J Fluoresc (2010) 20:733–738
DOI 10.1007/s10895-010-0615-3

ORIGINAL PAPER

Fluorescence Quenching Reaction of Polyvinylpyrrolidone-Eosin Y System for the Determination of Polyvinylpyrrolidone

Lihong Yu · Zhongfang Liu · Xiaoli Hu · Ling Kong · Shaopu Liu

Received: 14 April 2009 / Accepted: 7 February 2010 / Published online: 2 March 2010

Abstract In pH 1.8~2.8 weak acid medium, polyvinylpyrrolidone (PVP) and Eosin Y reacted to form complex that could result in Eosin Y (EY) fluorescence quenching. The maximum quenching wavelength was at 542 nm. The fluorescence quenching (ΔF) was proportional to the concentration of polyvinylpyrrolidone in a certain range. The linear range, the correlation coefficient and the detection limit were 0.33~2.0 μg•mL^{-1}, 0.9994 and 99.6 ng•mL^{-1}, respectively. The influences of the coexistence substances were tested and the results showed that the method had good selectivity. Therefore, a new method based on fluorescence quenching of eosin Y by PVP for the determination of trace PVP was developed. The method was sensitive, simple and rapid, which was applied to the determination of trace PVP in the beer with satisfactory results. The reaction mechanism was also discussed.

Keywords Eosin Y · Polyvinylpyrrolidone · Fluorescence quenching

Introduction

Polyvinylpyrrolidone (PVP) is a *N*-vinyl lactan non-ionic water-soluble polymer. Because of its unique characters such as good water solublity, film forming ability, adhesion, surface activity, stablity in acid or base as well as good biocompatibility and low toxicity, it has become one of the most important water-soluble polymers, and it has been more and more applied in medicines, foods, cosmetics and a variety of industries [1, 2]. So it is very important for the determination of PVP. However, there have been not many methods for the determination of PVP. At present, only a few methods such as titration method [3], turbidimetry [4], spectrophotometry [5, 6], gas chromatography [7, 8], HPLC [9, 10], infrared and fluorescence method [11] has been reported. These methods had some deficiencies in sensitivity, selectivity or simplicity, which affected their practical application. Therefore, further research and development of a new method for the determination of PVP is significance.

Some molecular structures and some chelating properties of PVP are similar to those of proteins. Therefore, it can provide some useful information to research the interactions of proteins with small molecules, and these reactions also can create conditions for developing some new analytical methods for PVP. Therefore, such researches not only are important study contents of polymer science, but also attract attentions of biochemistry, medicine chemistry and analytical chemistry. In recent years, the interactions PVP with various dyes such as some reduced vat dyes [12], methyl orange as a monoazo-dye [13], evans blue as a bisazo-dye [14] and bromphenol blue as a acid triphenylmethane dye [15] have been studied by absorption and fluorescence spectra. But these works only focused on the study of physical-chemical nature, or for the limitation of low sensitivity of the reaction systems having analytical application prospect have not been developed. So it has not been developed for a very useful analytical method from above reaction systems. Our experiments found that in pH 1.8~2.8 acid medium, the maximum excitation and emission wavelengths of eosin Y were located at 526 nm and 542 nm. When it reacted with PVP to form a complex, although it could not observe the changes of fluorescence spectral characteristics, it could cause the significant

L. Yu · Z. Liu · X. Hu · L. Kong · S. Liu (✉)
School of Chemistry and Chemical Engineering,
Southwest University,
Chongqing 400715, China
e-mail: liusp@swu.edu.cn

L. Yu
Guiyang Environmental Monitoring Central Station,
Guizhou 550002, China

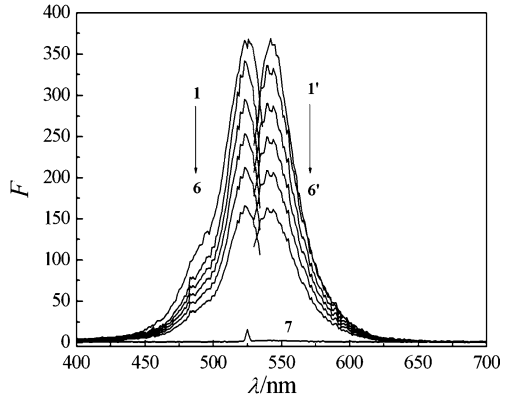


Fig. 1 Fluorescence spectra. 1~6: Excitation spectra; 1′~6′: Emission spectra; 1 and 1′: EY solution; 2~6 and 2′~6′: EY-PVP systems, PVP: from 2,2′ to 6-6′ are 0.4 μg•mL^{-1}, 0.8 μg•mL^{-1},1.2 μg•mL^{-1} respectively, 1.6 μg•mL^{-1}, 2.0 μg•mL^{-1}; 7: Emission spectrum of PVP (PVP: 1.6 μg•mL^{-1}), EY: 1.9×10^{-5} mol•L^{-1}, pH 2.0

fluorescence quenching of eosin Y. In a certain range, the fluorescence quenching value (ΔF) was directly proportional to the concentration of PVP. The linear range was 0.33~ 2.0 μg•mL^{-1}.The detection limit for PVP was 99.6 ng•mL^{-1}. In this work, the spectral characteristics, the optimum reaction conditions and influencing factors were studied and the effects of coexisting substances were tested, which showed that quenching the method had good selectivity. A new, high sensitive, easy and rapid fluorescence method for the determination of PVP based on the fluorescence quenching of eosin Y has been developed. The reaction mechanism was also been discussed.

Experimental

Apparatus and reagents

A RF-5301 spectrofluorophotometer (Hitachi Company, Japan) was used for recording fluorescence spectra and

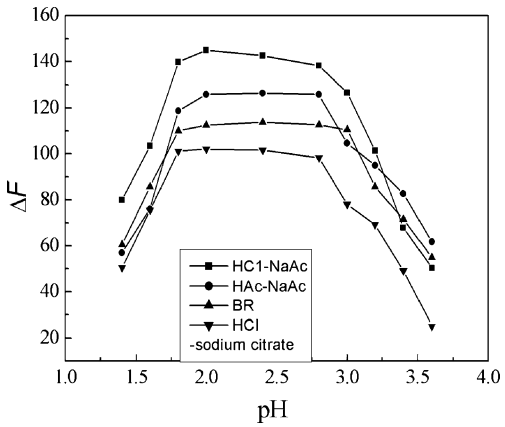


Fig. 2 Effect of pH. PVP: 1.6 μg•mL^{-1}, EY: 1.9×10^{-5} mol•L^{-1}

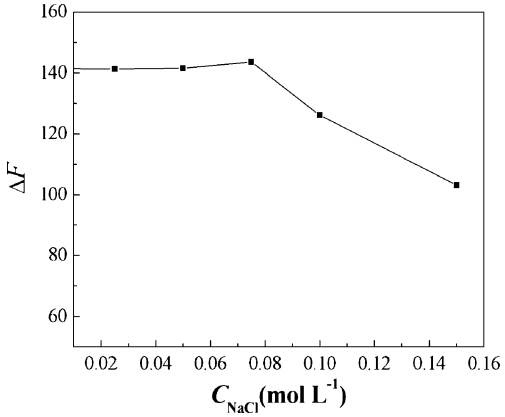


Fig. 3 Effect of ion strength. PVP: 1.6 μg•mL^{-1}, EY: 1.9×10^{-5} mol•L^{-1}

measuring the fluorescence intensity. A UV-2450 spectrophotometer (Shimadzu Corporation, Japan) was used for recording the absorption spectra. A PHS-3C meter (Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai) was used for adjusting pH values.

PVP (Polyyinylpyrrolidone, PVP-K-30, Shanghai Chemical Reagents Company, China, analytical reagent grade) standard solution: the concentration of PVP stock solution was 1.0 mg•mL^{-1} and the working solution was prepared by diluting the stock solution to 40.0 μg•mL^{-1}.

Eosin Y (EY, Guangzhou Chemical Reagent Plant, China, analytical reagent grade): The concentration of stock solution and working solution of EY water solution were 2.5×10^{-3} mol•L^{-1} and 2.5×10^{-4} mol•L^{-1}, respectively.

HCl-NaAc buffer solutions were prepared by mixing 1.0 mol•L^{-1} HCl and 1.0 mol•L^{-1} NaAc solution according to certain proportion and the pH values were adjusted by pH meter.

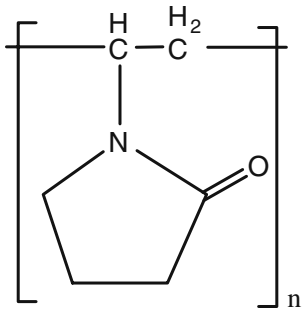
NaCl (The Chemical Reagent Plant in Beibei of Chongqing, analytical reagent grade) solution: 1.0 mol•L^{-1}.

All reagents were analytical reagent grade and doubly distilled water was used throughout.

General procedures

Into a 10.0 mL tube were added 2.0 mL of pH 2.0 HC1-NaAc solution, 0.75 mL of 2.5×10^{-4} mo•L^{-1} eosin Y

Fig. 4 The structure of PVP



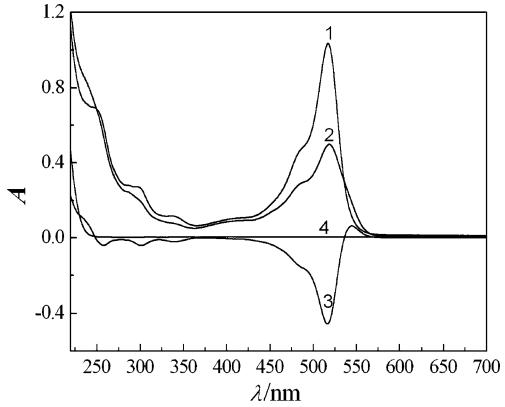


Fig. 5 Absorption spectra. 1. EY; 2. EY-PVP (measured against water); 3. EY-PVP (measured against the reagent blank); 4. PVP (measured against water), EY: 2.5×10^{-5}mol•L^{-1}, PVP: 3.2 μg•mL^{-1}

solution and suitable amounts of polyyinylpyrrolidone standard solution. The resulting solution was diluted to the mark with water and mixed thoroughly, then it was set aside for 25 min. Fluorescence spectra of the system were recorded and the fluorescence intensity (F) for binding product and F_o for reagent blank at $\lambda_{ex}/\lambda_{em}$=526 nm/542 nm in 1 cm crystal cell were measured, $\Delta F=F-F_o$.

Results and discussion

Fluorescence spectra

The fluorescence spectra of PVP-EY system are shown in Fig. 1. Under experimental conditions, PVP was non-fluorescence, but EY had strong fluorescence. The maximum excitation (λ_{ex})and emission wavelengths (λ_{em})for EY were located at 526 nm and 542 nm, respectively. When PVP and EY reacted with each other to form complexes, the fluorescence spectral characteristics of eosin Y was no changed, but its fluorescence intensity decreased obviously. And the fluorescence quenching ΔF was directly proportional to the concentration of PVP. So, this fluorescence quenching reaction could be applied for the determination of PVP.

Optimum reaction conditions

Effect of acidity

Four buffer solutions such as citrate sodium-NaOH, HC1-NaAc, HAc-NaAc, BR were used to investigate the effects of acidity on the fluorescence quenching of EY-PVP system. The results showed that HC1-NaAc buffer solution was the best, and the optimum pH range was 1.8~2.8 showed in Fig. 2. When pH value was beyond the range, ΔF decreases obviously. The pH 2.0 HCl-NaAc buffer was selected as reaction medium for the following experiments. The suitable amount of the buffer solution was 1.5 mL.

Effects of the eosin Y concentration

The effect of concentration of EY was examined. The results showed that when the concentration of EY was too low, the reaction was incomplete and ΔF was low. Increasing the concentration of EY to 1.25×10^{-5}mol•L^{-1}, ΔF reached the maximum and kept stable until 2.5×10^{-5} mol•L^{-1}. However, when the concentrations of EY is larger than 2.5×10^{-5}mol•L^{-1}, ΔF decreased gradually. And the absorption and fluorescence spectra would change gradually. So, in this work, 1.9×10^{-5}mol•L^{-1} of EY was selected as the experiment concentration.

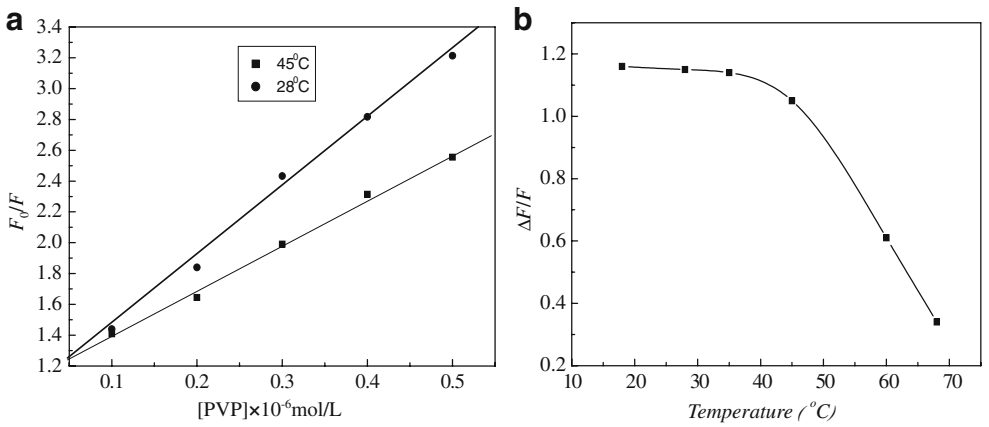


Fig. 6 Sterm-volmer plot and effect of temperature. **a** S-V plots of fluorescence quenching of EY by PVP in different concentration, PVP: 0.4μg•mL^{-1}, 0.8μg•mL^{-1}, 1.2μg•mL^{-1}, 1.6μg•mL^{-1}, 2.0 μg•mL^{-1}, EY: 1.9×10^{-5}mol•L^{-1}. **b** Effect of temperature on the fluorescence intensity, PVP: 1.6μg•mL^{-1}, EY: 1.9×10^{-5}mol•L^{-1}

Table 1 Parameters for the stern-volmer plot of PVP-EY system

T/ ^{0}C	Linear regression equations	Correlation coefficient	Ksv
28	$F_0/F=1.0\pm4.5\times10^5[Q]$	0.997	4.5×10^5
45	$F_0/F=1.0\pm3.0\times10^5[Q]$	0.998	3.0×10^5

Reaction speed and the stability of fluorescence quenching value (ΔF)

The experimental results showed that the reaction of PVP and EY at room temperature (20~25 °C) could complete in 25 min or so. Then ΔF values remained constant within 12 h, it can seen that the fluorescence signal had good stability. The fluorescence quenching value (ΔF) was measured after 25 min of the reaction in the following experiment.

Effects of ionic strength

The influences of ionic strength on fluorescence quenching (ΔF) were investigated using NaCl solution (see Fig. 3). It can be seen from Fig. 3 that when the concentration of NaCl is lower than 0.075 mol•L^{-1}, the effect of ionic strength on ΔF is weak. But, increased the concentration of NaCl is higher than 0.075 mol•L^{-1},ΔF decreases gradually, this shows that the ionic strength in higher concentration on the interaction of PVP with EY has effect, but a lower concentration (≤0.075 mol•L^{-1}) of NaCl was allowed.

Calibration graph

Under the optimum experimental conditions, the calibration graph of ΔF versus the concentration of PVP was constructed. The linear regression equation was $\Delta F=-26+105.4c$ (μg•mL^{-1}). The linear range was 0.33~2.0 μg•mL^{-1}. The correlation coefficient was 0.9994 and the detection limit was 99.6 ng•mL^{-1}.

The formation of complex and its effect on fluorescence

PVP is N-vinyl pyrrolidone polymer, of which the structure is as follows (see Fig. 4):

Its each monomer contains a strong polar (dipole moment is 40) lactam group [1], and the N atom easily protonation. Therefore, in acid medium, it mainly exists as large cation with much positively charge, and it is presence of as an irregular helical in the aqueous solution [10]. While in pH 2.0 acid medium, according to the dissociation constants of eosin Y (H_2L) (pKa_1=3.2, pKa_2=3.6 [16]), H_2L species occupies 93.8%. When H_2L reacted with PVP to form the complex, the fluorescence of EY was quenched and the new absorption spectra appeared (see Fig. 5). EY occurred obvious fading at the maximum wavelength of 517 nm. This showed that EY and PVP formed a binding product.

We considered the fluorescence quenching is a static quenching reaction due to change of the absorption spectrum when formation of the complex and the fluorescence quenching value (ΔF) and the binding constant (K) are decreased when temperature increased (see Fig. 6 and Table 1). We determined the composition ratio of the complex by Job's method of continuous variation and mole ratio methods. Results are PVP: EY=1:2. So the composition of the binding product is [PVP] $[EY]_2$.

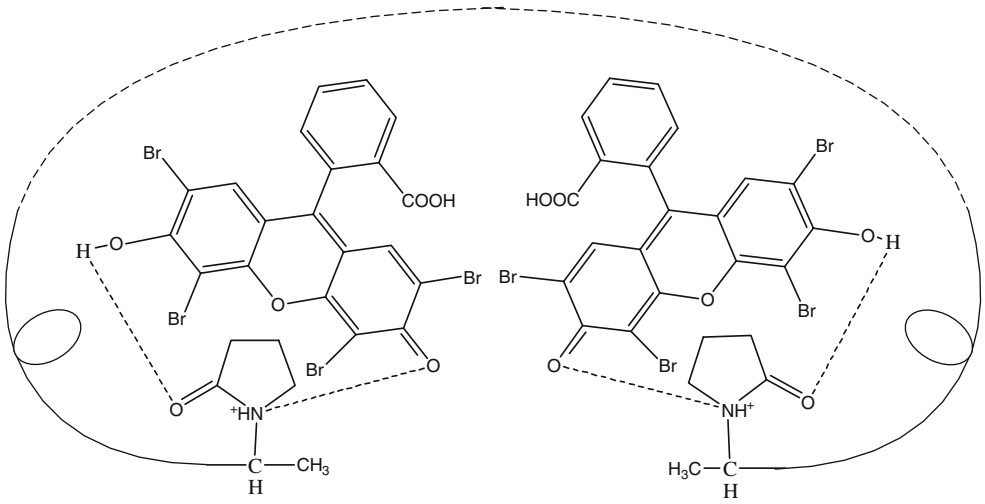


Fig. 7 Schematic diagram of the structure of the binding product of PVP with EY

Table 2 Effects of coexisting substances (PVP: 1.2 μg•mL^{-1})

Foreign substance	Coexisting concentration (μg•mL^{-1})	Relative error (%)	Foreign substance	Coexisting concentration (μg•mL^{-1})	Relative error (%)
Na^+,Cl^-	4300	5.0	HSA	10	−2.6
K^+,Cl^-	1000	−4.2	BSA	5	−4.0
Mg, SO_4^{2-}	180	−3.8	Ethanol	13	−5.0
Al^{3+},SO_4^{2-}	100	−3.6	Lactose	3000	−4.5
Cu^{2+}, SO_4^{2-}	48	4.3	Glucose	1000	−5.2
Ca^{2+},SO_4^{2-}	30	−2.5	Maltose	3000	4.8
Na^+,HPO_4^{2-}	50	−4.1	Sucrose	1000	4.5
Zn^{2+},Ac^-	50	3.9	Diabetin	1000	−4.3
NH_4^+,Fe^{3+},SO_4^{2-}	5	−4.3	L-Glycine	63	−0.8
NH_4^+, Fe^{2+},SO_4^{2-}	100	3.6	L-Tryp	50	4.3
Cd^{2+}, SO_4^{2-}	52	−4.5	DL-Half-cystine	20	4.7
Hg^{2+}, Cl^-	16	−4.1	L-Ganimalon	5	−4.6
Pb^{2+}, Ac^-	16	3.9	L-Histidine	20	5.6
Mn^{2+}, SO_4^{2-}	50	−5.0	DL-Aspartate	100	3.1
Citric acid	100	−4.2	Vitamin B_1	40	3.6
Tartaric acid	80	4.5	Vitamin C	600	5.1
Dextrin	600	−4.2	Soluble starch	300	5.0
β-Cyclodextrin	800	−4.6	CMC-Na	200	4.6

PVP exists as an irregular helical structure in aqueous solution. The eosin Y molecule (H_2L) is larger, and it is difficult to enter the interior of PVP molecule to bind with protonated lactan in helical structure due to the sterical hindrance. However, because the protonated lactan unit and the ketone group oxygen atom at two ends in PVP molecule have a larger degree of freedom, H_2L molecule can close to the two ends of PVP and form a complex through hydrogen bond and electrostatic attraction [17–26]. In which the carbonyl oxygen atom of PVP and the hydroxyl of eosin Y mainly combine by hydrogen bond, while the protonated lactan group of PVP interacts with the carbonyl oxygen atom of eosin Y binds mainly through electrostatic attraction. The inferred binding mode was speculated as follows (see Fig. 7):

Table 3 Determination results of PVP in beer samples

Sample		Found	Added	Total found					Average	Recovery	RSD
		(g•L^{-1})	(g•L^{-1})	(g•L^{-1}, n=5)					(g•L^{-1})	%	%
Dark beer	NO.1	0.13	0.08	0.20	0.20	0.21	0.22	0.21	0.208	97.5	4.0
	NO.2	0.15	0.16	0.30	0.30	0.32	0.29	0.31	0.304	96.3	3.8
	NO.3	0.14	0.24	0.40	0.37	0.34	0.38	0.39	0.376	98.3	6.1
Pure beer	NO.1	0.12	0.08	0.20	0.21	0.21	0.22	0.19	0.206	107.5	5.5
	NO.2	0.11	0.16	0.27	0.24	0.26	0.29	0.25	0.262	95.0	3.3
	NO.3	0.11	0.24	0.34	0.37	0.35	0.39	0.37	0.364	105.8	5.4
Qingdao beer	NO.1	0.10	0.08	0.19	0.17	0.21	0.15	0.18	0.180	100.0	1.2
	NO.2	0.10	0.16	0.28	0.26	0.26	0.26	0.25	0.262	101.3	4.2
	NO.3	0.10	0.24	0.33	0.35	0.38	0.32	0.36	0.348	103.3	6.9
Snowflake beer	NO.1	0.07	0.08	0.16	0.14	0.16	0.15	0.14	0.150	100.0	6.7
	NO.2	0.09	0.16	0.27	0.24	0.26	0.23	0.25	0.250	100.0	6.3
	NO.3	0.07	0.24	0.31	0.33	0.31	0.32	0.29	0.312	100.8	4.8

Selectivity of the method and its analytical application

Effects of foreign substances

The influences of coexisting substance were investigated (see Table 2). The results showed that a large amounts of common metal ions such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Al (III), Cu^{2+}, Mn^{2+}, Cd^{2+}, etc. were tolerant, a certain amount of Hg^{2+}, $Pb^{2+}$and relative large amounts of sugars, such as 1000 $\mu g \cdot mL^{-1}$ of glucose, sucrose and fructose; 3000 $\mu g \cdot mL^{-1}$ of maltose, lactose, and 300 $\mu g \cdot mL^{-1}$ of starch and 600 $\mu g \cdot mL^{-1}$ of dextrin were permitted; The method tolerated the existence of a certain amounts of organic acids (e.g. citric acid) and a small number of amino acids as well as natural surfactants (e.g. β-cyclodextrin). The interference of Fe (III) was serious, but 100 μg of Fe (II) did not interfere. So, the interference of Fe (III) could be eliminated by reducing it to Fe (II) using ascorbic acid. Thus, the method had a good selectivity.

Analytical application: determination of PVP in beer

Pipette 25.0 mL of beer sample into 100.0 mL beaker. Evaporate the solution to dry in a boiling water bath. Dissolve the residues and transfer to 500.0 mL flask and dilute to the mark with water, mix thoroughly. Pipette 1.0 mL of solution into 10.0 mL flask and determine the concentration of PVP according to the experimental method. The results, the recovery and the relative standard deviation (RSD) were listed in Table 3. It can be seen from Table 3 that the recovery and the relative standard deviation are in the range of 95.0~107.5% and 1.2~6.9%, respectively, which shows that the method has high accuracy and good reproducibility. It is suitable for the determination of PVP in beer.

Acknowledgement This work was supported by the National Natural Science Foundation of China (NO.20875078), and which was supported by Chongqing Municipal Key Laboratory on Luminescence and Real-Time Analysis,Southwest University.

References

1. Yan RX (1998) Water soluble polymer. Chemical Industry, Beijing, pp 586–699
2. Editorial Committee of Fine Chemicals, Yu MZ (1989) Dictionary of fine chemicals. Chemical Industry, Beijing, p 272
3. Dwyer RF, Lewandowski RJ (1964) Determination of polyvinyl-pyrrolidone by an Iodine titration method. Anal Biochem 9:133–138. doi:10.1016/0003-2697(64)90094-6
4. Hagel L, Andersson R (1976) Automated nephelometric determination of polyvinyl-pyrrolidone in salazopyrin. Anal Chim Acta 86:69–77. doi:10.1016/S0003-2670(01)83019-1
5. Chemileko FA, Kgarun MV, Chmilenko TS (2001) Spectrophotometic detemination of poly(Vinyl pyrrolidinone) in wasterwater. KhimTekhnol Vody 23:167–171
6. Ahmad AKS, El-Gendy AE, El-Naga NHA (1994) Spectrophotometric studies on molecular interactions III. complexation of chloranil with polyvinylpyrrlidone polymer and with the monomer N-methy-2-pyrrolidone. Bull Fac Pharm 32:5–31
7. Inger E, Lennard L (1990) Trace determination of high molecular weight polyvinylpyrrolidone by pyrolysis-gas chromatography. J Anal Appl Pyrolysis 17:251–260. doi:10.1016/0165-2370(90)85014-E
8. Liao LW, Kang Z, Cui YD, Yi GB (2001) Determination of residual N—vinylpyrrolidone in polyvinylpyrrolidone by gas chromatography. Chem World 6:298–300
9. Ziller KH, Rupprecht H (1981) Determination of poly(vinlypyrrolidone) in pharmaceutical solutions by liquid chromatography. Arch Pharm 314:970–972. doi:10.1002/ardp. 19813141113
10. Jones SA, Martin GP, Brown MB (2004) Determination of polyvinylpyrrolidone using high-performance liquid chromatography. J Pharm Biomed Anal 35:621–624. doi:10.1016/j.jpba.2004.01.024
11. Ovsepyan AM, Kobyakov VV, Dubrovin VI, Panov VP (1978) Determination of polyvinylpyrrolidone in aqueous solutions by IR-spectrophotometric and spectrofluorimetric methods. Pharm Chem J 12:1517–1520. doi:10.1007/BF00772661
12. Geeta N, Sheth (1991) Studies of interaction of polyvinyl pyrrolidone with reduced vat dyes using visible spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc 47:63–68. doi:10.1016/0584-8539(91)80179-M
13. Toru T, Shigeo F, Nobuhiko K (1983) Aggregation of methyl orange homologs carrying long alkyl groups in aqueous solution and their binding behaviors to poly(vinylpyrrolidone). J Coll Inter Sci 94:114–122. doi:10.1016/0021-9797(83)90240-0
14. Ahmad B (2004) Interaction of poly (N-vinyl 2-pyrrolidone) with a bisazo dye in aqueous solution. J Chem Soc Pak 26:349–354
15. Liu HP, Wang XM, Li HB, Hu YM (2006) Study on the reaction of bromophenol blue with polyvinlpyrrolidone. J Xingyang Normal Univ (NatSci) 19:174–177
16. Zhou GD (2003) Dictionary of chemistry. Chemical Industry, Beijing, p 654
17. Yang YL, Xu GY, Cheng AM, Yu H, Sui H (2000) The formation of HPAM/ PVP supramolecular complexes. J Shandong Univ 35:315–320
18. Li NW, Zhu Q (2007) Soaping process of reactive dying with PVP. Prog Textile Sci Technol 2:47–48
19. Robet BL (1996) The surface acting agent with pyrolidone group (I). Detergent Cosmetics 1:40–41
20. Cao WL, Zhang KH, Zhang JC (2002) Two important influential factors to the preparation and structure characterization of surface-capped CdS nanocrystals. Chin J Inorg Chem 997–102
21. Ping ZH, Yie JF, Ding YD, Hu XH, Ding ZM (1997) Investigation of polymer blends compatibility by FTIR. J Fudan Univ Nat Sci 36:439–444
22. Long YS, Kang Z, Liao LW, Cui YD (1999) Properties and determination of polyvinylpyrrolidone. Guangzhou Chem Ind 27:33–35
23. Cheng XD, Wang XB (2004) Fluorescence spectra of poly (vinylpyrrolydone) in aqueous solution. Acta Sci Nat Univ Sunyatseni (NatSci) 43:54–57
24. Li GZ, Sui WP, Xu GY, Tang JA, Jiang L (1995) Study on the interaction of poly (vinylpyrrolydone) and surface acting agent by some methods like brewster angle microscope. Chin Sci Bull 20:2248–2252
25. Gao GL, Fang Y, Zhu XH, Liu SX, Cui YL (2002) Fluorescence studies of the conformational behavior of poly (vinylpyrrolydone) in dilute aqueous solution. Chem J Chin Univ 23:2177–2181
26. Yu GY, Sui WP, Li GZ (1997) The interaction between PVP and C_{14}BE. Acta Chimi Sin 55:1179–1184