Investigation of the Microprecipitation of Folic Acid from Slightly Acidic Injection Solutions Using Dynamic Light Scattering

Sándor KÉKI,[a] Tamás SIPOS,[b] György DEÁK,[a] Géza MEZEY,[b] and Miklós ZSUGA*,[a]

Department of Applied Chemistry,[a] and Department of Pharmaceutical Technology,[b] Lajos Kossuth University, H-4010 Debrecen, Egyetem tér 1., Hungary. Received February 4, 1998; accepted April 27, 1998

Microprecipitation of folic acid was monitored by the dynamic light scattering (DLS) method. The pH was changed by CO_2 absorption and addition of HCl. The folic acid solutions were examined over the pH range 8.5—4.0. Over the pH range 5.4—6.0, microprecipitation was observed and at pH $\leq$5.2 crude precipitation of folic acid occurred. The dependence of the effective diameter on the pH over the pH range 5.4—6.0 is also reported.

Key words dynamic light scattering; folic acid injection; folic acid precipitation; microprecipitation

Administration of folic acid is indicated in malabsorption, during pregnancy and nursing, as well as in therapy associated with considerable loss of folic acid, *e.g.*, in artificial kidney dialysis. Folic acid can also be used as an antidote to methotrexate.[1)]

The injections prepared according to the USP XXIII method are basic solutions (pH 9.2 in the case of NaOH, pH 8.2 in the case of $NaHCO_3$)[2,3)] and, thus, their stability is not appropriate (hydrolytic decomposition). Neutralisation increases the stability of folic acid injections, making them more tolerable physiologically.[4)]

A crucial part of the preparation is saturation with CO_2, since oversaturation may cause the precipitation of folic acid due to pH-lowering.[5)] This is usually visible to the naked eye, but, sometimes, the seemingly clear solution contains particles. According to literature data,[6)] precipitation begins at pH 4.5—5.

The solutions for parenteral applications must not contain particles 4 μm in diameter or larger,[7)] *i.e.*, the size of red blood cells, because the larger particles may cause blockage of capillaries, or react with the cellular or humoral immune system. Thus, the consequence of their administration may be a cerebral and/or pulmonary embolus, initiation of thrombotic process, granulomatosis, macrophage reactions, phlebitis and free radical reactions. The particles are also believed to play a role in the development of acute respiratory distress syndrome. They can also affect the determination of pharmaceutical parameters.

Our primary goal was to establish the critical, lowest pH to avoid precipitation of folic acid. Microprecipitation of folic acid was monitored by dynamic light scattering (DLS).

Experimental

Sample Preparation for Light Scattering Measurements 1.5 g Folic acid (Takeda, Japan) and 2.25 g $NaHCO_3$ were dissolved in 100 ml double-distilled water by gentle warming to 35 °C, and the alkaline solution obtained was neutralized by CO_2 absorption. To reduce the pH, a 0.1 mole/dm^3 HCl-solution was added to the folic acid solution and the process was carefully checked by measuring the pH. Loading ampules was carried out in a CO_2 atmosphere.[6)] With this method a chemically stable, physiologically tolerable, sterilizable product was obtained. Prior to the light scattering measurements, the samples were passed through a 1 μm filter (Acrodisc CR PTFE, GELMAN, U.S.A.). The viscosity of the solution was determined with an Ostwald-type viscosimeter. There was no significant dependence of the viscosity on the pH of the folic acid solutions. The difference in viscosity of the double-distilled water and folic acid solutions was within 3%, thus $\eta = 0.9$ cP was used in the calculations.

Instrument The samples were examined by DLS performed on a Brookhaven light scattering instrument equipped with a BI-9000 digital correlator (Brookhaven Instruments Corp., U.S.A.). The light source was a vertically polarized Ar-laser operating at 488 nm. All measurements were performed at 25 °C.

Theoretical Background of Measurements and Data Analysis The autocorrelation function of the scattered light intensity[8)] was acquired in the homodyne mode. The normalized autocorrelation function is:

$$C(\tau) = [\langle I(0)I(\tau)\rangle - \langle I\rangle^2]/\langle I\rangle^2 \quad (1)$$

where at long delay times (τ) all correlation is lost, and the measured autocorrelation function is equal to the square of the average intensity ($\langle I\rangle^2$). In our calculations a measured baseline of four baseline channels was used for normalization.

In the simplest case (monodisperse, compact, rigid particle):

$$C(\tau) = A + Be^{-2\Gamma\tau} \quad (2)$$

where $\Gamma = 1/\tau_c$, and τ_c is the characteristic decay time of the autocorrelation function. Γ can be determined by a single exponential fit, and contains information on the temporal fluctuations of the concentration, which are related to the motion of the particles. So the diffusion coefficient is:

$$D = \Gamma/q^2 \quad (3)$$

where q is the magnitude of the scattering vector, which can be specified by the Bragg condition:

$$q = 4\pi n/\lambda_i \sin(\Theta/2) \quad (4)$$

where n is the refractive index of the solvent, λ_i is the wavelength of the incident light and Θ is the scattering angle. Under certain conditions (Newtonian fluid, spherical particles), the Stokes–Einstein relation between the diffusion coefficient and the hydrodynamic diameter (d_H) of particle can be used to determine d_H:

$$D = \boldsymbol{k}T/(3\pi\eta d_H) \quad (5)$$

where $\boldsymbol{k}$ is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solvent.

In the case of polydisperse systems, the experimentally determined autocorrelation function can be expressed in a more complex way:

$$C(\tau) = \int G(\Gamma)e^{-\Gamma\tau}d\Gamma \quad (6)$$

where $G(\Gamma)$ represents the distribution of decay times owing to the distribution of sizes. The moments of decay time distribution (and particle size distribution) can be determined by a simple data analysis technique, by the method of cumulants:

$$\ln C(\tau) = -\Gamma_{avr}\tau + (\mu_2\tau^2)/2 \quad (7)$$

where Γ_{avr} and μ_2 are the first and the second moments of the distribution, respectively. From these moments the polydispersity index of the particle size distribution can be easily expressed:

$$\text{polydispersity} = \mu_2/\Gamma^2_{avg} \quad (8)$$

* To whom correspondence should be addressed.

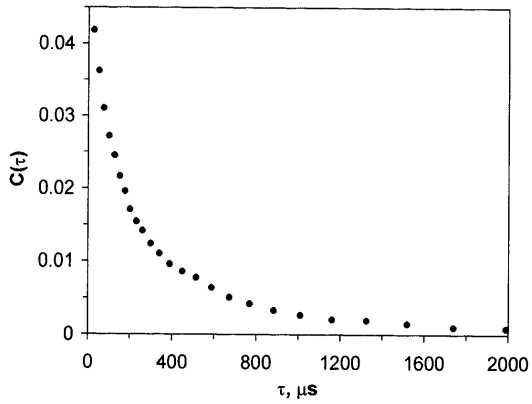

Fig. 1. A Typical Correlation Function Obtained for Folic Acid Solution at pH 5.8

Experimental conditions: $T=25\,^{\circ}C$, $\Theta=90^{\circ}$ and $\lambda=488\,nm$. Θ=scattering angle.

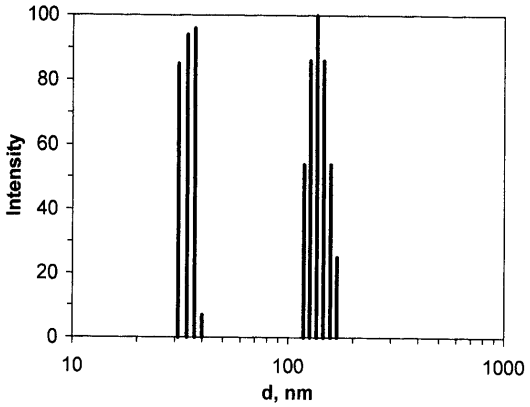

Fig. 2. The Particle Size Distribution of Folic Acid Solution at pH 5.8

Polydispersity=0.21. Experimental conditions: see Fig. 1.

For full particle-size distribution studies we used the NNLS (non-negative constraint least squares) calculation combined with multiple pass analysis.

Results and Discussion

In our studies, precipitation of folic acid *versus* pH was followed by the DLS, which has never before been reported in the literature. The pH was changed by CO_2 absorption and HCl addition. The investigated folic acid solutions seemed to be homogeneous at pH > 5.6 and slightly opaque over the pH range 5.4—5.6. These solutions were examined over the pH range 8.5—5.3 by the DLS. No particle size could be determined down to pH 6.0, indicating that the solutions did not contain particles larger than 5 nm in diameter. On reducing the pH to 5.8 and below, microprecipitation occurred. A typical correlation function of folic acid solution is shown on Fig. 1.

Analysis of the correlation function by NNLS yielded a bimodal particle size distribution with an effective diameter of 130 nm at pH 5.8. (Fig. 2.). The size distribution clearly demonstrated that the sample contained microcrystalline particles. By lowering the pH the intensity of the lower peaks, *i.e.*, 30—40 nm in size, tends to decrease with respect to the upper peaks.

The effective diameters were calculated by Eq. 5., *i.e.*, the effective diameters are average values. The effective diameter *versus* pH plot of folic acid solutions is shown on Fig. 3. As demonstrated by Fig. 3, the effective diameter increases as the pH is reduced. At pH ≤ 5.2, crude precipitation with sedimentation occurred which not allowed the determination of particle size by DLS. A sudden increase in particle size can also be recognized on the effective diameter *versus* pH plot, showing that the folic acid solutions cannot be used due to their physical instability if the pH < 5.6. This plot also indicates that care should be taken to adjust the pH of folic acid solutions to avoid microprecipitation and crude precipitation due to the low buffer capacity of folic acid solutions.

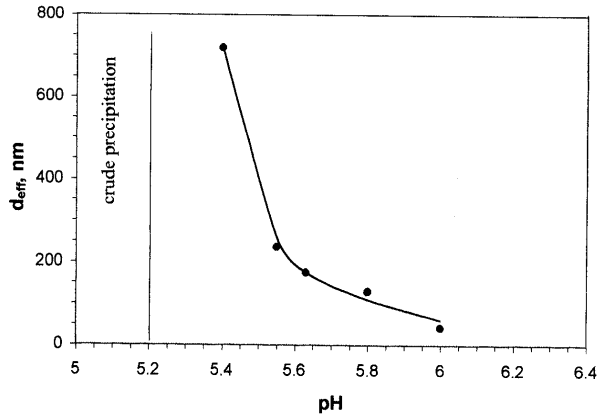

Fig. 3. The Effective Diameter *versus* pH Plot of the Folic Acid Solutions

Experimental conditions: see Fig. 1.

Conclusion

The microprecipatation of folic acid solutions as a function of pH was monitored by DLS. According to the results, microprecipitation of folic acid takes place over the pH range 5.4—6.0. DLS is a useful tool for monitoring the progress of precipitation (microcrystallization), and it is also capable of determining the size and size distribution of various samples in pharmaceutical technology. In addition, this methodology offers a very fast control of the size and size distribution of particles during processing.

Acknowledgments This work was financially supported by grants Nos. T 019508, T 025379 and T 025269 given by OTKA (National Found for Scientific Research Development, Hungary), and by grant No. FKFP 04441/1997.

References

1) Aslam M., Mezey G., "Drug Interactions," Springer Hungarica, Budapest, 1993.
2) The United States Pharmacopoeia XXIII, p. 692.
3) Martindale, The Extra Pharmacopoiea, 30th Edition, The Pharmaceutical Press, London, 1993, p. 1039.
4) Smith S. G., *The Pharm. J.*, **108**, 216 (1976).
5) Trissel L. A., "Handbook of Injectable Drugs," 7th Edition, Division of Pharmacy University of Texas M. D., Anderson Cancer Center Houston, Texas, 1992.
6) Mezey G., Török J., Abstact of Papers, Congressus Pharmaceuticus Hungaricus, p. 22; "A Novel Method for the Preparation of Folic Acid Injections and Investigation of Their Stability," Budapest, 1979.
7) Garvan J. M., Gunner B. W., Med. *J. Australia*, **2**, 1—6 (1964).
8) Schmitz K., "An Introduction to Dynamic Light Scattering by Macromolecules," Chapter 2, Academic Press Inc., San Diego, 1990, pp. 11—42.