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Photostability profiles of the experimental antimetastatic ruthenium complex NAMI-A

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Abstract

NAMI-A is a novel ruthenium complex with selective activity against metastases currently in Phase I clinical trials in The Netherlands. The photostability of this new agent in solid state and in solution has been investigated utilizing a stability-indicating reversed-phase high performance liquid chromatographic (HPLC) assay and ultraviolet/visible (UV/VIS) light spectrophotometry. In solid state, NAMI-A proved to be photostable. In solution, however, the compound degraded rapidly, in a pH-independent manner in the pH range of 2–5. At alkaline pH, the degradation rate was higher than at acidic pH. The type of buffer species had little influence. NAMI-A concentration was inversely related to the photostability. Addition of photostabilizers (5% DMSO, 2% benzyl alcohol, 0.001% curcumin) marginally increased the half-life. NAMI-A's photostability in solution was influenced to the greatest extent by addition of an alcohol, with the least polar solvent system (50% propylene glycol) providing the most stable medium. Based on the presented results, it is recommended to store NAMI-A solutions in the dark.
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1. Introduction

NAMI-A (imidazolium *trans*-tetrachloro(dimethylsulfoxide)imidazoleruthenium(III), $H_2im[trans\text{-}RuCl_4(DMSO)Him]$, Fig. 1 [1]) is a novel ruthenium anticancer agent. Preclinical pharmacological and toxicological studies showed selective activity against lung metastases of murine tumors [1–4] and low toxicity in mice and dogs [5,6]. Its action seems to be independent of the origin (type of primary tumor) and stage of growth of the metastases [4]. NAMI-A possesses no direct tumor cell cytotoxicity [3,4,7], although an interaction with cell cycle regulation has been observed

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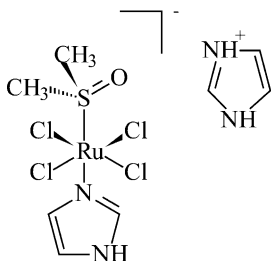

Fig. 1. Chemical structure of NAMI-A. Molecular weight = 458.18 g/mol. Molecular formula: $C_8H_{15}Cl_4N_4ORuS$.

in a transient accumulation of cells in the G_2/M phase [3,7,8]. This effect, and the capacity of NAMI-A to inhibit tumor cell matrix metalloproteinases, with important anti-angiogenic consequences [9], suggests this compound to be active in controlling metastasis growth. Correspondingly, host toxicity was mainly related to renal alterations, as expected for a heavy metal-derived compound, although it reversed within 3 weeks from the end of treatment [5].

Based on its promising activity and toxicity profile, NAMI-A is developed as a potential anticancer agent and has currently entered Phase I clinical trials. Before a suitable pharmaceutical formulation can be developed for a new drug substance, it is important to gain insight into its stability. Together with heat, moisture and atmospheric oxygen, light is an important external factor in drug stability. Therefore, according to the ICH Guidelines, photostability testing should be an integral part of stress testing of new drug substances and pharmaceutical products [10]. Information obtained from photostability studies can be used in specifying appropriate storage instructions and packaging materials. Previously, it was shown that hydrogen peroxide catalyzes the degradation reaction of NAMI-A in solution or causes a different type of degradation reaction to occur, possibly oxidation of ruthenium (III) [11]. Another study revealed that temperature affects the degradation of NAMI-A in solution, which can be described by the Arrhenius equation [12].

This study was initiated with the objective to obtain detailed knowledge on the photostability of NAMI-A drug substance in solid state and in aqueous solution. The effects of several parameters such as concentration, pH, buffer composition, solvent system, and photostabilizers on the degradation rate of NAMI-A in solution were investigated.

2. Experimental

2.1. Chemicals

NAMI-A was supplied by SIGEA Srl (Trieste, Italy). Sterile water for injections was purchased from B. Braun Medical (Melsungen, Germany). Methanol (HPLC grade) and ethanol 100% were obtained from Biosolve Ltd (Amsterdam, The Netherlands). Trifluoromethanesulfonic acid, sodium acetate trihydrate, acetic acid 96% (v/v), sodium dihydrogen phosphate dihydrate, sodium citrate monohydrate, dimethylsulfoxide, perchloric acid (70–72% (w/v)), benzyl alcohol, and sodium hydroxide pellets were purchased from Merck (Darmstadt, Germany). Sodium dodecylsulphate and quinine hydrochloride dihydrate were obtained from Fluka Chemica GmbH (Buch, Switzerland), curcumin from Sigma-Aldrich (St. Louis, MO), and polypropylene glycol from BUFA BV (Uitgeest, The Netherlands). All reagents were of analytical grade and used without further purification.

2.2. Light exposure

All samples and dark controls (samples placed alongside the exposed samples wrapped in aluminum foil) were exposed in triplicate in a Suntest CPS+ equipped with a xenon lamp (NXE 1500 B) with a coated quartz dish and window glass filter to allow only light with a wavelength of 320–800 nm and a spectral distribution similar to the ID 65 standard, as required in the ICH Guideline [10] (all Atlas Material Testing Technology LLC, Chicago, IL). Samples were exposed to light at the highest irradiation level of the Suntest apparatus, (765 W/m^2, after passage of the filters: 68.9 W/m^2 at <400 nm and 170 klux at >400 nm). The Suntest unit was connected to a Suncool^{TM} chiller (Atlas). Liquid samples and the quinine actinometric solution were exposed in standard 1 cm quartz

cells with two frosted sides and tight-fitting PTFE caps (4 ml total volume, HELLMA GmbH & Co, Müllheim, Germany). Solid samples were exposed in glass petri dishes ($\varnothing = 10$ cm). The temperature during exposure was monitored using a minimum–maximum thermometer (Merck).

2.3. Quinine hydrochloride dihydrate actinometric system

A 2% (w/v) quinine hydrochloride dihydrate solution served as the actinometric system to monitor the intensity of the ultraviolet (UV) radiation around 330 nm [10,13]. The untoward effects of light are generally due to the UV ranges of sunlight acting directly on the compound [14]. However, in order to evaluate the changes induced by light exposure under normal circumstances, exposure to light with a wavelength of 320–800 nm and a spectral distribution similar to the ID 65 standard, as required in the ICH Guidelines [10], was employed. The quinine actinometric system was used to monitor the UV energy received by the exposed samples, as measured by the change in absorbance at 400 nm (ΔA_{400}) between the exposed samples and dark controls. The ΔA_{400} was linear with duration of exposure.

Christensen et al. showed that after light exposure, the quinine actinometric system shows dark reactions, which can contribute significantly to the results obtained [15]. Therefore, samples (both NAMI-A and quinine) were measured immediately after being taken, thus minimizing the chance of distortion of results due to dark reactions taking place upon storage of samples.

2.4. High performance liquid chromatography

The high performance liquid chromatography (HPLC) system consisted of a model SP8800 ternary pump (Thermo Separation Products (TSP), Fremont, CA), a model 996 photo diode array (PDA) detector (Waters, Milford, MA) and a model SP8880 autosampler (TSP). Chromatograms were processed using Millennium® software (Waters). Separation was achieved using a μBondapak C18 column (300 mm × 3.9 mm internal diameter, particle size 10 mm, Waters), protected with a C8 guard column (Security Guard, Phenomenex, Torrance, CA). The mobile phase consisted of 0.50 mM sodium dodecylsulphate in 3% methanol, acidified to pH 2.5 using trifluoromethanesulfonic acid (triflic acid). The flow rate was 0.5 ml/min and the system was operated at ambient temperature. The detection wavelength was 358 nm and on-line spectral analysis was carried out with the PDA system. The injection volume was 20 μl. A run time of 10 min was employed for both the standard samples (calibration curve and quality control) and of 30 min for the samples under investigation. Employing this HPLC system, NAMI-A produces a peak with a retention time of approximately 4.1 min.

Calibration curves of standard NAMI-A solutions in distilled water were linear ($r > 0.98$) in the concentration range of interest (1–600 μg/ml). Samples were prepared by diluting the solution under investigation with sufficient mobile phase to produce 1.00 ml of a solution with a theoretical NAMI-A concentration of 500 μg/ml in an autosampler vial.

Degradation of NAMI-A can be followed using the presented HPLC method, which was proven to be stability-indicating, precise and accurate [11].

2.5. Ultraviolet/visible light spectrophotometry

Ultraviolet/visible light (UV/VIS) spectra were recorded with a Model UV/VIS 918 spectrophotometer (GBC Scientific Equipment, Victoria, Australia). The absorbance of the quinine actinometric samples was measured at 400 nm. For the NAMI-A samples, the absorbance at 390 nm was measured and spectra were recorded from 800 to 225 nm. Measurements were performed by diluting the solution under investigation to a theoretical concentration of 100 μg/ml with water for injections.

2.6. Kinetic experiments

2.6.1. NAMI-A in solid state

One hundred milligram of NAMI-A was spread in a layer of no more than 3 mm thickness, and irradiated for 24 h, alongside a dark control and the quinine actinometric system. The quinine actinometric system showed a ΔA_{400} of 0.75

between the dark controls and exposed samples. Before and after light exposure, NAMI-A was dissolved in water to a concentration of 1 mg/ml and further diluted as appropriate for immediate analysis by HPLC and by UV/VIS spectrophotometry.

2.6.2. NAMI-A in solution

2.6.2.1. Influence of concentration. The influence of concentration was studied in water for injections, at NAMI-A concentrations of 1, 10, and 20 mg/ml. Samples of all NAMI-A solutions were taken at appropriate time intervals ($t = 0$, 30 min, and 1, 2, 4, and 6 h) to be able to monitor the degradation in a quantitative manner. All other experiments were carried out in 10 mg/ml NAMI-A solutions in the appropriate solvent.

2.6.2.2. Influence of pH and buffer species. For both pH and buffer effect studies, buffers at a concentration of 0.15 M were employed. pH studies were carried out at pH 7.4 (phosphate buffer), pH 5, pH 3.5 (both acetate buffer), and pH 2 (perchloric acid). The effect of buffer species was investigated in citrate and phosphate buffers at pH 3.5.

The pH values of all test solutions in time were measured by a Model 654 pH meter (Metrohm AG, Herisau, Switzerland) and adjusted as necessary with perchloric acid or 1 M sodium hydroxide.

2.6.2.3. Influence of solvent system. Besides water, four solvent systems were investigated: 50% (v/v) propylene glycol, 50% (v/v) ethanol, 0.9% (w/v) NaCl, and 5% (w/v) dextrose.

2.6.2.4. Influence of photostabilizers. The influence of addition of three different photostabilizers was investigated by preparing solutions of NAMI-A in 5% (v/v) DMSO, in 2% (v/v) benzyl alcohol, and in 0.001% (w/v) curcumin in 50% ethanol. The concentration curcumin was set at 0.001%, as in this concentration, curcumin's UV/VIS spectrum overlapped that of NAMI-A. Curcumin is insoluble in water and therefore was dissolved in 100% ethanol in a concentration of 0.002%, then diluted 1:1 with a 20 mg/ml solution of NAMI-A in water.

3. Results and discussion

3.1. Degradation pathway and general observations

A degradation pathway for NAMI-A in solution as depicted in Fig. 2 has been proposed [1,2,16]. The structural elucidation of the various degradation products was based on UV/VIS spectrophotometry and nuclear magnetic resonance (NMR) spectroscopy. Degradation of NAMI-A consists of stepwise hydrolysis of the chloride ligands, in acidic media accompanied by hydrolysis of the DMSO group, followed by polymerization to poly-oxo or -hydroxy species [1,2,16]. This degradation mechanism, however, does not take into account specific light-catalyzed degradation of NAMI-A.

Upon photodegradation in water, the UV/VIS spectrum of NAMI-A 10 mg/ml in water changed as depicted in Fig. 3. Up to 4 h of exposure, three isosbestic points were present, at 277, 300, and 365 nm, suggesting a single transformation in the chromophore in the early stages of irradiation, although it cannot be excluded that other transformations not involving the chromophore take place. After this time, however, the spectrum began to distort the isosbestic points, providing evidence of subsequent stages of photodegradation. The loss of isosbestic points was accompanied by a general loss in specific absorbance and darkening of the solutions. Similar changes were observed in a solution of NAMI-A in water stored in the dark, but these changes occurred at a much lower rate.

Whereas immediately after dissolution, all NAMI-A solutions were dark yellow, upon photolysis, darkening to a brown and subsequently black color took place. Darkening of NAMI-A in solution has been ascribed to formation of poly-oxo or -hydroxy species [1,2,16].

HPLC analysis revealed that in all solutions one major degradation product was formed upon light exposure, which has previously been identified as the mono-hydroxy or -aqua species of NAMI-A

Fig. 2. Proposed degradation mechanism of NAMI-A.

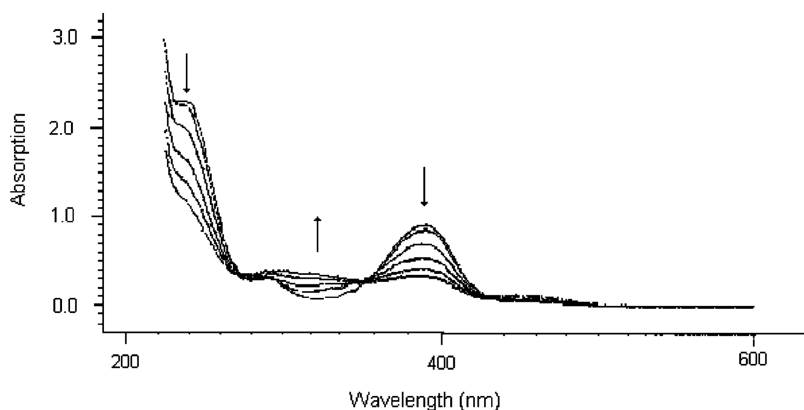

Fig. 3. Change in UV/VIS spectrum of a solution of 10 mg/ml NAMI-A in distilled water in time from $t = 0$ to 24 h upon light exposure. The directions of the arrows indicate an increase or decrease of absorption upon degradation.

(compound B in Fig. 2) [14]. One other degradation product was also formed in all solutions except the solution at pH 7.4 and the solution with 5% DMSO. It has maxima in its UV/VIS spectrum at 326 and 374 nm and has not yet been positively identified, although it has been observed that it is only formed under acidic conditions and thus may be any one of the DMSO-less structures in Fig. 2 [11]. At least six other degradation products were formed in the different solutions, but all in low amounts (up to 5% of the total peak area). Thus, upon photodegradation of NAMI-A, many different products are generated, with initially (in the first 6 h of light exposure), the mono-hydroxy or -aqua product being mainly formed. NAMI-A in solution when stored in the dark degrades in the same manner [12], indicating that exposure to high intensity light affects the rate, but not the mechanism, of degradation.

3.2. NAMI-A in solid state

After exposure for 24 h of NAMI-A drug substance in solid state, no degradation was observed in either the exposed samples or the dark controls. This indicates that NAMI-A drug substance is photostable under the tested conditions and does not need to be stored protected from daylight.

3.3. NAMI-A in solution

3.3.1. Dark controls

In general, the degradation of NAMI-A under the influence of light was monitored for 6 h. During this time period, all dark controls, except for the solution at pH 7.4, showed marginal amounts of degradation (2–10%), whereas the exposed samples degraded substantially ($>80\%$). The dark control at pH 7.4 degraded completely within 2 h, whereas the exposed samples at pH 7.4 were completely degraded within 1 h. Thus, light exposure accelerated the rate of degradation substantially.

3.3.2. Influence of concentration

Regression analysis (Fig. 4) showed that photodegradation of NAMI-A in water follows first-order kinetics ($r^2 \geqslant 0.994$ for the ln(concentration)–time plots).

The rate of degradation of NAMI-A in water is inversely proportional to the NAMI-A concentration. This effect has been described previously for several other drugs and has been ascribed to a reduced fraction of the sample being exposed to light at higher drug concentrations [17,18].

3.3.3. Influence of pH

Similar rate constants were found for NAMI-A in water and in the acidic buffers. All buffered solutions of NAMI-A showed (pseudo-)first-order degradation kinetics, as indicated by the linearity of the ln(concentration)–time plots ($r^2 > 0.98$), except for the solution at pH 7.4, which shows zero-order degradation kinetics ($r^2 = 1$ for the concentration–time plot). At pH >8 the degradation of NAMI-A was too fast to follow in our experimental design. These orders of reaction are identical to those found during storage under dark conditions [12]. However, for acidic solutions of NAMI-A stored in the dark, reaction rates are pH-dependent, with a pH of 3–4 providing the most stable environment [12]. This was not observed in the solutions exposed to light: all solutions of acidic pH exhibit similar reaction rate constants (Table 1). Only the solution at pH 7.4 degraded at a significantly faster rate than the acidic solutions. Thus, the influence of light on the degradation of NAMI-A in solution is larger than the influence of pH in acidic solutions. The acidic solutions stored in the light show reaction rate constants which are approximately a factor 20 higher than those for the solutions stored in the dark. On the other hand, although light enhances the degradation rate in alkaline solution by a factor 2 as compared to storage under dark conditions, the pH effect is larger than the effect of light under these conditions.

3.3.4. Influence of buffer species

Table 1 shows that neither the type nor the concentration of buffers influences the light-catalyzed degradation of NAMI-A. The small increase in the half-life of NAMI-A in the citrate and phosphate buffers (pH 3.5) is most likely a result of the temperature difference.

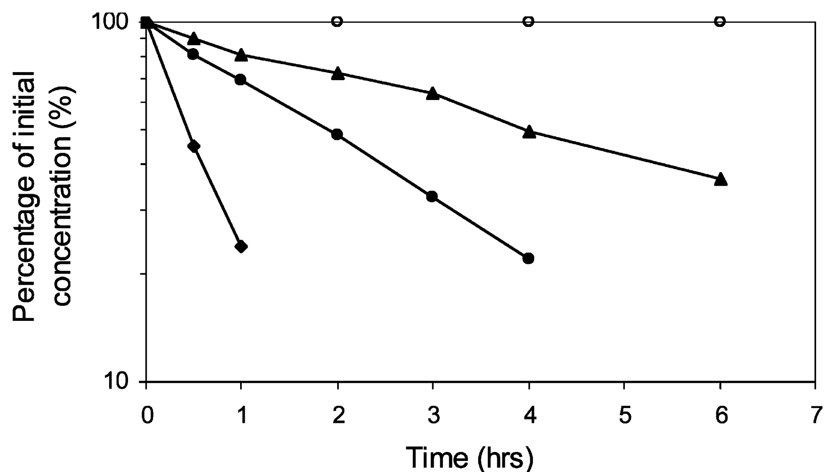

Fig. 4. Effect of NAMI-A concentration on the photodegradation in water. ▲ 20 mg/ml: $y = -0.1649x + 4.5931$, $r^2 = 0.9940$; ● 10 mg/ml: $y = -0.3746x + 4.6031$, $r^2 = 0.9992$; ◆ 1 mg/ml: $y = -1.4354x + 4.5791$, $r^2 = 0.9960$; ○ Reference: 10 mg/ml stored in the dark: $y = -0.002x + 4.6203$, $r^2 = 0.9950$.

3.3.5. *Influence of solvent systems*

Although NAMI-A is soluble in water (50 mg/ml), the influence of several co-solvents on its photostability in solution was investigated, as the polarity of the solvent could be of influence. For example, for minoxidil solutions it has been shown that an increase in solvent polarity resulted in a decrease in the rate of photodegradation, with water providing a more photostable medium than 25% ethanol and 25% propylene glycol [19]. The influence of the same co-solvents was examined on the photostability of NAMI-A, but their proportion was increased to 50% in order to enlarge the differences in polarity. Furthermore, as the for-

Table 1
Observed rate constants and half-lives of NAMI-A in solution exposed to light of high intensity

Effect	Solvent	C_0 (mg/ml)	k (s^{-1})	Half-life (h)	T_{av} (°C)
Concentration	Water	1	3.99×10^{-4}	0.5	27
		10	1.04×10^{-4}	1.9	27
		20	4.58×10^{-5}	4.2	30
pH	Acetate buffer pH 2	10	9.60×10^{-5}	2.0	28
	Acetate buffer pH 3.5	10	1.03×10^{-4}	1.9	25
	Acetate buffer pH 5	10	1.00×10^{-4}	1.9	25
	Phosphate buffer pH 7.4	10	5.58×10^{-6} M s^{-1}	0.6	25
Buffer species	Citrate buffer pH 3.5	10	6.32×10^{-5}	3.0	21
	Phosphate buffer pH 3.5	10	6.32×10^{-5}	3.0	21
Solvent	50% propylene glycol	10	2.59×10^{-5}	7.4	21
	50% ethanol	10	3.90×10^{-5}	4.9	21
	0.9% NaCl	10	5.81×10^{-5}	3.3	22
	5% dextrose	10	6.34×10^{-5}	3.0	22
Photostabilizer	5% DMSO	10	8.10×10^{-5}	2.4	30
	2% benzyl alcohol	10	8.03×10^{-5}	2.4	26
	0.001% curcumin in 50% ethanol	10	7.83×10^{-5}	2.5	26

C_0, initial NAMI-A concentration; T_{av}, average temperature.

mulated product of NAMI-A will be administered to patients intravenously dissolved in an isotonic medium such as normal saline or 5% dextrose, the photostability in these two solvents was also examined.

NAMI-A in the four different solvents showed first-order degradation kinetics, as was indicated by the linearity of the ln(concentration)–time plots ($r^2 > 0.97$). As the pH of all solutions was slightly acidic (pH 4–5), this substantiates the previous observation that at acidic pH, photodegradation of NAMI-A occurs according to first-order degradation kinetics. Fig. 5 shows the degradation profiles of NAMI-A in the different solvents. From the results in Table 1 and Fig. 5, it is obvious that the photostability of NAMI-A depends on the solvent system and the least polar solvent provides the most photostable environment for NAMI-A. Normal saline and 5% dextrose showed similar photodegradation rates to those in water (taking into account the experimental temperature differences), whereas some degree of photostability was provided by 50% ethanol and more by 50% propylene glycol.

Besides propylene glycol's low polarity, other factors may also play a role in its photoprotective effect on NAMI-A. It raises the viscosity, thus reducing molecular mobility, and also lowers the dielectric constant of the medium. Furthermore, the temperature difference provides a partial explanation of the decrease in reaction rate constant. All of these effects could contribute to the photoprotection of NAMI-A by propylene glycol.

3.3.6. *Photostabilizer effect*

Previously, DMSO has been reported to act as a photoprotective agent for FD & C Red No.3 (erythrocine, a dye) [20]. Benzoic acid derivatives, such as benzyl alcohol, have been used as light absorbers and photoprotective agents [21], while food colorants such as curcumin (maximum absorption at 430 nm) protect colored drug substances by spectral overlay [22]. Addition of 5% DMSO, 2% benzyl alcohol, or 0.001% curcumin resulted in a marginal increase in the half-life of NAMI-A upon light exposure. No differences between the photoprotectants were observed, although the average temperature during exposure of the solution containing 5% DMSO was higher than during exposure of the other two solutions, indicating that DMSO might be more effective than benzyl alcohol or curcumin.

3.3.7. *Anti-oxidants, chelating agents*

Many studies have shown photostabilizing effects of anti-oxidants and chelating agents on

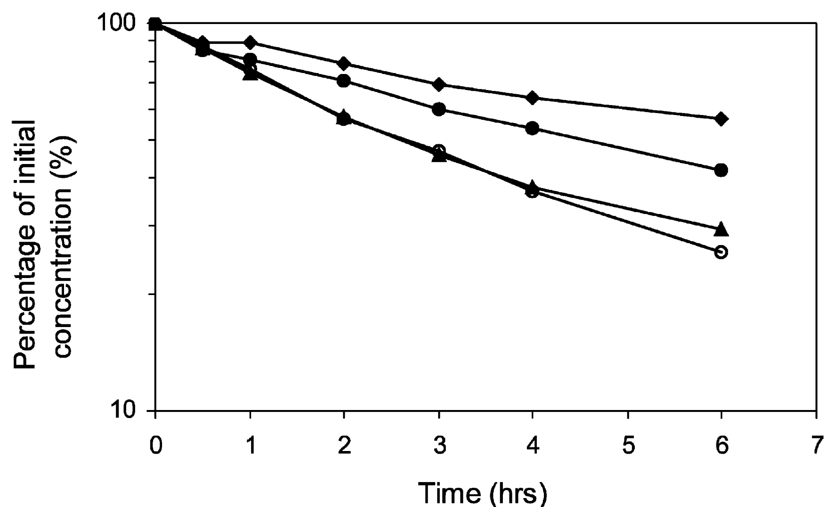

Fig. 5. Effect of solvent on NAMI-A photodegradation. ◆ 50% propylene glycol: $y = -0.0934x + 4.5637$, $r^2 = 0.9713$; ● 50% ethanol: $y = -0.1403x + 4.5459$, $r^2 = 0.9885$; ▲ normal saline: $y = -0.2090x + 4.5278$, $r^2 = 0.9742$; ○ 5% dextrose: $y = -0.2282x + 4.5571$, $r^2 = 0.9913$.

pharmaceutical substances in solution [17,19,21,23]. These studies, however, were not executed for NAMI-A, as anti-oxidants by nature are reducing agents and reduce the Ru (III) in NAMI-A to Ru (II) [1,2]. Chelating agents bind heavy metals, and thus will bind the ruthenium atom of NAMI-A, leading to its decomposition.

4. General discussion

None of the approaches attempted for stabilizing NAMI-A in solution to light exposure provided a very large photoprotective effect. Whereas addition of curcumin to a nifedipine solution increased the photostability by a factor of 60 [22], the largest effect observed on the photostability of NAMI-A was with 50% propylene glycol as solvent, which increased the photostability by a factor 3.5. Preparation of a product for intravenous use is preferentially performed employing as few excipients as necessary, due to possible intrinsic toxicity or activity of the excipient itself or to interactions between the new drug substance and the additives, leading to unknown effects on the activity of the new drug substance [24].

Previously, it was shown that NAMI-A in solution degrades in compliance with the Arrhenius equation [12]. It was, however, not feasible to keep the temperature constant during all experiments (Table 1), but the duration of the experiments was relatively short (6 h), so that the temperature could only have a marginal influence.

Instead of adding excipients to the NAMI-A solutions during manufacture of the pharmaceutical product, which might provide a slight degree of photoprotection, based on this study, it is preferred to store NAMI-A solutions protected from light.

5. Conclusion

NAMI-A drug substance in solid state is photostable. In aqueous solution, however, NAMI-A degrades rapidly upon light exposure. The degradation rate of samples exposed to light was greatly increased as compared to dark controls, indicating light catalysis. However, the degradation mechanism had probably not been altered: the same orders of reaction were observed and the same degradation products were generated.

NAMI-A concentration was inversely proportional with photostability. As opposed to solutions stored in the dark, lowering the pH did not influence the degradation rate for solutions exposed to light. The type of buffer used did not appear to influence the rate of degradation upon light exposure. Addition of DMSO, benzyl alcohol, and curcumin as photoprotectants showed a minimal photostabilizing effect. Addition of alcohol to the solvent system decreased the photodegradation rate slightly. The least polar solvent (50% propylene glycol) provided the most photostable medium for NAMI-A in solution, increasing the half-life by a factor 3.5.

It is concluded that NAMI-A solutions are photolabile and therefore should be stored protected from light.

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