

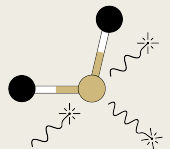
Raman Microspectroscopy: Articaine Hydrochloride 4% with EPI 1:200,000

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Report prepared for Dr. Jim Lundstrom (www.fargodentist.net)

Sample Preparation Methodology

- Ten samples arrived in tamper-resistant packaging
- 1 mL of anesthetic was pipetted from each vial into 2 mL centrifuge tubes
- Large magnet was placed underneath centrifuge tubes overnight to pull potential graphene/graphene oxide particles to the base of the tube
- Several methods of particle extraction were tested, though the anesthetic solution did not fully dry, leaving a liquid film that confounded the micro-Raman signal from potential particles. The following outlines the successful method for sample preparation for Raman microspectroscopy measurements:
 - *0.5 mL of anesthetic was pulled from one sample of anesthetic and pushed through a 0.2 μm filter into a separate tube, resulting in 0.25 mL of filtered anesthetic liquid*
 - *0.75 mL of MQ water was added to filtered anesthetic tube, resulting in a 1:3 ratio of anesthetic to water.*
 - *Filtered anesthetic tube was placed in a centrifuge for 15 minutes at 13.4 rpm.*
 - *10 μL of centrifuged anesthetic was pipetted onto an ethanol-cleaned glass microscope slide*
 - *Microscope slide was placed under a fume hood on a hot plate at 70 °C with a tube of air blowing through an 18 G needle located ~ 1 cm above the sample*



Raman Microspectroscopy Methodology

- The microscope slide of dried filtered anesthetic was examined for particles with characteristics synonymous with graphene (*i.e.*, dark carbon-like opaque bodies)
- Raman spectra from graphene-like particles were collected with the following instrument parameters:
 - *Instrument: Horiba LabRAM HR Evolution Raman Spectrometer*
 - *256x1024 pixel CCD detector*
 - *CCD Temperature: -70 °C*
 - *Laser wavelength: 532 nm*
 - *Grating: 600 gr/mm*
 - *Objective lens: 100x (N.A. 0.9)*
 - *Spectral range: 500-3200 cm⁻¹*
 - *Acquisition time: 5 sec*
 - *Accumulations: 5*



Results

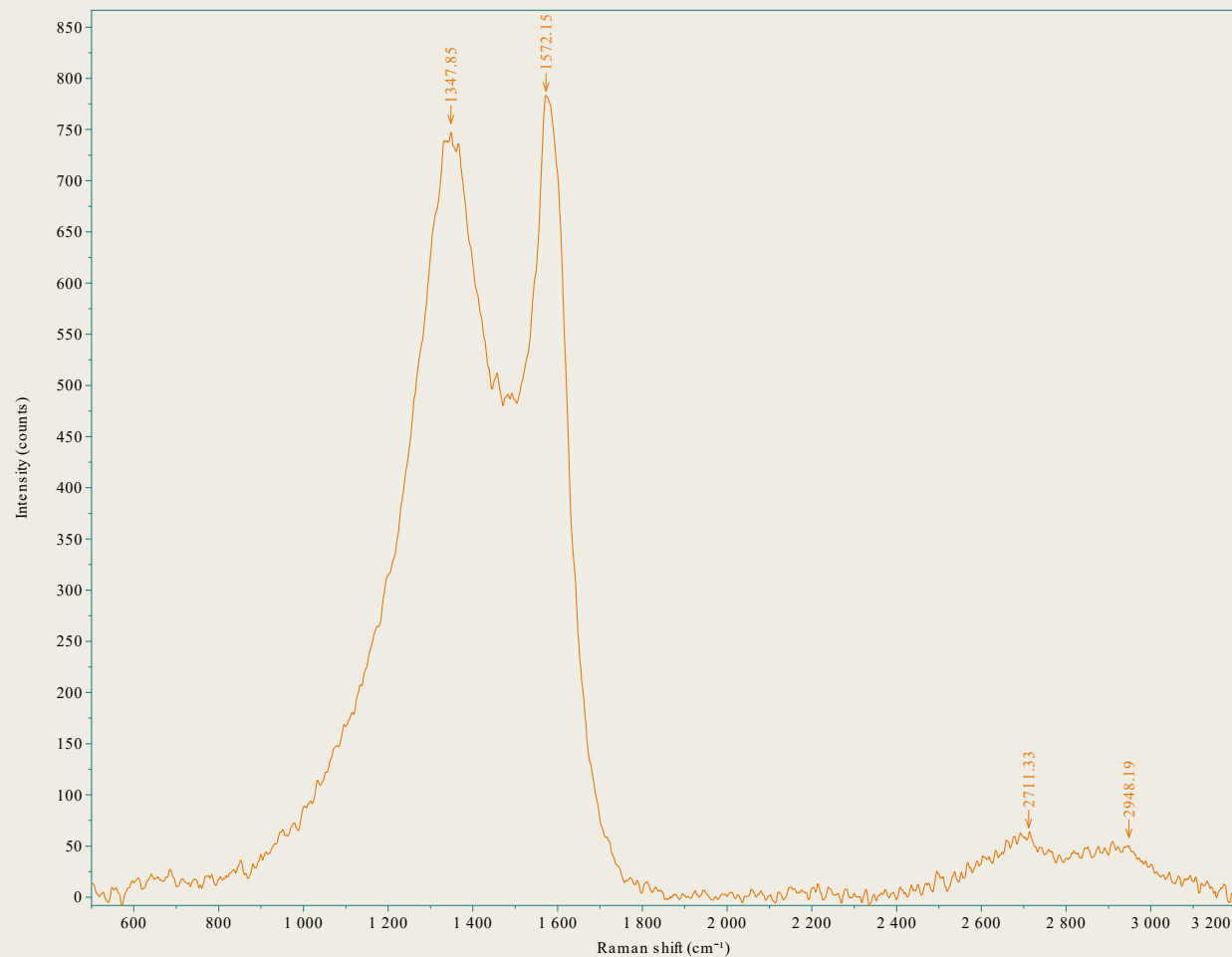
- Out of six particles examined, three particles resulted in spectra characteristic of graphene oxide (shown in following slides)
 - *Alternate three particles resulted in spectrum characteristic of the dried anesthetic liquid*
- Graphene oxide is characterized by two dominant peaks, D at $\sim 1350\text{ cm}^{-1}$ and G at $\sim 1585\text{ cm}^{-1}$, and a second-order band, 2D centered around $\sim 2900\text{ cm}^{-1}$
- Shifts in peak position and intensity (as shown by labeled peaks in following slides) are attributed to disorder and structural defects in crystal orientation
 - *D band peak intensity and width will decrease significantly when crystallinity is high and oxygen content is low, as this band is directly a measure of crystal disorder*
 - *2D band of graphene will be replaced by a “bump” (as seen in results) if there is a high degree of structural disorder*



Spectrum Results: Particle 1/3



▲ Graphene-like particle image acquired with transmitted light and 100x objective

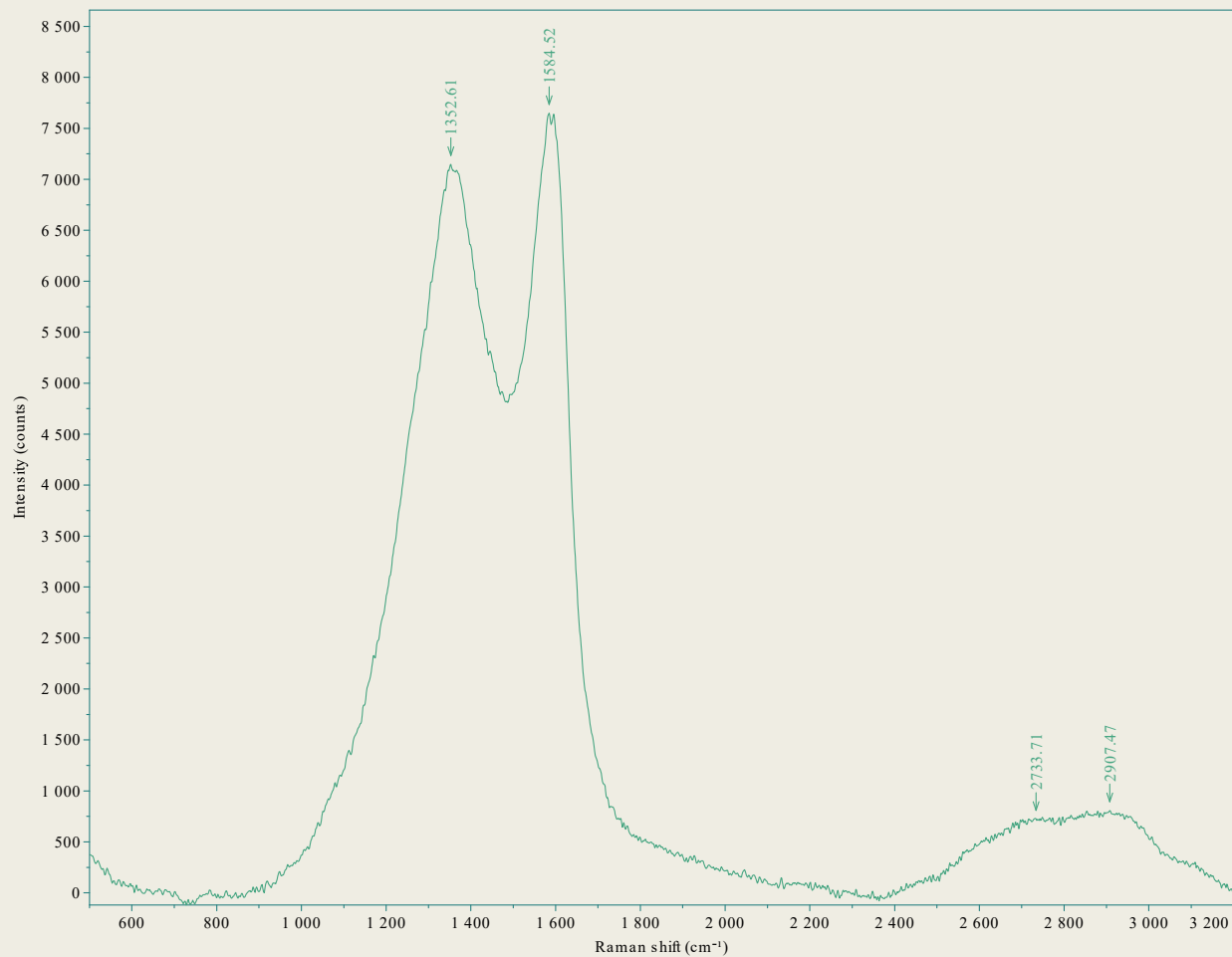


▲ Raman spectrum acquired from graphene-like particle. Peak positions (labeled) are associated with graphene oxide.

Spectrum Results: Particle 2/3

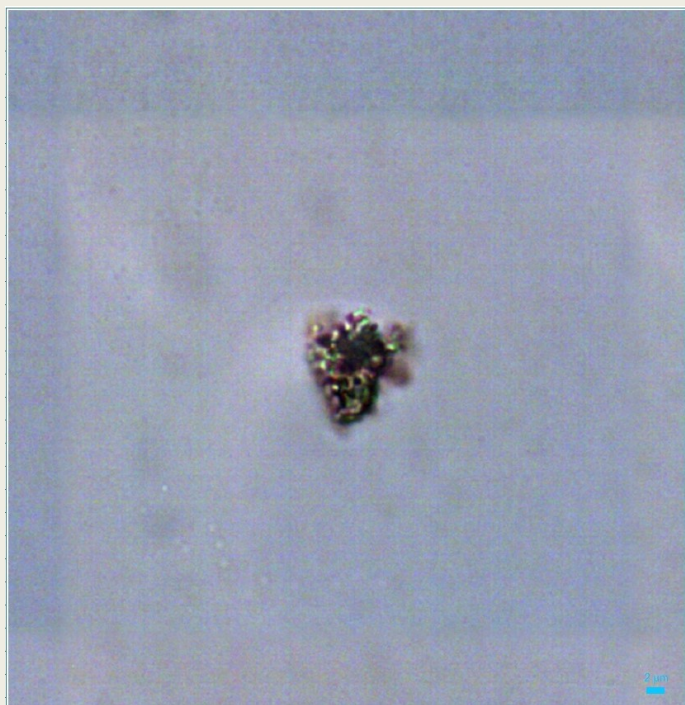


▲ Graphene-like particle image acquired with transmitted light and 100x objective

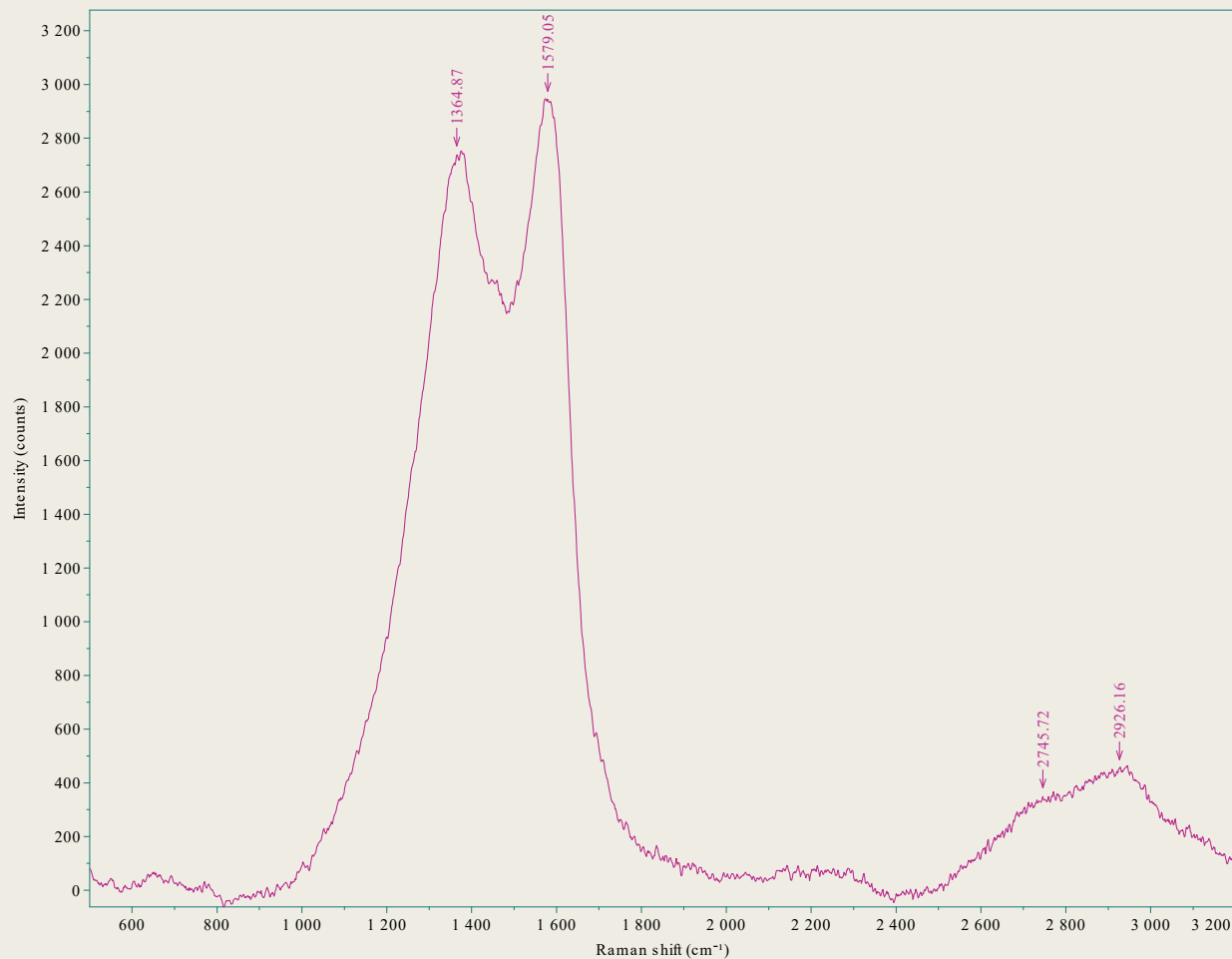


▲ Raman spectrum acquired from graphene-like particle. Peak positions (labeled) are associated with graphene oxide.

Spectrum Results: Particle 3/3



▲ Graphene-like particle image acquired with transmitted light and 100x objective



▲ Raman spectrum acquired from graphene-like particle. Peak positions (labeled) are associated with graphene oxide.

Conclusions

- Based on spectral results portraying D, G, and 2D peaks, I can affirm the presence of graphene oxide particles in this anesthetic with high confidence.
 - *Although I have confirmed the presence of graphene oxide in this anesthetic, the concentration or amount of graphene oxide particles in the anesthetic is unclear.*
- Spectrum acquired from the dried-liquid anesthetic produced inconclusive results, indicating a need for further testing for determination of lipid nanoparticles or hydrogels in the solution.

