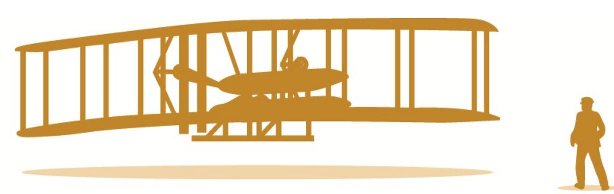

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Determination of the Intracellular Fate and Trafficking of Chelerythrine, a Potent BH3 Mimetic and Na^+/K^+ ATPase Inhibitor, in Human Lens Epithelial Cell Cultures via Surface-Enhanced Raman Spectroscopy (SERS)

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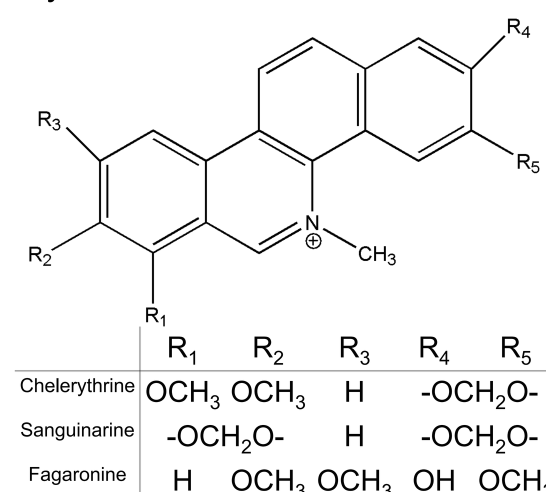
Introduction

Chelerythrine and QBAs

- Quaternary benzophenanthridine alkaloids (QBAs) have been known for centuries for their beneficial biological activity and use in a variety of medicinal treatments.[1]



- Chelerythrine (CET), in particular, has gained much attention in recent years as it has been shown to induce apoptosis in a variety of cancer cell lines.[2]
- Despite many reports on the potential therapeutic properties exhibited by this compound, little is known of the exact mechanism or intracellular fate of CET:[2,3]
  1. Protein kinase C (PKC) binding and inhibition.
  2. Pro-survival protein inhibition and activation of mitochondrial apoptotic mechanisms.
  3. Intranuclear trafficking with DNA binding.
  4. **Direct binding and inhibition of K^+ influx via the Na^+/K^+ ATPase.**

SERS and Biochemical Sensing

- SERS is a powerful, non-invasive, nanoscale sensing technique capable of detecting small molecules or proteins in biological matrices at ultralow concentrations.[4]

Main Scientific Goals

- *Aim 1:* Treat human lens epithelial cells (HLECs) with known concentrations of CET.
- *Aim 2:* Extract cellular components and determine the presence and concentration of CET via SERS.
- *Aim 3:* Quantify the amount of CET per µg of protein to determine potential targeting and affinities.

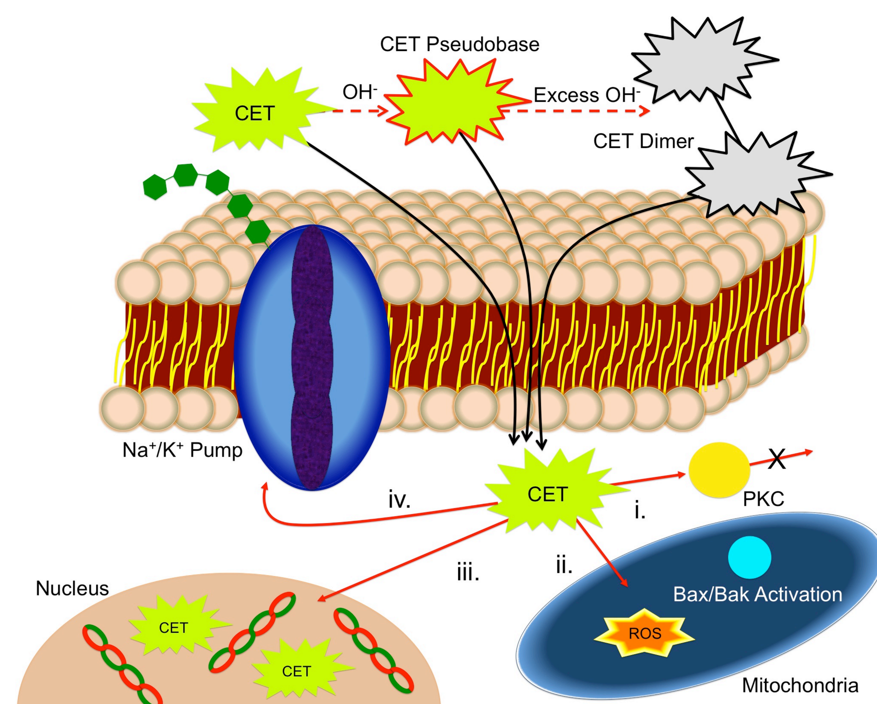


Figure 1. Possible cellular trafficking mechanisms and destinations for CET-induced apoptosis.

Experimental Methods

AgNP Synthesis and Characterization

- A large batch of colloidal AgNPs (ORI, 3.85 L) were synthesized via a modified Creighton method, employing the aqueous reduction of $AgNO_3$ by $NaBH_4$ in a 1:2 mM ratio, respectively.[5]
- AgNPs were then subjected to a one-step tangential flow filtration procedure to concentrate and purify the original colloid for optimum SERS-based sensing (Ag50R).[6]
- The resulting colloids were characterized by ultraviolet-visible absorption spectroscopy (UV-VIS), Raman spectroscopy, and transmission electron microscopy (TEM).

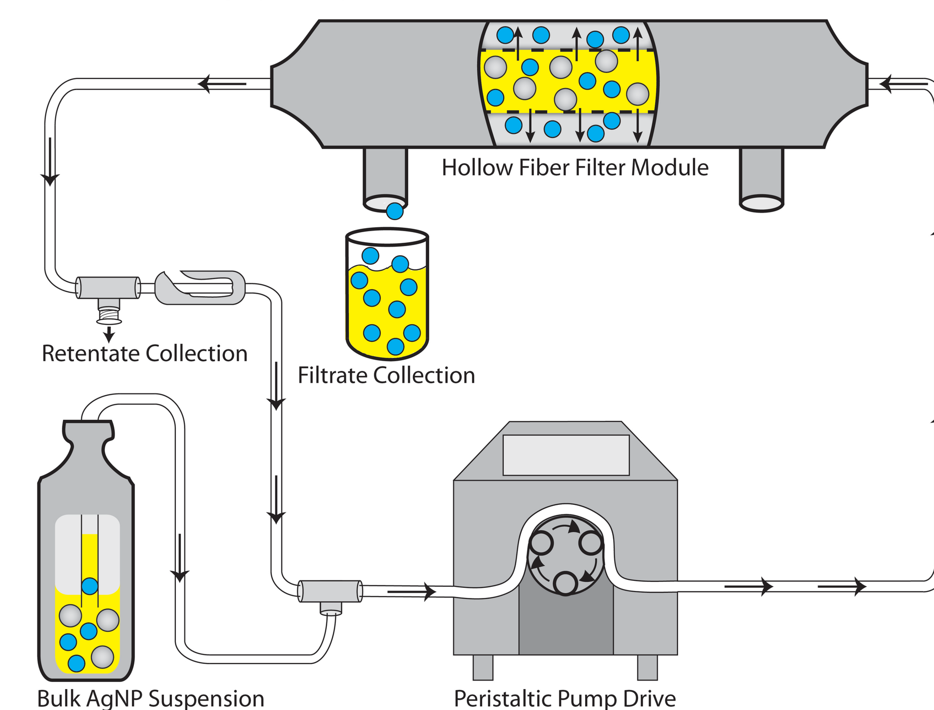


Figure 2. Schematic of the dynamic tangential flow filtration process utilized to concentrate and purify the ORI colloid.

HLEC Culture and Preparation of Cellular Extracts

- HLECs were obtained as a primary fetal human cell line (FHL124) courtesy of Dr. John Reddan, Oakland University, Michigan, and cultured on gelatin support media in 10% of a 1:1 mixture of heat-inactivated horse serum and 10% FBS.
- Once fully confluent, HLECs were treated with and without 50 µM of CET in PBS (DMSO control) for 30 minutes at 37 °C, a time sufficient to **induce** apoptosis and completely inhibit the NKA.
- Cytosolic, crude and purified plasma membrane, whole cell, and NKA extracts were obtained from the primary culture and stored at – 20 °C for further analysis.

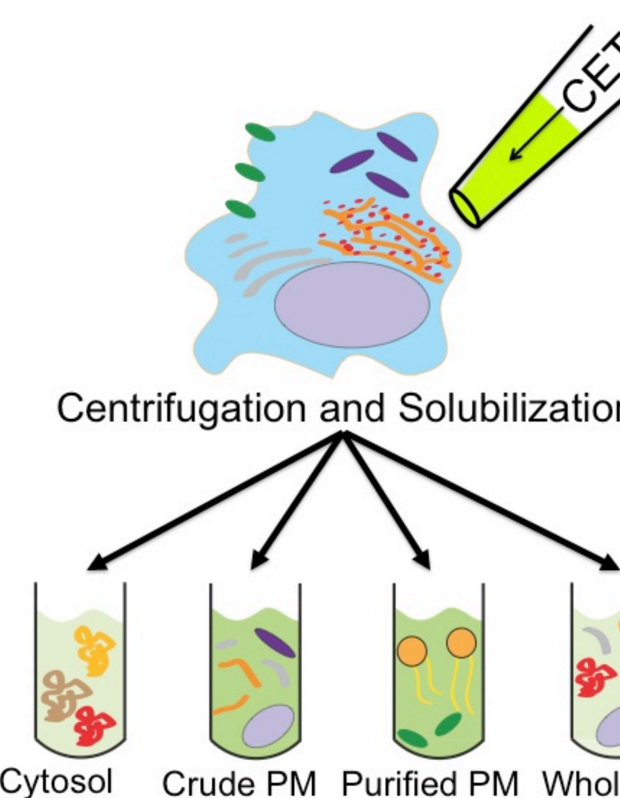


Figure 3. Schematic depicting the cellular harvesting and extraction procedure.

Spectroscopic Measurements and Analysis of CET

- Stock solutions of monomeric CET chloride in high quality water were subjected to UV-VIS analysis prior to cellular treatments to determine molar extinction coefficients for the two most prominent absorption features.

Figure 4. Cary Bio 50 UV-VIS Spectrophotometer (Varian, Inc.)

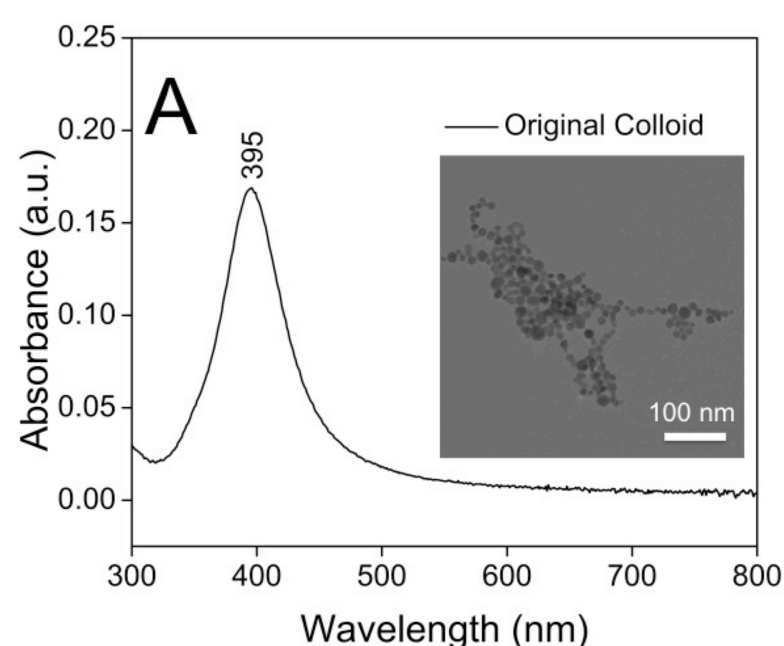
- Successive dilutions of a CET stock solution were performed (10^{-5} – 10^{-10} M) and analyzed via SERS. A linear calibration function was generated to quantitatively determine the CET contentment in each cellular extract.

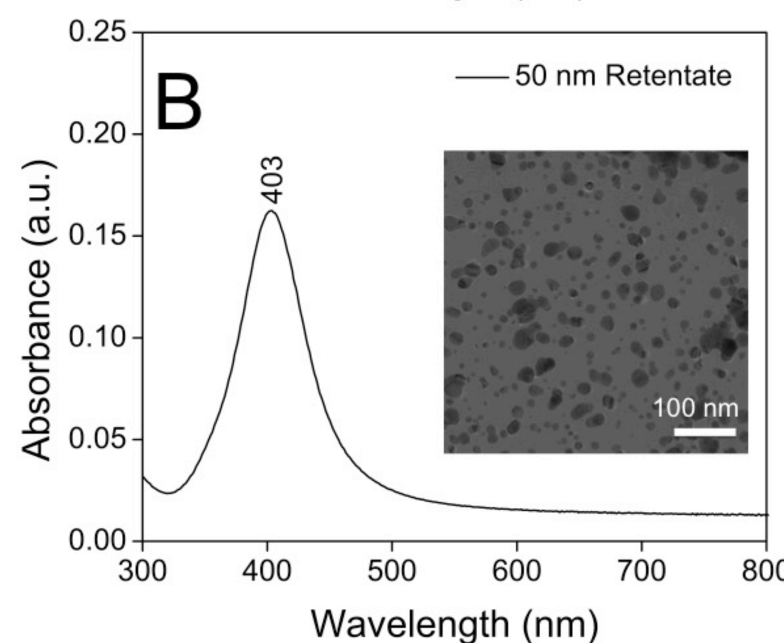
Figure 5. LabRamHR 800 (Horiba, Inc.) utilized to collect the Raman and SERS spectra of CET.

Results & Discussion

AgNP Characterization

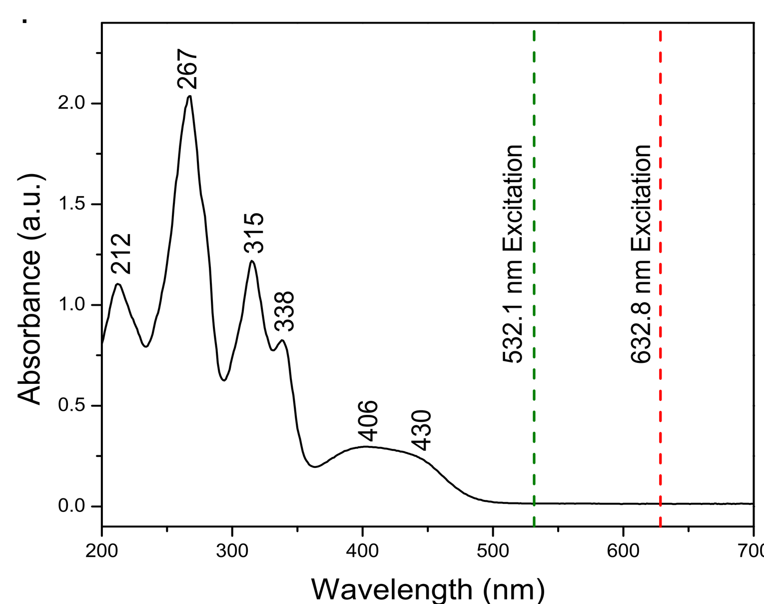
- The formation of spherical AgNPs of moderate size distribution (1- 150 nm in diameter) and aggregation were confirmed by UV-VIS and TEM analysis.



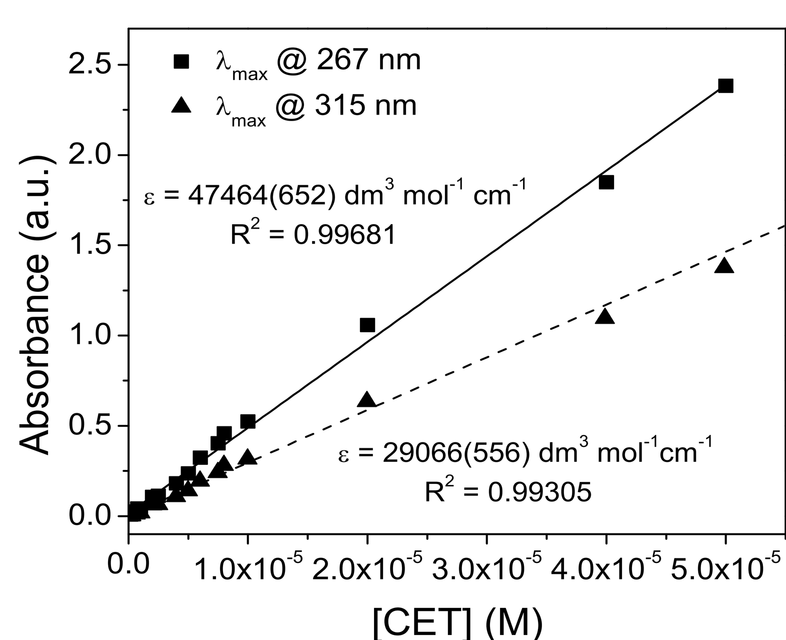


UV-VIS Analysis of Monomeric CET

- The UV-VIS spectrum of CET displayed 5 – 6 prominent absorption peaks, mostly in the UV.

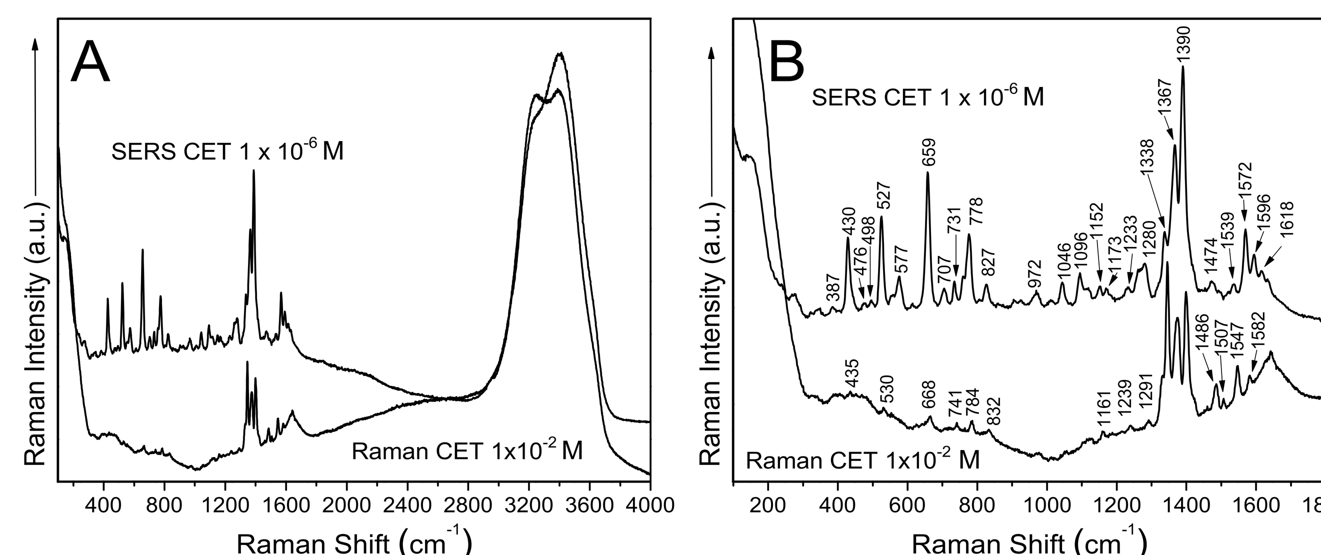


- Despite a strong extinction, CET could not be detected reliably below 10^{-6} M due to low signal and biological interferences.

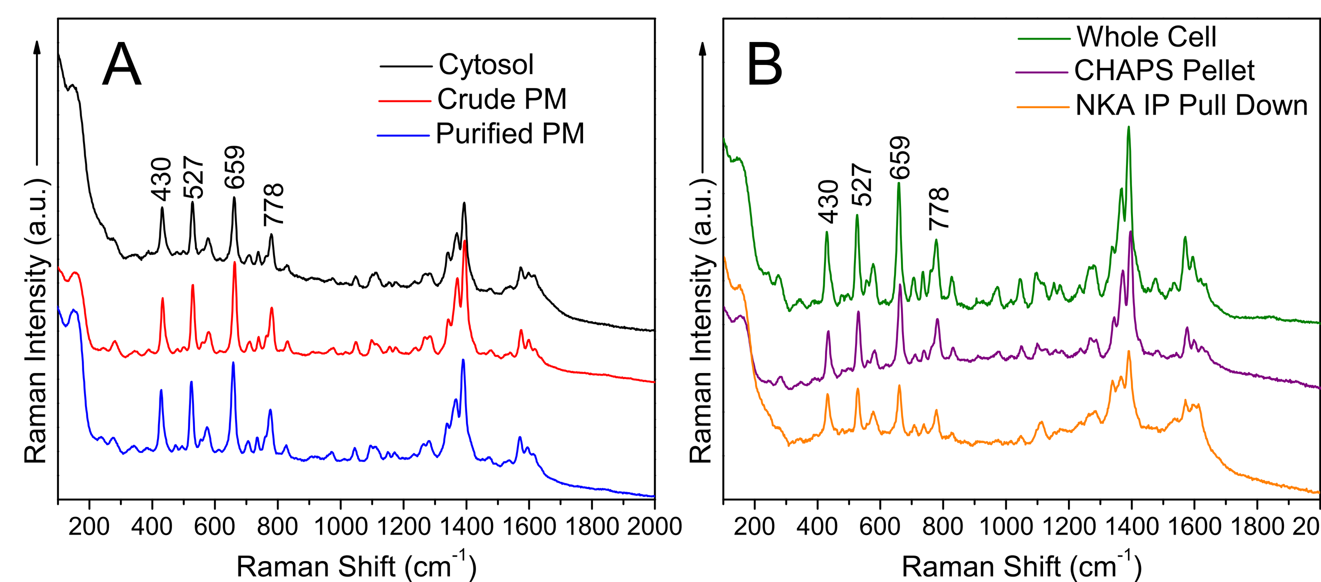


Raman and SERS Analysis of Monomeric CET and Cellular Extracts

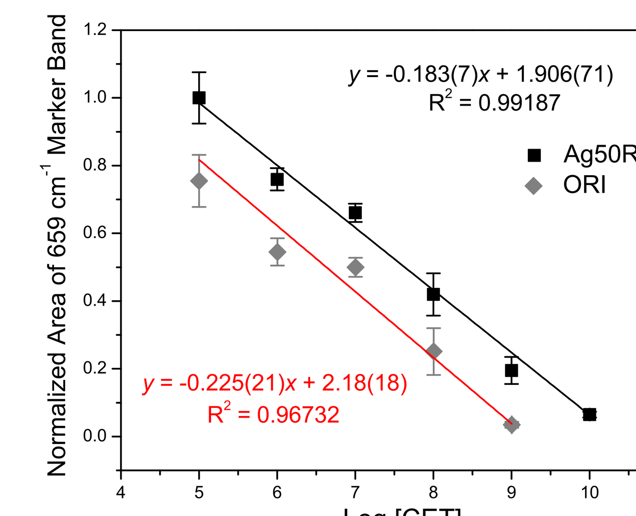
- The Raman spectrum of CET (without AgNPs) exhibited few vibrational modes and a LOD of ~ 10^{-2} M. Upon the addition of AgNPs, a detailed vibrational spectrum was obtained.



- The presence of CET in HLEC extracts was rapidly confirmed (3 s acquisition), even with very minute sample volumes (50 - 100 µL in each), and in he presence of an extensive biological matrix.



- A linear calibration curve was constructed based upon the integrated SERS area of the 659 cm^{-1} "marker band"; however, the Ag50R substrate yielded a much better LOD.



- The total monomeric CET content in each extract was interpolated from the Ag50R linear calibration function.
- Total protein content in each extract was determined by the BIO-RAD® technique.

HLEC Extract	[CET] in Extract (M)	# CETs / µg Protein
Cytosol	$1.04(18) \times 10^{-6}$	3.11×10^{11}
Crude PM	$2.70(2) \times 10^{-4}$	1.01×10^{14}
Purified PM	$8.19(37) \times 10^{-5}$	6.08×10^{13}
Whole Cell	$1.16(16) \times 10^{-7}$	1.67×10^{11}
Whole Cell Pellet	$8.23(22) \times 10^{-7}$	1.12×10^{13}
NKA Pull Down	$2.69(25) \times 10^{-9}$	3.86×10^{10}

Conclusions

- SERS proved to be a rapid, precise, and facile technique for determining CET content despite the presence of an extensive biological matrix.
- The CET content in the crude PM and cytosol suggest a rapid, Nerstian equilibrium (244 µM calculated from Nerst equation).
- These data also suggest that CET has a higher affinity for membrane proteins, which have a higher abundance of P-type ATPases, such as the NKA.
- On going experiments will attempt to further track CET in the NKA and other protein extracts.
- Furthermore, novel cellular extraction techniques are being developed to prevent CET loss in insoluble cell fractions.

Acknowledgments

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