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Article

Evaluating the Bio-Efficacy and Environmental Sustainability of Pyridinium Chlorochromate: Synthesis and Anti-Diabetic Screening via α-Amylase Inhibition

Seplappatty Kalimuthu Periyasamy[1], V. Rethinagiri[2], M. Venkatesan[3], R. Ramkumar[4], K. A. Mohamed Akhil[5], A. Mohamed Anas[6]

[1]PG & Research Department of Chemistry, Jamal Mohamed College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli – 620020, Tamil Nadu, India.
Email: skps@jmc.edu
[2]Department of Chemistry, M.A.M. College of Engineering and Technology, Tiruchirappalli – 621005, Tamil Nadu, India.
[3]Department of Chemistry, J.J. College of Engineering & Technology (Sowdambika Group of Institutions), Tiruchirappalli – 620009, Tamil Nadu, India.
[4]Madras Centrifugal Castings, Chennai – 600058, Tamil Nadu, India.
[5]Jamal Mohamed College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli – 620020, Tamil Nadu, India.
[6]Jamal Mohamed College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli – 620020, Tamil Nadu, India.
Corresponding Author: Seplappatty Kalimuthu Periyasamy, skps@jmc.edu

Abstract: This study aims to describe the synthesis and evaluation of the anti-diabetic activity of pyridinium chlorochromate (PCC). The structure of the synthesized compound was confirmed by determining its melting point, as well as by analyzing its IR, UV–Visible, ^{1}H NMR, and ^{13}C NMR spectral data. The anti-diabetic activity was assessed based on its biological properties using the α-amylase inhibition assay.
Keywords: PCC, Cr(VI) reagents, anti -diabetic,α-amylase inhibition method

1.INTRODUCTION

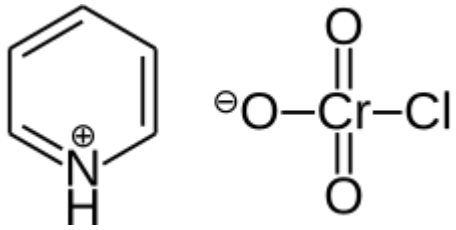
Anti-diabetic activity refers to the capacity of a substance to lower blood glucose levels and aid in the management of diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia due to defects in insulin secretion, insulin action, or both. Insulin, produced by pancreatic β-cells, regulates blood glucose by facilitating its uptake into cells; however, in diabetes, either insufficient insulin production (Type 1 diabetes) or insulin resistance combined with inadequate insulin secretion (Type 2 diabetes) leads to elevated blood sugar levels. This condition is often associated with symptoms such as increased thirst, frequent urination, fatigue, and weight loss, and if uncontrolled, can result in complications affecting the heart, kidneys, nerves, and eyes. Anti-diabetic agents, including synthetic drugs and natural compounds, help control glucose levels by enhancing insulin secretion, improving insulin sensitivity, promoting glucose uptake, or inhibiting carbohydrate digestion, and their efficacy is commonly evaluated through *in vitro* and *in vivo* experimental models.

Chromium compounds have been widely utilized in both aqueous and non-aqueous media for the oxidation of a variety of organic compounds.[1–11] Among these, hexavalent chromium [Cr(VI)] reagents are particularly important due to their versatility and ability to oxidize a wide range of organic functional groups.[12–18] The development of newer Cr(VI) reagents for efficient and selective oxidation of organic substrates continues to be an area of significant research interest.

Pyridinium chlorochromate (PCC), also known as the Corey–Suggs reagent, is one of the most commonly used Cr(VI) oxidizing agents in organic synthesis.[19] It is primarily employed for the oxidation of alcohols to the corresponding aldehydes and ketones. PCC offers several advantages over other oxidizing agents: it is an air-stable yellow-orange solid, only slightly hygroscopic, and requires relatively mild conditions for reactions. Chemically, PCC is represented by the formula $[C_5H_5NH]^+[CrO_3Cl]^-$.

A key advantage of PCC is its selectivity, as it efficiently oxidizes primary alcohols to aldehydes without further oxidation to carboxylic acids, unlike many other oxidizing agents. This selectivity makes PCC a valuable reagent in synthetic organic chemistry.

Structure of Pyridinium Chlorochromate



Pyridinium chlorochromate (PCC) is a commercially available reagent that was discovered accidentally. It was originally prepared by the addition of pyridine to a cold solution of chromium trioxide in concentrated hydrochloric acid. PCC has since become an important reagent in organic synthesis due to its efficiency and selectivity.

Previous studies have explored the biological activities of PCC. Emmanuel Olumuyiwa *et al*.[20] reported the antibacterial activity of the synthesized compound, pyridinium chlorochromate, against four bacterial isolates (GBB418, GBB420, GBB421, and GBB422) obtained from infected pepper plants. Similarly, S. K. Periyasamy[21] described the synthesis and evaluation of the antibacterial activity of pyridinium chlorochromate.

However, a survey of the available literature indicates that no systematic study has been conducted to evaluate the anti-diabetic potential of PCC using *in vitro* methods. Therefore, the present study focuses on determining the **anti-diabetic** activity of PCC by employing the α-amylase inhibition assay.

2. **Main objective of the work**

The objective of this study is to synthesize the chromium(VI) oxidant pyridinium chlorochromate (PCC), to characterize the synthesized compound using appropriate analytical techniques and to evaluate the anti-diabetic activity of the prepared PCC.

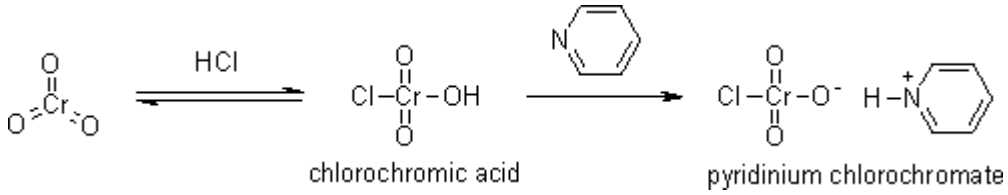
3. **EXPERIMENTAL METHOD**

3.1 Materials:

Analar grade of reagents used for the preparation of Pyridinium chlorochromate.

3.2 Preparation of Pyridinium Chlorochromate (PCC) Corey-Suggs Reagent

Chlorochromic acid is prepared *in situ* by dissolving chromium(VI) oxide in 6 M aqueous hydrochloric acid. Subsequent addition of pyridine to the cooled solution yields pyridinium chlorochromate (PCC) as bright orange crystals.



Pyridinium chlorochromate (PCC) shares several key advantages when compared with pyridinium dichromate (PDC): it is stable, commercially available, not particularly hygroscopic, and can be stored for extended periods. PCC

exhibits excellent solubility in many organic solvents; dichloromethane (DCM) is the preferred choice at room temperature for most applications, whereas dimethylformamide (DMF) can promote the over-oxidation of primary alcohols to carboxylic acids. Although PCC is more acidic than PDC, acid-labile substrates can still be safely oxidized by performing the reaction in the presence of a buffer, such as sodium acetate (CH3COONa) or calcium carbonate (CaCO3).

3.3. Characterization of Pyridinium chlorochromate:

The structure of the synthesized pyridinium chlorochromate (PCC) was confirmed by elemental analysis and melting point determination. Further structural characterization was carried out using vibrational (FT-IR), electronic (UV-Vis), 1H NMR and 13C NMR spectroscopy.

Fourier-transform infrared (FT-IR) spectra were recorded using an attenuated total reflection (ATR) method on a Bruker Alpha II spectrophotometer (Bruker, Germany) at the Jamal Mohamed College Instrumentation Centre, Tiruchirappalli. A small amount of the solid compound was loaded directly onto the spectrometer and data were collected across the wavenumber range of (4000 - 400 cm-1. The resulting spectrum, managed *via* computer interface, plotted percent transmittance (%T) against wavenumber (cm-1), where the sharp absorption peaks confirmed the characteristic functional groups present in the compound.

Electronic spectra were obtained using a Laboindo UV-3000+ UV-Vis spectrophotometer (Germany), also located at the Jamal Mohamed College Instrumentation Centre. A small sample of PCC was evaluated *via* the absorbance method to identify the characteristic chromophores, electronic transitions, and extent of conjugation within the complex.

Finally, 1H(500 MHz) and 13C(125MHz) NMR spectra were recorded on a Bruker Avance spectrometer. The analysis was performed using deuterated dimethyl sulfoxide (DMSO-d6, (CD3)2SO) as the solvent to provide final structural confirmation of the pyridinium cation.

3.4. Biological activtiy - Anti-inflammatory Screening:

The anti-diabetic activity of the synthesized compound was evaluated via an α-amylase inhibition assay. The screening was conducted at the Bio Techno Solutions Training and Research Institute, Ponmalaipatti, Tiruchirappalli.

3.5 α-Amylase Inhibitory Assay Method

The assay mixture (total volume: 3 mL) consisted of 1 mL of alpha-amylase enzyme solution (1mL), 1 mL of the test sample or standard drug (acarbose) at various concentrations (20--100µg/mL), and 1 mL of sodium phosphate buffer (0.02M, pH 6.9 containing 0.006 M NaCl. This mixture was incubated at 37° C for 10 min.

Following the initial incubation, 1 mL of a 1% starch solution (prepared in the same phosphate buffer) was added to the reaction mixture, which was then incubated at 37° C for 10 min

The enzymatic reaction was terminated by the addition of 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent. The mixture was immediately heated in a boiling water bath for 5 min, subsequently cooled to room temperature, and diluted with 10 mL of distilled water.

The absorbance of the final solution was measured at 540 nm using a UV-Vis spectrophotometer (Optima, SP-3000, Tokyo, Japan). A control was prepared following the same procedure using the phosphate buffer solution instead of the test sample.

Percent (%) inhibition activity = [(A-B)/A] x 100.

Where A and B – absorbance values of blank and sample, respectively. A curve of concentration versus percentage inhibition was plotted and concentration required for 50% inhibition was determined.

4. **RESULTS AND DISCUSSIONS**

4.1 Characterization of Pyridinium chlorochromate (PCC)

Pyridinium chlorochromate (PCC) was synthesized and comprehensively characterized using melting point determination, elemental analysis, and spectroscopic techniques, including Fourier-transform infrared (FT-IR), ultraviolet-visible (UV-Vis), 1HNMR, and 13C NMR spectroscopy. Furthermore, the biological profile of the synthesized compound was evaluated by screening its *in vitro* anti-diabetic potential using the alpha-amylase inhibition assay.

4.1.1 Determination of Melting Point of Pyridinium Chlorochromate (PCC)

The melting point of the synthesized pyridinium chlorochromate (PCC) was determined to assess its purity and confirm its identity. A small quantity of the dried PCC sample was finely powdered and packed into a capillary tube sealed at one end. The tube was placed in a digital melting point apparatus and heated gradually at a controlled rate. The temperature at which the compound initiated melting and the temperature at which it completely liquefied were carefully recorded to establish the melting range. This observed value was subsequently compared with literature data to evaluate sample purity; a sharp melting range signifies a high degree of purity, whereas a broad or depressed melting range indicates the presence of impurities or residual solvent.The observed melting point of PCC is 206°C

4.1.2 Determination of elemental analysis of Pyridinium Chlorochromate (PCC)

Elemental analysis of the synthesized pyridinium chlorochromate (PCC) was performed to verify its chemical composition and evaluate its purity by comparing experimentally derived values with theoretical percentages.(Table-1) Typically, carbon, hydrogen, and nitrogen contents are determined via CHN combustion analysis, whereas the constituent chromium and chlorine percentages are quantified using independent analytical techniques, such as atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS).

Table :1 Experimental elemental analysis Values of PCC

Element	**Calculated (%)**	**Experimental (%)**
Carbon (C)	30.7	**30.4**
Hydrogen (H)	3.1	**3.2**
Nitrogen (N)	7.2	**7.0**
Chromium (Cr)	26.6	**26.3**
Chlorine (Cl)	18.1	**18.3**

4.1.3 Interpretation of Experimental Results

The experimentally derived elemental values are in close agreement with the theoretically calculated percentages, confirming the expected molecular composition of the synthesized PCC. Minor variations may be attributed to instrumental detection limits, sample handling artifacts, or trace impurities. Crucially, the CHN data validate the successful integration of the pyridinium moiety, while the quantified chromium and chlorine contents confirm the structural integrity of the chlorochromate counter-anion.

4.1.4 Vibrational Spectroscopy of Pyridinium chlorochromate:

Pyridiniumchlorochromate (PCC) contains a Cr(VI), oxo chromate unit and a protonated pyridinium cation .Its IR spectrum reflects vibrations from: Cr=O bonds, Cr–Cl bond , C–N and C=C of pyridinium ring N–H stretching of protonated nitrogen.(Table-2)

Table: 2 IR Absorption Bands of PCC

Wavenumber (cm^{-1})	**Assignment**	**Interpretation**
3334	N–H stretching	Protonated pyridinium (NH^+)
2973, 2927,2884	Aromatic C–H stretching	Pyridinium ring
1650	C=C / C=N stretching	Aromatic ring vibrations

1452, 1416,1380,1327,1274	C–N stretching	Pyridinium group
1087,1044,952	Cr=O stretching	Terminal oxo groups
879	Asymmetric Cr=O stretch	Strong band, characteristic of Cr(VI)
521	Cr–Cl stretching	Confirms chloride coordination

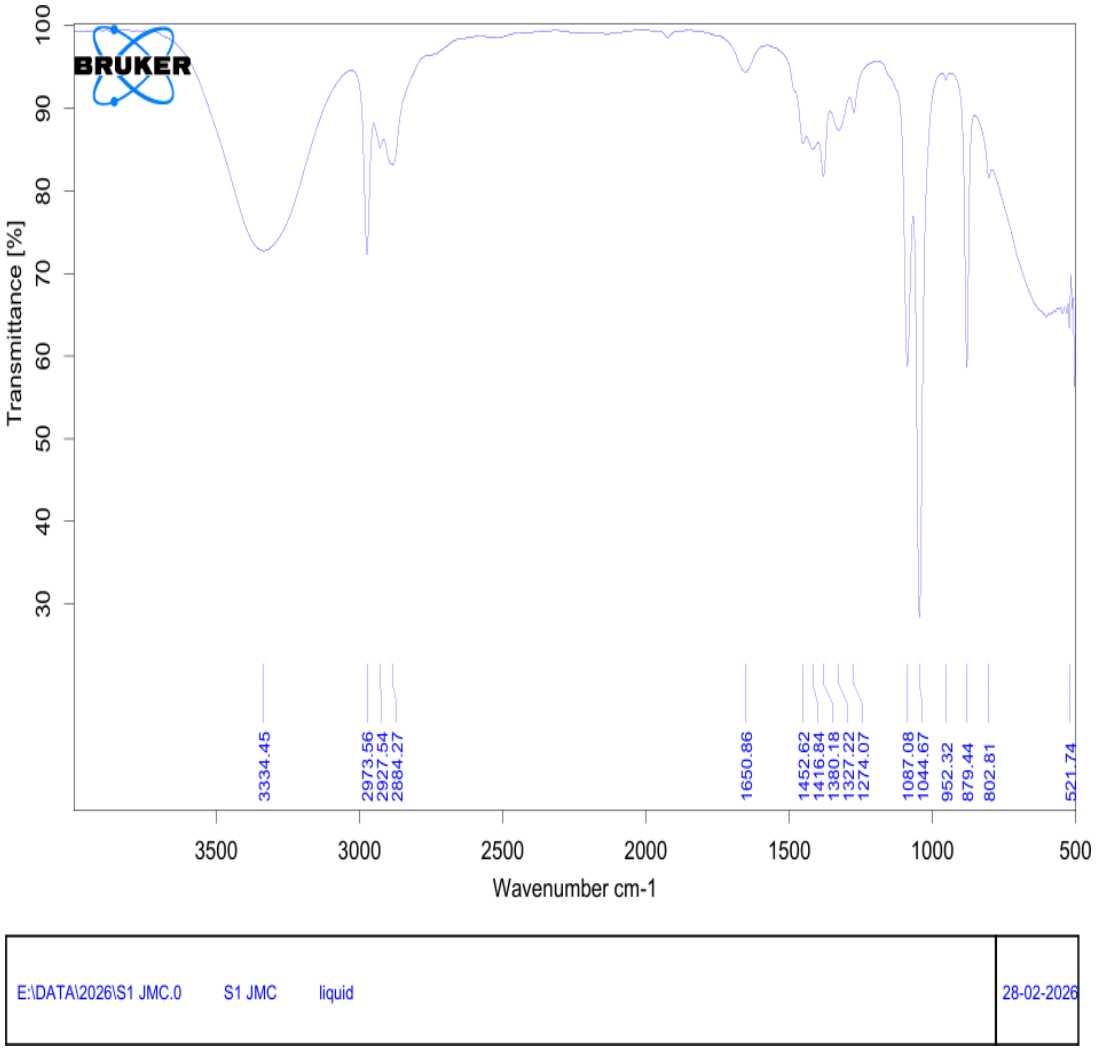


Fig: 1 IR spectrum of PCC

The FT-IR spectrum of pyridinium chlorochromate (PCC) provides definitive evidence of its structural components and functional groups. A strong, sharp absorption band observed in the range of 952 -- 1087 cm-1 is assigned to the asymmetric and symmetric Cr=Ostretching vibrations, confirming the presence of terminal oxo groups and a chromium center in the +6 oxidation state. The Cr—Cl stretching vibration appears in the lower wavenumber region at 521 cm-1, confirming the coordination of the chloride ion to the metal center. A broad band of medium intensity centered around 3334 cm-1corresponds to the N--H stretching frequency, validating the protonation of the pyridine nitrogen. Furthermore, characteristic sharp peaks in the 1600--1500cm-1region are attributed to the aromatic C= C and C= N ring stretching vibrations of the pyridinium core, while additional C--N stretching bands observed between 1350 and 1400 cm-1 further support this architecture. Collectively, these vibrational frequencies confirm the integrity of the chlorochromate anion [CrO3Cl]-and its ionic pairing with the protonated pyridinium cation.

4.1.5 Electronic Spectroscopy of Pyridinium chlorochromate:

Pyridinium chlorochromate (PCC) is a Cr(VI)-based oxidizing agent containing the chromate ion coordinated with chloride and pyridinium cation. Its UV–Visible spectrum mainly arises from **charge transfer transitions**, as Cr(VI) has a d0 electronic configuration (no d–d transitions)(Table-3)

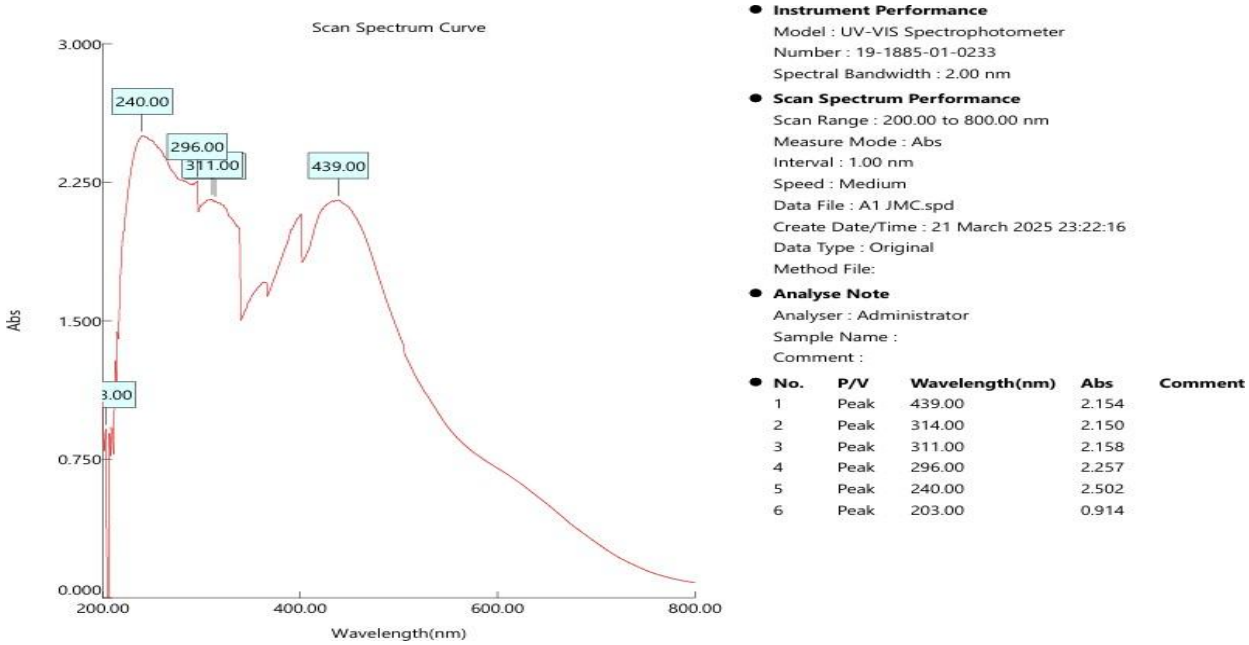


Fig 2: UV Spectrum of Pyridinium Chlorochromate

Table : 3 Important Absorption Bands of PCC in UV spectrum

Wavelength (nm)	Type of Transition	Assignment
203 & 296 nm	$\pi \rightarrow \pi^*$	Pyridinium ring transition
~311 and 314 nm	$n \rightarrow \pi^*$	Chromate oxygen to chromium transition
~439 nm	LMCT	$O^{2-} \rightarrow$ Cr(VI) charge transfer

The UV–Visible spectrum of Pyridinium chlorochromate exhibits several important electronic transitions that help in understanding its structure and properties. The $\pi \rightarrow \pi^*$ transitions are observed in the ultraviolet region at around 203 nm and 296 nm, which arise from the aromatic pyridinium ring and are characterized by high intensity due to strong absorption (high molar absorptivity). In addition, $n \rightarrow \pi^*$ transitions occur due to the excitation of lone pair electrons present on the oxygen atoms, resulting in bands of moderate intensity. A prominent feature of PCC is the ligand-to-metal charge transfer (LMCT) transition, which appears as a strong and broad band in the visible region around 439 nm. This transition involves the transfer of electrons from the filled oxygen 2p orbitals to the empty chromium 3d orbitals and is responsible for the characteristic orange color of PCC. The high molar absorptivity (ε) of this band confirms the presence of charge transfer transitions. Furthermore, the broad nature of the visible band is typical of chromate species, and its position may show slight shifts depending on solvent polarity, indicating a solvatochromic effect.

4.1.6 H1 NMR Spectrum of Pyridinium chlorochromate

Pyridinium chlorochromate is an oxidizing reagent composed of a pyridinium cation ($C_5H_6N^+$) and a chlorochromate anion (CrO_3Cl^-). The ^1H NMR spectrum of Pyridinium chlorochromate shows characteristic signals arising from the aromatic protons of the pyridinium ring. These protons appear in the chemical shift range of approximately 7.5–9.5 ppm. Due to the presence of the positively charged nitrogen (N^+), which is strongly electron-withdrawing, the aromatic protons are deshielded and resonate downfield. The pyridinium ring contains five aromatic protons, which can be categorized based on their positions. The H-2 and H-6 protons, located ortho to the nitrogen, are the most deshielded and typically appear in the range of 8.8–9.5 ppm. The H-3 and H-5 protons, in the meta position, resonate around 8.0–8.7 ppm, while the H-4 proton at the para position appears slightly upfield at 7.5–8.2

ppm. The splitting pattern of these signals is generally observed as complex multiplets due to coupling interactions between adjacent aromatic protons. The typical coupling constants include ortho coupling in the range of 6–8 Hz and weaker meta coupling of about 1–2 Hz. Integration of the signals corresponds to a total of five protons, consistent with the five hydrogen atoms present on the pyridinium ring.

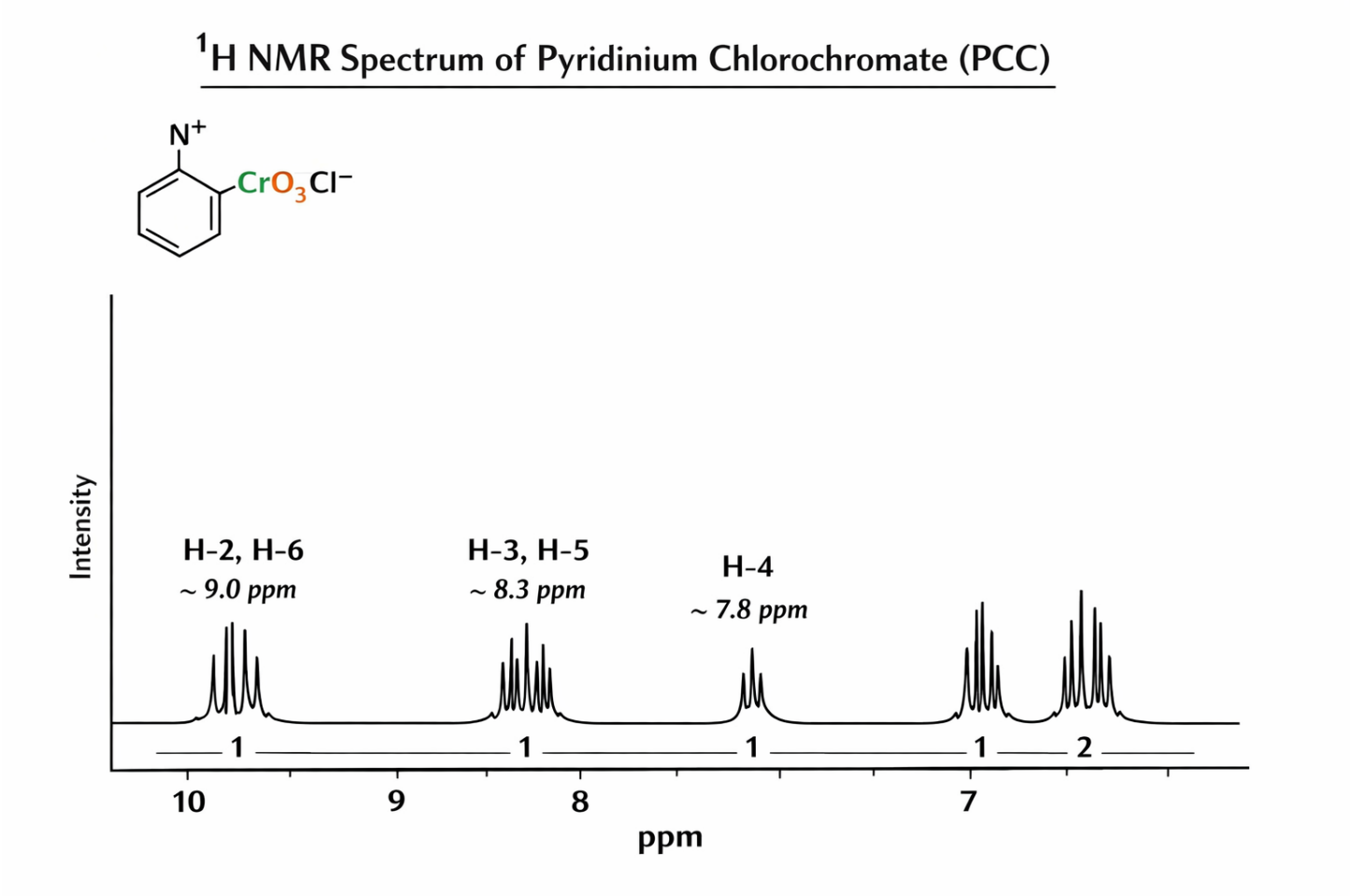


Fig 3: H[1] NMR Spectrum of Pyridinium Chlorochromate

4.1.7 C13 NMR Spectrum of Pyridinium chlorochromate:

The [13]C NMR spectrum of Pyridinium chlorochromate reflects the carbon environments of the pyridinium ring. Although the ring contains five carbon atoms, fewer distinct signals (typically 3–5 peaks) are observed due to symmetry. All carbon signals appear in the aromatic region (~120–160 ppm) and are shifted downfield because of the electron-withdrawing effect of the positively charged nitrogen (N^+). The C-2 and C-6 carbons, which are ortho to the nitrogen, resonate in the range of 145–155 ppm and are highly deshielded. The C-3 and C-5 carbons (meta position) appear around 130–140 ppm, while the C-4 carbon (para position) is the least deshielded and resonates at 120–130 ppm. Typically, three signals are observed corresponding to C-2/C-6, C-3/C-5, and C-4; however, in high-resolution spectra, up to five separate signals may be distinguished. The peaks are generally sharp, though slight broadening can occur due to the presence of paramagnetic Cr(VI), and no aliphatic carbon signals are present in the spectrum.

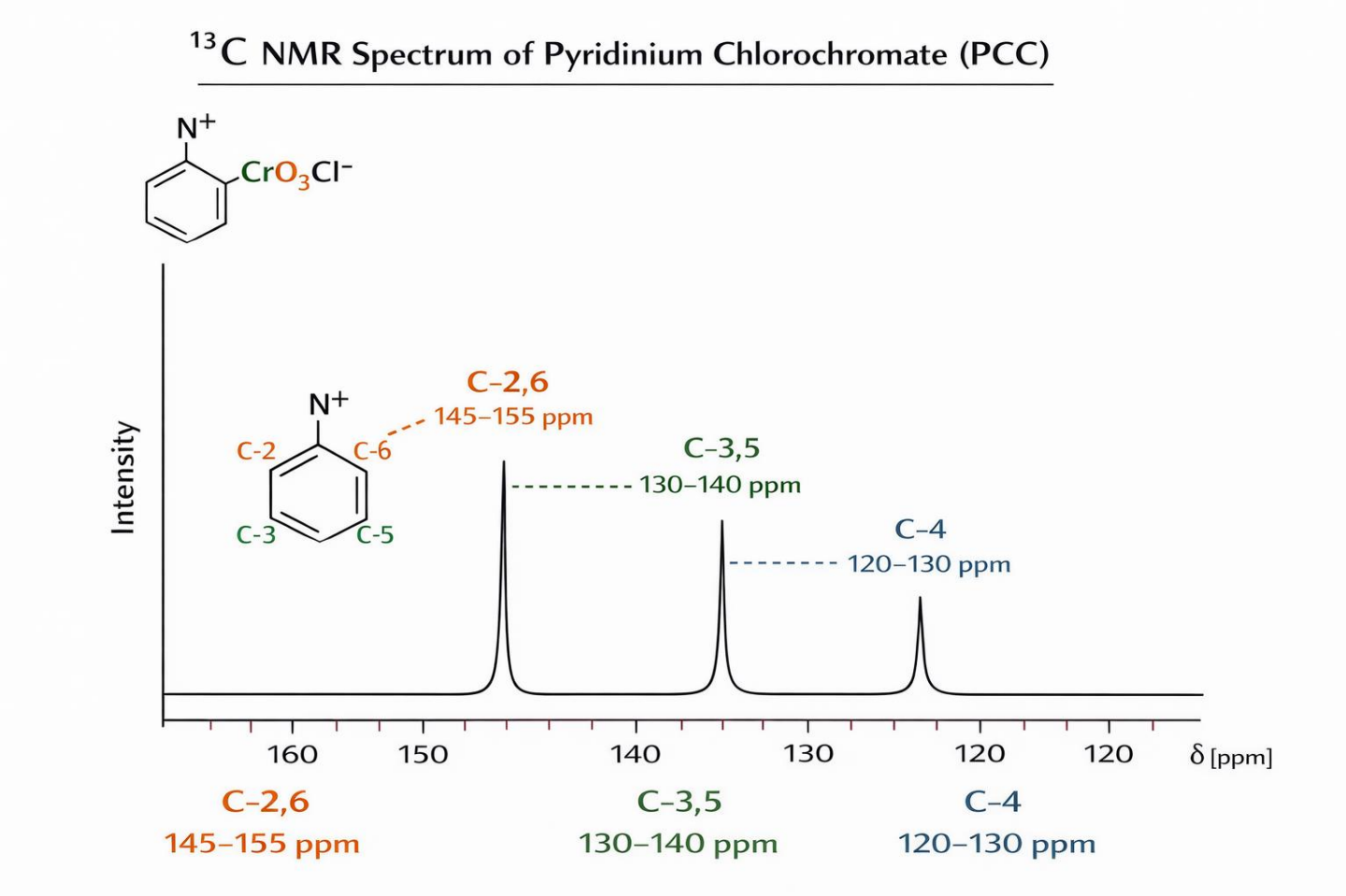


Fig 4: C^{13} NMR Spectrum of Pyridinium Chlorochromate

4.2 Biological activity of Pyridinium Chlorochromate:

Pyridinium chlorochromate has been reported to exhibit various biological activities. In the present study, the anti-diabetic activity of pyridinium chlorochromate was investigated.

4.2.1. Anti-diabetic activity of Pyridinium Chlorochromate:

The anti-diabetic activity of pyridinium chlorochromate was determined using the α-amylase inhibition assay.

4.2.2 α-amylase inhibition method.

α-Amylase is secreted by the pancreas and salivary glands. It is a key enzyme in carbohydrate digestion that catalyses the initial hydrolysis of starch by acting on the interior α-D-1,4-glucosidic linkages. Amylase converts starch into α-limit dextrins, maltose, and maltotriose. The α-D-(1,4) glycosidic linkages in carbohydrates are cleaved by α-amylase to produce oligosaccharides, which are further cleaved to the monosaccharide glucose by α-glucosidase. Therefore, inhibitors of these enzymes can delay the increase in blood glucose levels in people who consume carbohydrate-rich food, thereby keeping hyperglycemia under control. The ethanolic extract of pyridinium chlorochromate showed a maximum inhibition of 71.30%, as compared with the standard drug acarbose (80%), at a concentration of 100 µg/mL.(Table 4)

TABLE 4: Anti-Diabetic Activity Of Standard Acarbose Vs Pcc By Alpha-Amylase Assay Method

S.N O	CONCENTRAT ONS	ALPHA AMYLASE (%)	
		SAMP LE S3	ACARB OSE
1.	20 (µg//ml)	45.21	50.43
2.	40 (µg//ml)	50.43	55.65
3.	60 (µg//ml)	57.39	63.47
4.	80 (µg//ml)	63.47	71.30
5.	100 (µg//ml)	71.30	80
	IC50 Value	36.84	22.91

The α-amylase inhibitory assay is commonly used to evaluate the anti-diabetic potential of plant extracts or compounds. The enzyme α-amylase is responsible for breaking down starch into simple sugars during digestion. Inhibiting this enzyme helps reduce the rate of glucose release and absorption, thereby controlling postprandial (after-meal) blood glucose levels. In this study, the inhibitory activity of PCC was compared with that of the standard anti-diabetic drug acarbose at different concentrations (20–100 µg/mL).

The results presented in Table 4 show that both PCC and acarbose exhibited concentration-dependent inhibition of α-amylase activity. At 20 µg/mL, PCC showed 45.21% inhibition, whereas acarbose showed 50.43% inhibition. As the concentration increased to 40 µg/mL, inhibition increased to 50.43% for PCC and 55.65% for acarbose. At 60 µg/mL, the inhibition further increased to 57.39% (PCC) and 63.47% (acarbose). At 80 µg/mL, PCC exhibited 63.47% inhibition, while acarbose showed 71.30% inhibition. The highest inhibition was observed at 100 µg/mL, where PCC showed 71.30% inhibition and acarbose showed 80% inhibition.

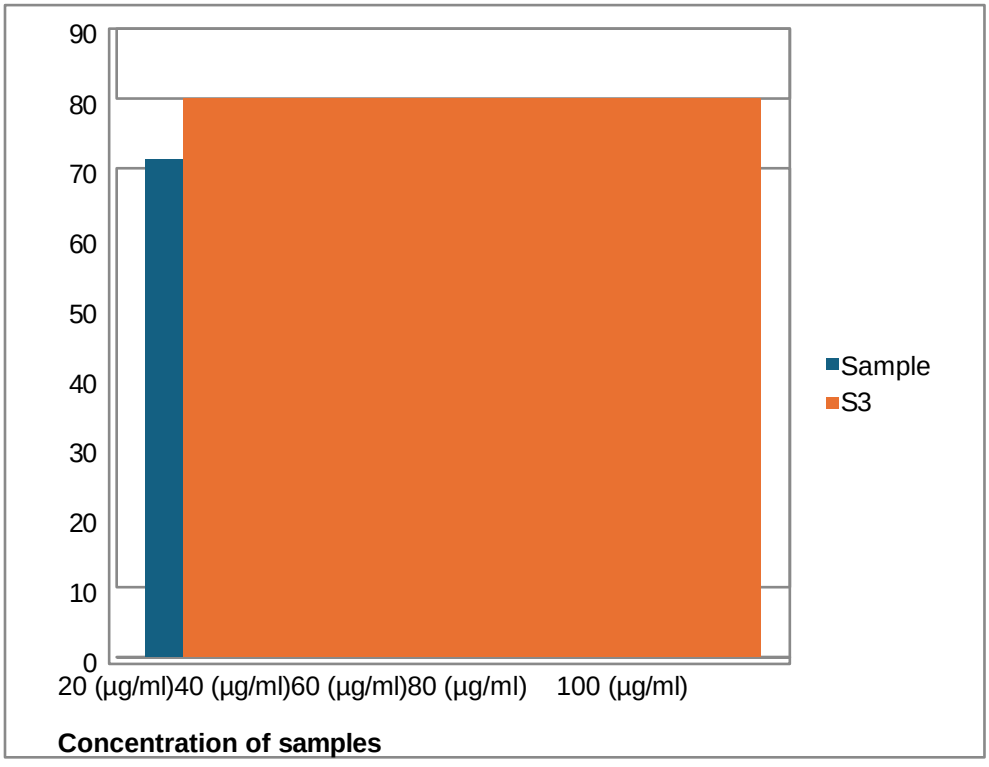


Figure 5: ***In vitro Anti-diabetic Activity of PCC Compared with Standard Drug Acarbose Using α-Amylase Method***

Figure 5 illustrates the α-amylase inhibitory activity of PCC in comparison with that of the standard anti-diabetic drug acarbose at different concentrations (20–100 µg/mL). The figure shows that the percentage inhibition of α-amylase increases as the concentration increases for both the sample and the standard drug. At lower concentrations (20 µg/mL), PCC exhibits moderate inhibition of the enzyme, while acarbose shows slightly higher inhibitory activity. As the concentration increases to 40, 60, 80, and 100 µg/mL, (Figure 6 & 7) the inhibition gradually increases for both, indicating a dose-dependent inhibitory effect. However, throughout the tested concentrations, acarbose demonstrates higher α-amylase inhibition than PCC, indicating that the standard drug is more potent in controlling enzyme activity. Despite this, PCC shows considerable inhibitory activity, suggesting the presence of bioactive compounds capable of reducing the activity of α-amylase. The ability of PCC to inhibit α-amylase suggests that it may help slow down the breakdown of starch into glucose, thereby potentially reducing postprandial blood glucose levels. Thus, the chart indicates that PCC possesses promising *in vitro* anti-diabetic activity, although its effectiveness is lower than that of the standard drug acarbose.

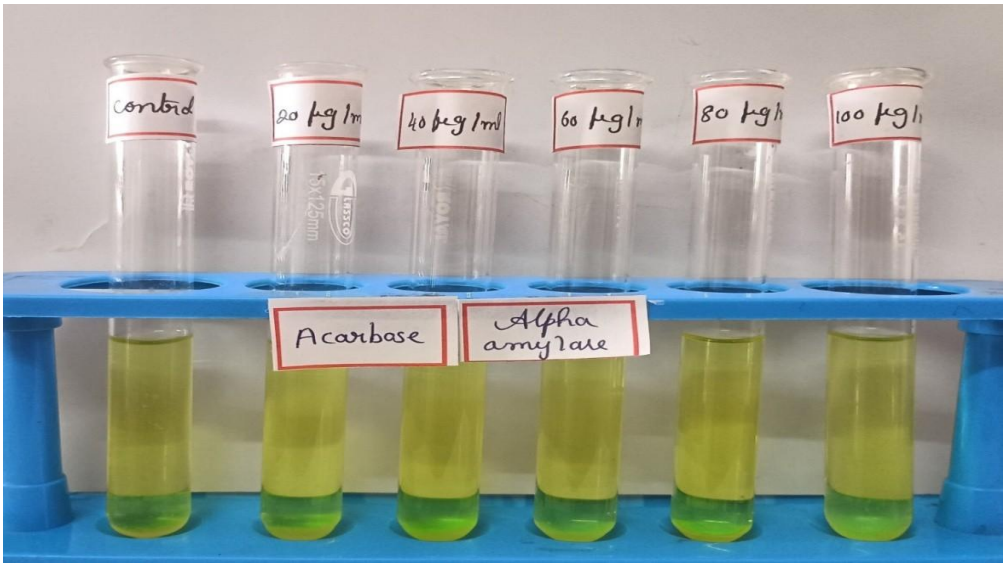


Figure 6: Anti-Diabetic Activity of Standard Acrobose by Alpha- Amylase Assay Method

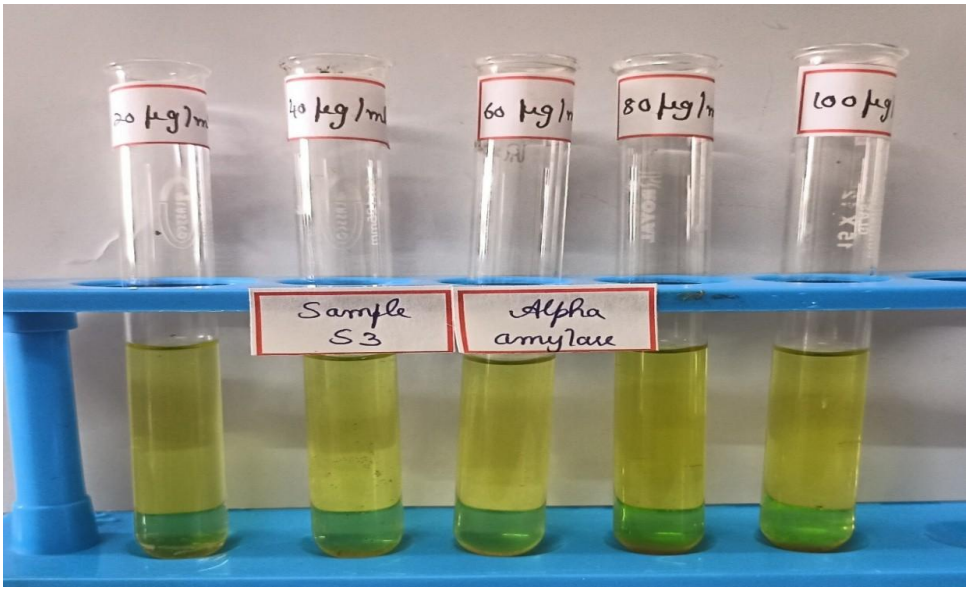
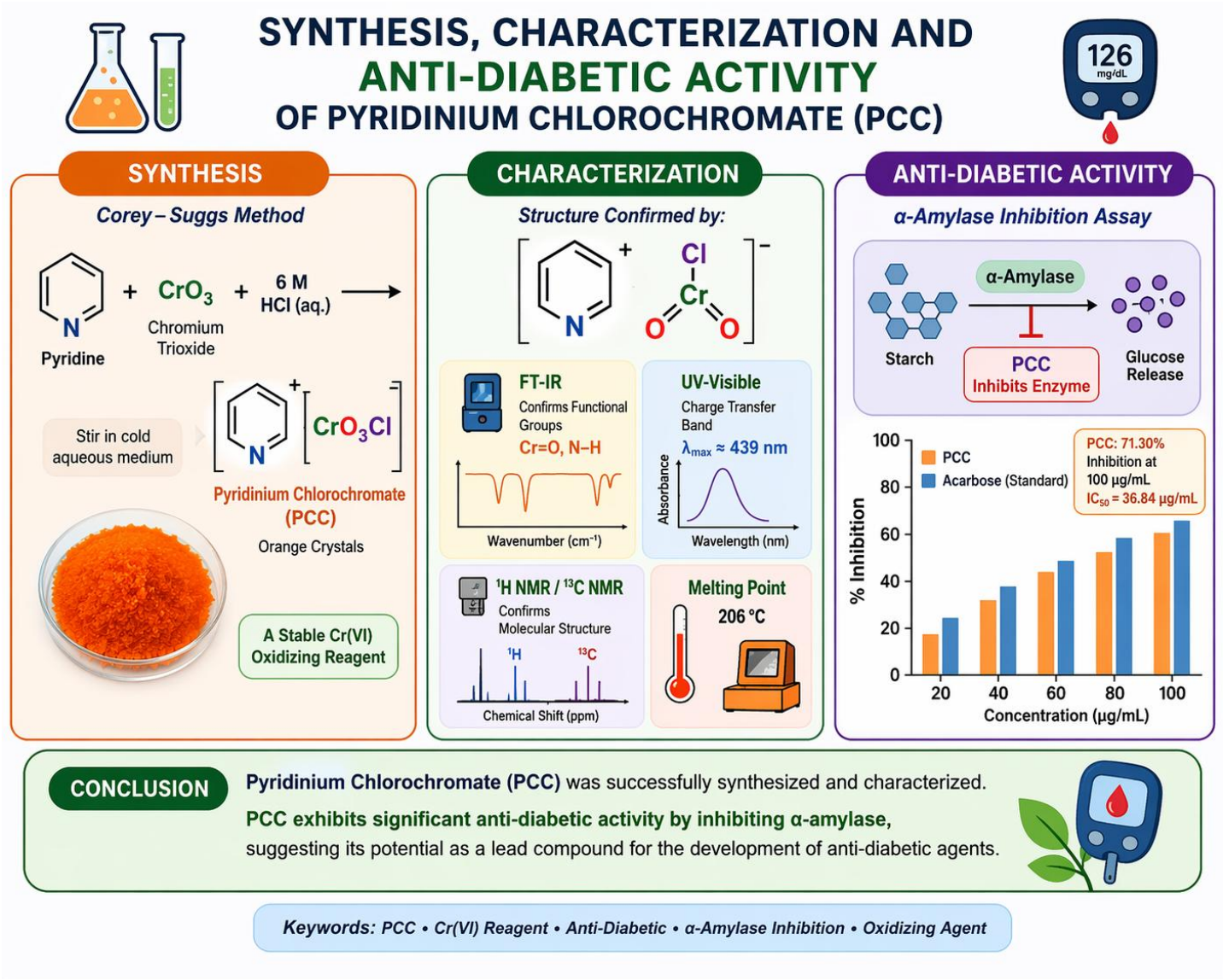


Figure 7: Anti-Diabetic Activity of PCC by Alpha-Amylase Assay Method

5. GRAPHICAL ABSTRACT:



6. CONCLUSION

The present study successfully demonstrates the synthesis, characterization, and evaluation of the anti-diabetic activity of pyridinium chlorochromate (PCC). The compound was confirmed by melting point determination, elemental analysis, and FT-IR, UV–Visible, and NMR spectroscopic studies, validating its structural integrity and purity. The *in vitro* α-amylase inhibition assay revealed that PCC exhibits significant, concentration-dependent anti-diabetic activity, with a maximum inhibition of 71.30% at 100 µg/mL, although this was slightly lower than that of the standard drug acarbose. The observed inhibitory effect suggests that PCC can effectively delay carbohydrate digestion and reduce postprandial glucose levels. These findings indicate that PCC possesses promising anti-diabetic potential and may serve as a lead compound for further pharmacological investigations, including detailed *in vivo* studies and mechanism-based evaluations.

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