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Research Paper

Specialized Manufacturing of Calcium Lactate Gluconate (CLG) by *West Bengal Chemical Industries Ltd (WBCIL)*: A Comprehensive Analytical Perspective

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Abstract
Calcium, the most prevalent mineral in the human body, is crucial for maintaining bone and dental health. Adequate calcium intake is essential, with the Indian Council of Medical Research recommending 600-800 mg/dayfor adults and 1200 mg/day for pregnant and lactating women. Insufficient intake can lead to osteopenia and osteoporosis, making supplementation important. Among various forms, calcium lactate gluconate (CLG) standsout for its high solubility and bioavailability. This paper explores the synthesis, properties, and benefits of CLG, particularly a novel formulation by West Bengal Chemical Industries (WBCIL) where lactate and gluconate molecules are bonded to a single calcium ion, enhancing stability and absorption. WBCIL employs advanced analytical techniques such as FT-IR and NMR spectroscopy, alongside rigorous testing for heavy metals and microbial contaminants, ensuring the highest standards of safety and efficacy. The paper discusses the superior solubility, transparency, and stability of CLG, validating its suitability for pharmaceutical and industrial applications. The findings underscore the importance of comprehensive analytical testing in guaranteeing the quality of CLG and highlight the pivotal role of advanced spectroscopic techniques in the development and qualitycontrol of chemical compounds.

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I. Introduction

Calcium is the most prevalent mineral in the human body, with about 99% stored in bones and teeth.[1] It can be found in both plant-based and animal-based foods, with milk being a particularly rich source. The Indian Council of Medical Research (ICMR) recommends a daily calcium intake of 600-800 mg for adults. This requirement increases to 1200 mg per day for pregnant and lactating women.[2] Chronic insufficient calcium intake can result in conditions such as osteopenia and osteoporosis.[3] To address these deficiencies, calcium supplementation is essential. There are several forms of calcium supplements available, including organic salts such as tricalcium citrate, calcium lactate, calcium lactate gluconate, and calcium gluconate, as well as inorganic salts like calcium chloride, calcium carbonate, and calcium phosphate.[4] The selection of a calcium salt is influenced by factors such as calcium content, solubility, taste, and bioavailability, with higher solubility generally leading to better calcium absorption. Notably, calcium lactate gluconate (CLG), formed from the combination of calcium lactate and calcium gluconate, stands out as a highly soluble calcium form.[4] Some supplements are available in the name of calcium lactate gluconate as a mixture of calcium lactate and calcium gluconate which is chemically different from calcium lactate gluconate. Calcium lactate gluconate is a bidental complex where one ligand is lactate ion and another ligand is gluconate ion and both these ions are attached to the single calcium ion. With its chemical formula $C_9H_{16}CaO_{10}$, calcium lactate gluconate (CLG) demonstrates high bioavailability and water solubility, making it an excellent option for supplementation. CLG is essential for providing elemental calcium, which is vital for numerous physiological processes such as bone health, muscle function, and nerve communication.[5]

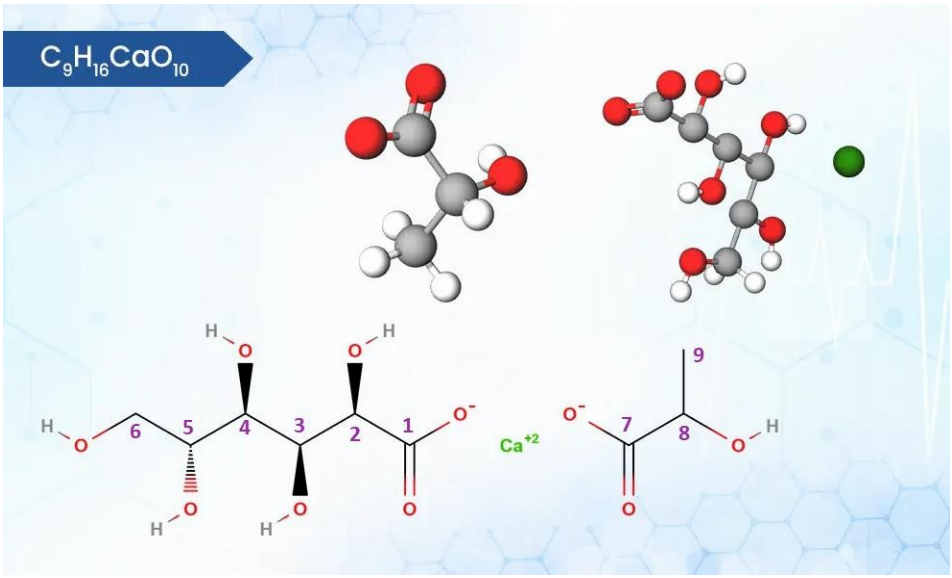


Figure 1: The chemical structure of Calcium Lactate Gluconate [Source: WBCIL]]

Within the realm of pharmaceuticals and food industries, the synthesis and production of calcium lactate gluconate (CLG) demand meticulous attention to detail to ensure both safety and efficacy. West Bengal Chemical Industries (WBCIL) stands at the forefront of specialized manufacturing, employing advanced techniques and stringent analytical methods to guarantee the highest standards of quality in CLG production. The introduction of a new innovative material by WBCIL, which incorporates both lactate and gluconate ion bonded to a single calcium ion, marks a significant advancement in the realm of calcium lactate gluconate (CLG) supplementation. Commercially available CLG supplements typically combine separate calcium lactate and calcium gluconate molecules. However, the material introduced by WBCIL integrates both lactate and gluconate ions directly bonded to a single calcium ion. This novel structure holds promise for addressing stability issues commonly encountered in commercially available CLG supplements, while also offering potential enhancements in calcium absorption by the body.

Superiority of pre-formed CLG (lactate and gluconate bonded to a same calcium molecule) over the mixture of calcium lactate and calcium gluconate

In the realm of drug development, formulation constitutes the pivotal transition of a prospective drug candidate into a viable pharmaceutical product. In the early stages of drug development, a variety of potential candidate molecules are typically under consideration, each distinguished by its own distinct physicochemical characteristics and efficacy towards specific biological targets. However, the selection of the most promising drug candidate goes beyond mere pharmacological effectiveness. In reality, the physicochemical properties of the molecule play a crucial role in dictating its pharmaceutical processing, stability, compatibility with excipients, solubility, and eventual bioavailability. These factors collectively shape the decision-making process, guiding the selection of the most suitable candidate for further development.[6] Calcium-lactate gluconate (CLG) exhibits significant advantages over plain calcium-lactate-gluconate blends in calcium concentration stability. The blends can exhibit large variability in calcium concentration, as low as 90% or greater than 100%. Additional calcium use (addition or subtraction) is often necessary to achieve acceptable levels. Notably, some blends may also exceed permissible calcium levels (e.g., 108%), making them unsuitable for use. WBCIL's method proposed the use of CLG, where lactate and gluconate ions are bound to a calcium cation in a 1:1 stoichiometric ratio. This study explores a facile method for the synthesis of CLG through the reaction of glucono-delta-lactone and lactic acid with a calcium source. The reaction is conducted in aqueous media under controlled temperature and pH conditions. The influence of various reaction parameters, such as the type and amount of calcium source, on the yield and purity of the final product was investigated. This defined system ensures predictable and constant calcium levels, generally the 98% and 99.9% respectively. For practical use, the desirability would be 99.5%. This inherent stoichiometry eliminates the need for additional calcium, simplifies drug synthesis, and increases product stability.

In terms of stability and consistency, pre-formed compounds guarantee even distribution and stability, minimizing the potential for dosage and efficacy variations compared to mixtures. This uniformity is critical for pharmaceutical formulations, ensuring reliable therapeutic effects. Research indicates that some pre-formed compounds may offer enhanced bioavailability compared to combinations of individual components.[7] This can result in improved absorption and utilization of calcium within the body, which is crucial for various physiological

functions. Although direct clinical trials directly comparing these formulations may be sparse, research on the clinical effectiveness of calcium supplementation often examines outcomes like bone health, cardiovascular health, and overall calcium balance. Such studies indirectly endorse the use of pre-formed calcium compounds due to their enhanced stability and bioavailability.[8] Pre-formed compounds are typically well-tolerated and come with established safety records, which are critical factors in clinical practice and patient adherence. While cost-effectiveness may vary based on specific formulations and applications, pre-formed compounds can often provide economic advantages in terms of manufacturing efficiency, storage requirements, and precise dosage administration.[9]

Specialised manufacturing process of WBCIL

WBCIL's manufacturing process for CLG begins with the meticulous selection of raw materials and proceeds through a series of steps designed to yield a product of exceptional purity and consistency. Aqueous solutions of glucono-delta-lactone and lactic acid were combined in a reaction vessel equipped with a stirring apparatus and temperature control. The desired calcium source was then added slowly while maintaining the pH using a pH meter and titrator with either additional glucono-delta-lactone or lactic acid solution. The reaction mixture was stirred on heating. The completion of the reaction was monitored by IR. The choice of calcium source in the synthesis of CLG plays an important role and it's a detrimental factor for the reaction kinetics. During the synthesis, pH plays a crucial role and proper modulation of pH from alkaline to acidic zone with addition of ligand is a critical process parameter. Central to this process is the extensive employment of analytical techniques, each serving a specific purpose in characterizing the synthesized CLG. Initial visual observation of the CLG sample obtained from WBCIL provides valuable insights into its physical characteristics, which align with established standards for CLG. Water solubility is an early indicator of quality. A high solubility indicates that CLGs are well formulated, as poorly formed or damaged samples may exhibit reduced solubility. Fourier-transform infrared (FT-IR) spectroscopy provides a non-destructive method for evaluating the functional groups in a CLG sample. Comparing the obtained spectrum with a standard reference (US7781407B2), FT-IR confirms the molecular identity and structural integrity of CLG. This method identifies distinctive vibrational signals associated with specific chemical interactions, enabling rapid and reliable analysis.[10] In pharmaceutical research, elucidation of the molecular structure of a compound is of paramount importance. Nuclear magnetic resonance (NMR) spectroscopy provides powerful tools for this purpose. Due to the complex molecular formula of CLG (C H CaO), NMR spectroscopy plays an important role in structural elucidation.[11,18] Analysis of the observed chemical shifts in the NMR spectrum can provide detailed information about the specific hydrogen and carbon environment of the CLG molecule (Fig. 4). This technique validates the presence of expected functional groups and provides crucial data for structural confirmation. Apart from this, the risk posed by the quantity of heavy metals present in Ca supplements is of grave concern. Calcium supplements inevitably get contaminated with heavy metals having the same charge density.[12] Therefore, it is imperative to indicate the level of this toxic metal in these supplements to create awareness among consumers.[13] Stringent testing for arsenic content and heavy metal contaminants ensures the safety of the CLG product of WBCIL, with results consistently falling well below regulatory limits. With an ever-increasing population utilizing calcium supplements to improve bone health and vitality, it is essential that these products are safe for human consumption. A very critical indicator of safety is the bacteriological and mycological quality of these compounds. There is a need for investigation of mycological/bacteriological contamination in these supplements.[14,20] Microbiological testing further confirms the safety profile of WBCIL-produced CLG, with negligible levels of microbial contaminants detected. Thus, the specialized manufacturing procedure employed by WBCIL for CLG underscores its commitment to delivering a product of unparalleled quality and safety.

Why CLG is water soluble

Calcium lactate gluconate readily dissolves in water, forming a transparent solution. Intermolecular forces and hydration favour the high solubility of CLG. This phenomenon can be explained by considering the key factors:

The dissolution behaviour of a mixed salt is governed by the interplay between lattice energy and the thermodynamics of solvation. Initial solvent-solute interactions influence the overall solvation energy. However, a more comprehensive approach considers the entire solvation process. This includes dissociation of the constituent ions from the crystal lattice and subsequent solvation of the dissociated ions in solution. The resulting net solvation energy, encompassing both dissociation and solvation enthalpic and entropic contributions, competes favourably with the lattice energy to determine the dissolution rate. Solvents with favourable physicochemical properties that promote stronger solute-solvent interactions (e.g., dipole-dipole interactions, hydrogen bonding) enhance the solvation of individual ions. This effectively reduces the lattice energy's influence and accelerates the dissolution process.

The high aqueous solubility of calcium lactate gluconate can be attributed to the interplay between intermolecular forces and the energetic favourability of dissolution. The crystal lattice of calcium lactate gluconate is primarily stabilized by ionic interactions between Ca^{2+} cations and the polyanionic lactate ($C_3H_5O_3^-$) and gluconate ($C_6H_{11}O_7^-$) moieties. However, the dissolution process is driven by the collective effect of overcoming the lattice energy and harnessing favourable interactions with water molecules.

The disruption of the lattice energy is facilitated by extensive hydrogen bonding and dipole-dipole interactions within the calcium lactate gluconate structure. The multiple hydroxyl (OH) and carboxylate (COO^-) groups present in the lactate and gluconate moieties readily participate in hydrogen bonding with neighbouring molecules, weakening the overall packing efficiency of the crystal lattice.[15] Additionally, the presence of these polar groups generates significant dipole-dipole interactions, further destabilizing the lattice structure.

On the other hand, dissolution in water is energetically favourable due to the formation of strong hydration shells around the dissociated ions. The Ca^{2+} cation interacts favourably with the lone pairs on surrounding water molecules, releasing a substantial amount of hydration energy.[16] Furthermore, the multiple oxygen atoms within the lactate and gluconate moieties readily form hydrogen bonds with water molecules, further contributing to the overall hydration energy gain. This combined effect of a relatively low lattice energy and high hydration energy surpasses the energy required for dissociation, resulting in the high solubility of calcium lactate gluconate in water.

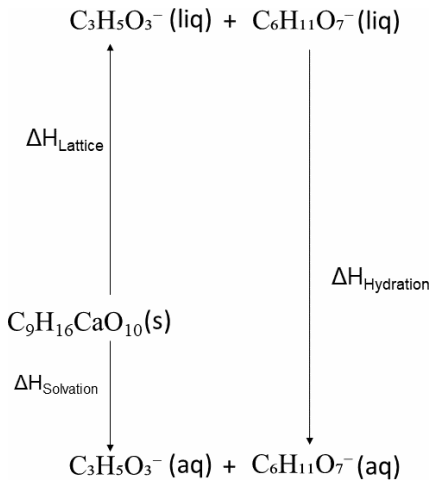


Figure 2: The dissolution profile of CLG. (Due to the complex structure and mixed salt nature of calcium lactate gluconate, this graphic depicts lattice energy ($\Delta H_{Lattice}$) (energy holding the crystal structure together) for easier visualization, although dissociation energy ($\Delta H_{Dissociation}$) is technically involved during dissolution.)

Study transparency of CLG

The transparency of calcium lactate gluconate solutions arises from the interplay between light scattering and absorption by the dissolved species. Due to their small size (on the order of angstroms), the constituent ions (Ca^{2+}, lactate$^-$, and gluconate$^-$) fall within the Rayleigh scattering regime. In this regime, particles are much smaller than the wavelength of visible light, leading to minimal interaction and negligible deflection of the light waves. Additionally, the lack of chromophores, coloured molecules that preferentially absorb certain wavelengths, within the dissolved calcium lactate gluconate eliminates selective absorption across the visible spectrum. Consequently, all wavelengths of visible light propagate through the solution with minimal attenuation, resulting in a transparent appearance.

Conversely, the solid state of calcium lactate gluconate exhibits a crystalline structure with particle diameters exceeding the wavelength of visible light. Upon interaction with this crystalline lattice, light undergoes multiple scattering events in various directions. This incoherent, multidirectional scattering disrupts the passage of light through the solid, leading to reflection at numerous angles relative to the observer. Due to this phenomenon, the solid scatters all visible wavelengths of light equally, appearing white to the human eye. In essence, the dissolution process breaks down the large crystalline structure into significantly smaller individual ions, effectively reducing their light scattering cross-section. This minimal scattering allows the unimpeded transmission of visible light through the solution, resulting in a transparent liquid state.

Analytical Techniques used by WBCIL

The CLG sample obtained from WBCIL manifested as a white or yellow-white tinged powder with minimal odour. This visual observation aligns with established physical characteristics of CLG. Additionally, the sample exhibited good solubility in water, further corroborating its identity as CLG. Quantitative analysis revealed an assay of 96-102%. This value signifies the percentage of the target CLG compound present in the sample and falls within theacceptable range for CLG products.

1. Fourier-Transform Infrared (FT-IR) Spectroscopy:

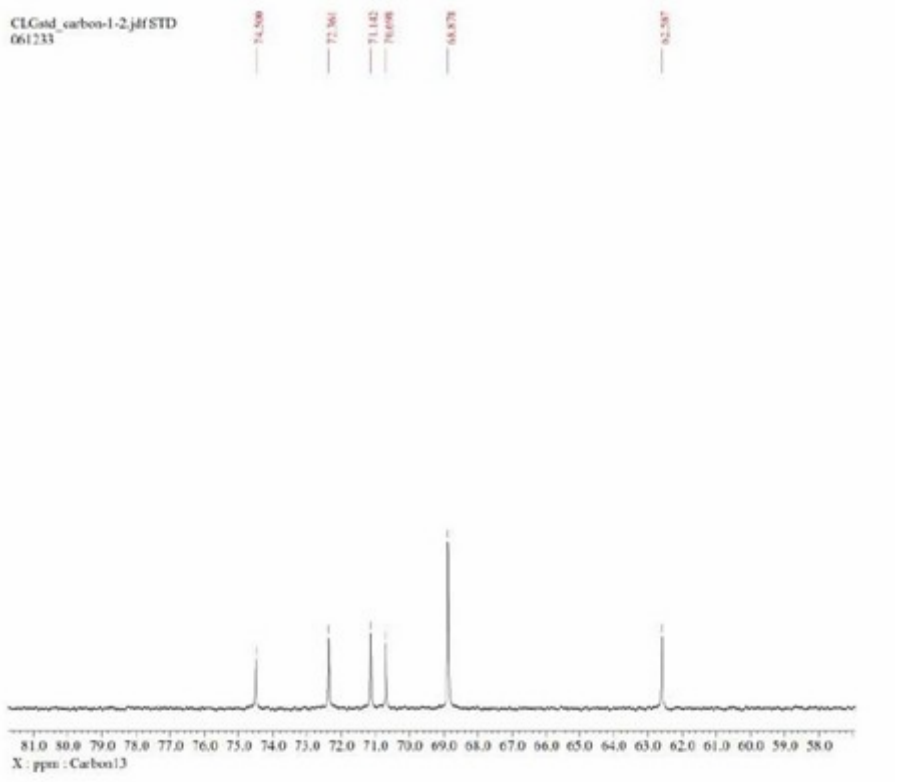
Fourier-Transform Infrared (FT-IR) spectroscopy was employed to characterize the functional groups present in the synthesized calcium lactate gluconate (CLG) sample. This technique probes the sample with infrared radiation, and functional groups within the molecule absorb specific frequencies, resulting in a characteristic absorption pattern. The FT-IR spectrum of the CLG sample was then compared to a standard reference spectrum of calcium lactate gluconate.[17] A high degree of similarity between the two spectra was observed. This concordance confirms the presence of the anticipated functional groups in the synthesized CLG, signifying a successful production process that yielded a product consistent with the desired molecular structure.

2. Proton Nuclear Magnetic Resonance (H-NMR) Spectroscopy and Carbon Nuclear Magnetic Resonance (C-NMR) Spectroscopy:

Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy offers a powerful tool for elucidating the specific chemical environments of hydrogen atoms within a molecule. In the context of this study, ^{1}H-NMR analysis aimed to identify and characterize the unique hydrogen environments present in the CLG molecule. This data serves as a crucial piece of evidence to verify its molecular structure. The ^{1}H-NMR spectrum exhibited distinct peaks for protons H-2, H-8, and H-9. A doublet peak at 4.15 ppm was assigned to H-2, while a quartet peak at 4.06 ppm was attributed to H-8. Three protons belonging to H-9 resonated as a distinct doublet at 1.32 ppm. Additionally, multiplate peaks were observed around 3.6 ppm, likely corresponding to protons in a more complex environment (Fig. 4).

Furthermore, Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectroscopy complements ^{1}H-NMR by providing information about the carbon framework of the molecule (Fig. 3). This analysis provided valuable insights into the carbon-carbon and carbon-hydrogen connectivity within the CLG molecule.

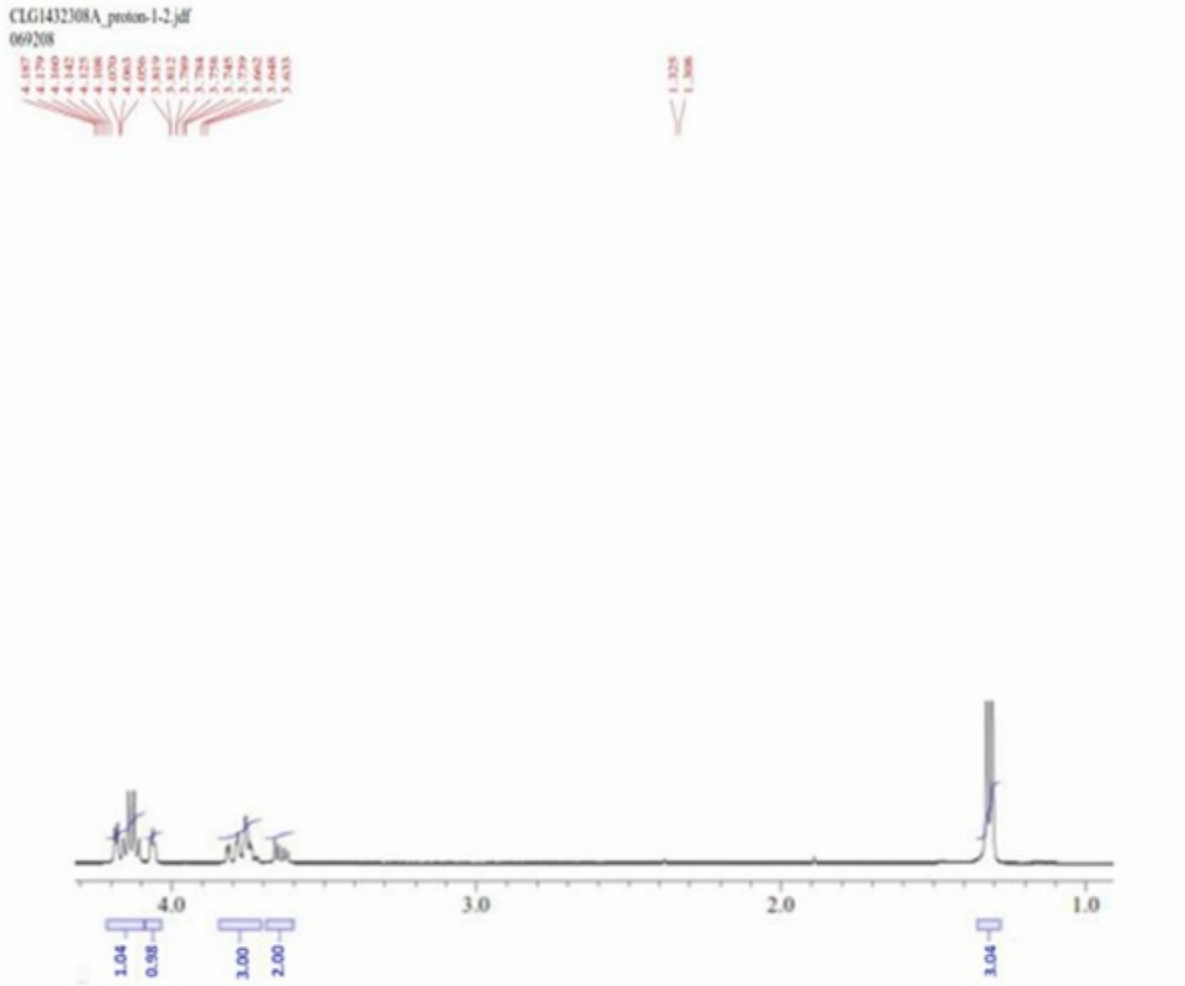
Collectively, ^{1}H-NMR and ^{13}C-NMR analyses provided in-depth information about the specific hydrogen and carbon environments of the CLG sample. These data corroborated the findings from FT-IR spectroscopy, solidifying the structural integrity of the synthesized CLG. While a detailed interpretation of the NMR spectra falls outside the scope of this discussion, the observed chemical shifts and peak multiplicities confirm the presence of the expected functional groups, validating the overall molecular composition of the CLG sample.



Discussions:

Std Chemical Shift (ppm)	**Plant Batch Chemical Shift (ppm)**	**Carbon *Assignments***
182.7	182.7	CO
179.0	179.0	CO
74.5	74.5	CH
72.361	72.360	CH
71.142	71.141	CH
70.698	70.688	CH
68.87	68.87	CH
62.587	62.587	CH_2
20.39	20.39	CH_3

Figure 3: ^{13}C NMR spectra of Calcium Lactate Gluconate (CLG)



Position	Integral	Pattern	J
4.15 [ppm]	1	d	J1= 5.4 [Hz]
4.06 [ppm]	1	q	J1=2.7 [Hz]
3.78 [ppm]	3	m	
3.64 [ppm]	2	d	J1=11.5[Hz]
1.32 [ppm]	3	d	J1=6.9 [Hz]

Figure 4: ^{1}H NMR spectra of calcium Lactate Gluconate (CLG) in D_2O

3. Mass spectroscopy:

Mass spectrometry analysis corroborated the successful synthesis of calcium lactate gluconate. The spectrum revealed key structural information, with prominent peaks at m/z 323.1 and 325.1 corresponding to the

deprotonated molecular ion $[M-H]^+$ and protonated molecular ion $[M+H]^+$ of the target molecule, respectively. These peaks confirm the expected molecular mass of calcium lactate gluconate. Furthermore, a fragment ion peak observed at m/z 195.1 aligns with the presence of a gluconate sub-unit within the molecule (Fig. 5). The combined presence of these diagnostic peaks strongly supports the successful incorporation of both lactate and gluconate moieties into the synthesized calcium salt it also proves the absence of calcium lactate and calcium gluconate as a single entity.

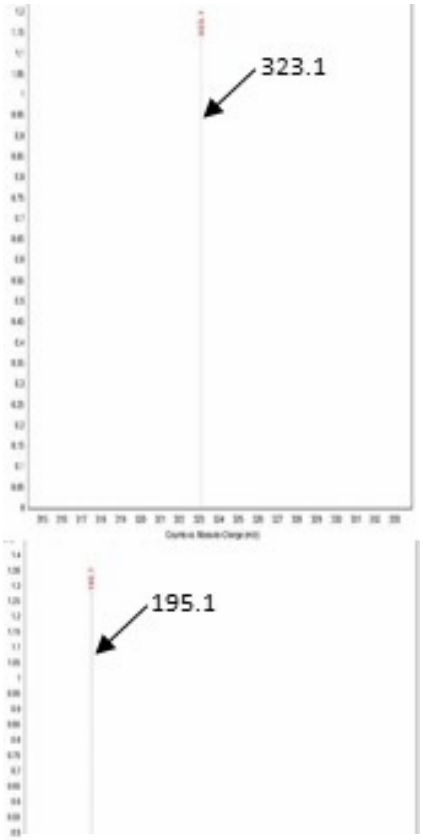


Figure 5: Electrospray ionization-mass spectra of CLG

4. Arsenic Content Analysis:

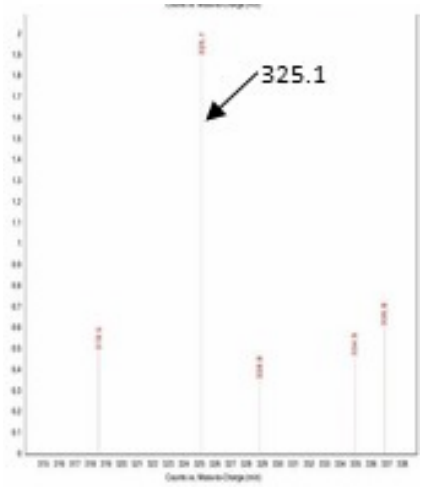
Arsenic content is a critical parameter for Calcium Lactate Gluconate safety due to its potential toxicity. The analysis employed treatment with arsenic trioxide, followed by quantification to determine the arsenic concentration in the sample. The arsenic content analysis revealed a very low concentration, well below the regulatory limit of 3 ppm. This result ensures the safety of the CLG product for intended uses.

5. Heavy Metal Analysis:

The presence of heavy metals, including lead, can compromise CLG safety. This analysis aimed to identify and quantify any heavy metal contaminants present in the sample. The heavy metal analysis confirmed the absence of lead and other potential contaminants, further emphasizing the product's purity.

6. Microbiological Analysis:

Microbiological testing is crucial for ensuring the safety of CLG, particularly for applications in the pharmaceutical and food industries. The CLG sample demonstrated negligible yeast and mold content, indicating low levels of these potential microbial contaminants. Furthermore, the absence of Escherichia coli (E. coli) and Salmonella spp. was confirmed, signifying the absence of these pathogenic bacteria. These microbiological findings contribute to the overall safety profile of the WBCIL-produced CLG.



II. Conclusion

The comprehensive analytical characterization of the CLG sample from WBCIL demonstrated its compliance with rigorous quality standards. The low arsenic content, absence of heavy metals, and adherence to established physical properties ensure the safety and efficacy of the product for various applications. The utilization of advanced analytical techniques like FT-IR and NMR spectroscopy provided robust evidence of the CLG's identity, purity, and structural integrity. These findings support the suitability of the WBCIL-produced CLG for pharmaceutical and industrial applications. This study highlights the importance of thorough analytical testing in guaranteeing the quality and safety of CLG. The results contribute to a deeper understanding and utilization of CLG across diverse industries, emphasizing its value as a high-quality and safe compound. Furthermore, the application of advanced spectroscopic techniques underscores their critical role in characterizing chemical compounds and facilitating the development and quality control of such products. Through the integration of advanced analytical techniques and rigorous testing protocols, WBCIL ensures that each batch of CLG meets or exceeds industry standards, paving the way for its utilization across diverse pharmaceutical and industrial applications. This comprehensive analytical perspective not only validates the excellence of WBCIL's manufacturing process but also underscores the pivotal role of advanced spectroscopic techniques in ensuring the quality and integrity of chemical compounds.

References

[1]. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington (DC): National Academies Press (US); 1997. PMID: 23115811.

[2]. Trailokya A, Srivastava A, Bhole M, Zalte N. Calcium and Calcium Salts. J Assoc Physicians India. 2017 Feb;65(2):100-103. PMID: 28457049.

[3]. Shlisky J, Mandlik R, Askari S, Abrams S, Belizan JM, Bourassa MW, Cormick G, Driller-Colangelo A, Gomes F, Khadilkar A, Owino V, Pettifor JM, Rana ZH, Roth DE, Weaver C. Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes. Ann N Y Acad Sci. 2022 Jun;1512(1):10-28. doi: 10.1111/nyas.14758. Epub 2022 Mar 5. PMID: 35247225; PMCID: PMC9311836.

[4]. Palacios C, Cormick G, Hofmeyr GJ, Garcia-Casal MN, Peña-Rosas JP, Betrán AP. Calcium-fortified foods in public health programs: considerations for implementation. Ann N Y Acad Sci. 2021 Feb;1485(1):3-21. doi: 10.1111/nyas.14495. Epub 2020 Sep 28. PMID: 32986887; PMCID: PMC7891425.

[5]. Selgas MD, Salazar P, García ML. Usefulness of calcium lactate, citrate and gluconate for calcium enrichment of dry fermented sausages. Meat Sci. 2009 Aug;82(4):478-80. doi: 10.1016/j.meatsci.2009.04.001. Epub 2009 Apr 7. PMID: 20416674.

[6]. Elian M, Srianta I, Trisnawati CY, Arisasmita JH. Effects of calcium fortification (calcium lactate gluconate) on the physicochemical and sensory properties of soy-corn milk. International Journal of Food, Nutrition and Public Health. 2012;5(1/2/3):91-104.

[7]. Gaisford S. Pharmaceutical preformulation. Aulton's Pharmaceutics E-Book: Aulton's Pharmaceutics E-Book. 2021 Apr 23:360.

[8]. Chaurasia G. A review on pharmaceutical preformulation studies in formulation and development of new drug molecules. Int J Pharm Sci Res. 2016 Jun 1;7(6):2313-20.

[9]. Vilegave K, Vidyasagar G, Chandankar P. Preformulation studies of pharmaceutical new drug molecule and products: An Overview. The American Journal of Pharmacy. 2013;1(3):1-20.

[10]. Coates J. Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry. 2000 Sep 15;12:10815-37.

[11]. Wong KC. Review of spectrometric identification of organic compounds.

[12]. Rehman S, Adnan M, Khalid N, Shaheen L. Calcium supplements: an additional source of lead contamination. Biol Trace Elem Res. 2011 Oct;143(1):178-87. doi: 10.1007/s12011-010-8870-3. Epub 2010 Oct 15. PMID: 20953844.

[13]. Gopinath R, Naidu RA. Pharmaceutical preformulation studies–current review. International Journal of Pharmaceutical and Biological Archives. 2011;2(5):1391-400.

[14]. He X, Liu X, Nie B, Song D. FTIR and Raman spectroscopy characterization of functional groups in various rank coals. Fuel. 2017 Oct 15;206:555-63.

[15]. Grabowski SJ. Understanding hydrogen bonds: theoretical and experimental views. Royal Society of Chemistry; 2020 Nov 13.

[16]. Atkins PW, De Paula J, Keeler J. Atkins' physical chemistry. Oxford university press; 2023.

[17]. Buendia MT, inventor. Calcium gluconolactate compositions and methods of making same. United States patent US 7,781,407. 2010 Aug 24.

[18]. Elyashberg M. Identification and structure elucidation by NMR spectroscopy. TrAC Trends in Analytical Chemistry. 2015 Jun 1;69:88-97.

[19]. Rehman S, Adnan M, Khalid N, Shaheen L. Calcium supplements: an additional source of lead contamination. Biological trace element research. 2011 Oct;143:178-87.

[20]. TOURNAS VH. Microbial contamination of select dietary supplements. Journal of food safety. 2009 Aug;29(3):430-42.