International Journal of Pharmaceutics, 24 (1985) 27–41
Elsevier

IJP 00804

Investigation of the photochemical and thermal degradation of aqueous nitroprusside solutions using liquid chromatography

O.R. Leeuwenkamp *, E.J. van der Mark, W.P. van Bennekom and A. Bult

Department of Pharmaceutical Analysis and Analytical Chemistry, Subfaculty of Pharmacy, State University Leiden, Gorlaeus Laboratories, 2300 RA Leiden (The Netherlands)

(Received June 28th, 1984)
(Modified version received November 25th, 1984)
(Accepted December 3rd, 1984)

Summary

Nitroprusside is in aqueous solutions sensitive to light. A rapid ion-pair reversed phase chromatographic method was employed for selective determination of nitroprusside in photodegraded solutions.

Solutions of nitroprusside (50 μg/ml) in water, normal saline, 5% dextrose and autoclaved 5% dextrose were subjected to 350 nm light and to irradiation of normal daylight fluorescent tubes, respectively. Almost identical, non-linear decay curves were obtained for nitroprusside in these solutions in 350 nm light and under normal daylight fluorescent tubes, respectively. Addition of citric acid or disodium edetate (0.25 mg/100 ml) to non-sterilized and autoclaved 5% dextrose admixtures did not improve the stability of nitroprusside. A 10-fold concentration of citric acid promoted the degradation. By contrast the addition of cyanocobalamin (1 mg/100 ml) to 5% dextrose solutions resulted in a significantly slower rate of degradation, particularly upon exposure to 350 nm light because of the high molar absorptivity of cyanocobalamin at that wavelength.

The non-linearity of the nitroprusside decay curves, i.e. an initial rapid loss followed by a phase of slower decomposition, can be at least in part explained by the occurrence of a screening effect caused by the gradually developing yellow colour of the solutions due to formation of pentacyanoaquoferrate(III). Secondly, back-formation of nitroprusside from the degradation products pentacyanoaquoferrate(II) and nitrite comes into play. Regeneration of nitroprusside was found to be most effective in the pH range 3.5–5.5.

Correspondence: O.R. Leeuwenkamp, Department of Pharmaceutical Analysis and Analytical Chemistry, Subfaculty of Pharmacy, State University of Leiden, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

Finally the effect of autoclaving (15 min, 121°C) on nitroprusside solutions was studied with the chromatographic method. In water and normal saline essentially no thermal degradation of nitroprusside could be found after sterilization. However, substantial loss of nitroprusside (~ 40%) in 5% dextrose solutions was found upon autoclaving.

Introduction

Nitroprusside (disodium pentacyanonitrosylferrate(II) dihydrate) is a potent hypotensive drug with a rapid onset of action when administered by injection or infusion (Tinker and Michenfelder, 1976). It has long been recognized that nitroprusside is susceptible to photodegradation, particularly in solution (Overbeck, 1853; Kolthoff, 1923; Justin-Mueller, 1935; Baudisch, 1948). The photodecomposition — a disadvantage of its clinical use — has been subject of study in order to formulate more stable pharmaceutical preparations (Van Loenen and Hofs-Kemper, 1979; Schumacher, 1966; Anderson and Rae, 1972; Tol, 1976; Martin and Patel, 1969).

Upon exposure to light the absorbance at about 395 nm strongly increases, possibly due to formation of the pentacyanoaquoferrate(III) ion which exhibits a relatively high molar absorptivity ($\epsilon = 750\ M^{-1} \cdot cm^{-1}$) at that wavelength (Kenney et al., 1961; Espenson and Wolenuk, 1972; Toma, 1975). Based on the unproven assumptions that the unstable pentacyanoaquoferrate(III) species is the primary photodecomposition product and that its concentration is a reliable measure for the degree of photodegradation, the increase of the absorbance at 395 nm was used a criterium for the extent of decomposition (Van Loenen and Hofs-Kemper, 1979; Schumacher, 1966; Anderson and Rae, 1972; Tol, 1976; Martin and Patel, 1969). In addition, the ratio of the absorbances at 395 nm and 440 nm was used by Van Loenen and Hofs-Kemper (1979) to obtain an impression of the concentration of pentacyanoaquoferrate(II), which is possibly the primary photodecomposition product (Lodzinska and Gogolin, 1973; Frank et al., 1976; Mitra et al., 1979) and absorbs rather strongly at 440 nm ($\epsilon = 640\ M^{-1} \cdot cm^{-1}$). In view of the inadequacy of direct spectrophotometry to quantitate nitroprusside selectively in (photo-) degraded solutions, lack of consensus on the point of photostability is therefore not surprising. Vesey and Batistoni (1977) employed a colorimetric method based on the formation of a purple-coloured adduct in the reaction of sulphide with nitroprusside. Because of the transient nature of the developed colour the method is not very reliable. Furthermore, no insight is simultaneously obtained in the nature and fate of the photodecomposition products.

Recently, Leeuwenkamp et al. (1984) have developed an ion-pair reversed-phase chromatographic system for the separation of nitroprusside from the photodecomposition products, including hexacyanoferrate(III) which is one of the degradation products (Leeuwenkamp et al., 1984; Van Loenen and Hofs-Kemper, 1979). The chromatographic procedure described by Baaske et al. (1981) is not suitable because nitroprusside and hexacyanoferrate(III) are not resolved. Primary aim of

this study, using our chromatographic method, was to examine whether or not the additives, citric acid and disodium edetate, which are recommended in literature (Anderson and Rae, 1972; Tol, 1976; Van Loenen and Hofs-Kemper, 1979), improve stability of nitroprusside solutions. We were particularly interested in the photostability of infusion solutions (~ 50 μg/ml), since these preparations are susceptible to photodecomposition because of inevitable exposure to light during use in the clinic. Moreover, decomposition occurs at a much higher rate than in the more concentrated injection solutions (1 g/100 ml), as was already observed by Van Loenen and Hofs-Kemper (1979). Additionally, the influence of cyanocobalamin, proposed as a photoprotective agent (Tasuhara, 1982), was investigated.

Finally, the chromatographic method was also utilized in order to examine the effect of sterilization on various nitroprusside preparations. Effect of autoclaving has already been investigated by some authors (Anderson and Rae, 1972; Van Loenen and Hofs-Kemper, 1979) employing direct spectrophotometry.

Experimental

Reagents

Disodium pentacyanonitrosylferrate(II) dihydrate, potassium hexacyanoferrate(II) and (III), sodium nitrite, potassium nitrate, sodium chloride, glucose monohydrate (dextrose), citric acid, disodium edetate, cyanocobalamin, sodium bicarbonate and hydrochloric acid were all Merck analytical grade and used as received. Mixtures of pentacyanoaquoferrate (II) and (III) were prepared by dissolving solid trisodium pentacyanoamineferrate(II) trihydrate (Kenney et al., 1961) in a 0.01 M phosphate buffer (pH 5.0) resulting in a total concentration of $\sim 10^{-4}$ M (Toma, 1975). The resulting yellow colour of the obtained mixtures indicated the presence of pentacyanoaquoferrate(III), whereas formation of a purple colour upon addition of cyanamide pointed at the presence of the pentacyanoaquoferrate(II) species (Nieman et al., 1976).

For preparation of all solutions and eluent, deionized water obtained from a Milli-Q Purification System was used.

Chromatography

Apparatus

The liquid chromatograph consisted of a Constametric I solvent delivery system (Laboratory Data Control, Riviera Beach, FL, U.S.A.), a Rheodyne 7125 injection valve equipped with a fixed 20-μl loop and a PU 4020 LC multiwavelength detector (Pye Unicam) set at a detection wavelength of 220 nm (0.16 AUFS).

A Valco stainless-steel column (25 cm, 3.0 mm i.d.) was packed with 2.2 g μ-Bondapak phenyl-bonded 10 μm pellicular material (Waters Ass.) suspended in isopropanol utilizing the upflow pressurized slurry technique (Snyder and Kirkland, 1979). The packed column was flushed with 100 ml methanol at a pressure of about 350 bar (Haskel MCP-71 pump, HPLC Technology, Macclesfield, U.K.). Throughout

the experiments, a flow rate of 1.0 ml/min was maintained (pressure < 140 bar). The response of the UV detector was recorded with a flat bed Kipp BD-40 y-t recorder (10 mV f.s.d.) (Kipp, Delft, The Netherlands).

Mobile phase

The aqueous part of the eluent contained 0.01 M potassium dihydrogen phosphate (Merck analytical grade), 0.005 M of the ion-pairing agent tetrabutylammonium phosphate (Eastman Kodak) and 0.0011 M *n*-octylamine (Baker). By dropwise addition of 1 M potassium hydroxide (Merck analytical grade) the pH was adjusted to 7.0. Tetrabutylammonium phosphate (0.005 M) and *n*-octylamine (0.0011 M) were also added to the methanolic part of the mobile phase. The aqueous and methanolic part were mixed under stirring in the ratio 65 : 35; the resulting eluent was filtered through 0.45 μm membrane filter (Sartorius) and finally degassed in vacuo. The column was conditioned by recycling overnight of the mobile phase at a flow rate of 0.5 ml/min. Chromatography was performed at ambient temperature. Consequently, some day-to-day variations in the observed retention times occurred, necessitating frequent calibration of the chromatographic properties by injecting mixtures of nitroprusside and the possible degradation products, viz. nitrite, nitrate, hexacyanoferrate(II) and hexacyanoferrate(III). For the same reason mixtures of fresh in solution generated pentacyanoaquoferrate(II) and (III) (vide supra) were injected frequently. Furthermore, the retention times decreased slowly upon prolonged use of the liquid chromatographic system. The initial properties of the system could be restored by flushing with 50 : 50 methanol–water followed by methanol.

Photolysis and autoclaving experiments

All nitroprusside solutions were prepared immediately before the start of the experiments under subdued-light conditions and the pH of the solutions was determined according to the method of the USP Ed. XX for dextrose solutions. It is noteworthy that the ratio nitroprusside/adjuvant (citric acid or disodium edetate) is the same as in the injection formulation recommended by Van Loenen and Hofs-Kemper (1979), whereas the concentration of cyanocobalamin (1 mg/100 ml) was based on a ~30-fold absorbance at 350 nm and a ~10-fold absorbance at 395 nm with respect to nitroprusside. The two mentioned wavelengths are important for the following reasons: (1) photolysis experiments were inter alia conducted in 350 nm light; and (2) according to the MO diagrams of Manoharan and Gray (1965) and Golebiewski and Wasielewska (1980) an important photoactive band of nitroprusside is located at about 395 nm.

Nitroprusside solutions in clear Pyrex glass containers were subjected in a Rayonet Photochemical Reactor (Southern New England Ultraviolet, Middletown, CT, U.S.A.) to the photo-irradiation of two, diametrically positioned, 350 nm tubes or placed at a distance of 120 cm on a white sheet of paper under two 40 W daylight fluorescent tubes (Philips). At selected time intervals, samples were taken from the solution and analyzed immediately. The heights of the symmetric nitroprusside peaks were measured and related to the peak height for the unphotolyzed solution (t = 0 min, ~ 50 μg/ml) in order to calculate the percentage undegraded nitroprus-

side at the times of sampling. In our previous study concerning the assay design (Leeuwenkamp et al., 1984) linear calibration graphs were obtained for nitroprusside in the concentration range 10–100 μg/ml. At the level of 50 μg/ml a relative standard deviation (n = 5) of 0.54% was calculated. After taking the last sample, the pH was measured once again and the resulting colour was observed visually.

For the autoclaving experiments (15 min, 121°C) 100 ml glass infusion bottles, wrapped in aluminium foil and filled with 80 ml of the freshly prepared solutions, were used. Samples were withdrawn from the solutions before and after sterilization in order to calculate the percentage undegraded nitroprusside. Initial and end pH values were measured and furthermore the colours of the autoclaved solutions were observed visually.

Results and Discussion

Initially the effect of 350 nm light on nitroprusside (~ 50 μg/ml) dissolved in water and in an iso-osmotic sodium chloride solution was studied by means of the developed chromatographic method (Leeuwenkamp et al., 1984). In Fig. 1 typical chromatograms for nitroprusside in water (0, 22 and 55 min) are shown. The chromatograms for normal saline solutions of nitroprusside are very similar. Under the chromatographic conditions used the possible photodegradation products of nitroprusside, viz. nitrite and nitrate (Mitra et al., 1963; Buxton et al., 1969; Mitra et al., 1972; Wolfe and Swinehart, 1975; Mitra et al., 1979), hexacyanoferrate(II) (Van Loenen and Hofs-Kemper, 1979; Leeuwenkamp et al., 1984) and hexacyanoferrate(III) (Van Loenen and Hofs-Kemper, 1979; Leeuwenkamp et al., 1984) have retention times of 1.7, 4.1 and 4.8 min, while unaltered nitroprusside elutes at 6.0 min. Upon injection of a mixture of the relatively unstable penta-

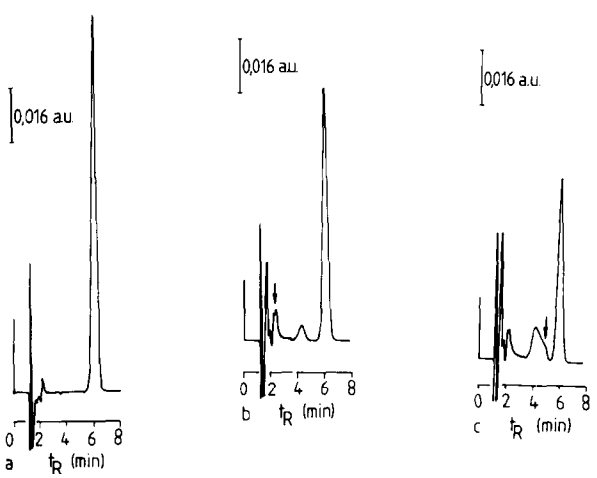

Fig. 1. Typical chromatograms for nitroprusside (50 μg/ml) in water (pH 5.5) upon photo-irradiation with 350 nm light during: (a) 0 min; (b) 22 min; (c) 55 min. Chromatographic conditions as given under Experimental.

cyanoaquoferrate(II) and pentacyanoaquoferrate(III) species, both generated in solution (see Experimental), a comparatively small chromatographic peak appears in the chromatogram at a retention time of 2.5 min. Cyanide, which is also a potential decomposition product (Wolfe and Swinehart, 1975; Frank et al., 1976; Vesey and Batistoni, 1977; Van Loenen and Hofs-Kemper, 1979; Bisset et al., 1981) is not observable because of the low absorptivity at 220 nm. In Table 1 the observed retention times of the potential photodegradation products are summarized together with the upper and lower limits for the individual retention times observed in the present study. Thus it can be stated that nitroprusside is separated from its photodecomposition products by the employed chromatographic system and consequently selective determination of nitroprusside in photolyzed solutions is achievable. Furthermore, simultaneously an impression of the formed degradation products during the course of photolysis is obtained. The following qualitative observations could be made on photoirradiation of the aqueous and normal saline nitroprusside solutions: (1) initial rapid decrease of the nitroprusside peak; (2) initial rapid increase of the peak at the retention time of nitrite and nitrate, and the pentacyanoaquoferrate species; (3) the peak at the retention time of the aquo species decreases slowly after $t = 20$ min; (4) hexacyanoferrate(II) and especially hexacyanoferrate(III) are formed in a later stage of the photodegradation process and both peaks increase gradually; and (5) at about $t = 20$ min the colourless solution becomes yellow and after 55 min the solution becomes turbid in contrast to solutions of nitroprusside in 5% dextrose.

For nitroprusside in 5% dextrose the same observations were made on the point of the peak at the retention time of the pentacyanoaquoferrate species during the course of photolysis. As can be seen in Fig. 2, this peak has decreased strongly after $t = 120$ min. The turbid aqueous and normal saline nitroprusside solutions obtained after $t = 55$ min, were not analyzed in order to avoid plugging of the column.

Primary aim of the present study was, however, the systematic evaluation of the stability of nitroprusside in various preparations at the dilution of infusion solutions (50–200 μg/ml).

For nitroprusside in water, in 0.9% sodium chloride, in 5% dextrose, and in 5%

TABLE 1

RETENTION TIMES (t_R) FOR NITROPRUSSIDE AND ITS OBSERVABLE PHOTODEGRADATION PRODUCTS

	t_R (min) [a]
nitrite/nitrate	1.7 (1.5–1.8)
pentacyanoaquoferrate(II)/(III)	2.5 (2.3–2.7)
hexacyanoferrate(II)	4.1 (4.0–5.2)3
hexacyanoferrate(III)	4.8 (4.8–5.8)
nitroprusside	6.0 (5.9–8.0)

[a] Between parentheses the lower and upper limits for the individual retention times, observed in the course of the present study, are given.

Fig. 2.

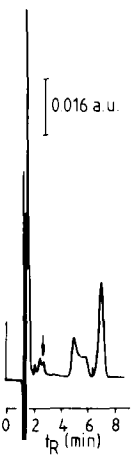

Fig. 3.

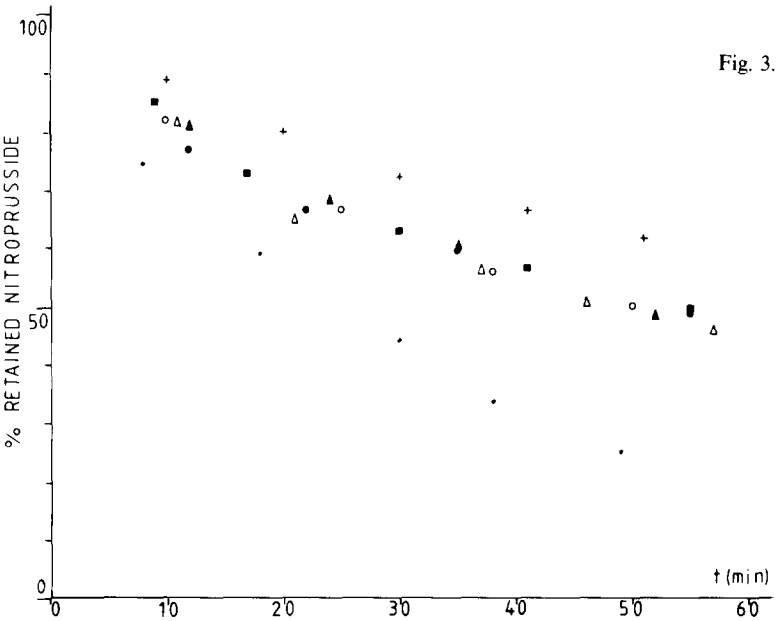

Fig. 2. Chromatogram for nitroprusside with an initial concentration of 50 μg/ml in 5% dextrose (pH 5.6) after 120 min photo-irradiation with 350 nm light.

Fig. 3. Decay curves (% vs time) for various nitroprusside solutions with an initial concentration of 50 μg/ml upon photo-irradiation with 350 nm light. ○, H_2O (5.6; 6.9); ●, 0.9% sodium chloride (5.6; 5.5); ■, 5% dextrose (5.6; 5.8); △, 5% dextrose containing 0.25 mg/100 ml citric acid (5.6; 5.0); ▲, 5% dextrose containing 0.25 mg/100 ml disodium edetate (6.0; 5.5); +, 5% dextrose containing 1 mg/100 ml cyanocobalamin (5.5; 6.3); ·, 5% dextrose adjusted to pH 3.0 with hydrochloric acid (3.0; 3.5). Between parentheses the initial and end pH values are given.

dextrose-containing citric acid or disodium edetate as adjuvant (0.25 mg/100 ml) very similar chromatograms and decay curves were obtained (Fig. 3). This indicates that sodium chloride, dextrose and the aforementioned adjuvants in the given concentration do not substantially affect the rate of decomposition and the nature of the products formed. Although citric acid and disodium edetate do not affect the stability of nitroprusside, a somewhat more delayed and less pronounced formation of hexacyanoferrate(III) (arrowed) was found (Fig. 4) in comparison to the solutions without these additives (Fig. 1c). Apparently, oxidation of pentacyanoaquoferrate(II) resulting in the pentacyanoaquoferrate(III) species, which is subsequently converted into hexacyanoferrate(III) due to replacement of coordinated water by cyanide, is inhibited by the adjuvants as a result of chelation of traces Fe(II) ions which act as powerful catalysts in the oxidation (Toma, 1975). No observable hexacyanoferrate(III) (arrowed) is produced as shown in Fig. 4, when citric acid or disodium edetate is present in a concentration of 2.5 mg/100 ml supporting the above-delineated role of these substances in the degradation process. Moreover, the peak at the retention time of the pentacyanoaquoferrate species rapidly diminishes from t = 25 min in contrast to the solutions containing 0.25 mg/100 ml additive.

Fig. 4.

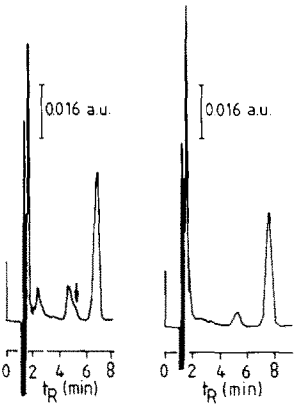

Fig. 5.

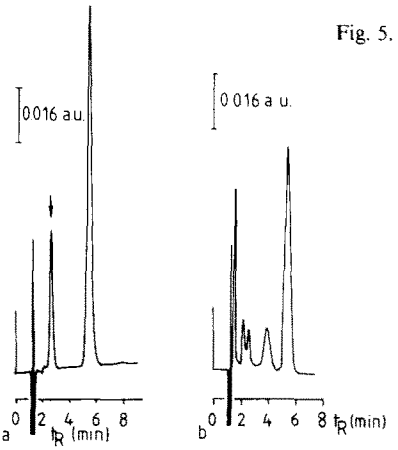

Fig. 4. Typical chromatograms for nitroprusside with an initial concentration of 50 μg/ml in 5% dextrose containing 0.25 mg/100 ml citric acid (350 nm, t = 58 min) and 2.5 mg/100 ml citric acid (350 nm, t = 58 min), respectively.

Fig. 5. Typical chromatogram for nitroprusside with an initial concentration of 50 μg/ml in 5% dextrose containing 1 mg/100 ml cyanocobalamin upon photo-irradiation with 350 nm light for: (a) 0 min; (b) 10 min.

This might be the result of replacement of ligands in the rather unstable pentacyanoaquoferrate species under photo-irradiation conditions by citrate or edetate which are powerful complexing agents with respect to iron (Sillen and Martell, 1980). In addition, the observation was made that the solutions turn rapidly blue most probably due to Prussian Blue formation and that nitroprusside degrades at a faster rate in the presence of 2.5 mg/100 ml citric acid. In conclusion, the additives, citric acid and disodium edetate, do not improve the photostability of nitroprusside solutions. On the contrary, in a concentration of 1 mg/100 ml (see Experimental) cyanocobalamin, proposed as a photoprotective agent by Tatsuhara (1982), exerts a significant stabilizing effect on the degradation upon exposure to 350 nm light because of the high absorptivity of cyanocobalamin at that wavelength ($\epsilon = 1.4 \times 10^4$ $M^{-1} \cdot cm^{-1}$) (Kirschbaum, 1981) in comparison to nitroprusside ($\epsilon = 40$ $M^{-1} \cdot cm^{-1}$). In summary, only the addition of cyanocobalamin is advantageous. In Fig. 5 typical chromatograms for nitroprusside in 5% dextrose containing 1 mg/100 ml cyanocobalamin (arrowed) are shown. It is obvious that cyanocobalamin, which is photosensitive (Kirschbaum, 1981), degrades rapidly, under formation of aquocobalamin and solutes slightly earlier than hexacyanoferrate(II). At t = 51 min about 20% of the initial amount of cyanocobalamin is left.

After about 20 min of photo-irradiation all solutions, not containing cyanocobalamin, become yellow due to formation of the yellow pentacyanoaquoferrate(III) ion. Since this species exhibits in comparison to nitroprusside a high molar absorptivity ($\epsilon = 600$ $M^{-1} \cdot cm^{-1}$) at 350 nm, inhibition of the photodecomposition due to a screening effect is reasonable. Furthermore, backformation of nitroprusside from

the photodegradation products nitrite and pentacyanoaquoferrate(II) according to:

$$Fe(CN)_5H_2O^{3-} + NO_2^- + 2H^+ \rightarrow Fe(CN)_5NO^{2-} + 2H_2O$$

was also considered as a possible cause for the observed non-linearity of the nitroprusside decay curves (Fig. 3). Mitra et al. (1979) suggested the occurrence of nitroprusside backformation via the above-given reaction in order to explain reversibility of the pH decrease after placing photolyzed solutions in the dark. Davies and Garafalo (1976) reported formation of nitroprusside from nitrite and pentacyanoaquoferrate(II) at pH < 9. The possibility of backformation was examined by adding nitrite ($\sim 10^{-4}$ M) to a 10^{-4} M mixture of pentacyanoaquoferrate(II) and (III) and analyzing the reaction mixture chromatographically after 10 min. A peak with a height corresponding to 3.7×10^{-5} M nitroprusside ($\sim 40\%$ backformation) was observed at the retention time of nitroprusside (Fig. 6), while upon addition of a sodium sulphide solution a purple colour developed characteristic for nitroprusside (Swinehart, 1967). Evidently, a substantial regeneration of nitroprusside possibly in combination with a screening effect due to presence of pentacyanoaquoferrate(III) accounts for the observed non-linearity of the nitroprusside decay curves. The decrease in the rate of disappearance is accompanied by a levelling off of the nitrite/nitrate peak in the chromatograms, which is directly related to the cleavage of the nitrosyl moiety in the first step of photodecomposition.

In view of the proton-dependent nature of the reaction between nitrite and pentacyanoaquoferrate(II) regeneration of nitroprusside in relation to pH of the pentacyanoaquoferrates/nitrite solution was also investigated. At pH 3.0 a negligi-

Fig. 6.

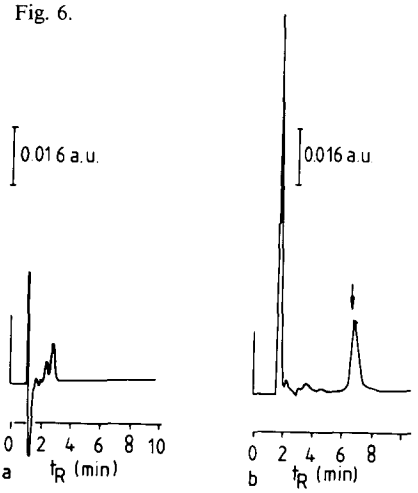

Fig. 7.

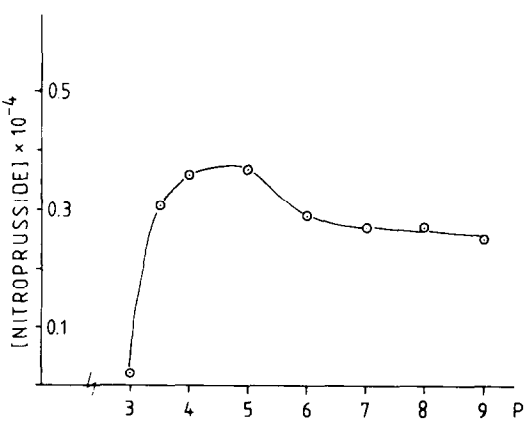

Fig. 6. Typical chromatograms for 10^{-4} M mixture of pentacyanoaquoferrate(II) and (III) containing 10^{-4} M nitrite: (a) 0 min; (b) 10 min.

Fig. 7. pH-dependence of the nitroprusside backformation (0.01 M phosphate buffers).

ble formation of nitroprusside was observed in contrast to pH $\geq$ 3.5 solutions (Fig. 7). A very similar relationship was reported for the second-order rate constant in the reaction between pentacyanoaquoferrate(II) and thioureum (Macartney and McAuley, 1981). According to Malin and Koch (1978), the low reactivity at pH $\leq$ 3 can be ascribed to monoprotonated pentacyanoaquoferrate(II) (pK $\cong$ 2.7). Because of the absence of nitroprusside regeneration at pH $\leq$ 3.0, decay of nitroprusside in a 10^{-3} M hydrochloric acid solution was studied. A more rapid decline of the amount of nitroprusside (Fig. 3) was found in combination with a green/yellow coloured (t = 58 min) solution. This represents evidence for occurrence of backformation in the other solutions (pH $\cong$ 5.5). In addition the stability in a 5% dextrose solution adjusted with 10^{-2} M sodium bicarbonate to pH 7.4 was examined: the resulting decay curve did not deviate considerably from the curve for 5% dextrose (pH 5.5). However, far more hexacyanoferrate(II) is formed in the pH 7.4 solution (Fig. 8). This finding is in agreement with the observation of Tol (1976). Most probably cyanide evolves to a smaller extent from the pH 7.4 5% dextrose solution and therefore more hexacyanoferrate(II) is formed. Furthermore it is striking that Tol recommended a pH value between 3 and 5 for nitroprusside injection solutions. The improved stability at these pH values is explainable in terms of the already described pH-dependent regeneration of nitroprusside. Since the backformation of nitroprus-

Fig. 8.

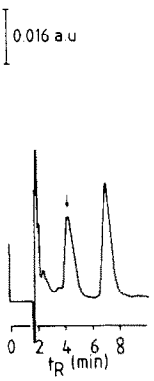

Fig. 8. Typical chromatogram for nitroprusside with an initial concentration of 50 μg/ml in 5% dextrose adjusted to pH 7.4 with 10^{-2} M sodium bicarbonate and photo-irradiated for 55 min with 350 nm light. End pH 6.75.

Fig. 9.

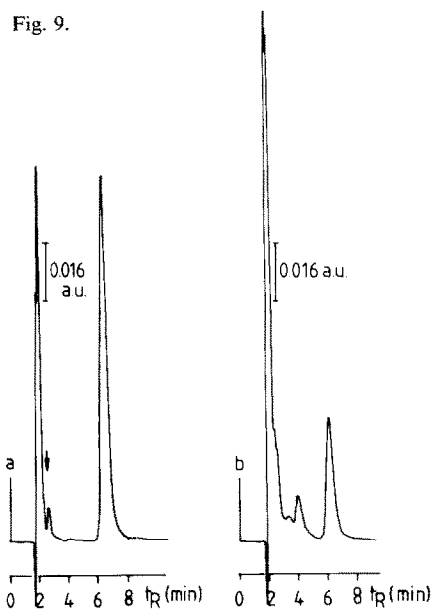

Fig. 9. Chromatograms for nitroprusside with an initial concentration of 50 μg/ml in autoclaved 5% dextrose upon photo-irradiation with 350 nm light for: (a) 0 min; (b) 50 min.

side is optimal in the pH range 3.5–5.5 (Fig. 7), we recommend for nitroprusside solutions a pH value between 3.5 and 5.5.

Additionally, the stability of nitroprusside added to (prior) autoclaved 5% dextrose solutions in absence or presence of additives was investigated. The autoclaved 5% dextrose solutions used meet the specifications of the USP Ed. XX on the point of absorptivity at $\lambda = 284$ nm and the pH. For nitroprusside in sterilized 5% dextrose ($E_{284nm} = 0.126$, pH 4.6) almost identical decay curves were found as for the drug in the corresponding non-autoclaved solutions (pH 5.5 or adjusted to pH 4.6). However, the solutions become green at about t = 10 min (20% decomposition) and subsequently blue due to Prussian Blue formation. The adjuvants with exception of cyanocobalamin were again ineffective. Obviously, the thermal degradation product of glucose 5-hydroxymethylfurfural and its acidic oxidation products (Durham et al., 1982) do not exert significant influence on the rate of photodegradation. In Fig. 9 a typical chromatogram for nitroprusside added to an autoclaved 5% dextrose admixture is shown. The higher degree of degradation at t = 50 min in comparison to photodegradation experiments concerning nitroprusside added to non-sterilized 5% dextrose solutions at an earlier stage of the present study is related to a replacement of one of the tubes in the Rayonet light chamber. The 5-hydroxymethylfurfural peak, which is higher at a detection wavelength of 254 nm, is arrowed in Fig. 9.

Not only the effect of 350 nm light was studied, but also the decomposition of nitroprusside under normal daylight fluorescent tubes. In general the same observations were made as upon exposure to 350 nm light. However, a much slower degradation occurred due to a considerable lower light intensity. In Table 2 the data for several nitroprusside solutions (t = 25 h) are summarized. At about t = 20 h the decrease of the nitroprusside concentration has completely levelled off for the same reasons as already discussed. Identical results ($\pm 2\%$) were obtained for nitroprusside added to autoclaved 5% dextrose solutions.

Surprisingly, in all cases with the exception of the cyanocobalamin-containing solutions, green/yellow solutions were produced. The nature of the primary photodegradation products depends on the wavelength (Golebiewski and Wasielewska, 1980; Jarzynowski et al., 1981) and possibly on the intensity of the incident light.

TABLE 2

DATA FOR NITROPRUSSIDE SOLUTIONS WITH AN INITIAL CONCENTRATION OF 50 μg/ml UNDER NORMAL DAYLIGHT FLUORESCENT TUBES (t = 25 h)

Solution	Initial pH	End pH	% nitroprusside	Observed colour
0.9% sodium chloride	5.5	5.0	65	green/yellow
5% dextrose	5.6	5.3	62	green/yellow
5% dextrose containing 0.25 mg/100 ml citric acid	4.9	4.9	62	green/yellow
5% dextrose containing 1 mg/100 mg cyanocobalamin	5.5	5.2	67	violet/yellow

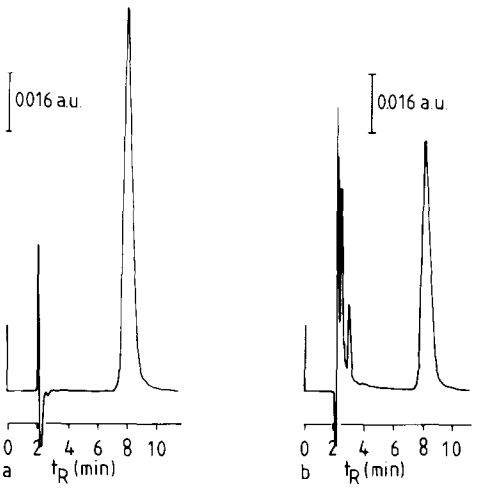

Fig. 10. Typical chromatograms for nitroprusside with an initial concentration of 50 μg/ml autoclaved (15 min, 121°C) in water and in 5% dextrose, respectively.

Furthermore, this might also be the case for the consecutive reactions. In Table 2 the resulting colours and initial as well as end pH values are given.

Finally, the chromatographic method was employed for analysis of autoclaved (15 min, 121°C) nitroprusside solutions. In Fig. 10 typical chromatograms are shown. Only the solutions without dextrose appeared to be autoclavable. In the case of nitroprusside in 5% dextrose admixtures a loss of about 40% was found. A slight improvement was obtained when the solutions were purged for 20 min with nitrogen immediately before sterilization or when the solution was acidified to pH 3.0. The latter observation points at involvement of thermal decomposition products of dextrose in the degradation process of nitroprusside, since at low pH values production of the thermal degradation products is limited (Martindale, 1977). The observed thermal degradation of nitroprusside in 5% dextrose solutions can be explained in two ways. In the first place it is known from literature that nitroprus-

TABLE 3

DATA FOR NITROPRUSSIDE SOLUTIONS WITH AN INITIAL CONCENTRATION OF 50 μg/ml AFTER AUTOCLAVING (15 min, 121°C)

Solution	Initial pH	End pH	% nitro-prusside
in water (50 μg/ml)	5.5	6.4	100
0.9% sodium chloride	5.5	6.8	100
aqueous nitroprusside solution containing 0.25 mg/100 ml citric acid	5.2	5.0	99
5% dextrose	5.2	3.9	59
5% dextrose, pH 3.0	3.0	3.1	72

side possesses the ability to react with a wide variety of aldehydes leading to unstable intermediates (Swinehart, 1967). Secondly, reduction of nitroprusside by the aldehydes resulting in unstable reduced nitroprusside under autoclaving conditions might occur (Swinehart, 1967). In Table 3 observations concerning autoclaving experiments are summarized. In contrast to photodegradation the thermal decomposition of nitroprusside does not give rise to formation of either pentacyanoaquoferrates or hexacyanoferrates as can be seen in Fig. 10.

Conclusions

The employed chromatographic method is a versatile tool for selective determination of nitroprusside in (photo-)degraded solutions as well as for qualitative purposes.

Upon exposure to 350 nm light and under normal daylight fluorescent tubes non-linear decay curves were found for the aqueous nitroprusside solutions (50 μg/ml) studied. The additives citric acid and disodium edetate (0.25 mg/100 ml) do not improve the stability of nitroprusside. On the contrary, addition of the photoprotective agent cyanocobalamin (1 mg/100 ml) to nitroprusside solutions resulted in a significantly lower decomposition rate, particularly in 350 nm light because of the high molar absorptivity at that wavelength.

The non-linearity of the decay curves can be explained by a combination of two phenomena. In the first place yellow solutions were produced due to formation of the pentacyanoaquoferrate(III) ion. This degradation product exhibits a relatively high molar absorptivity at the wavelength of photoirradiation (350 nm). Furthermore, the relatively high molar absorptivity of the photodegradation products at 395 nm plays an important role during photolysis under normal daylight fluorescent tubes. Nitroprusside has at that particular wavelength a photoactive absorption band. Secondly, in conjunction with a screening effect a substantial pH-dependent backformation of nitroprusside from pentacyanoaquoferrate(II) and nitrite occurs. Regeneration of nitroprusside appeared to be most effective in the pH range 3.5–5.5 and consequently we recommend for nitroprusside solutions a pH within this range.

Nitroprusside can resist autoclaving (15 min, 121°C) in solutions without dextrose. After sterilization of nitroprusside in 5% dextrose solutions substantial loss was found. Probably dextrose and its degradation product 5-hydroxymethylfurfural are responsible for the observed degradation.

References

Anderson, R.A. and Rae, A., Stability of sodium nitroprusside solutions. Austr. J. Pharm. Sci., 1 (1972) 45–46.

Baaske, D.M., Smith, M.D., Karnatz, N. and Carter, J.E., High-performance liquid chromatographic determination of sodium nitroprusside. J. Chromatogr., 212 (1981) 339–346.

Baudisch, O., Radical Reactions with certain nitrogen compounds. The conversion of benzene (toluene, etc.) in other compounds at low temperature. Science, 108 (1948) 443–444.

Bisset, W.I.K., Burdon, M.G., Butler, A.R., Glidewell, C. and Reglinski, J., Photochemistry of the nitroprusside ion and the consequences for the detection of cyanide in mixtures of nitroprusside and blood: use of sodium nitroprusside as a hypotensive agent. J. Chem. Res., (S) (1981) 229.

Buxton, G.V., Dainton, F.S. and Kalecinsky, J., Radiation chemistry of aqueous solutions of sodium nitroprusside. Int. J. Radiat. Phys. Chem., 1 (1969) 87–98.

Davies, G. and Garafalo, A.R., Stoichiometry and kinetics of the substitution-controlled oxidation of pentacyanoaquo iron(II) species by hydrogen peroxide and by tertbutylhydroperoxide. Inorg. Chem., 15 (1976) 1101–1106.

Durham, D.G., Hung, C.T. and Taylor, R.B., Identification of some acids produced during autoclaving of D-glucose solutions using HPLC. Int. J. Pharm., 12 (1982) 31–40.

Espenson, J.H. and Wolenuk, S.G., Kinetics and mechanisms of some substitution reactions of pentacyanoferrate(III) complexes. Inorg. Chem., 11 (1972) 2034–2041.

Frank, M.J., Johnson, J.B. and Rubin, S.H., Spectrophotometric determination of sodium nitroprusside and its photodegradation products. J. Pharm. Sci., 65 (1976) 44–48.

Golebiewski, A. and Wasielewska, A., New interpretation of spectral and photochemical properties of nitroprusside. J. Mol. Struct., 67 (1980) 183–187.

Jarzynowski, T., Senkowski, T. and Stasicka, Z., Flash photolysis of nitrosylpentacyanoferrate(II) complex. Pol. J. Chem., 3 (1981) 55–61.

Justin-Mueller, E., Sur la transformation du nitroprussiate de Na en bleu cyanoferrique. Bull. Soc. Chim. Fr., 2 (1935) 1932.

Kenney, D.J., Flynn, T.P. and van Gallini, J.B., Reactions of ferropentacyanamines. J. Inorg. Nucl. Chem., 20 (1961) 75–81.

Kirschbaum, J., Analytical Profiles of Drug Substances, Vol. 10, Academic Press, New York, 1981, pp. 183–288.

Kolthoff, I.M., Leitfähigkeitstitrationen mit Nitroprussidnatrium. Z. Anal. Chem., 62 (1923) 216–217.

Leeuwenkamp, O.R., Mark, E.J. Van der, Klauw, P.M. Van der, Bennekom, W.P. Van and Bult, A., Reversed-phase ion-pair chromatographic method for determination of nitroprusside in photolyzed solutions. Anal. Chim. Acta, 161 (1984) 211–219.

Lodzinska, A. and Gogolin, R., Photochemical reactions of sodium nitrosoferricyanide in aqueous solutions. Roczniki Chem. 47 (1973) 881–888.

Macartney, D.H. and McAuley, A., Kinetics of formation of (thiourea) pentacyanoferrate(II) complexes in aqueous perchlorate media. Inorg. Chem., 20 (1981) 748–751.

Malin, J.M. and Koch, R.C., Protonation of the pentacynoaquoferrate(II) ion, $Fe(CN)_5OH_2^{3}$ (aq.) Inorg. Chem., 17 (1978) 752–754.

Manoharan, P.T. and Gray, H.B., Electronic structure of nitroprusside ion. J. Am. Chem. Soc., 87 (1965) 3340–3348.

Martin, T. and Patel, J.A., Determination of sodium nitroprusside in aqueous solution. Am. J. Hosp. Pharm., 26 (1969) 51–53.

Martindale, The Extra Pharmacopeia, 27th Ed., The Pharmaceutical Press, 1977, p. 55.

Mitra, R.P., Jain, D.V.S., Banerjee, A.K. and Chari, K.V.R., Photolysis of sodium nitroprusside and nitroprussic acid. J. Inorg. Nucl. Chem., 25 (1963) 1263–1266.

Mitra, R.P., Sharma, B.K. and Mittal, S.P., Photolysis of sodium nitroprusside in the presence and absence of air. J. Inorg. Nucl. Chem., 34 (1972) 3919–3920.

Mitra, R.P., Sharma, B.K. and Mittal, S.P., Redox titration of photolysed solutions of sodium nitroprusside and the nature of the primary photochemical reaction of the nitroprusside ion. Indian J. Chem., 18 (1979) 351–353.

Nieman, T.A., Holler, E.J. and Enke, C.G., Reaction rate method for determining trace concentrations of cyanamide. Anal. chem., 48 (1976) 899–902.

Overbeck, A., Zersetzung des sog. Nitroprussidnatrium. Z. Anal. Chem., 62 (1853) 216–217.

Schumacher, G.E., Sodium nitroprusside injection. Am. J. Hosp. Pharm., 23 (1966) 532.

Sillen, L.G. and Martell, A.E., Stability Constants, Special Publications No. 17, London, 1980, The Chemical Society, pp. 477, 636–637.

Snyder, L.R. and Kirkland, J.J., Introduction to Modern Liquid Chromatography, 2nd Ed., Wiley-Interscience, New York, 1979, p. 216.

Swinehart, J.H., The nitroprusside ion. Coord. Chem. Rev., 2 (1967) 385–402.

Tatsuhara, T., Stabilization of sodium nitroprusside injection solutions. CA 1982, 97, P203257 q, Jpn. Kokai Tokkyo Kokai Jp. 83, 146, 714. (Cl A61 K33/26), 10 Sep 1982, Appl. 81/32, 519, 09 Mar 1981: 4pp.

Tinker, J.H. and Michenfelder, J.D., Sodium nitroprusside: pharmacology, toxicology and therapeutics. Anaesthesiology, 45 (1976) 340–354.

Tol, A., Nitroprussidenatrium Chemie-Farmacologie-Toxicologie-Injectiebereiding en Analyse. Med. Ned. Ver. Ziekenhuisapothekers, 33 (1976) 205–218.

Toma, H.E., Iron(II) catalysis in the oxidation of the aquopentacyanoferrate(II) complex by molecular oxygen. Inorg. Chim. Acta, 15 (1975) 205–211.

Van Loenen, A.C. and Hofs-Kemper, W., Stabiliteit en ontleding van natrium nitroprusside oplossingen I. Literatuuroverzicht Pharm. Weekbl., 113 (1979) 1080–1088.

Vessey, C.J. and Batistoni, G.A., The determination and stability of sodium nitroprusside in aqueous solutions (determination and stability of SNP). J. Clin. Pharm., 2 (1977) 105–117.

Wolfe, S.K. and Swinehart, J.H., Photochemistry of pentacyanonitrosylferrate (2-), nitroprusside. Inorg. Chem., 14 (1975) 1049–1053.