Research Article

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Received: 7 January 2008, Revised: 18 April 2008, Accepted: 6 May 2008, Published online 9 September 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI 10.1002/bio.1068

Determination of propafenone hydrochloride by flow-injection analysis coupled with resonance light scattering detection

Xiaoli Hu*, Dongpo Xu, Shaopu Liu, Zhongfang Liu and Sa Liu

ABSTRACT: A simple, sensitive and rapid flow injection analysis (FIA) method with resonance light scattering (RLS) was described for the determination of propafenone (PPF). The method was based on the ion-association reaction of 12-tungstophosphoric acid (TP) with propafenone. In pH 1.0 acidic medium, TP reacted with PPF to form an ion-associate complex, which resulted in a significant enhancement of RLS intensity. The maximum scattering peak was located at 340 nm, the RLS intensity was proportional to the concentration of PPF in the range 0.003–9.0 μg/mL, and the detection limit (3σ) of 1.0 ng/mL was obtained at a sampling rate of 60 samples/h. The feasible reaction conditions and FIA parameters for the system were optimized. The method proposed in this paper shows satisfactory reproducibility with a relative standard deviation (RSD) of 2.1% for 10 successive determinations of 2.0 μg/mL PPF. The present method had been successfully applied to the determination of PPF in serum samples and pharmaceutical samples. The results obtained were in agreement with the method used in the *Chinese Pharmacopoeia*.

Keywords: resonance light scattering; 12-tungstophosphoric acid; propafenone hydrochloride; flow injection analysis; determination

Introduction

Propafenone hydrochloride (PPF) is a class Ic antiarrhythmic agent with slight β-adrenergic-antagonist properties, which is effective in the treatment of supraventricular and ventricular arrhythmias (1). It is able to depress intracardiac conduction velocity as a consequence of its binding to the open state of cardiac Na channels (2). PPF is administered as racemate and the enantiomers have different pharmacological activities and kinetics of disposition. In the interest of avoiding toxicity and using PPF scientifically and securely, it is very important to develop an accurate and quick determination technique for PPF serum concentration, which can help clinical doctors gain more information and choose the best curative programme that most benefits patients. Nowadays, several methods are available for the determination of PPF and its metabolites in human plasma. Most authors have used liquid–liquid extraction (LLE) as the sample clean-up step (3–7). A simple solid-phase extraction (SPE) as the clean-up procedure was published for the determination of PPF derivatives in liver microsomes (8) and plasma and urine samples (9). The stabilities of PPF intravenous solutions were tested by Dupuis *et al.* (10).

Automatic and user-friendly analytical methods are of great interest. Flow-injection analysis (FIA) offers distinct advantages over manual procedures, owing to its speed, simplicity, enhanced reproducibility and versatility. Also, the flow-injection system allows conjunction with a variety of detection systems, such as spectrophotometry (11), ISE (12), ICP–MS (13) and so on. Resonance light scattering (RLS) is a relatively new analytical technology that has received much attention because of its sensitivity and simplicity. Up to now, there are reports describing the hyphenated technique (FIA–RLS) (14–22). The aim of present work was to develop a simple, rapid, selective, sensitive and reproducible FIA–RLS method for the determination of PPF and pharmaceutical samples by TP.

In this study, we found that in an acid medium TP or PPF produced very weak RLS signals. However, when the two agents reacted with each other electrostatically to form ion-association complexes, the RLS intensity could be greatly enhanced. Due to this characteristic of the system, a new analytical procedure is proposed for the determination of PPF by flow injection analysis. The RLS spectral characteristics, optimum reaction conditions and analytical properties have been studied. The method has high sensitivity and good selectivity and has been applied to the determination of real samples. In addition, the influencing factors and the reasons for RLS enhancement are discussed. To our knowledge, this is the first method for the analysis of PPF based on RLS detection.

Experimental

Reagents

All reagents used were of analytical reagent grade and solutions were prepared with deionized distilled water. The stock concentration of propafenone hydrochloride (Guangzhou Mingxing Pharmaceutical Co. Ltd, China) was 10.0 μg/mL and a series of working concentrations were prepared by diluting the stock solution to an appropriate volume with distilled water.

* Correspondence to: X. Hu, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, People's Republic of China.
E-mail: xiaolihu@swu.edu.cn

School of Chemistry and Chemical Engineering, Southwest University, Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, Chongqing 400 715, People's Republic of China

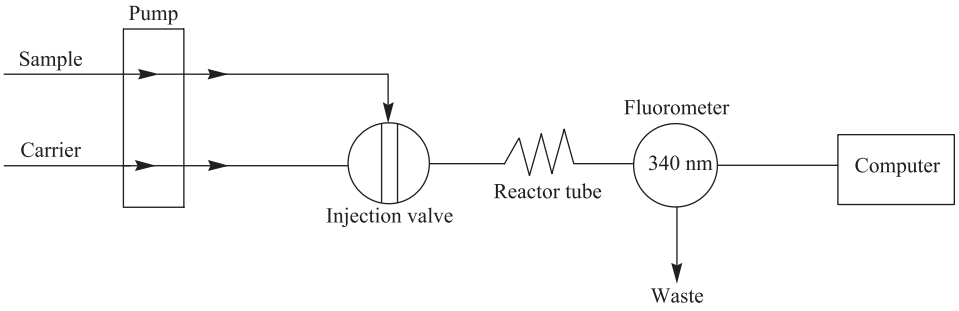

Figure 1. Schematic diagram of FIA system for PPF determination with RLS detector. Reactor length, 60 cm; carrier flow rate, 2.6 mL/min; injection volume, 86 μL.

12-Tungstophosphoric acid was synthesized as previously described (23). As it exists stably in pH 0–1.5 acid conditions (24), it was dissolved in 1.0 mol/L HCl as stock solution for use, and its concentration was 1.0×10^{-3} mol/L. The stock solution was diluted 10 times with water to 1.0×10^{-4} mol/L as the working solution.

Propafenone hydrochloride tablets (Jiangsu Yunyang Pharmaceutical Co. Ltd, China) were prepared for the quantity determination by the method described in this paper.

Apparatus

A Hitachi F-2500 spectrofluoremeter with a 90 μL quartz flow cell (Tokyo, Japan) was used throughout as the detector for recording RLS spectra by scanning synchronously with the same excitation and emission wavelengths and measuring RLS intensity by time scan pattern. The determination parameters were the slit (Ex/Em) of 5.0/5.0 nm and PMT voltage 400 V.

Figure 1 indicates a scheme of the FIA apparatus for the determination of PPF. The flow system used consisted of a peristaltic pump (Shanghai Electrical Machine Factory, Shanghai, China) and an eight-way rotary valve with exchangeable sample loop Polytetrafluoroethylene (PTFE) tubing, 1.0 mm i.d., was used to connect all components in the flow system. The method was tested with model solutions before its application to real samples.

Procedure

12-Tungstophosphoric acid solution of 1.0×10^{-4} mol/L as carrier solution was continuously pumped through the system until a stable baseline was obtained. For the analysis of PPF, appropriately diluted solutions were directly prepared in deionized water and injected into the carrier stream in the FIA manifold by an eight-way injection valve. The RLS intensity (I) was detected at $\lambda_{ex} = \lambda_{em}$. $\Delta I = I - I_0$, where I and I_0 are the RLS intensities of the carrier in the presence and absence of PPF, respectively. Typically, three repeated injections of standards and sample were made and the results are reported as the mean value. In the present study, the major flow-through parameters were 60 cm, 2.6 mL/min and 86 μL for the length of mixing tubing, flow rate of carrier stream and injection loop volume, respectively. The sample throughput was 60 samples/h.

Results and discussion

RLS spectra

Figure 2 shows the RLS spectra of TP, PPF and TP-PPF, which were obtained by stopping the sample segment at a FI peak in flow cell in detector. It can be seen that the RLS of TP or PPF alone is very weak over the wavelength range 220–600 nm. When PPF reacted with TP to form an ion-associate, it could lead to a remarkable enhancement in the intensity of RLS and the maximum RLS peak was at 340 nm. Therefore, 340 nm was chosen as the determination wavelength in further experiments.

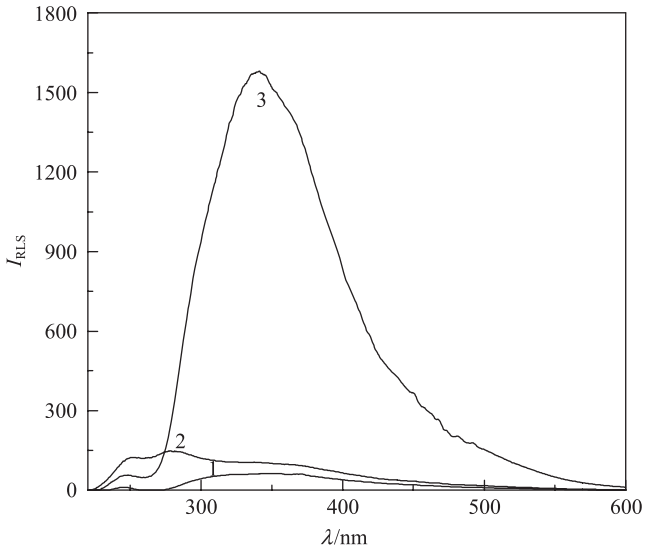

Figure 2. RLS spectra. 1, TP (1.0×10^{-4} mol/L); 2, PPF (2.0 μg/mL); 3, TP + PPF; pH 1.0.

Optimum reaction conditions

In order to investigate the optimal experimental conditions for the reaction of TP and PPF and the performance of the employed FIA system, a univariate experimental design procedure was adopted, including all the parameters which could influence the analytical features of the developed method. The effect of the various experimental variables was studied with respect to the detection limit, precision and sample throughput rate.

Effect of carrier acidity. As TP is exists stably in pH 0–1.5 acid conditions, we considered the effect of acidity in a strong acid medium. TP was dissolved in HCl solutions of different concentrations; the results showed that this acidity range had little influence on the experiment, so 0.1 mol/L HCl was employed as the acidity of the carrier in this study.

Effect of ionic intensity. The effect of ionic strength on the intensity of RLS was investigated by adding NaCl solution. The

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

results showed that only when the concentration of NaCl solution was low did the ionic intensity have a small effect on ΔI_{RLS}, e.g. the changes of ΔI_{RLS} was 4.4% when the concentration of NaCl was 0.01 mol/L.

Effects of TP concentration. The effects of different TP concentrations on relative RLS intensities were investigated in the range 1.0×10^{-5}–1.0×10^{-3} mol/L. The results showed that the relative RLS intensity first increased and then decreased with increasing TP concentration. The optimum concentration of TP was in the range 5.0×10^{-5}–5.0×10^{-4} mol/L, and therefore 1.0×10^{-4} mol/L was selected as the optimum concentration of TP.

Influence of FIA variables. We investigated the effect of different parameters on the signal, including the carrier flow rate, the injected sample volume, and the reactor length, to optimize the FIA–RLS system and improve the sensitivity of the FIA approach for the determination of PPF.

The effect of the flow rate on the determination of PPF was studied at a concentration of PPF 2.0 μg/mL by injecting aliquots of 86 μL of the working sample solution into a TP 1.0×10^{-4} mol/L carrier solution, and by performing the repetitive experiments at four different fixed flow rates in the range 1.0–4.0 mL/min. It was found that the flow rate through the system dramatically influenced the performance of the system. The choice of the flow rate must take into account the change in peak height as well as peak width and the return rate to the baseline with flow rate, which influenced the sampling throughput. The experiment results showed that the RLS intensity increased with increasing the flow rate below 3.0 mL/min. However, lower flow rates led to broader peaks, due to the sample dispersion in the flow process, and a flow rate >3.0 mL/min caused poor reproducibility. So a flow rate of 2.0–3.0 mL/min was considered as optimal for the determination. Based on the results, a flow rate of 2.6 mL/min was chosen for the desirable sample frequency.

The injection volume had strong influence on the sensitivity and dynamic range of the method. The effect of the sample volume was studied over the range 40–100 μL. It was found that the higher the sample volume, the higher were the peak heights and the longer was the residence time of the sample in the detector, requiring a longer time to reach a steady state and greater consumption of sample. Although smaller volumes have the advantage of lower sample consumption and shorter sample residence time (i.e. enhanced sampling frequency), the desired sensitivity and precision must also be considered. In the present study, a volume of 86 μL was selected as a reasonable compromise.

The effect of the length of reactor was examined by injecting a sample solution of the analyte (2.0 μg/mL) over the range 40–80 cm. The experiments showed that the sensitivity was independent of the length of reactor. The interaction between TP and PPF was incomplete when the reaction coils length was too short, whereas the RLS intensity could decrease when it was too long. A reasonable compromise was a length of 60 cm.

Relationship between RLS intensity and PPF concentration

PPF at different concentrations reacted with TP and the RLS intensities were measured at the optimized experimental conditions, as reported. A typical recording of a calibration run for PPF is shown in Fig. 3, which is described by the linear regression equation: $\Delta I = -834.3 + 1039.2c$, where c is μg/mL, the correction coefficient (γ) is 0.9998, the linear range is 0.003–9.0 μg/mL and

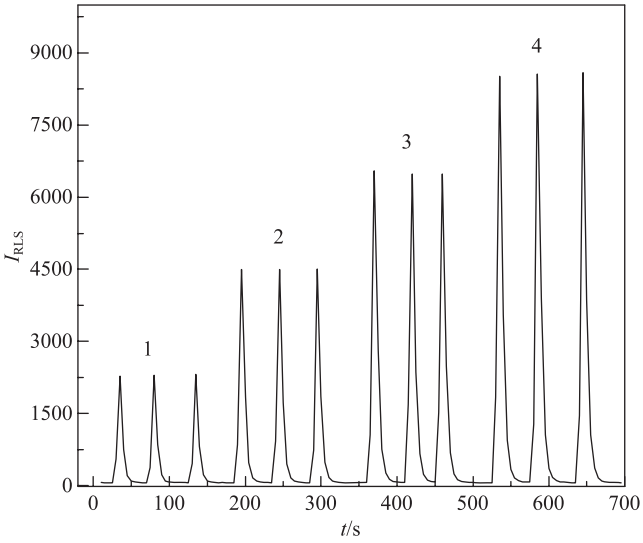

Figure 3. Calibration peak signals for the determination of PPF by FIA. Carrier flow rate, 2.6 mL/min; reactor length, 60 cm; injection volume, 86 μL; PPF concentrations (1–4), 3.0, 5.0, 7.0 and 9.0 μg/mL.

the detection limit (3σ) is 1.0 ng/mL. The figure demonstrates the high reproducibility of the measurements and the short response and washout times. The repeatability, expressed as the relative standard deviation (RSD) of 10 replicate injections (each containing PPF 2.0 μg/mL) was 2.1%. These results indicate that the precision is good enough for the determination of PPF at ultra-low concentrations.

Discussion of the reaction mechanism of TP with PPF

Reasons for RLS enhancement. (a) *The hydrophobicity of the enhanced ion-association*. Either PPF^+ or $(PW_{12}O_{40})^{3-}$ has good hydrophilicity and can easily form hydrates by dissolving them in reaction medium. The scattering intensities are very weak under this condition. When they react with each other to form an ion-association complex by electrostatic force, their charges are neutralized to form neutral compounds and the hydrophobic interface of the ion-association complex and aqueous phase forms because of the hydrophobicity of the aryl and alkyl frameworks, which is advantageous to the enhancement of RLS (25). (b) *Effect of molecular volume*. It is known that the bigger the molecular volume (26), the higher the RLS intensity. While the molecular volume is calculated with difficulty, it can be estimated generally according to the variation of molecular weight. When PPF^+ reacts with $(PW_{12}O_{40})^{3-}$ to form the ion-association complex, the molecular weight increases from 378 (PPF^+) to 4016 [PPF_3 ($PW_{12}O_{40}$)], which would result in great enhancement of RLS intensity. Moreover, the aggregations of the ion-association complex are also an important reason for RLS enhancement.

Selectivity of the RLS–FIA method

Under optimum conditions, several cations and anions and saccharide, as potential interferents, were studied in detail. Table 1 gives the tolerance limits of interfering substances on the determination of PPF. As shown, common metal ions and inorganic anions do not interfere with the determination of PPF. The RLS–FIA method therefore has good selectivity.

Table 1. Effect of coexisting substances[a]

Coexisting substance	Concentration (μg/mL)	Relative error (%)	Coexisting substance	Concentration (μg/mL)	Relative error (%)
NaCl	584.4	4.4	Pepsin	25.0	1.9
KBr	100.0	−2.7	Urea	100.0	4.4
NH_4NO_3	50.0	2.1	Starch	260.0	2.8
$MgSO_4$	150.0	4.0	Lactose	500.0	3.9
$CaCl_2$	100.0	−4.3	Sucrose	50.0	−3.0
$BaCl_2$	100.0	3.2	DL-Threonine	10.0	−3.0
$CuCl_2$	100.0	3.3	Glucose	100.0	3.0
$Fe_2(SO_4)_3$	22.5	−3.2	Maltose	100.0	0.8
$AlCl_3$	15.8	2.7	Vitamin C	36.0	2.9

[a]Concentration of PPF was 2.0 μg/mL; under optimal conditions, average of five measurements.

Table 2. Results for the determination of PPF in tablets[a]

Method	Found mean value (mg/one tablet; $n = 5$)	Specified amount (mg/one tablet)	Recovery (%, $n = 5$)	RSD (%)
RLS–FIA	55.4	50.0	110.7	1.3
Pharmacopoeia method	56.7	50.0	113.4	3.5

[a]Carrier flow rate, 2.6 mL/min; reactor length, 60 cm; injection volume, 86 μL.

Table 3. Results for the determination of PPF in serum samples

No.	Found (μg/mL)	Added (μg/mL)	Found ($n = 5$) (μg/mL)	Recovery (%)	RSD (%)
1	ND	2.0	2.0 1.91 1.98 2.02 2.08	99.9	3.1
2	ND	3.0	2.88 2.92 2.95 2.96 2.93	97.6	1.1
3	ND	4.0	4.07 4.01 4.04 3.93 4.09	100.7	1.6

ND, not detected.

Analytical application

The proposed method was applied to the analysis of PPF in commercial tablets so as to prove the precision and accuracy of the proposed method to real sample analysis. Ten tablets were weighed, crushed and combined. An amount of powder equivalent to about 1.0 mg PPF was accurately weighed, then dissolved with ethanol, filtred and finally diluted with water into a 100 mL volumetric flask as the working solution for the experiment. The method was compared with the standard method of *The Pharmacopeia of the People's Republic of China* (27). The results are listed in Table 2. As can be seen, no significant difference was found between the two methods.

A 2.0 mL aliquot of fresh serum sample (from a healthy person) and 3.5 mL methanol were mixed thoroughly and centrifuged at 4500 r.p.m. for 15 min. A 1.0 mL aliquot of the supernatant fluid was diluted to 20.0 mL to be used for the determination. The results are listed in Table 3, where it is shown that the method has good reproducibility. The RSD of human serum is in the range 1.1–3.1%, and the recovery is from 97.6–100.7%. From these results, it is suggested that the present FIA method with RLS detector can be applied successfully to the determination of propafenone concentration in serum samples.

Conclusions

The present paper describes for the first time a highly convenient, rapid and accurate RLS–FIA method for the quantification of PPF. Using FIA with RLS detection, the results demonstrated that not only was the analysis time shortened but high reproducibility was obtained. The method was successfully applied to the determination of PPF in tablet dosage forms and serum samples and compared with the *Pharmacopoeia* method. Therefore, the proposed technique can be developed further to monitor PPF in clinical studies.

Acknowledgements

The authors gratefully acknowledge financial support for this study by the National Natural Science Foundation of China (Grant No. 20475045), Chongqing Municipal Key Laboratory on Luminescence

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

and Real-Time Analysis (Grant No. CSTC 2006CA8006) and the Natural Science Foundation Project of CQ CSTC (Grant No. CSTC 2007BB1359).

References

1. Bryson HM, Palmer KJ, Langtry HJ, Fitton A. Propafenone. A reappraisal of its pharmacology, pharmacokinetics and therapeutic use in cardiac arrhythmias. *Drugs* 1993; **45**: 85–130.
2. Benz I, Kohlhardt M. Responsiveness of cardiac Na channels to antiarrhythmic drugs: the role of inactivation. *J. Membr. Biol*. 1991; **122**: 267–278.
3. Afshar M, Thormann W. Validated capillary electrophoresis assay for the simultaneous enantioselective determination of propafenone and its major metabolites in biological samples. *Electrophoresis* 2006; **27**(8): 1517–1525.
4. Fromm MF, Botsch S, Heinkele G, Evers J, Kroemer HK. Influence of renal function on the steady-state pharmacokinetics of the antiarrhythmic propafenone and its phase I and phase II metabolites. *Eur. J. Clin. Pharmacol*. 1995; **48**: 279–283.
5. Kern R. Internally standardized simultaneous assay of propafenone and 5-hydroxypropafenone enantionmers in human plasma by means of high performance liquid chromatography. *Methods Find. Exp. Clin. Pharmacol*. 1994; **16**(3): 203–210.
6. Kunicki PK, Paczkowski D, Sitkiewicz D. High-performance liquid chromatographic method for the simultaneous determination of propafenone and 5-hydroxypropafenone in human serum. *Pol. J. Pharmacol. Pharm*. 1992; **44**: 161–171.
7. Tracqui A, Kintz P, Mangin P. Systematic toxicological analysis using HPLC/DAD. *J. Forens. Sci*. 1995; **40**: 254–262.
8. Spatzenegger M, Ecker G, Jaeger W. Determination of the new MDR-modifier PFP 6 and its metabolites in human liver microsomes by high-performance liquid chromatography. *Biomed. Chromatogr*. 1996; **10**: 127–130.
9. Hofmann U, Pecia M, Heinkele G, Dilger K, Kroemer HK, Eichelbaum M. Determination of propafenone and its phase I and phase II metabolites in plasma and urine by high-performance liquid chromatography–electrospray ionization mass spectrometry. *J. Chromatogr. B* 2000; **748**: 113–123.
10. Dupuis LL, Wong B, Trope A. Stability of propafenone hydrochloride in i.v. solutions. *Am. J. Health Syst. Pharm*. 1997; **54**: 1293–1295.
11. Nakano S, Nakaso K, Noguchi K, Kawashima T. Catalytic flow-injection determination of copper at nanogram levels by using color formation of N-phenyl-*p*-phenylenediamine with *m*-phenylenediamine in the presence of pyridine and ammonia as activators. *Talanta* 1997; **44**: 765–770.
12. Zolotov YA, Shpigun LK, Kolotyrkina IY, Novikov EA, Bazanova OV. The trace determination of some heavy metals in waters by flow-injection spectrophotometry and potentiometry. *Anal. Chim. Acta* 1987; **200**: 21–33.
13. Falkner KK, Edmond JM. Determination of gold at femtomolar levels in natural waters by flow-injection inductively coupled plasma quadrupole mass spectrometry. *Anal. Chem*. 1990; **62**: 1477–1481.
14. Li Y, Dong LJ, Wang WP, Hu ZD, Chen XG. Flow injection analysis–Rayleigh light scattering detection for online determination of protein in human serum sample. *Anal. Biochem*. 2006; **354**: 64–69.
15. Vidal E, Palomeque ME, Lista AG, Fernández Band BS. Flow injection analysis–Rayleigh light scattering technique for total protein determination. *Anal. Bioanal. Chem*. 2003; **376**: 38–41.
16. Dai XX, Li YF, Huang CZ. Analysis of heparin by combining technique of flow injection analysis with resonance light scattering. *Chin. J. Anal. Chem*. 2005; **33**: 1535–1538.
17. Hu XL, Liu SP, Liu ZF. Determination of kanamycin using flow injection analysis coupled with resonance Rayleigh scattering detection. *Bull. Chem. Soc. Jpn* 2006; **79**: 247–251.
18. Xu DP, Liu SP, Liu ZF, Hu XL. Determination of verapamil hydrochloride with 12-tungstophosphoric acid by resonance Rayleigh scattering method coupled to flow injection system. *Anal. Chim. Acta* 2007; **588**: 10–15.
19. Huang CZ, Pang XB, Li YF. Determination of heparin using azure B by flow injection analysis–resonance light scattering coupled technique. *Anal. Lett*. 2005; **38**: 317–330.
20. Li Y, Dong LJ, Zhang YH, Hu ZD, Chen XG. Determination of proteins by flow injection analysis coupled with the Rayleigh light scattering technique. *Talanta* 2007; **71**: 109–114.
21. Dong LJ, Li Y, Zhang YH, Chen XG, Hu ZD. Determination of proteins in human serum by combination of flow injection sampling with resonance light scattering technique. *Microchim. Acta* 2007; **159**: 49–55.
22. Dong LJ, Li Y, Zhang YH, Chen XG, Hu ZD. A flow injection sampling resonance light scattering system for total protein determination in human serum. *Spectrochim. Acta A* 2007; **66**: 1317–1322.
23. Rocchiccioli-Deltcheff C, Fournier M, Franck R, Thouvenot R. Vibrational investigations of polyoxometalates. 2. Evidence for anion–anion interactions in molybdenum(VI) and tungsten(VI) compounds related to the keggin structure. *Inorg. Chem*. 1983; **22**: 207–216.
24. Liu SZ, Wang EB, Xu L. Studies on redox properties and stability of 1:12 heteropoly complexes with rare earth elements. *Chin. J. Inorg. Chem*. 1994; **10**: 40–46.
25. Liu SP, Jiang ZL, Kong L, Liu Q. Absorption and Rayleigh scattering and resonance Rayleigh scattering spectra of $(HgX_2)_n$ nanoparticles. *Sci. China Ser. B* 2002; **45**: 616–624.
26. Zhang YH. *Physical Chemistry*. Shanghai Traffic University Press: Shanghai, 1988; 538.
27. Editorial Committee of the Pharmacopoeia of the People's Republic of China. *The Pharmacopoeia of the People's Republic of China*, Part D. Chemical Industry Press: Beijing, 1995; 707.

