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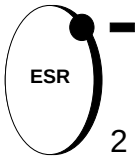

At the Forefront of
Free Radical Research

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Chelating Buffers to Remove Adventitious Metals [1]

1. Introduction/Background

Trace amounts of adventitious transition metals are naturally present in buffer solutions. The approach below can minimize this contamination. Our experience is that the level of contaminating metals can be less than 1 nM with this approach.

2. Reagents and Materials

Chelex resin

Chelex 100 (200-400 mesh sodium form) BioRad Lab CAT#142-2842
or
Chelex 100 sodium form (50 – 100 mesh) Sigma CAT# C7901

Buffer

For example phosphate-buffered saline.

Ascorbic acid (0.100 M)

We prepare ascorbate stock solutions using only the diacid. It is prepared as a 0.100 M stock solution (typically 10 mL) using high purity water. This solution is colorless, having a pH of ≈2. It is stored in a volumetric flask with a tight-fitting plastic stopper, thus access of oxygen to the solution is limited during long-term storage. As the solubility of oxygen in air-saturated water is 0.25 mM, the solution will become anaerobic with loss of <1% of the original ascorbate. If the flask is indeed clean, we have found that the solution can be kept for several weeks without significant loss of ascorbate due to the low pH and lack of oxygen. The appearance of any hint of a yellow color is an indication of ascorbate deterioration. We avoid the use of sodium ascorbate as it invariably contains substantial quantities of oxidation products as evidenced by the yellow color of the solid and of the solution.

3. Protocol to "clean" buffers

1. Use plastic ware to spoon out Chelex resin so that no metal is introduced into the resin.
2. In general use 5 - 10 mL resin for 1 liter of buffer.

3. Transfer resin into a 50 mL tube and add some buffer to wash the resin. (Resin is acidic and the wash will adjust the pH).
4. Centrifuge, gently, the 50 mL tube or let the resin settle and discard the supernatant.
5. Repeat steps 3 and 4 one more time.
6. Add washed resin to the batch of buffer that needs to be de-metalled.
7. Stir solution very slowly for approximately 24 h (resin needs time to chelate all the metals). We stir as slowly as possible so any moving and tumbling of the resin is as minimal as possible. This is to minimize mechanical breakage of the resin.
8. If buffer needs to be sterile, then filter through a 0.22 μm filter. Otherwise, buffer can be stored over the resin for several months. (Clean resin has a bright white appearance. (When the resin begins to show a rust-red (iron) or blue (copper) tinge, the resin should be renewed. The resin is expensive; it is easily recycled with acid washes, then pH adjustment.)

4. Ascorbate test for adventitious metals *via* UV spectrophotometer [1]

This test takes advantage of the fact that metals catalyze the oxidation of ascorbate. The test works well for buffers in the pH rage of approximately 6 - 7.5. Buffers with pH $\geq$ 7.8 can be problematic because the background rate of the true autoxidation reaction is high enough to not allow this test to be reliable.

The loss of ascorbate is followed by its UV visible absorbance at 265 nm, ε=14,500 M^{-1} cm^{-1} [1].

1. A meticulously clean standard 1 cm UV-cuvette is needed. Cleaning with HCl or HNO_3 is suggested to ensure that all catalytic metals are removed from the surface of the cuvette.
2. Transfer 3.00 mL de-metalled buffer into the UV-cuvette.
3. Add 3.5 μL of 0.10 M ascorbic acid solution; this will yield an absorbance of approximately 1.8 at 265 nm.
4. Monitor loss of ascorbate for 15 min at 265 nm. Interrogate the solution only a few times as the UV-light can initiate photo-oxidation of the ascorbate, this is especially true if a diode array UV-Vis spectrometer is used.
5. A loss of more than 0.5 % over 15 min indicates significant metal contamination.

1988-12; Rev 2007-04-17; Rev 2007-07-31; Rev 2008-01-02; Rev 2008-06-19; Rev 2008-07-01

References

1 Buettner GR. (1988) In the absence of catalytic metals ascorbate does not autoxidize at pH7: ascorbate as a test for catalytic metals. *J Biochem Biophys Meth*. **16:** 27-40. http://dx.doi.org/10.1016/0165-022X(88)90100-5 or http://www.healthcare.uiowa.edu/corefacilities/esr/publications/buettnerpubs/pdf/JBBM-1988-16-27-GRB-Ascorbate_test.pdf