# 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*

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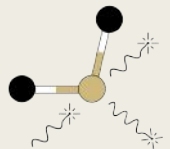
Raman Microspectroscopy Methodology

- 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*
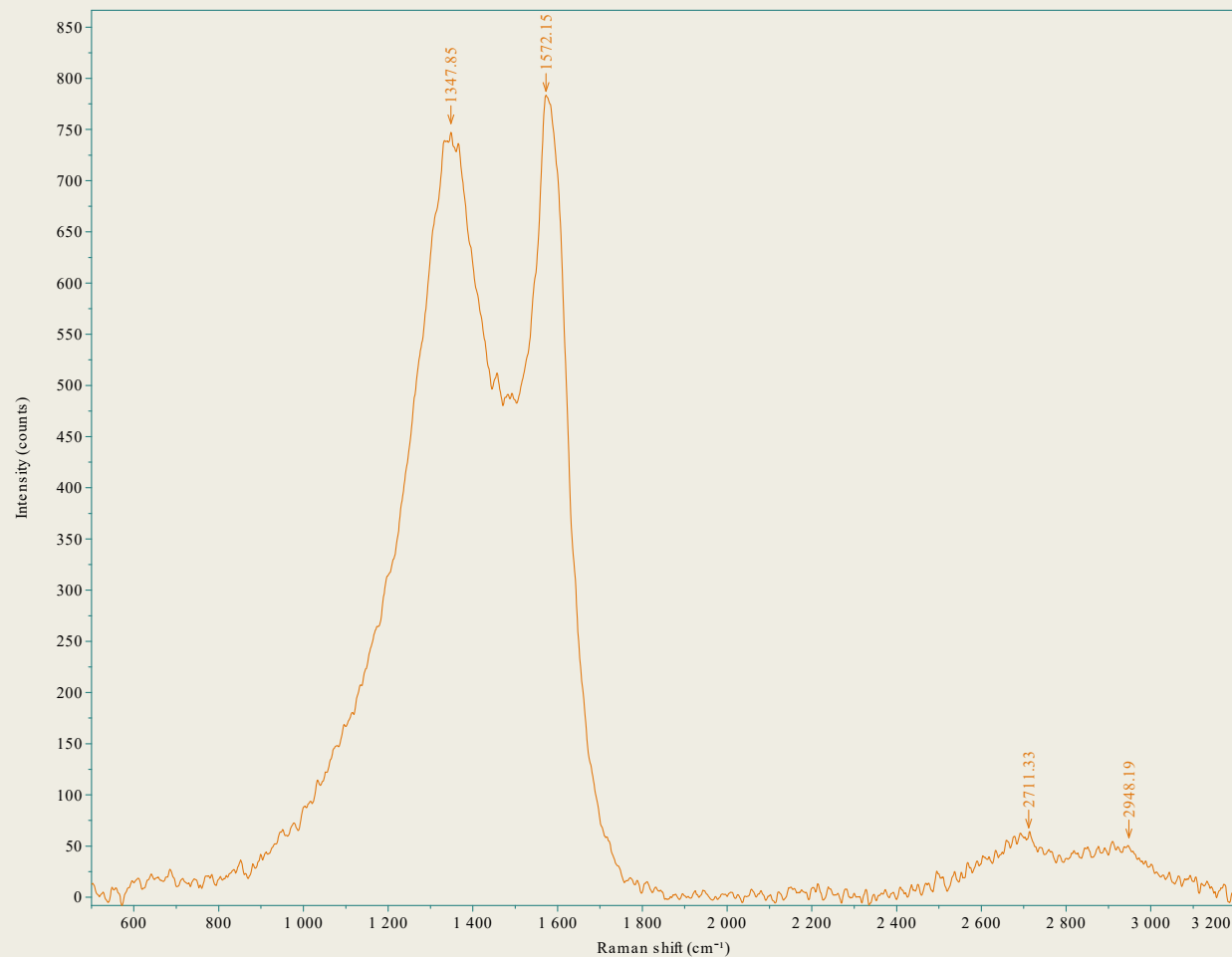
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Results

- 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 ~1350 cm-1 and G at ~1585 cm^{-1}, and a second-order band, 2D centered around ~2900 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*

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Spectrum Results: Particle 1/3

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

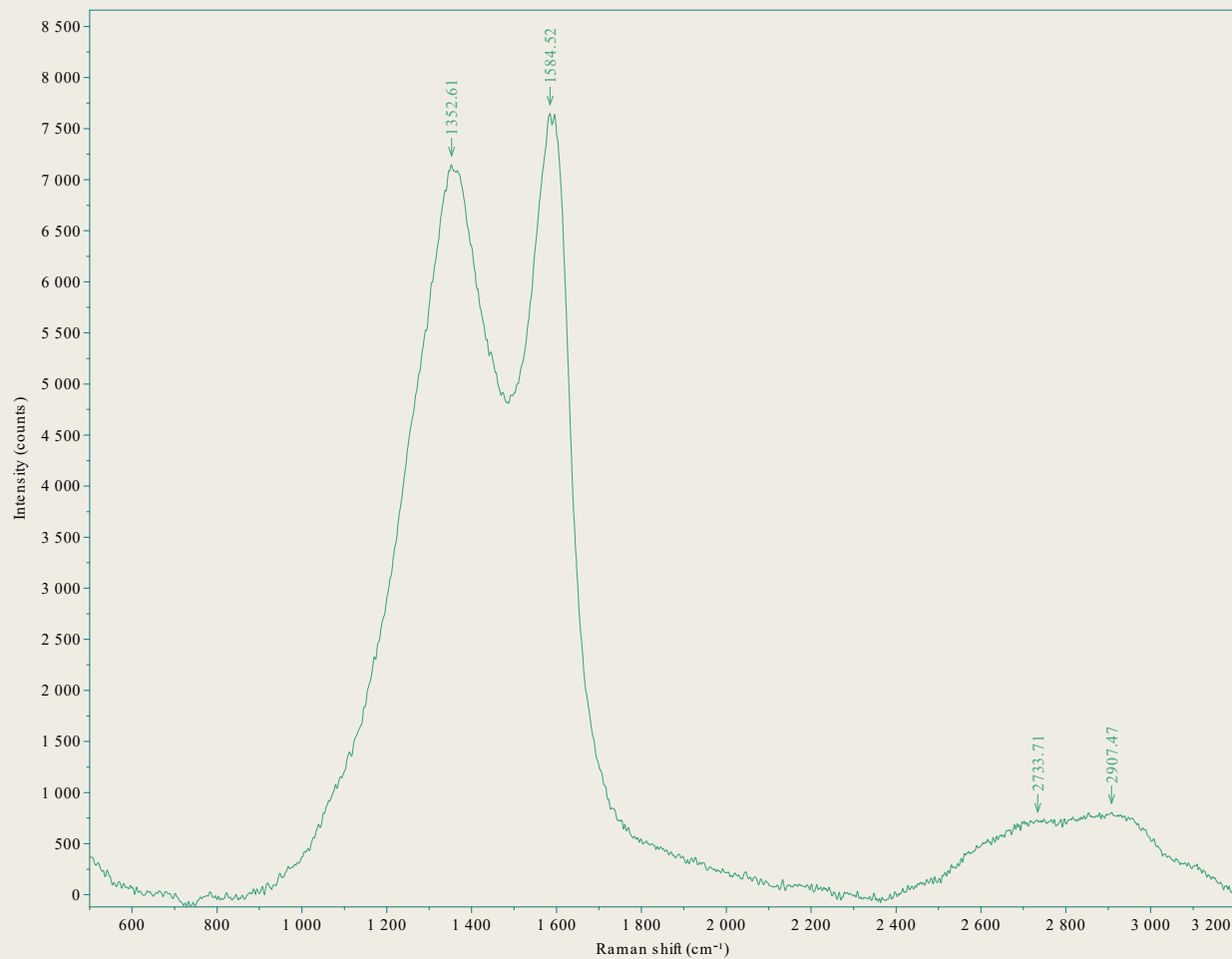▲ Raman spectrum acquired from graphene-like particle. Peak positions (labeled) are associated with graphene oxide.

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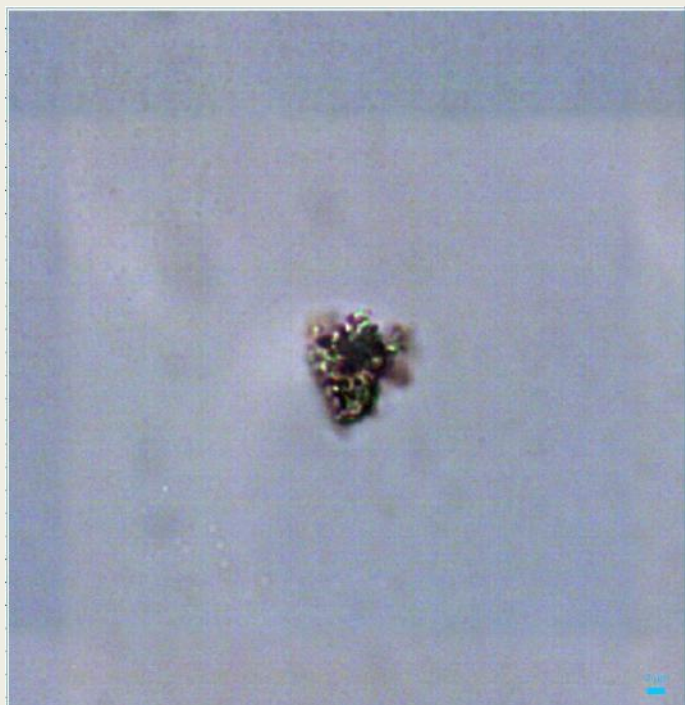Lab

Spectrum Results: Particle 2/3
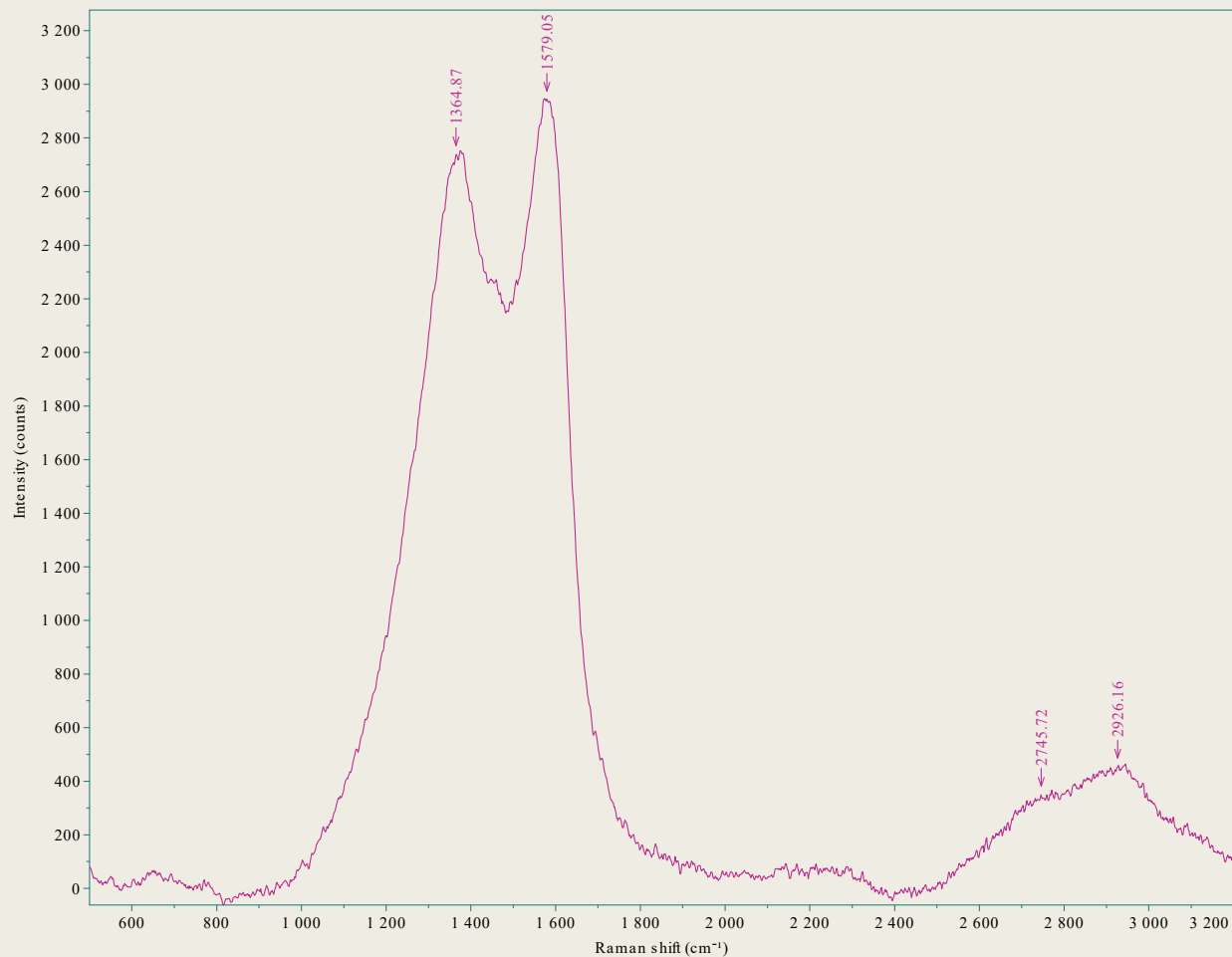

▲ Graphene-like particle image acquired with transmitted light and 100x objective

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

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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.

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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.

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