Pharmaceutical Chemistry Journal, Vol. 47, No. 10, January, 2014 (Russian Original Vol. 47, No. 10, September, 2013)

QUANTITATIVE DETERMINATION IN AQUEOUS SOLUTIONS OF PHYSIOLOGICALLY ACTIVE COPOLYMERS OF *N*-VINYLPYRROLIDONE AND 2-METHYL-5-VINYLPYRIDINE

S. A. Kedik,[1] A. V. Panov,[1] I. V. Sakaeva,[2] Yu. V. Kochkina (Cherta),[1] D. V. Eremin,[1] V. V. Suslov,[1] and M. A. Zaitsev[3]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 47, No. 10, pp. 54 – 56, October, 2013.

Original article submitted November 9, 2011.

Techniques for quantitative determination of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymers in aqueous solutions were developed. The spectrophotometric technique allowed the copolymer concentration in dilute solutions (0.8 – 1.2 mg/mL) to be determined even in the presence of the interfering additive merthiolate. The refractometric technique was simpler and allowed the copolymer concentration in concentrated solutions (> 1 mass%) to be determined.

Keywords: quantitative determination, *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer, aqueous solutions.

We reported previously [1] on the synthesis of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymers and established their molecular-weight characteristics. It was found that copolymers containing up to 25 ± 5 mol% 2-methyl-5-vinylpyridine were very soluble in H_2O. The issue of quantitative determination of the copolymer in aqueous solutions became especially important because the synthesized copolymers were intended for use as physiologically active preparations. Recalling that the copolymer concentration in the preparations is usually low (at the level of 0.1 mass%), highly accurate techniques using modern physicochemical equipment should be utilized. Such methods include photometric [2] and refractometric [3] ones. Therefore, the present work aimed to develop a technique for quantitative determination of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymers in aqueous solutions.

EXPERIMENTAL PART

N-Vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymers were synthesized by radical copolymerization while maintaining a constant ratio of monomers in the reaction mixture [1]. The synthesis conditions were described in detail [1]. The products isolated after the synthesis were amorphous white powders with a specific aroma. Copolymers that were very soluble in H_2O (namely samples 1-4, the molecular weights and ratios of monomer units of which were published before [1]) were used for the investigations. Merthiolate (sodium ethylmercurythiosalicylate, thiomersal, Gihon, Argentina) was used as one of the aqueous solution components.

The copolymer concentrations in the aqueous solutions were determined by photometry and refractometry. Absorption spectra of copolymer solutions were recorded on an SF-104 spectrophotometer in quartz 1-cm cuvettes in order to perform the first technique. Absorption of purified H_2O (FS 42-2619-97) was taken as the baseline. The copolymer was dried to constant mass at 100-105°C in order to prepare the solutions. Then, an accurate weight was used to prepare an aqueous solution of concentration 100 mg/mL. This solution was diluted with purified H_2O to concentrations 0.005 – 0.030 mg/mL in steps of 0.005 mg/mL in order to prepare a series of solutions. The index of refraction (n_D) was determined on a URL-1 refractometer with a thermostatted prism unit by the traditional method [5] at constant temperature 25.0 ± 0.1°C in order to perform the second technique. Samples were thermostatted at 25°C for at least 15 min. The arithmetic average of two parallel measurements was taken

[1] Institute of Pharmaceutical Technologies, Moscow, 121353 Russia; E-mail: alpha602@mail.ru.
[2] State Scientific Center for Drug Expertise and Control, Ministry of the Russian Federation for Public Health, Moscow, 127051 Russia.
[3] Space Research Institute, Russian Academy of Sciences, 117997 Moscow.

as the index of refraction. The allowed discrepancy between these was less than 0.0002.

RESULTS AND DISCUSSION

Heterocyclic compounds containing pyridine are known to absorb strongly in the UV wavelength range. This allows quantitative determination of the content of 2-methyl-5-vinylpyridine copolymer units in solution even if its concentration is relatively low.

Figure 1 shows typical absorption spectra in the range 190 – 300 nm of aqueous solutions of the copolymer of various concentrations. The copolymer concentration was selected during preliminary experiments so that the solution optical density would be less than 0.5, i.e., obeyed the Lambert–Bouguer–Beer law [6]:

$$D_\lambda = \varepsilon_\lambda \times C \times l, \quad (1)$$

where D_λ is the optical density at wavelength λ of a solution of the substance; ε_λ, the molar absorption (extinction) coefficient of the substance at wavelength λ [L/(mol · cm)]; C, the molar concentration of the substance (mol/L); and l, the thickness of the solution absorbing layer (cm).

It was more convenient for our problem to use units of specific absorption and solution concentration related not to the amount but to the mass of substance (e.g., mg) and a defined thickness of absorbing layer (e.g., 1 cm). Then, the expression for the solution concentration would be:

$$X = \frac{D_\lambda}{E_\lambda}, \quad (2)$$

where X is the substance concentration (mg/mL of solution) and E_λ, the specific absorption (extinction) coefficient of the substance at wavelength λ [mL/(mg × cm)].

Figure 1 shows that the absorption maximum for all solutions was observed at 269 nm, which was selected as the characteristic value for determining the copolymer concentration of the aqueous solutions. Based on Eq. (2), the specific absorption coefficient E_{269} should be determined beforehand. For this, the optical density D_{269} was determined for solutions of four copolymer samples of known concentration. The average E_{269} = 12.5 mL/(mg · cm) was calculated from the slope of the linear concentration dependences D_{269} (Fig. 2, line through points 1 – 4).

A technique for quantitative determination of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer in aqueous solutions was developed by using the results. According to it, an aqueous solution (1.00 mL) of unknown concentration is placed into a 50-mL volumetric flask, adjusted to the mark with purified H_2O, and stirred. The optical density of the resulting solution is determined at the absorption maximum (269 nm) in a 1-cm quartz cuvette using purified H_2O as the reference solution. The copolymer content

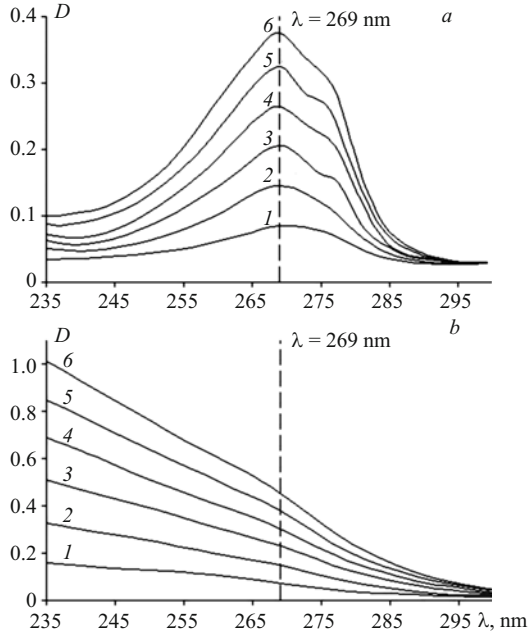

Fig. 1. Absorption spectra of aqueous solutions of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer (a) and merthiolate (b). Aqueous solution concentration of copolymer or merthiolate (mg/mL): 0.005 (*1*), 0.010 (*2*), 0.015 (*3*), 0.020 (*4*), 0.025 (*5*), 0.030 (*6*). Dashed lines denote characteristic wavelength.

(X_c) in mg per mL of preparation is calculated using the formula:

$$X_c = \frac{D_{269} \times V_1}{E_{269} \times V_2}, \quad (3)$$

where D_{269} is the optical density of the test solution at 269 nm that is measured relative to the reference solution; E_{269}, the specific absorption coefficient of aqueous solutions of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer (E_{269} = 12.5 mL/mg · cm); V_1, the dilution volume (50 mL); and V_2, the preparation solution volume (1 mL).

The recommended range of copolymer aqueous solution concentrations is 0.8 – 1.2 mg/mL.

A specific version of the proposed technique is quantitative determination of the copolymer in the presence of interfering components in the aqueous solutions. Merthiolate, which is used as a preservative in many vaccines, is one such compound. Figure 1*b* shows that merthiolate absorbs at λ = 269 nm, i.e., in the same region as the copolymer. This fact makes it necessary to determine the specific absorption coefficient of aqueous solutions of merthiolate (E^{m}_{269}). The quantity E^{m}_{269} was calculated from the slope of linear concentration dependences D_{269} for solutions with a known merthiolate concentration (Fig. 2, line *5*) and was E^{m}_{269} = 15.3 mL/mg · cm.

Quantitative determination of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer in aqueous solutions in the presence of merthiolate was carried out by the technique

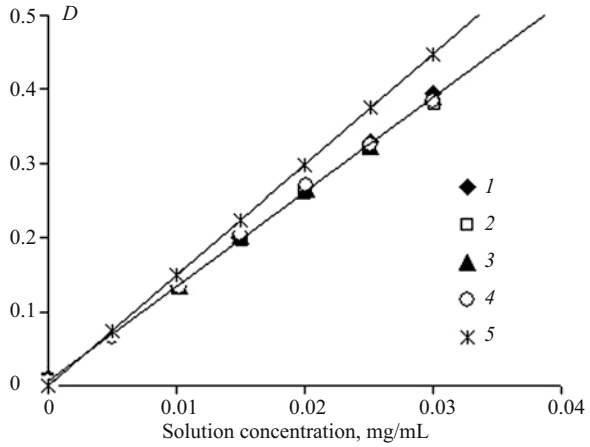

Fig. 2. Optical density at 269 nm of aqueous solutions of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer (*1* – *4*) and merthiolate (*5*) as functions of concentration. Copolymer sample numbers from the literature [1] correspond to numbers of points on the plot.

described above. However, the copolymer content (X_c^m) in mg per mL of preparation was calculated using a different formula:

$$X_c^m = \frac{D_{269}V_1 - Y_m E_{269}^m}{E_{269}V_2}, \quad (4)$$

where D_{269} is the optical density of the test solution at 269 nm that is measured relative to the reference solution; E_{269}, the specific absorption coefficient of aqueous solutions of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer (E_{269} = 12.5 mL/mg · cm); E_{269}^m, the specific absorption coefficient of aqueous solutions of merthiolate (E_{269}^m = 15.3 mL/mg · cm); Y_m, the merthiolate concentration obtained from a standard spectrophotometric technique for its quantitative determination (FS 42-3874-99) (mg/mL); V_1, the dilution volume (50 mL); and V_2, the preparation solution volume (1 mL).

The recommended range of copolymer aqueous solution concentrations is the same as that without merthiolate (0.8 – 1.2 mg/mL).

Refractometry is a simpler method for quantitative determination of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer in aqueous solutions that is effective at higher concentrations. Investigations showed that the index of refraction of copolymer aqueous solutions was a linear function of concentration at constant temperature (Fig. 3). The concentration increment of the index of refraction per 1 mass% at 25°C was 0.00208. Based on these data, a refractometric technique for quantitative determination of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer in aqueous solutions was developed. According to it, a solution for the investigations is first prepared by transferring quantitatively an accurate weight of solution (1.25 g) into a 25-mL volumetric flask, adding purified H_2O (15 mL) and

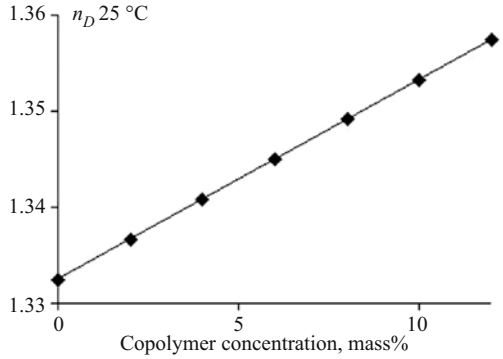

Fig. 3. Index of refraction of aqueous solutions of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer at 25°C as a function of concentration.

HOAc (0.5 mL, 1 M, to improve solubility), and dissolving the preparation with vigorous stirring. Then, the volume is adjusted to the mark with purified H_2O and stirred. The reference solution is prepared in parallel by adding HOAc (0.5 mL, 1M) to a 25-mL volumetric flask, adjusting the volume to the mark with purified H_2O, and stirring. The copolymer content in the solution (Y_c, mass%) is calculated using the formula:

$$Y_c = \frac{(n_D^{25} - n_{D,0}^{25})}{F}, \quad (5)$$

where n_D^{25} and $n_{D,0}^{25}$ are the indices of refraction of the copolymer and reference solutions at 25°C, respectively; *F*, the index of refraction increment of copolymer solution upon increasing the concentration by 1 mass% (*F* = 0.00208).

The recommended range of copolymer aqueous solution concentrations is > 1 mass%.

Thus, photometric and refractometric techniques are acceptable for quantitative determination of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine copolymer in aqueous solutions. The developed techniques can determine the copolymer content in concentrated (1 mass%) solutions (refractometry) and in solutions with lower concentrations (0.8 – 1.2 mg/mL, spectrometry) including in the presence of the interfering additive merthiolate.

REFERENCES

1. S. A. Kedik, A. V. Panov, I. V. Sakaeva, et al., *Khim.-farm. Zh.*, **46**(8), 19 – 22 (2012).
2. M. I. Bulatov and I. P. Kalinkin, *Practical Handbook of Photocolorimetric and Spectrophotometric Analytical Methods* [in Russian], Khimiya, Leningrad (1976).
3. B. V. Ioffe, *Refractometric Methods in Chemistry* [in Russian], Khimiya, Leningrad (1974).
4. E. K. Fedorov, O. E. Lobanov, L. F. Mosalova, et al., *Vysokomol. Soedin., Ser. A*, **36**(9), 1446 – 1451 (1994).
5. RF State Pharmacopoeia, XIIth Ed., Part 1, Scientific Center for Drug Expertise, Moscow (2008).
6. L. I. Tarutina and F. O. Pozdnyakova, *Spectral Analysis of Polymers* [in Russian], Khimiya, Leningrad (1986).