Dependence of the Rate Coefficient for Photochromic Relaxation of Mercury(II) Dithizonate on Excess Free Ligand

Anton E. Goodwin¹ and Horacio A. Mottola*

Department of Chemistry, Oklahoma State University, Stilliwater, Oklahoma 74078

The effect of different experimental parameters on the value of the rate coefficient in the photochromic process for the return of the excited form of mercury(II) dithizonate to the ground state is discussed. Special attention is devoted to the presence of free ligand under extraction conditions (from aqueous solutions) and nonextraction conditions. The observed effect is suggested as responsible for discrepancies of some published data on this photochromic relaxation. In the presence of excess ligand, a ligand attack favoring the ground-state form is proposed to explain the dependence.

Diphenylthiocarbazone (dithizone), C_6H_5N =NCSNHNH- C_6H_5 , was first prepared and studied by Emil Fischer as part of a now classical work on phenylhydrazine (1). It was also Fischer who noted its reactions with heavy metal ions to give brilliantly colored products (1). Analytical chemists turned their attention to dithizone as a unique reagent after Hellmut Fisher, in 1925, pointed out its value for detection and determination of metal ions (2). Since then, dithizone has been used in a myriad of analytical procedures both as a chromogenic and as a separation tool (3).

Although the photochromic property of mercury(II) dithizonate was recognized as such only in the last half of the 1940s (4-6), its effect on the absorptiometric determination of mercury with dithizone was reported by Winkler in 1940 (7). He wrote: "...under the influence of a strong light ...a rise in the photometric reading...occurred when a filter centered at 610 millimicrons (No. 61) was used, but with a No. 49 filter there appeared to be a drop in the reading." Since these early observations a few papers have appeared discussing the photochromism of mercury(II) dithizonate. The first detailed inquiry was reported by Meriwether et al. (8, 9) who prepared. characterized, and examined the photochemical behavior of 24 metal dithizonates in solution. Eleven of those 24 chelates were found photochromic; the one with mercury(II) has slow return to ground state, which permits a relatively easy study of its properties. More recently Geosling et al. (10) report a twelfth dithizonate (copper) as photochromic and also a series of contradictory observations: (1) even though the return of the excited species to the ground state follows first-order kinetics, irreproducibility for successive runs is observed, (2) large differences in reported values of observed rate coefficients are evident, (3) there is no agreement on the effects that different concentrations of water or the metal chelate itself have on the values of the rate coefficients. These and other conclusions of various authors, for example, that the rate of return of the excited species depends on the time elapsed since preparing the solution and on the exact treatment used to clean the glassware, pose several puzzling questions. During a study on the photochromic behavior under solvent-extraction conditions a series of observations were made that pointed to the presence of free ligand as playing a major role on the rate of return to the ground state. This invited a reexamination of the photochromic behavior of the mercury(II) dithizonate in general. Observations and interpretations that came from these studies are reported here.

EXPERIMENTAL SECTION

Apparatus. The experimental unit used in these studies was a custom-assembled one, details of which are shown in Figure 1. The modular components included: (1) as irradiation lamp a PN 100, 8-V, 50-W projection lamp (Riluma, Switzerland); (2) an absorptiometric unit consisting of a 6-V tungsten lamp (Chicago Miniature Lamp Works No. 1493 operated by a MP-1026 regulated power supply (Pacific Precision Instruments, Concord, CA), a H-10 Jobin-Yvon concave holographic grating monochromator (Instrument SA, Inc. Metuchen, NJ), a specially designed cell holder accommodating a 1-cm glass cell and equipped with a magnetic stirring system consisting of a miniature dc motor (0.35-2.5 V, minimum current for operation 25 mA; Edmund Scientific, Barrington, NJ) operated by an "Energel", 6-V rechargeable battery (Radio Shack, Ft. Worth, TX), and a HU-V-4000B pin photodiode-operational amplifier combination (EG&G Electro-Optics, Salem, MA) operated by a 915 dual power supply, ±15 V, 25 mA (Analog Devices, Norwood, MA); (3) a readout unit based on a Nicolet 2090A Explorer digital oscilloscope with 94A plug-in and Model D amplifier (Nicolet Instrument Corp., Madison, WI); (4) a signal conditioning unit: Oriel 7600 multifunction signal processor (Oriel Corp., Stamford, CT); and a computer/controller system built around a 9825A desktop computer (Hewlett-Packard, Palo Alto, CA) with the following Hewlett-Packard peripherals: 9878A I/O expander, 59306A relay actuator, 9885M flexible disk drive, 9871A printer, and 9862A plotter.

The cell holder was equipped with a water jacket for temperature control. Constant temperature was maintained by circulating water with a Lauda/Brinkmann Model K2/R circulator (Brinkman Instruments Inc., Westbury, NY).

The light from the irradiation source was filtered through 6-mm heat-absorbing glass to remove infrared radiation. Two shutters opened and closed by electrical solenoids (actuated by the relay actuator on command from the desktop computer) were used to control the irradiation and protect the detector from the intense light during irradiation.

Reagents and Solutions. All reagents used were of AR grade. The water used was purified by distilling deionized water in a borosilicate still equipped with a quartz-covered immersion heater. A commercial sample of mercury(II) dithizonate (Eastman Kodak, Co., Rochester, NY) was used in experiments involving direct dissolution of the metal chelate in the organic solvent. This sample was found to contain uncomplexed mercury(II) by photometric titrations with dithizone solutions. The needed correction was taken into account in experiments concerned with the effect of the ligand/metal ratio on the kinetics of the photochromic relaxation process. The dithizone used in extraction and dissolution experiments was also from Eastman Kodak Co. and was purified as recommended by Irving (11).

Cleaning of Glassware. Cleaning and conditioning of glassware in agreement with Geosling et al. (10) were found critical during the photochromic studies reported here. The procedure and usage that follow were found to provide reliable conditions for reproducibility.

All glassware was cleaned by soaking in 3 M hydrochloric acid for 1/2 to 1 h, rinsed with distilled water, dried, and then filled with a solution of dithizone in benzene. After a week the contents were removed and the glassware was rinsed several times with solvent, inverted, and air-dried. After this cleaning procedure, pipets, volumetric flasks, and amber glass bottles were marked,

¹Permanent address: CONOCO, Inc., Ponca City, OK 74601.



Figure 1. Experimental setup for photochromic studies: (A) power supply for W lamp; (B) W lamp; (C) monochromator; (D) cell holder; (E) magnetic stirrer; (F) detector; (G) multifunction signal processor; (H) digital oscilloscope; (I) I/O expander; (J) desktop computer; (K) disk drive; (L) plotter; (M) printer; (N) relay actuator; (O) power supply for irradiation lamp; (P) irradiation lamp; (Q) power supply (6-V battery) for stirrer; (R) power supply for detector; (S) shutter with solenoid.

used only for one service operation (e.g., a particular pipet was used only to transfer dithizone solutions), and then rinsed with solvent and air-dried again.

Procedures. Parallel studies involving solutions of mercury(II) dithizonate prepared by extraction of mercury(II) ions from aqueous solutions into benzene solutions of dithizone and dissolution of the solid metal chelate in benzene were carried out. The procedure for extraction consisted in equilibrating equal volumes of solutions of $HgCl_2$ in 0.0050 M sulfuric acid and dithizone in benzene. Shaking for 1 min was sufficient for attainment of equilibrium. After equilbration, the extract was filtered through hydrophobic MN617 WA filter paper (Macherey-Nagel Co., Duren, Germany) and collected into two 2-oz, amber glass bottles. Without further preparation the extracted samples were irradiated and the return to ground state was monitored.

In experiments involving direct dissolution of the dithizonate, the solutions were allowed to stand for 4 to 20 h in amber glass bottles and in the dark. Afterward the sample was irradiated and the return to ground state was monitored.

Empirical Approach To Ascertain the Reliability of Data in Direct Dissolution Experiments. Experiments involving the direct dissolution of metal complex and excess free ligand which were conducted immediately after sample preparation yielded irreproducible data. Such irreproducibility was observed even after accounting for the presence of an impurity in the solid chelate sample [probably mixed ligand complex such as ClHg-(HDz), which is photochromic (6, 12)]. The problem was traced to the variable content of water in the solvent, to the amount of ambient light present during the preparation and transfer of samples, and to the need of rather prolonged standing times in the dark. Data variation was observed in an unpredictable manner during the first 4 h of standing in the dark or even the first 20 h, depending on the experimental conditions.

Because of these complications the following control procedure was adopted which yielded reliable data: a series of six samples containing a single concentration of complex and varying concentrations of excess dithizone were prepared, stored in amber glass containers, and allowed to stand in darkness for at least 4 h. After data were gathered, the absorbances at 605 nm, for both the background and the maximum concentration of excited species produced, as well as the value of the observed return rate constant were examined by graphical examination to assess the smoothness in data trends. Points that obviously deviated from the data trend were not used subsequently. A preponderance of scattered points indicate an unstable system and the corresponding run was discarded. This procedure permitted a wider range of data points to be collected and examined statistically, which is a definite advantage of this experimental design.

RESULTS AND DISCUSSION

Photochromism in the mercury(II) dithizonate is observed as a reversible photoinduced change between two states having different absorption spectra

$$A (\lambda_1) \stackrel{n\nu}{\longleftarrow} A^* (\lambda_2) \tag{1}$$

The ground state produces orange solutions ($\lambda_1 = 490$ nm) and the excited state blue solutions ($\lambda_2 = 605$ nm). All collected evidence seems to support the postulate of Meriwether et al. (8, 9) that the photochromic transformation involves a trans-cis isomerization about the C=N bond and N-to-N hydrogen transfer



Of the references concerning photochromism of this dithizonate, only Meriwether et al. (8, 9) and Geosling et al. (10) discuss mechanisms, rate coefficients, and other experimental observations. Although these two studies agree that the relaxation process for the return of the excited species to the ground state is first order in the excited species and both indicate the possible existence of isomers in the excited state, other statements are either contradictory or indicative of a more complicated process. Because they are the focus of this paper, the points of particular interest are the value of the rate coefficient for the relaxation process being dependent on the concentration of metal chelate in the organic solution (Meriwether et al.), the lack of reproducibility in the rate of return to ground state (Geosling et al.), and the dependence of this rate on the presence of free ligand in the medium (this work). Experimental observations show that if sufficient care is taken in the cleaning/conditioning of glassware, in the preparation and handling of solutions, and in allowing the necessary time for total relaxation in the dark (4 h at least), reproducible rate data can be obtained. Under such conditions it can be concluded that (1) both the return rate coefficient, $k_{\rm obsd}$, and the absorbance of the excited species, A^* , vary depending on the metal/ligand ratio in the solution, (2) the photochromism of the mercury(II) dithizonate solutions obtained from extraction (into benzene) or from direct dissolution of the metal chelate in the solvent is characterized by the same results and observations, and (3) as a result of the irradiation process the excess ligand exists in two forms and this suggests that the ligand itself is photochromic

$$L \stackrel{h\nu}{\longleftrightarrow} L^*$$
 (3)

Effect of Irradiation Time on the Population of the **Excited State and k_{obsd}.** Figures 2 and 3 depict how A^* and $k_{\rm obsd}$ are affected by the time of irradiation. A photosteady state is established after about 60 s of irradiation under the experimental conditions described earlier, independently of the presence or absence of free ligand. About a 10% decrease in the concentration of molecules in the excited form is produced by addition of only 6.2×10^{-6} mol of dithizone/L of solution of metal chelate. The decrease in A^* with excess ligand present is paralleled by an increase in $k_{\rm obsd}$, with the interesting additional feature of larger values of this coefficient at short irradiation times. This can be attributed to the simultaneous existence of two equilibria, eq 1 and eq 3, and to the assistance of the ground state form of the ligand in the return of the metal chelate to the orange form in the dark. Experiments in which excess free ligand was injected into solutions of excited mercury dithizonate gave additional support for the postulation that the unexcited form of the ligand increases the relaxation rate. Although the two slopes of the logarithmic plot for such experiments were different, they were both linear. Irradiating the sample a second time



Figure 2. Effect of time of irradiation on the population of the excited state (temperature, 25.0 °C): (O) no excess free ligand present, (Δ) 4.3 × 10⁻⁶ M excess free ligand, (\odot) 6.2 × 10⁻⁶ M excess free ligand; dissolution experiment; monitoring at 605 nm, 25 °C; concentration of mercury(II) dithizonate, 1.10 × 10⁻⁵ M; data points are A_{max} values from individual experiments (background subtracted out).



Figure 3. Effect of time of irradiation on the rate coefficient for return to ground state: temperature, 25.0 °C; excess free ligand (A) 6.2 μ M, (B) 4.3 μ M, (C) 0.5 μ M, (D) 0.0 μ M; other experimental conditions as in Figure 2.

gave the typical pattern of deviation from linearity at the early relaxation times and a slope $[\log (A_t - A_{\infty})]$ at the later relaxation times that was the same as the slope in the injection experiments.

The slight curvature observed in the curve D without excess dithizone, in Figure 3, is the result of a slight excess of metal (as chloride) in the metal chelate. The results of Figures 2 and 3 were obtained by direct dissolution of the metal chelate in benzene and the presence of free metal (salt) was ascertained by photometric titration with ligand. The curve with 0.5×10^{-6} M dithizone added shows less curvature due to the complexation of the free metal by added ligand. In any event the effects are relatively minor and for all practical purposes, without excess free ligand or at low free ligand concentrations, k_{obsd} remains practically constant with irradiation time.

Effect of Ligand/Metal Ratio on the Population of the Excited State and k_{obsd} . Figure 4 shows that different amounts of chelate are excited under photosteady conditions of irradiation depending on the presence of a deficit or excess of ligand in the medium. Interestingly the maximum population is obtained when the stoichiometric 2:1 ligand:metal ratio is attained.

Figure 5 shows the effect of ligand:metal ratio on the value of k_{obsd} under extraction conditions while an identical effect of excess free ligand was observed when dithizone is added to solutions of mercury(II) dithizonate prepared by direct dissolution of the chelate in benzene.

The first point to glean from this figure is that the lowest value of $k_{\rm obsd}$ is observed when the stoichiometric ratio is realized. Excess metal (substoichiometric amounts of ligand) shows a slight effect on the rate coefficient but excess of ligand, on the other hand, dramatically increased the value of $k_{\rm obsd}$. The value of $k_{\rm obsd}$, however, is independent of the concen-



Figure 4. Dependence of the population of mercury(II) dithizonate in the excited state as a function of the metal:ligand ratio: temperature, 25.0 °C; extraction experiments at pH 2.00; monitoring at 605 nm, 40 s irradiation time; total mercury concentration (A): 30 μ M, (B) 20 μ M, (C) 15 μ M, (D) 10 μ M.



Figure 5. Effect of ligand:metal ratio on the value of the rate coefficient for the relaxation process: solvent extraction of the Hg(II) dithizonate; other experimental conditions as in Figure 4; total Hg(II) (O) 10 μ M, (D) 15 μ M, (Δ) 20 μ M, (\oplus) 30 μ M.



Figure 6. Logarithmic plots of $(A_t - A_{\infty})$ vs. relaxation time as a function of irradiation time.

tration of metal chelate in solution, confirming the observation of Geosling et al. and in contradiction to that of Meriwether et al. This figure strongly suggests the presence of free ligand as impurity in the preparations used by Meriwether et al. On the other hand, the need for longer times of standing in the dark before reproducible data can be obtained seems to explain the statement that results were irreproducible from run to run (10).

Effect of Excess Free Ligand and Irradiation Time on the Course of the Relaxation Process. Figure 6 shows that at short irradiation times the decay curves follow uncomplicated first-order kinetics but as the time of irradiation increases and the free ligand in excess also increases, deviation from uncomplicated first-order kinetics becomes evident. At sufficiently large irradiation times, relaxation starts more slowly than at short irradiation times and becomes faster as

Table I. Kinetics of the Photochromic Relaxation of Mercury(II) Dithizonate

at stoichiometric ligand-to-metal ratio



 a This interpretation is valid only at irradiation times of 60 s or longer.



Figure 7. Dependence of absorbance for excited state as a function of relaxation time and excess free ligand. Background due to excess ligand subtracted out.

it proceeds, in time collapsing to rate coefficient values equal to those at short irradiation times. The deviation from the first-order expected dependence is augmented by the presence of free ligand.

Proposed Mechanisms To Explain the Collected Experimental Observations. Table I summarizes the proposed mechanisms to explain the relaxation of the photochromic excited state of mercury(II) dithizonate under stoichiometric conditions and in the presence of free ligand (the normal situation under analytical extraction conditions). They take into account the combined dependence on time for irradiation and excess ligand. Figure 7 is a computer-generated surface where the background absorbance of the excess ligand has been subtracted out. It shows the dependence of absorbance change of the excited mercury(II) dithizonate on relaxation time and on excess free ligand.

When the mercury(II) chelate is present in solution without excess free ligand, the return to ground state follows an uncomplicated first-order kinetic path. In the presence of free ligand and as a result of a simultaneous relaxation process of the free ligand itself, the ground state form of the ligand assists the return of the metal chelate in a manner which results in an exponential acceleration of the rate of return as L* relaxes to L. This effect results in a dependence of k_{obsd} for the metal chelate relaxation on [L], which in turn is changing with time (actually increasing as the time of relaxation increases until it assumes the value of $C_{\rm L}$ and the rate becomes equal to that at relatively low irradiation times when for all practical purposes $C_{\rm L} = [L]$). Values of $k_{\rm obsd}$ in both the absence and presence of excess free ligand are slopes determined by a least-squares linear regression through data points of $\ln (A_t$ $-A_{\infty}$) vs. time during the second half-life of the relaxation. Values for k''_{obsd} , however, require an indirect approach as illustrated in Figure 8. Extrapolation to zero relaxation time of the linear portion of the first-order plot provides the re-



in presence of excess free ligand a

Figure 8. Calculation of k''_{obsid} from the deviation from first-order plots.



Figure 9. Characteristic rate profiles for the decay of photochromic exited state of the Hg(II) dithizonate: (curve A) 2.0×10^{-5} M Hg(HDz)₂, 8.3×10^{-6} M H₂Dz; (curve B) 1.0×10^{-5} M Hg(HDz)₂, 4.5×10^{-6} M dithizone, H₂Dz; dissolution experiments; irradiation time, 360 s.

action kinetics which would have been followed if the irradiation were not affecting the value of $C_{\rm L}$. Curvature at the earlier relaxation times is the result of conversion of L* to L in an exponential manner. The deviation D (Figure 8) at each point in time is equal to the difference between the observed value of the rate proportionality coefficient and the expected one if first-order kinetics were followed throughout the entire relaxation:

$$D = k_{\text{expected}} - k_{\text{obsd}} \tag{4}$$

The instantaneous values of $k_{\rm obsd}$ are calculated from experimental data by using the equation $k_{\rm obsd} = \ln [(A_t - A_{\rm bkgd})/A_{\rm max}]/t$. Because these values appear unchanged after the third half-life, an average value determined during 3 to 6 half-life interval is $k_{\rm expected}$. The slope from a least-squares linear regression through data points of $\ln D$ vs. time for the first two half-lives gave $k''_{\rm obsd} = (5.4 \times 10^3)[L^*]_t$, with

$$[L^*] = C_L - [L]_t \tag{5}$$

$$[\mathbf{L}]_t = [\mathbf{L}]_0 + [\mathbf{L}^*]_0 [1 - \exp(-tk''_{\text{obsd}})]$$
(6)

As a test of the validity of these interpretations, experimental data were superimposed on simulated data calculated



Figure 10. Relaxation profile for 2.0×10^{-5} M Hg(HDz)₂ with 1.2×10^{-5} M excess H₂Dz: (curve A) calculated by considering the excess ligand present as form L* through the run, equation used for absorbance measurement $A_t = A_{\infty} + A_0^* \exp(-0.018t)$; (curve B) solid line calculated with $A_t = A_{\infty} + A_0^* \exp(-1t)(2.7 \times 10^{-3}) + (2.6 \times 10^3)[L]_t)$], (open circles) selected experimental points; (curve C) calculated by considering the excess ligand present as form L through the run, equation used for absorbance measurement $A_t = A_{\infty} + A_0^* \exp(-0.034t)$ where A_t is the absorbance at time t, A_{∞} is the absorbance at infinity, A^*_0 is the absorbance of excited form of the metal chelate at zero relaxation time; dissolution experiments; irradiation time, 360 s.

with the coefficients and equations developed in the model. Figures 9 and 10 show excellent agreement between the three sets of data. The solid line is the calculated rate profile (absorbance vs. time) and the circles are selected points from the over 500 experimental points available from each run. Figure 10 also shows calculated data considering all the excited ligand remaining as L* through the entire run (curve A) and all the ligand remaining as L during the entire run (curve C). The excellent agreement displayed in these figures was observed with all the experimental data collected in this study, indicating at least the validity of the mathematical model to predict experimental trends. In turn this validity lends credibility to the mechanistic postulations.

Speculative Considerations Regarding Structural Forms of Ligand and Metal Chelate Involved in the Photochromic Studies Reported Here. Meriwether et al. have reported dithizone as photochromic without providing apparent experimental evidence for such behavior (8). Coleman et al. (13), on the other hand, reported spectral evidence for a photochromic change in the absorption spectrum of dithizone when its chloroform solutions were subjected to the near-infrared radiation of a Cary 14 W lamp. With the experimental setup and under the experimental conditions described here, no net photochromic changes were detected but this may very well be the result of the particular configuration of the cell and its geometry toward irradiation. Unfiltered irradiation with a General Electric Quartzline Lamp (ELH, 120 V, 300 W) and simultaneous acquisition of absorption spectra with a Hewlett-Packard HP8450A spectrophotometer, however, did show bleaching of the green color, with the associated absorbance decrease of the λ_{max} values of 450 and 620 nm characteristic of dithizone. Evidently radiant power and the energy of the photons used in the irradiation process are critical in characterizing the type of behavior observed and point to the need of a systematic study of these variables which is under way. Its results will be reported elsewhere. In any event sufficient evidence has been accumulated for a change in the dithizone species upon irradiation as well as a modifying effect on the relaxation of the excited dithizone species by the metal chelate itself. As such, the net effect of (a) excess free ligand in increasing the value of the rate coefficient for return to ground state of the metal chelate while (b) an increase in irradiation time, under the same conditions, decreases the same value, can be interpreted to



Figure 11. Ground state and excited forms of dithizone and mercury(II) dithizonate.

be the result of dithizone existing in two different species when irradiated.

Because the free ligand and the metal chelate are affected by light, one can speculate that a common mechanism exists for both species. That photochromism of the metal chelate resides in the attached ligand finds support in the literature (8, 9) in the observation that location of absorption bands for different dithizonates are independent of the metal ion. Structures such as I (Figure 11), which is reported for solid dithizone (11), show two protons available to form five-membered rings through hydrogen bonding. Structure II illustrates the proposed excited form of the ligand, which would result from the rearrangement of one of the two hydrogen bonds from N–H–S to N–H–N. Support for this structure is the fact that the same trans-cis photochromic isomerism is exhibited by S-alkylated dithizones (11). Structure III is proposed for the ground state and has been reported for solid mercury(II) dithizonate (14). Structure IV is proposed for the excited state and basically a similar trans-cis isomerism was suggested by Meriwether to account for the photochromism of the metal chelate (8, 9). Stereo models show that the nitrogen atom that accepts the proton in his model is the same one that couples through a hydrogen bond to form a five-membered ring in the dithizone molecule. Thus Meriwether's structure of the metal chelate in the excited form is a resonance form of the model resulting from trans-cis isomerism and is shown by structure IV. Since the trans-cis isomerization depends on the azomethine link in both ligand and metal chelate, a common mechanism for their photochromism seems reasonable.

Four experimental facts must be taken into account when the mechanism for the return to ground state is considered: (1) the rate of return is independent of the metal chelate concentration, (2) excess free ligand facilitates the return, (3) the rate coefficient for the return to ground state increases during the time of relaxation following long periods of irradiation in the presence of excess free ligand, and (4) after a standing time of 4 to 20 h, water does not affect the return process. The first of these experimental facts implies that the reverse isomerization process is not assisted through collisions with neighboring molecules and that the N-S protons of the complex do not participate to any great extent in an intermolecular transfer. The energy stored in the excited species is thermally released to the solvent.

The role that excess free ligand plays in the relaxation of the metal complex is explained in terms of its N and S protons. Either proton in structure I can transfer to the sulfur or nitrogen atoms of the excited molecule of structure IV and facilitate its return to the ground state.

For an explanation of the third experimental fact, it may be noted that following the trans-cis isomerization of the ligand due to irradiation, one proton is bonded between two nitrogen atoms. This bonding effectively restricts its participation in any exchange reaction and in turn stabilizes the ligand in such a way that the other proton is no longer labile. Hence, with longer irradiation periods more ligand is promoted to the excited state (where both protons are stabilized) and there is no exchange reaction.

Interpretation of the effect of water is not easy and additional experimental work is needed to clearly account for it. It seems related to the general fact that under certain experimental conditions, even in absence of water, the system needs to reach a steady-state situation with respect to the species involved if reproducibility is to be realized. Water may participate, through hydrogen bonding, in the alteration of compositional distribution of the different species involved.

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Determination of the Molar Substitution Ratio of Hydroxyethyl Starches by Gas Chromatography

Ying-Chi Lee, David M. Baaske,* and James E. Carter¹

Analytical Methods Development Group, Product Development Section, Pharmaceutical Development Department, Research & Development Division, American Critical Care, McGaw Park, Illinois 60085

An improved gas chromatographic method for the determination of the molar substitution ratios of hydroxyethyl starches has been developed. The hydroxyethyl groups are cleaved from the starch by hydriodic acid through heating in a sealed vial in the presence of adipic acid for 10 h. The reaction product is extracted into o-xylene and chromatographed at 100 °C on a column of 10% UCW-98. A percent relative standard deviation of less than 0.95% was obtained.

Hydroxyethyl starches (HES) have been used medically as plasma volume expanders, in the treatment of physiologic shock, during leukapheresis, and for cryogenic strorage of whole blood. They are even more widely used in nonmedically related industries as coating agents, thickeners, sizings, or glues.

Medical HES's are made from highly branched waxy-maize starch (amylopectin) by reaction with ethylene oxide. They are readily water soluble and in contrast to native starch aqueous solutions are stable over prolonged periods. As with other synthetic polymers, hydroxyethyl starch is a heterogeneous group of molecules. Its chemical, physical, and pharmacological properties are primarily dependent on molecular

 $^1\,\mathrm{Current}$ address: Ortho Pharmaceutical Corp., Research Laboratories, Raritan, NJ $\,$ 08869.

weight, molecular weight distribution, the amount of hydroxyethyl substituents, and the pattern of that substitution.

The molar substitution ratio (MS) is defined as the number of moles of ethylene oxide groups per mole of anhydroglucose units. It is one of the key determinants of the duration of pharmacological action, water solubility, and solution stability of HES. A second measure of the hydroxyethyl substitution on HES is the degree of substitution (DS) which is defined as the number of moles of anhydroglucose units with at least one hydroxyethyl substituent divided by the total number of moles of anhydroglucose. For extensively substituted HES the differences between DS and MS is significant due to the increasing percent of multiple substitution to a single anhydroglucose unit with increasing hydroxyethyl content.

The ability to rapidly and accurately determine the MS ratio is a critical factor in proper control of chemical, physical, and biological properties of HES. Several analytical methods have been developed over the years for the determination of the molar substitution ratio. All of them are based on the cleavage of hydroxyethyl anhydroglucose ether bonds in refluxing hydriodic acid. The reaction products derived from the hydroxyethyl group are then quantitated. They are all based on the method developed by Morgan (1) for ethers and esters of ethylene glycol and applied to (hydroxyethyl)cellulose. The method was subsequently modified by Lortz (2) for use with low-substituted starch ethers. The products are ethyl iodide and ethylene which are quantitated by reaction with