# SOME OBSERVATIONS ON THE CHEMISTRY OF $KO_2$–DMSO SOLUTIONS

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## 1. Introduction

Dimethyl sulfoxide (DMSO) is the most commonly used non-aqueous solvent for the superoxide ion ($O_2^-$). Three main sources for $O_2^-$ in DMSO are potassium superoxide ($KO_2$) [1], tetramethylammonium superoxide [2] ($(CH_3)_4N^+O_2^-$) and electrochemical reduction of dissolved $O_2$ in the presence of supporting electrolytes [3]. A comprehensive discussion of these solutions is given in [4]. The solubility of $KO_2$ in DMSO can be increased by a factor of 30 by addition of 18-*crown*-6 ether, which complexes $K^+$.

Recommended methods for determination of $O_2^-$ concentrations in DMSO include simple measurement of optical absorbance of the solution [5], and formation of color by addition of nitro-blue tetrazolium ($NBT^{2+}$) or related compounds [6]. We find these methods unsatisfactory, because of interference by absorption of $O_2$, dimsyl ion ($CH_3SOCH_2^-$), and (if crown ether is added to solubilize $KO_2$) the $K^+$–*crown*-ether complex, all of which appear to be present in variable amounts in $KO_2$ solutions. Also, dimsyl ion effectively mimics some of the chemistry of the superoxide radical. Methods which gave good results include determination of oxygen formed by iodine oxidation of $O_2^-$ [7], and optical determination of reduction of ferricytochrome *c* [8,9].

## 2. Materials and methods

$KO_2$ (purity <97%) was obtained from 3 suppliers: Pfaltz and Bauer; K and K Chemicals; Mine Safety Appliances (MSA). Material from the first 2 was granular, and was ground in a mortar before use; the MSA material was a fluffy powder. DMSO from Burdick and Jackson Labs. was redistilled over $CaH_2$ through a 20-plate column under reduced pressure. Materials were stored, solutions made up and manipulations carried out in a dry-box under purified nitrogen or helium. Cytochrome *c* from horse heart, and nitro-blue tetrazolium were from Sigma Chemical Co. The 18-*crown*-6 ether from Alfa products was purified by precipitation from dimethoxyethane [10]. Potassium hydride (KH) dispersed in mineral oil, was obtained from Aldrich Chemicals and was washed with hexane and dried under $N_2$. Spectra were obtained with a Cary 210 spectrophotometer.

## 3. Results and discussion

### 3.1. *Reactions with $NBT^{2+}$ and other reagents*

Superoxide in aqueous solution reacts quantitatively with $NBT^{2+}$ to give a blue color [11], and the method has been used for its determination in DMSO [6]. We find however that KOH dissolved in DMSO gives the same blue color with $NBT^{2+}$. Since $KO_2$ inevitably contains some KOH, the $NBT^{2+}$ reaction cannot be used for $KO_2$ solutions in DMSO. Other compounds which give color reactions with $O_2^-$, and were also found to react with solutions of KOH in DMSO, included *p*-iodonitrotetrazolium violet, *p*-benzoquinone, anthraquinone, duroquinone and tetranitromethane.

### 3.2. *Optical properties of $KO_2$ solutions*

Slurries of ground $KO_2$, stirred by bubbling with $N_2$ for different times (10 s to 90 min), yielded on filtration solutions which by the oxygen evolution method always showed 5.1 ± 0.4 mM (16 samples), but their optical absorbances (1 mm pathlength) at 270 nm ranged from 0.96–1.67. $KO_2$ exposed to ordinary humid air must inevitably contain some KOH and KOOH. Although DMSO is often referred to as an 'aprotic' solvent, it is well known to react

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with strong bases such as t-BuOK to form the dimsyl ion [12], $CH_3SOCH_2^-$. We suspect that some dimsyl may be present in $KO_2$ solutions. For example, in a typical experiment, a solution of $H_2O$ in DMSO was added to a 0.48 mM $KO_2$ solution to give a solution 5.56 M in $H_2O$. While there was a rapid drop of 26% in the absorbance at 265 nm, the cytochrome *c* titration showed a drop of only 2% in the $O_2^-$ concentration. Subsequently, the absorbance and $O_2^-$ concentration declined relatively slowly; a further drop of 75% in the absorbance and 77% in the $O_2^-$ concentration was found after 143 min. Similar results were found at lower $H_2O$ concentrations, and the rate constant for reaction of $H_2O$ with $O_2^-$ in DMSO was found to be about $\sim 1.2 \times 10^{-4}\ M^{-1} . s^{-1}$ (similar to the rate found in dimethylformamide [13]), but something in the solution reacts much faster with $H_2O$. Although we have some indications that this is dimsyl, we have no definitive proof since the well-known red color reaction with triphenylmethane [14] gives a faint coloration lasting only seconds. The rapid fading of the red color of the triphenylmethyl carbanion is most likely due to the presence of small amounts of oxygen in the system which bleaches the color.

We prepared solutions of dimsyl by reacting KH with DMSO [15], and found that the absorbance is proportional to the concentration (determined by formanilide titration [16]) at <1.4 mM; the quotient falls at higher concentrations, probably owing to aggregation [12]. The low-concentration spectrum is shown in fig.1B. These solutions are not very stable, and the values shown are reproducible to only ±20%. Solutions of KOH in DMSO showed considerable absorption, but it was neither reproducible nor stable.

Oxygen gas exhibits a charge-transfer spectrum in the ultraviolet in most organic solvents [17], and DMSO is no exception. We determined the solubility of oxygen in DMSO (by the gas chromatographic method described below) as $2.20 \pm 0.09\ mM . atm^{-1}$ at 22.5°C. The absorption spectrum of $O_2$ in DMSO (recorded *vs* argon-saturated DMSO) is given in fig.1C.

Since $KO_2$ solutions are often made up with 18-*crown*-6 ether added to solubilize the $K^+$, we determined the spectrum of KF in 0.3 M 18-*crown*-6 ether in DMSO, shown in fig.1A. Exactly the same spectrum was found in solutions of KCl, KCN and $KH_2PO_4$.

Since it seems impossible to eliminate traces of water, KOH, possibly dimsyl and/or other extraneous species, we were unable to determine an authentic spectrum of $O_2^-$ in DMSO. Published values of its extinction coefficient [7,18] must be regarded as suspect.

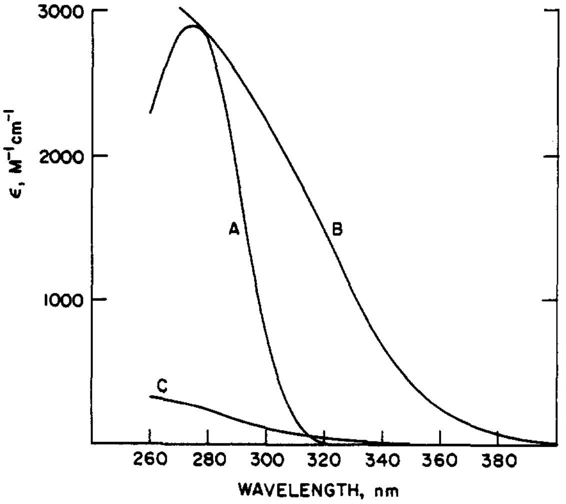

Fig.1. (A) KF in DMSO containing 0.3 M 18-*crown*-6 ether; (B) dimsyl potassium ($CH_3SOCH_2^-K^+$) in $Me_2SO$ at <1 mM; (C) oxygen dissolved in DMSO (1 atm).

### 3.3. *Oxygen evolution by gas chromatography*

$O_2^-$ is quantitatively converted to oxygen by reaction with an excess of iodine in DMSO [7]. He-Saturated dilute $KO_2$ in DMSO (1 ml) was injected from a syringe into a chamber containing He-swept 1 M $I_2$ in DMSO. The $O_2$ formed was swept through silica gel into a Varian series 1520 Aerograph equipped with a 3 m column (4 mm i.d.) packed with mixed 5A-13X molecular sieves, which gave well-separated peaks of nitrogen and oxygen. The area under the oxygen peak was determined, and calibrated using $O_2$ saturated water (1.30 mM). Determinations of $KO_2$ concentrations in DMSO were reproducible to ±2% at 1 mM and ±4% at 0.1 mM.

### 3.4. *Titration by ferricytochrome* c *(cyt.* c*)*

Oxidized cyt. *c* reacts quantitatively with $O_2^-$ in aqueous solution and the reaction can be very precisely monitored at 550 nm, where the change in molar extinction coefficient is $21\,100\ M^{-1} . cm^{-1}$ [9]. In DMSO solution, $O_2^-$ can be determined by adding the solution to a large volume of aqueous cyt. *c*, but great care must be taken to avoid the self-disproportionation reaction of $O_2^-$ which occurs in water as a result of partial conversion of $O_2^-$ to $HO_2$, and which competes with the reaction of $O_2^-/HO_2$ with the cyt. *c*. Since

Table 1
Comparison of cyt. *c* and $O_2$-evolution methods

| Dilution factor | $[O_2^-]$ (mM) | |
|---|---|---|
| | $O_2$ | Cyt. *c* |
| 1 | 0.386 | 0.407 |
| 2 | 0.198 | 0.196 |
| 4 | 0.092 | 0.103 |
| 8 | 0.045 | 0.051 |

the disproportionation is less rapid in alkaline solution, the cyt. *c* is phosphate-buffered to pH 9.75; the solution is rapidly stirred, and the DMSO solution added very slowly (<0.3 ml/min) to avoid any significant buildup of $O_2^-$ concentration. Dropwise addition must be avoided, since one small drop will carry too much $O_2^-$ at once into the solution; the biuret tip is immersed in the stirred solution, and the flow rate controlled by a Fisher-Porter Lab-Crest stopcock.

Table 1 shows results obtained by this method on a series of solutions obtained by successive 1:1 dilutions from a diluted stock solution of $KO_2$ in DMSO, together with determinations on the same solutions by the oxygen evolution method.

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