{zein}}$ first increases rapidly with the increase of $c_{\text{NaOH}}$ and reaches an upper limit. The solubility data can be well correlated as $S_{\text{zein}} = -65.35 \exp (c_{\text{NaOH}} / 2.1) + 62.3$.

The primary reason for the effect of $c_{\text{NaOH}}$ on zein solubility in Figure 7 is analyzed as follows. Figure S4 shows the comparison between H$^2$ NMR spectra of zein/DMSO solution and zein/DMSO/NaOD solution. As shown in Figure S4, a large proportion of zein hydrogen bonds in DMSO disappears after adding NaOD, which demonstrates that the chemical reaction occurs. At a low $c_{\text{NaOH}}$ (<0.15 mol/L), the structure of zein molecules is not damaged seriously, and NaOD only causes the rearrangement of hydrogen bonds, that is, reacts with −COOH in zein molecules, which increases the interaction between protein and solvents. However, at a high $c_{\text{NaOH}}$ (>0.15 mol/L), the second and third condensation of zein molecules will be damaged, which causes structural looseness of zein molecules, and inner hydrophobic groups will be exposed.$^{52}$ Therefore, the zein solubility increases with the increase of $c_{\text{NaOH}}$ and reaches an upper limit.

4.3.2. Effect of Temperature on Zein Solubility. Fitted curves at different temperature and fitting parameters are shown in Figure S5 and Table S4, the fitting parameters are substituted into eq 2 and zein solubility data at different temperature is obtained, as shown in Figure 8. It can be seen that $S_{\text{zein}}$ increased first, then decreases with the increasing of temperature. The solubility data are correlated as $S_{\text{zein}} = 24.54 + 4090 \times \frac{4.26}{x^2 - 29.68^2 + 14.26^2}$, $R^2 = 0.9051$, which follows the Lorentz equation.

At a constant pH and ionic strength, the reason for the zein solubility curve first increasing and then decreasing along with the increase of temperature is analyzed as follows. The activity of protein molecules increases along with the increase of temperature from 0 to 40 °C, which makes the protein solubility
In summary, the developed UDDCS method, which is based on the principle of protein dissolution, includes the following steps: (1) the protein is thoroughly dispersed into a certain solvent using ultrasonics and stirring and then quickly reaches the dissolution equilibrium; (2) the stability of the dispersion system, is determined as the solubility of protein; (3) the concentration of the centrifugate at different centrifugal speeds is tested using a UV technique; (4) the stability of the dispersion using ultrasonics and stirring and then quickly reaches the dissolution equilibrium; (2) the stability of the dispersion system, is determined as the solubility of protein.

4.3.3. Effect of Urea on Cas Solubility. Fitted curves at different urea concentrations and fitting parameters are shown in Figure S6 and Table S5, the fitting parameters are substituted into eq 2, and Cas solubility data is obtained, as shown in Figure 9. This demonstrates that $S_{\text{Cas}}(c_{\text{urea}})$ decreases with the increase of $c_{\text{urea}}$ and the possible reason is that urea decreases the hydrolyzing degree of Cas in NaOH aqueous solutions. The solubility data follows the exponential equation, which is correlated as $S_{\text{Cas}} = 268.33 \exp (-c/2.76) + 24.01$, $R^2 = 0.9915$.

5. CONCLUSIONS

In summary, the developed UDDCS method, which is based on the principle of protein dissolution, includes the following steps: (1) the protein is thoroughly dispersed into a certain solvent using ultrasonics and stirring and then quickly reaches the dissolution equilibrium; (2) the stability of the dispersion system is evaluated using the differential centrifugation technique; (3) the concentration of the centrifugate at different centrifugal speeds is tested using a UV–vis spectrometer; (4) the $S_i$ of the centrifugate concentration is calculated; (5) when protein dispersion systems with different added concentrations are treated by the steps mentioned above, a modified exponential equation is selected to correlate the relationship between $S_i$ and $c_{\text{add}}^i$; and (6) the curvature equation of the modified exponential equation is calculated, and the corresponding concentration of the maximum curvature value, which means the fastest deterioration of the stability for a protein dispersion system, is determined as the solubility of protein.

The verification results of Ly solubility in NaCl aqueous solutions indicate that the “solubility curve” measured by the UDDCS method is basically the ML curve, which completely accords with the original design of the UDDCS method. The verification results of zein solubility in Et aqueous solution also confirm the reliability of the UDDCS method.

The UDDCS method is successfully applied to evaluate zein solubility in NaOH solution and Cas solubility in urea aqueous solutions. The total time needed in the UDDCS method is approximately 110 min to obtain a solubility point, and it can evaluate the solubility curve of a certain protein within 12 h. Thus, the UDDCS method is suitable for fast and reliable assessment of protein solubility.

■ ASSOCIATED CONTENT

 Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03666.

Equations of protein added concentrations, standard curve equations of proteins in solvents, standard deviation, curvature equation, and first derivative of the curvature equation, $c_{\text{add}}$, eqs S2, S3, S4, and S5, $s_i$, $K$, and $K'_i$; effects of ultrasonic and centrifugal time on protein dispersion, $s_i$ as a function of $c_{\text{urea}}$ and $c_{\text{add}}^i$, H$^1$ NMR spectra of zein in deuterated DMSO solution and deuterated DMSO/NaOD solution; and fitting parameters of $a$, $b$, and $R^2$ in Figures 3 and 5 and Figures S3, S5, and S6 (PDF)

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Notes

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ABBREVIATION

\( a, b = \) fitting parameters of equations (−)
\( A = \) absorbance (1)
\( c_{\text{Cas}} = \) concentration of Cas (g/L)
\( c_{\text{add}} = \) protein concentration originally added (g/L)
\( c_p = \) protein concentration after differential centrifugation (g/L)
\( c_{\text{av}} = \) average value of the protein concentration after differential centrifugation (g/L)
\( c_{\text{ky}} = \) concentration of Ly originally added (g/L)
\( c_{\text{NaCl}} = \) concentration of NaCl (g/L)
\( c_{\text{NaOH}} = \) concentration of NaOH (g/L)
\( c_{\text{protein}} = \) concentration of the protein (g/L)
\( c_{\text{zein}} = \) concentration of zein originally added (g/L)
\( i = \) number of protein samples (−)
\( j_0 = \) centrifugal speeds (rpm)
\( K = \) curvature (−)
\( K' = \) first derivative (−)
\( m_i = \) mass of protein added (g)
\( n = \) number of centrifugal tubes (−)
\( \text{rpm} = \) revolutions per minute
\( R^2 = \) correlation index (−)
\( s_i = \) standard deviation values (−)
\( s'_i = \) first derivative of \( s_i \) (−)
\( s''_i = \) second derivative of \( s_i \) (−)
\( S = \) protein solubility (g/L)
\( S_{\text{Cas}} = \) Cas solubility (g/L)
\( S_{\text{Ly}} = \) Ly solubility (g/L)
\( S_{\text{zein}} = \) zein solubility (g/L)
\( t_c = \) centrifugal time (min)
\( t_u = \) ultrasonic time (s)
\( T = \) temperature (°C)
\( \lambda = \) characteristic absorbance peak (nm)

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