Determination of Phenytoin Sodium Injection and Tablets by Highly Accurate Nephelometric Titration

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A highly accurate nephelometric titration method was developed for the quantitative analysis of phenytoin sodium injection and tablets. The titration operating conditions and the validation of the method were studied. Five batches of phenytoin sodium injection and tablets were determined by the proposed method and the control experiment methods, respectively. The results of the titration are comparable to those of control experiments. The proposed method is accurate and reproducible, which is considered suitable for the quantitative analysis of a large number of samples.

Key words highly accurate nephelometric titration; phenytoin sodium; colloid

Titrimetry is often applied in analytical chemistry for its superior speed and simplicity with little sacrifice in accuracy and precision. Phenytoin sodium (DPH) forms phenytoin silver collosol quantitatively with silver nitrate solution. The reaction between phenytoin sodium and silver nitrate reveals a 1 : 1 molar ratio[1] as illustrated in Chart 1.

However, there was no accurate method available to determine the titration end-point. Therefore, gravimetric,[2] potentiometric titration,[3] high-performance liquid chromatography (HPLC),[4] and other complicated methods[5—8] are often used. The instrumental method is generally not as accurate and precise as the titrimetry in macro analysis. Besides, gravimetric is now rarely used in the laboratory for its inconvenience.

Our previous study revealed a possibility of using highly accurate nephelometric titrimetry to determine phenytoin sodium.[9] Now, as a continuous study, this accurate, rapid and simple titration method was developed for the quantitation of phenytoin sodium injection and tablets. An internal nephelometric sensor was immersed in the titrate to monitor the change in the intensity of the scattered light during the titration. After adding the first drop of the silver nitrate solution, the phenytoin sodium solution became cloudy, and, as the titration continued, the scattered light detected increased gradually. As more titrant was added after passing the point, the solution would not become cloudier and the scattered light would not increase; on the contrary, the scattered light would decrease in intensity because of the dilution of the solution. A peak would appear at the stoichiometric point in the profile of relative intensity of scattered light *versus* the volume of titrant and therefore the end-point of the titration could be determined accurately.

The titration operating conditions were optimized in the present study. The proposed method has been validated and found to be precise and accurate. The results acquired by highly accurate nephelometric titrimetry are comparable to those by the control experiments under the operating conditions.

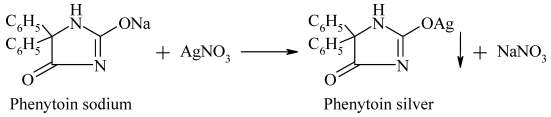

Chart 1. The Reaction between Phenytoin Sodium and Silver Nitrate

Experimental

Instrumentations The nephelometric sensor and the assembly of the nephelometric titrator were self-made[9] and are shown in Fig. 1. A pH meter with a glass indicating electrode and a saturated calomel reference electrode system was used for the potentiometric titration (pHS-3A; Chengdu Instruments Factory, China). An HPLC system (Shimadzu, Kyoto, Japan) comprising a UV detector was used for HPLC analysis.

Regents and Chemicals Phenytoin sodium injections (local supplier I, Jiangsu, China), phenytoin sodium tablets (local supplier II, Jiangsu, China), phenytoin sodium standard substance (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China), and silver nitrate (standardized according to The Pharmacopoeia of the People's Republic of China) were used. Methanol and deionized water used in HPLC analysis were HPLC grade (Dikma, U.S.A.). The other reagents were all analytical grade.

Optimizing Colloid Stabilizers and Titration Concentration The scattered light detected is unstable for phyentoin silver colloid coagulating during the titration, thus a colloid stabilizer is added. To assess the protective capability of colloid stabilizers, different amounts of starch, dextrin and sodium carboxymethylcellulose (CMC-Na) solutions were added in each 25 ml of 0.01 mol/l phytoin sodium standard solution, and then 25 ml of 0.01 mol/l silver nitrate solution was added slowly, respectively.

It was reported[9] that the titration error depends on the sharpness of the peak in the titration curve and the sharpness is related to the concentration of the solutions. To study the influence of the concentration on the sharpness of the peak and the titration error, phyentoin sodium standard solutions with six different concentrations (0.1, 0.05, 0.025, 0.01, 0.005, 0.001 mol/l) were

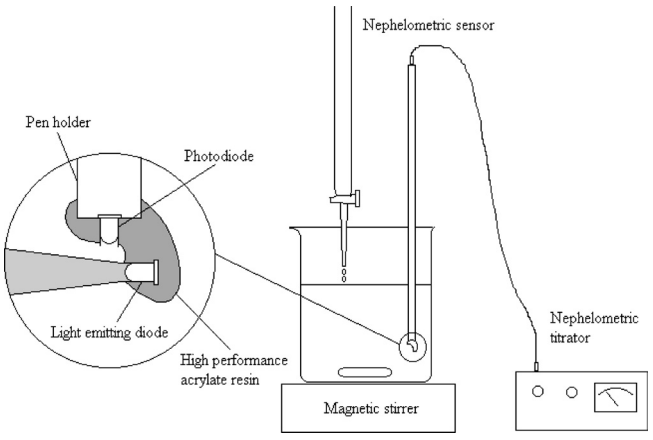

Fig. 1. The Nephelometric Sensor and the Assembly of the Nephelometric Titrator

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Table 1. Capability of the Colloid Stabilizers to Protect Phenytoin Silver Colloid

Concentration (%)	Volume of starch solution (ml)				Volume of dextrin solution (ml)				Volume of CMC-Na solution (ml)			
	1	2	5	10	1	2	5	10	1	2	5	10
1	+	+	+	+	+	+	−	−	+	+	+	+
2	+	+	+	+	+	−	−	−	+	+	+	+
5	+	+	+	+	−	−	−	−	+	+	+	+

+, colloid coagulated; −, colloid uncoagulated.

prepared and titrated with 0.01 mol/l silver nitrate solutions, respectively. The content of phyentoin sodium standard substance was determined for each sample.

Validation of the Proposed Method and Determination of Phenytoin Sodium Injection and Tablets The assay was developed for the quantitative analysis of phenytoin sodium injection and tablets. Repeatability was evaluated in the intra- and inter-day assays. Recovery of phenytoin sodium was determined by self-made preparations at different concentration levels using a mixture of standards with 50, 100 and 200% of the quantified levels of phenytoin sodium in the preparations. To study the specificity of the proposed method, intentional degradation was achieved by exposing phenytoin sodium preparations to stress conditions of heat (60 °C) in order to test the ability of the proposed method to separate phenytoin sodium from the degradation products. The residual phenytoin sodium concentrations were approximately 75% after degradation. After the degradation treatments were completed, the degraded samples were determined by both highly accurate nephelometric titration and HPLC.[4)]

Five batches of phenytoin sodium injection and tablets were determined by the proposed method and HPLC, respectively. Blank experiment was employed to eliminate systematic errors. Statistical analysis of the determined values using the Student's *t*-test[10)] at 95% confidence interval was performed.

Results and Discussion

Optimizing Colloid Stabilizers and Titration Concentration Table 1 shows the coagulation of the phenytoin silver colloid. It is seen that 2 ml of 2% or more dextrin solution protects the colloid effectively, therefore the dextrin solution was used as the colloid stabilizer in the experiment.

To study the influence of the amount of dextrin on the determined results, 2, 5, and 10 ml of 2% dextrin solution was added in each 25 ml of 0.01 mol/l phenytoin sodium solution, respectively. The determined content of phenytoin sodium was 98.64±0.15% (mean±RSD, n=6), 98.82±0.10% (mean±RSD, n=6) and 98.75±0.13% (mean±RSD, n=6), respectively. It is observed that the amount of dextrin solution affects the precision insignificantly. Therefore, an amount of 5 ml of 2% dextrin solution was used in the proposed procedure.

Phyentoin sodium solutions with six different concentrations (0.1, 0.05, 0.025, 0.01, 0.005, 0.001 mol/l) were titrated with 0.01 mol/l silver nitrate solutions, respectively. Colloid coagulation happened, as the concentration of phyentoin sodium is higher than 0.05 mol/l. For other four different concentrations (0.025, 0.01, 0.005, 0.001 mol/l), the determined contents were 97.54±0.17% (mean±RSD, n=6), 98.82±0.10% (mean±RSD, n=6), 98.51±0.18% (mean±RSD, n=6) and 98.38±0.47% (mean±RSD, n=6), respectively. It was seen that the optimum titration concentration leading to a minimum error is 0.01 mol/l.

The Proposed Procedure Transfer accurately measured phenytoin sodium injection or powered phenytoin sodium tablets, equivalent to about 300 mg phenytoin sodium, to a 100-ml volumetric flask. Dilute with water to volume, filter the mixture through a syringe filter (0.45 μm). Transfer 25.0 ml of the resulting solution to a 100-ml beaker, add 5 ml of 2% dextrin solution, place the beaker on a magnetic stirrer, immerse the nephelometric sensor in the beaker, and titrate with 0.01 mol/l silver nitrate solution. Record both the volume of the titrant and the relative intensity of the scattered light. Determine the end-point by the peak in the profile of relative intensity of scattered light versus the volume of titrant. Each milliliter of 0.01 mol/l silver nitrate is equivalent to 2.7425 mg $C_{15}H_{11}N_2NaO_2$.

Validation of the Proposed Method and Determination of Phenytoin Sodium Injection and Tablets A validation of the proposed method was conducted, and the results showed that the RSD values were within the range of 0.11—0.14% for intraday assays and 0.14—0.23% (n=3) for interday assays. The average recovery rates were 98.87±1.72% (mean±RSD, n=3) and 98.15±2.23% (mean±RSD, n=3) for phenytoin sodium injection and tablets, respectively. For the proposed highly accurate nephelometric titration, good precision and accuracy were obtained.

For specificity study of the proposed method, the determined content of degraded phenytoin sodium injection were 71.37±0.12% (mean±RSD, n=6) and 71.28±0.27% (mean±RSD, n=6) by highly accurate nephelometric titration and HPLC,[4)] respectively. The determined content of degraded phenytoin sodium tablets were 76.63±0.15% (mean±RSD, n=6) and 76.48±0.32% (mean±RSD, n=6) by highly accurate nephelometric titration and HPLC,[4)] respectively. Statistical analysis of the determined values using the Student's *t*-test,[10)] was performed. The t value at 95% confidence interval is less than the Student's t critical ($t_{0.05,10}$=2.228), which indicates that there is no significant difference in the determined values between highly accurate nephelometric titration and HPLC, and the proposed method was not interfered from the degradation products.

Five batches of phenytoin sodium injection and tablets were determined by the present method, HPLC and potentiometric titration, respectively. The representative titration curves are illustrated in Fig. 2, and the determined values are listed in Table 2.

The results determined by the proposed method and by the control experiments are analyzed using the Student's *t*-test[10)] at 95% confidence interval, and no significant difference was found between them.

Conclusion

An accurate, rapid and simple method, highly accurate nephelometric titration has been described and validated for quantitative determination of phenytoin sodium injection and

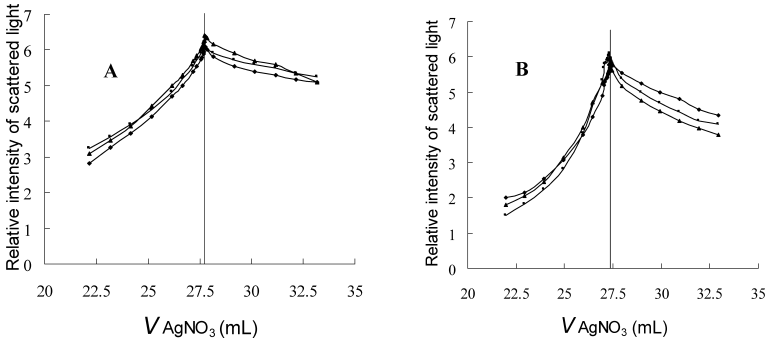

Fig. 2. Typical Titration Curves of Phenytoin Sodium Injection (A) and Tablets (B) with 0.01 mol/l $AgNO_3$ Solution

Table 2. The Results Determined by Highly Accurate Nephelometric Titration, HPLC, and Potentiometric Titration

Sampler number	Percent of labeled claim of phenytoin sodium injection (%)			*t*-test values	
	Highly accurate nephelometric titration	HPLC	Potentiometric titration		
1	101.48±0.10[a)]	101.33±0.19	101.51±0.09	1.688	0.538
2	99.70±0.11	99.53±0.22	99.76±0.10	1.700	0.991
3	100.25±0.07	100.11±0.20	100.31±0.11	1.616	1.124
4	99.75±0.07	99.61±0.21	99.79±0.09	1.555	0.861
5	100.43±0.10	100.27±0.20	100.48±0.12	1.747	0.780
1	101.52±0.09	101.34±0.21	101.59±0.11	1.904	1.188
2	102.63±0.12	102.44±0.19	102.72±0.13	2.021	1.214
3	99.12±0.11	98.93±0.21	99.21±0.13	1.984	1.305
4	98.24±0.14	98.07±0.18	98.36±0.14	1.861	1.510
5	102.47±0.13	102.26±0.22	102.6±0.13	1.967	1.689

a) Mean±RSD of six measurements.

tablets. Acceptable assay precision and accuracy were obtained at the analytical concentration. In addition, the results of specificity indicated that there was no interference from the degradation products.

The overall procedure is accurate and reproducible which is considered suitable for quantitative analysis of a large number of samples.

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