In situ Generation of Ruthenium Tetroxide and Osmium Tetroxide for the Physical Sciences and Their Reaction Indicators

Paul Beauregard
Chemist and Electron Microscopist
beauregа@westol.com

Introduction:

Recently, there was a suggestion on the MSA listserver about the use of osmium tetroxide (OsO_4) and how to handle it. One suggestion was that ampoules be scored, placed in a glass jar, and the ampoule smashed to release the contents. This seemed like a very unsafe way to use osmium tetroxide or ruthenium tetroxide. The purpose of this article is to suggest a way to generate smaller amounts of these compounds in a safer manner than smashing ampoules and wondering about what to do with the unused portion after staining or storing. Another purpose is to discuss a new reaction indicator for mainly osmium tetroxide. The use of a reaction specific indicator was mandatory for judging the level or degree to which staining had proceeded in thin sections for the transmission electron microscope (TEM).

Tetroxide Backgrounds:

The book *Polymer Microscopy*[1], shows a reaction where ruthenium trichloride reactes with sodium hypochlorite to produce ruthenium tetroxide (RuO_4). This reaction could be used to generate *in situ* RuO_4 and its vapors at a fairly fast rate. The safest way to proceed is to minimize the amount of RuO_4 generated, but still generate enough to stain thin sections. A method used to do this in the past is outlined below.

While using gloves, 1 to 2 mgs of $RuCl_3.xH_2O$ were typically weighed out, to which were added a few mLs of ~5% sodium hypochlorite solution. The generated RuO_4 gas was contained inside a nearly sealed glass container like a Petri dish or ground glass covered dish. This "minimal use" procedure limited accidental human exposure to a very minute amount of the dangerous fumes generated if anything went wrong. One to two milligrams (mgs) was enough RuO_4 material to stain most TEM grids in less than 5-10 minutes. Since this is a vapor-state staining process, one needs a reaction indicator and various materials that can be used, and are listed as reacting with RuO_4 in the book *Polymer Microscopy*[1]. After experimentation, 3M tape 665 was found to turn dark enough to indicate the degree of reaction. 665 does not work with OsO4.

Looking at the periodic table of the elements, it seemed reasonable that OsO_4 could be generated by using an analogous reaction equivalent to the reaction of ruthenium trichloride hydrate with sodium hypochlorite or Clorox® bleach. However, these two tetroxides did not react at the same rates.

Various reactions for RuO_4 generation were listed in the book *Advanced Inorganic Chemistry, A Comprehensive Text*[2] and could be reviewed. The use of heat and nitric acid were described. What was needed was an OsO_4 analog of the *in situ* RuO_4 generation at room temperature.

Discussion of Results:

During the listserver exchange mentioned above, it was suggested that sodium or potassium periodate be used for *in situ* generation of OsO_4. That reaction was stated or hinted at in *Advanced Inorganic Chemistry* by Cotton & Wilkinson[2]. On the Internet there was a strange statement about osmium dioxide. Paraphrasing, "When you opened a bottle of osmium dioxide you could smell traces of OsO_4." This air oxidation statement suggested that *in situ* generation of OsO_4 should be possible with a more powerful oxidizer. A review of microscopy textbooks yielded nothing of value about this statement, neither about *in situ* generation of OsO_4, nor about how to gauge the progress of the staining reaction with an indicator. This gauge was something that was needed. If one stained with fumes from a 2% or 4% solution, the reaction times would vary and one risked over-staining thin sections on TEM grids. Not only that, the concentration of the fumes would vary with the apparatus volume used. If one used an opened ampoule to suspend the grids in or over the OsO_4, then air currents could dilute the vapors and give variable results. Clearly, any procedure needed a reaction indicator gauge for either tetroxide. Furthermore, a way was needed to partially control the way the tetroxides were produced and their concentration in a closed system like that mentioned above.

The next step was to find a good reaction indicator for OsO_4. An old dilute solution of osmium tetroxide in sulfuric acid from G. Fredrick Smith was used to conduct experiments to test for a good OsO_4 reaction indicator. It was quickly discovered that OsO_4 did not stain the 665 indicator tape because the tape did not have enough double bonds, if any.

Having worked on rubber samples, some obvious indicator choices existed. One was a gum stock butadiene rubber known as Budene® 1207 (Goodyear Tire & Rubber). It was relatively colorless and transparent. Natural rubber worked fine, but it was not pure and was colored depending on the grade used. The Budene 1207 was dissolved in cyclohexane as a thick saturated solution. This took about a week of occasional shaking. This solution could then be spread out on a Fisher Superfrost® microscope slide to form a thin indicator film. Sometimes a thicker localized spot of deposited rubber would also be created. This Budene indicator gradually turned darker with increased exposure to the GFS osmium tetroxide solution when placed in the Petri dish apparatus. This was a fairly good indicator but a better commercial indicator might be found by someone else.

Osmium dioxide and osmium chloride were chosen for the starting osmium materials. It was very clear that *in situ* generation of OsO_4 was not going to lead to a cheaper way to make OsO_4. None of this material was cheap. On the other hand, one only had to buy a small amount, such as five grams for testing.

For oxidizers, I chose 30% hydrogen peroxide, sodium periodate and Clorox® bleach (sodium hypochlorite). Both osmium compounds produced a darkening of my indicator and natural rubber pieces in the Petri dish with selected oxidizers. The bad news was that the reaction did not proceed at a very fast rate. Instead of 1 to 2 minutes, the osmium compounds required 1 to 2 hours or longer to stain thin sections using 1 to 2 mgs. The good news was that it did stain the double bond indicator at room temperature. I did not try to heat any of the reactants to see if a quicker reaction occurred. I did not want to generate hypochlorous acid fumes by heating bleach, for example.

OsO_2 reacted very slowly and could take hours or overnight to generate enough OsO_4 to stain grids. OsO_2 powder also caused hydrogen peroxide to almost instantly decompose *very violently*. *DO NOT* use 30% H_2O_2 with OsO_2. The other two oxidizers worked fine. OsO_2 had some drawbacks. One was that it never reacted totally, and thus one could be left with a dangerously fine powder of OsO2 at the end of the reaction to clean up. This could be messy, so use gloves.

$OsCl_3$ reacted with all three oxidizers and did generate OsO_4 at a faster rate than OsO_2, but at a much slower rate than $RuCl_3$ generated RuO_4. $OsCl_3$ did generate enough OsO_4 over a 1 to 3 hour time period to stain thin sections. One only needed a few milligrams to do the job, just like $RuCl_3$. Using more $OsCl_3$ would

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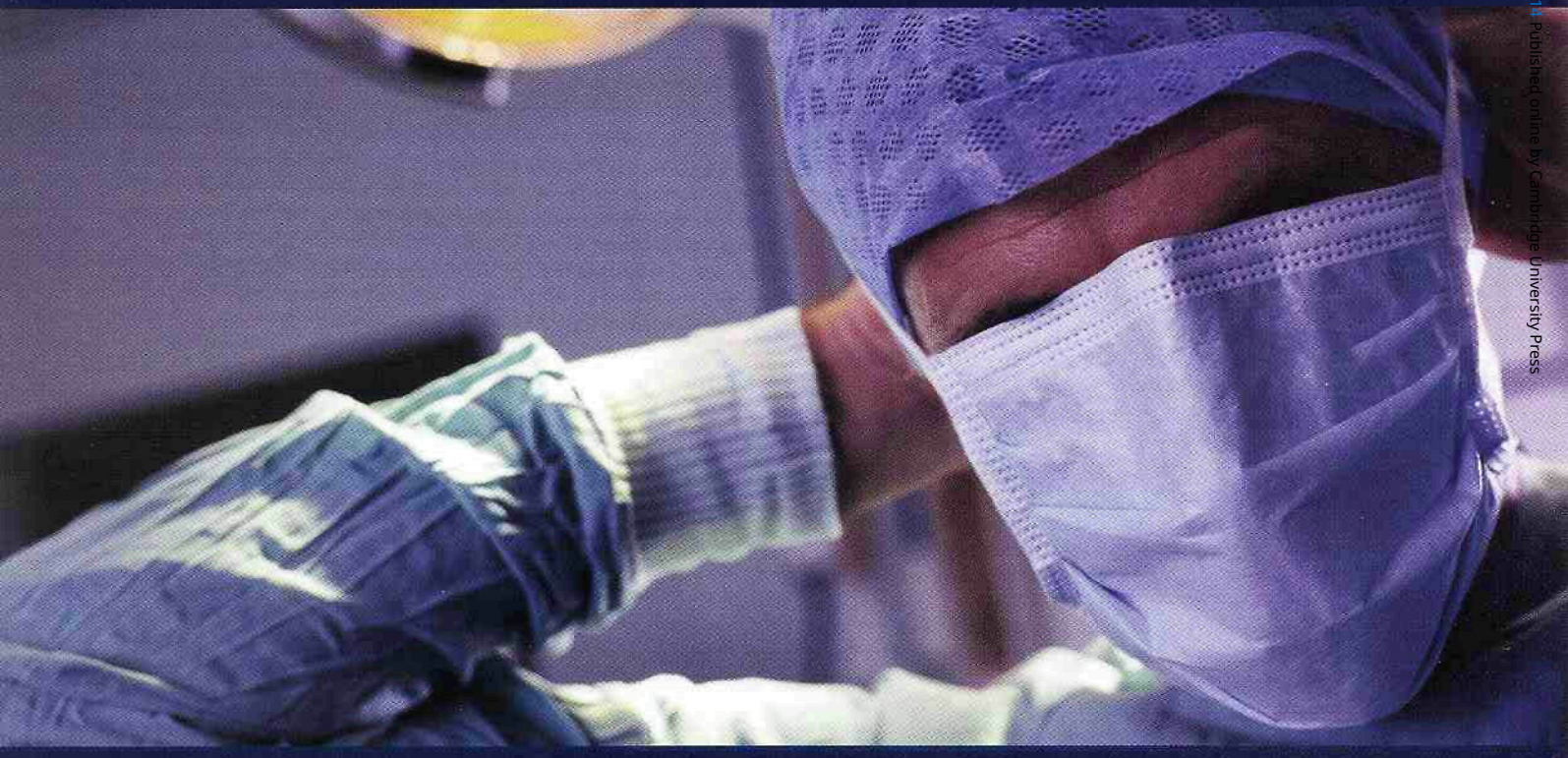

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only make clean up and waste disposal more complicated, but it would speed up the staining. One should only use the amount that the apparatus would require to stain the desired grids in a reasonable time period. The Budene 1207 glass microscope slide was used to gauge the degree of staining of the thin sections or polymer cast films. Gloves were used at all times to avoid skin absorption and reaction. Different manufacturer's MSDS sheets were read to get a better viewpoint of the toxic nature of OsO_4, $OsCl_3$ and OsO_2. These MSDS sheets were a bit scary to read.

In summary, these osmium reactions were truly analogs of the RuO_4 *in situ* generation scheme. *In situ* generation of OsO_4 was much safer to handle in my opinion than ampoules. Hypochlorite and $OsCl_3$ were the preferred materials for staining of thin sections by *in situ* generation of OsO_4.

All MSDS sheets for OsO_4, OsO_2, $OsCl_3$, RuO_4, $RuCl_3$, *etc.*, were read. Gloves were required at all times. An unobstructed fume hood airflow was required. Testing of the hood airflow was done with ammonium hydroxide and hydrochloric acid fumes that were used to create white smoke at the hood inlet face.

Disclaimers:

Osmium compounds are **highly toxic**, not just hazardous. Extreme care should be used in handling osmium compounds or ruthenium compounds. All the precautions and handling precautions for ruthenium and its compounds should apply to osmium and its compounds. Osmium absorbs more through the skin and travels further down into the skin than does ruthenium. MSDS sheets suggest that osmium is more hazardous to use. Read the MSDS sheet(s) in detail. Think about the consequences of using poor techniques. Nobody can be responsible for your handling of these compounds but you. You use them at your own risk.

Conclusions:

1) *In situ* generation of both RuO4 and OsO4 was possible and practical for staining things like polymer thin sections, *etc.*.
2) Sodium hypochlorite was the easiest oxidizer to use and was easily obtained.
3) RuO_4 was more hazardous, more widely reactive, and was easier to generate *in situ*.
4) OsO4 was more toxic, hazardous, and was harder to generate *in situ*.
5) Use only the smallest amount needed to do the staining job in the apparatus used.
6) The use of a reaction indicator gauge was mandatory.
7) In severe cases, like sterically hindered reactions, an indicator made up of one's sample or its components would be needed for proper stain reaction gauging.
8) Samples were done in batches to avoid repeated exposure and toxic accumulations.
9) Use these chemicals only if needed.
10) It was easiest to generate RuO_4 from $RuCl_3.xH_2O$. One could generate OsO_4 vapors using OsO_2 or $OsCl_3$ but they were relatively slow to react versus $RuCl_3$.
11) Corn oil, Budene 1207, ethanol, DOP (dioctylphthalate) or some other appropriate tetroxide scavenger should be used at the end of the staining process to react with any excess tetroxides.
12) Safety was number one. Talking with a previous user was very helpful. These compounds are hazardous, toxic, poisonous and had very low exposure limits. The TWA exposure was about 2000 times lower than for phosgene gas that could kill. ■

References:

1) Sawyer Linda C., Grubb David, *Polymer Microscopy*, Chapman and Hall, p105 to 124, 1987.
2) Cotton F. Albert, Wilkinson Geoffrey, *Advanced Inorganic Chemistry A Comprehensive Text*, second edition, Interscience, p 1005, 1966.