

A novel and stable formulation of powdered high-assay calcium hypochlorite as a solid precursor of the antimicrobial compound hypochlorous acid: a preformulation study

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ABSTRACT

Hypochlorous acid (HOCl) has been strongly recognized in the wake of the COVID-19 pandemic. It is a well-known antimicrobial compound with a broad-spectrum effect that demonstrates excellent clinical safety results in wounds. However, stable HOCl products remain unavailable due to challenges in maintaining storage stability, particularly at higher concentrations ($\geq 200 \mu\text{g g}^{-1}$) at room temperature. To address this limitation, we investigated calcium hypochlorite ($\text{Ca}(\text{OCl})_2$) as a stable solid precursor for *in-situ* HOCl generation. This paper presents comprehensive preformulation characterization data, including powder stability analysis and release kinetics, establishing the foundational parameters necessary for future product development. We have demonstrated that commercial $\text{Ca}(\text{OCl})_2$ granules dissolves in a buffered medium and converts to HOCl at the time of use. However, the granules convert too slowly and may jeopardize user-friendliness. Through a mixed multi-variate experimental design, we found that grinding the granules into a powder (particle sizes between 45 and 500 μm) significantly improved the conversion rates, achieving $\geq 99.4\%$ conversion within 10 s of shaking in acetic acid buffer pH 4.3 for samples equivalent to $2000 \mu\text{g g}^{-1}$ HOCl. Particles sized 125–150 and 180–250 μm showed rapid dissolution, with $\geq 98.4\%$ conversion within just 1 s of shaking. Powdered $\text{Ca}(\text{OCl})_2$ was stable for at least 24 months at $23^\circ\text{C} \pm 2$ when stored dry ($\leq 5\%$ degradation), though higher temperatures and moisture negatively affected the storage stability. Flow behavior of powdered $\text{Ca}(\text{OCl})_2$ was also studied by the angle of repose, Hausner's ratio and compressibility index for future production purposes of the novel formulation. Good flow was observed for powders above 250 μm in particle size, whereas the tests failed for particle size below 150 μm . In summary, these findings demonstrate that powdered $\text{Ca}(\text{OCl})_2$ is highly stable under the proper storage conditions, making it a promising and novel HOCl precursor formulation for combating bacterial biofilm and other topical microbial infections.

1. Introduction

There is a global need for effective and safe antimicrobial and biofilm-eradicating formulations due to the spread of antimicrobial resistance [1,2]. Literature indicates growing evidence of bacterial biofilm being present in non-healing wounds and their adverse role in delaying normal wound healing [3]. The biofilm barrier reduces antibiotic susceptibility by limiting antibiotic access to bacterial cells and protecting the microbial community from the host immune response. Consequently, bacterial biofilms are 100–1000 times more tolerant to antibiotics compared to planktonic cells [4]. Biofilm formation also

poses challenges in other healthcare areas, such as medical devices and dental health, and in industrial settings such as fish tanks, water treatment systems, and food processing equipment [5,6].

Hypochlorous acid (HOCl) has been strongly recognized in the wake of the COVID-19 pandemic in its role as one of the human body's first line of defense against invading pathogens. This has created an exceptional opportunity in the field of infection control. The use of HOCl is not new and has been extensively documented and reviewed in literature. The first form of HOCl was produced over 200 years ago in France as potassium hypochlorite, a liquid chlorine solution [7]. It has therefore been extensively documented as a promising compound in the fight

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against microbial infections [8–11]. In more recent literature, Fazli et al. [12] and Burian et al. [13,14] have demonstrated that combining HOCl and acetic acid ($\text{HOCl} + \text{HOAc}$) yields promising clinical results in the treatment of acute and biofilm-infected chronic wounds.

Produced naturally in humans by the enzyme myeloperoxidase, HOCl is mainly expressed in neutrophils and, to a lesser degree, in monocytes as a first line of defense against invading pathogens via oxidative stress [15–19]. It is a highly reactive and non-toxic oxidizer at low concentrations [20]. The efficacy of HOCl is influenced by contact time, concentration and pH value of the solution [19,21–24]. Its decomposition will therefore result in a loss of antimicrobial activity [25]. Ready-to-use HOCl aqueous solutions, such as electrolyzed water, face long-term stability issues due to the compound's propensity for oxidation reactions [15,23,26]. This instability poses pharmaceutical development challenges, including accelerated degradation in non-buffered systems and under ultraviolet light or elevated temperature. The degradation rate of HOCl is concentration-dependent and occurs more rapidly at higher concentrations as well [8]. Additionally, organic compounds (e.g., proteins and carbohydrates) and inorganic metal ions (e.g., ferrous iron and copper ions) readily consume HOCl through oxidation reactions [27–31]. On-site equipment for producing HOCl solutions addresses the stability issue; however, such apparatuses can be impractical for deployment in certain contexts, such as on a battlefield.

Currently available ready-to-use HOCl solutions for skin disinfection and wound irrigation are regulated as biocides or medical devices, depending on their intended use and claims. These commercial products usually contain lower HOCl concentrations ($\sim 50\text{--}300\ \mu\text{g g}^{-1}$) due to stability limitations in liquid formulations. However, effective biofilm eradication typically requires HOCl concentrations of $100\text{--}200\ \mu\text{g g}^{-1}$ for simple biofilms with extended contact times [32–34], while complex or mature biofilms in non-healing wounds may require high concentrations of $1000\text{--}3000\ \mu\text{g g}^{-1}$ for reliable eradication [35]. Higher-concentration HOCl formulations intended for biofilm eradication in chronic wounds may require medicinal product/drug classification due to stricter regulatory requirements, and at least medical device class II regulation in the U.S and class III within the EU [36,37]. To develop a stable high-concentration formulation of HOCl, it is crucial to address the aforementioned challenges, and a solid precursor of HOCl like calcium hypochlorite ($\text{Ca}(\text{OCl})_2$) could be a viable candidate.

Commercially available as a granule formulated product, $\text{Ca}(\text{OCl})_2$ is mainly used in water-treatment and pool disinfection worldwide. The high-concentration variant of commercial $\text{Ca}(\text{OCl})_2$ granules is called high-assay calcium hypochlorite. It contains approximately 70 % (w/w) $\text{Ca}(\text{OCl})_2$, and 30 % of other compounds, such as sodium chloride (NaCl), calcium chloride (CaCl_2), calcium hydroxide ($\text{Ca}(\text{OH})_2$), chlorate salts (NaClO_3 and $\text{Ca}(\text{ClO}_3)_2$) and moisture [38–40]. This composition results from the industrial production method where moist slaked lime ($\text{Ca}(\text{OH})_2$) reacts with chlorine gas (Cl_2) [39,41]. Consequently, higher purity of $\text{Ca}(\text{OCl})_2$ is difficult to achieve since the process reaction inevitably produces CaCl_2 as a byproduct alongside the desired calcium hypochlorite. However, higher purity also increases the risk of explosion and fire accidents during storage and transportation of $\text{Ca}(\text{OCl})_2$ [42].

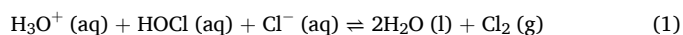
Using solid $\text{Ca}(\text{OCl})_2$ over liquid hypochlorites, such as sodium hypochlorite (NaOCl), presents several advantages. Firstly, solid $\text{Ca}(\text{OCl})_2$ offers better storage stability as liquid hypochlorites degrade more rapidly [43]. Consequently, higher concentration formulations are not limited by stability issues since $\text{Ca}(\text{OCl})_2$ is a solid prior to activation. Secondly, it reduces chlorate (ClO_3^-) formation, an unwanted byproduct, which occurs during production and storage degradation [41,44]. Lastly, the solid form and higher concentration of $\text{Ca}(\text{OCl})_2$ (approximately 70 % active chlorine content) simplify handling and storage compared to NaOCl, which typically contains only 10–15 % active chlorine.

The problem related to storage stability of HOCl and liquid chlorine

has also been addressed by commercial sodium dichlorocyanurate (NaDCC) tablets by releasing HOCl and OCl^- upon dissolution in water. Furthermore, these tablets have addressed the issue of liquid transportation entirely, while the use of $\text{Ca}(\text{OCl})_2$ proposes the transportation of suitable buffer media. Although NaDCC has proven effective for water disinfection and surface cleaning [45,46], clinical evidence for skin disinfection and wound biofilm applications remains limited. In contrast, $\text{Ca}(\text{OCl})_2$ -based formulations eliminate cyanuric acid accumulation, which can reduce chlorine efficacy over time [47] and create regulatory challenges in pharmaceutical development due to uncertainty regarding toxicity in wounds. The use of a solid $\text{Ca}(\text{OCl})_2$ /buffer system can potentially address the unique requirements of chronic wound care, including storage stability, precise pH control, rapid antimicrobial action, and compatibility with wound healing processes. Clinical safety testing of HOCl + acetate buffer in chronic venous leg ulcers has been reported by Fazli et al. with promising results [12]. This targeted approach positions $\text{Ca}(\text{OCl})_2$ -based formulations to fill a distinct clinical niche that complements rather than directly competes with established NaDCC applications. $\text{Ca}(\text{OCl})_2$ -based formulations may also offer distinct advantages over NaDCC for wound irrigation and intact skin disinfection applications. Importantly, this approach eliminates cyanuric acid/cyanurate formation, unlike NaDCC.

The aim of this study was to evaluate the use of high-assay calcium hypochlorite granules as a stable, solid precursor of HOCl. As stated above, although HOCl is highly effective against many pathogens, it is unstable and readily undergoes oxidation reactions [15,23,26]. For $\text{Ca}(\text{OCl})_2$ granules to serve as a suitable HOCl precursor, they must dissolve easily in aqueous media and convert to HOCl *in situ*. Preliminary tests indicated that the granular form did not dissolve instantly. Therefore, two hypotheses were proposed: 1) storing the active pharmaceutical ingredient as a solid precursor will extend the formulation's shelf life, and 2) grinding $\text{Ca}(\text{OCl})_2$ granules into a powder will enable instant dissolution of the powder in aqueous media, without compromising storage stability under the appropriate conditions.

The solubility and dissolution rates of basic compounds such as $\text{Ca}(\text{OCl})_2$ are high in acetic media. However, maintaining the pH above 4.0 is essential to avoid significant formation of Cl_2 [15,26,48–50]. Previous studies [51] have shown that the formation of Cl_2 takes place at low pH following the reaction (Eq. (1)):



At higher pH levels, studies indicate that the decomposition and reaction rates of HOCl increase with pH, peaking at pH 6.9–7.1 [23,26]. ClO_3^- becomes a significant degradation product at these pH values. Theoretical calculations suggest that $\text{Ca}(\text{OCl})_2$ dissolved in water at neutral pH (around pH 7) yields approximately 50 % of HOCl, and 50 % of its corresponding base, hypochlorite (OCl^-) [28,50]. Adjusting the pH to weakly acidic levels ($\sim 4\text{--}5$) increases HOCl conversion while keeping the pH high enough to prevent chlorine gas formation.

To optimize the dissolution of $\text{Ca}(\text{OCl})_2$ and its conversion to HOCl, a mixed full-factorial experimental design was implemented with the aforementioned challenges in mind. The commercial $\text{Ca}(\text{OCl})_2$ granules were ground to a powder and sieved. The experimental variables included particle size, buffer strength of acetic acid, HOCl concentration, and shaking time.

Additionally, the reproducibility and user-friendliness of powdered $\text{Ca}(\text{OCl})_2$ as a HOCl precursor were investigated. Characteristics such as physical appearance and powder flow were examined. Given the alteration from granules to powder, a 24-month stability study at room temperature under different storage conditions was conducted. A short-term test was also conducted, investigating the stability of powder compared to granules at different temperatures.

2. Materials and methods

2.1. Materials

Commercial calcium hypochlorite granules ($\leq 70\%$; $\text{Ca}(\text{OCl})_2$) was purchased from Barchemicals (MO, Italy). Glacial acetic acid (100 %; CH_3COOH), sodium thiosulfate solution (0.1 M; $\text{Na}_2\text{S}_2\text{O}_3$), potassium iodide ($\geq 99.5\%$; KI), iodine solution (0.05 M) and soluble starch were acquired from Merck (Darmstadt, DE). Sodium hydroxide ($\geq 98.5\%$ pellets; NaOH) was purchased from VWR Chemicals (Radnor, PA, USA). Purified water was purchased from Fagron (Rotterdam, Netherlands) or prepared by a Milli-Q® water purification system for ultrapure water by Merck Millipore (Darmstadt, DE).

In the present work, commercial high-grade calcium hypochlorite with a 65–70 % available chlorine content will be referred to as $\text{Ca}(\text{OCl})_2$ granules, to differentiate it from the grinded calcium hypochlorite granules, herein referred to as powdered $\text{Ca}(\text{OCl})_2$ or $\text{Ca}(\text{OCl})_2$ powder. HOCl and $\text{Ca}(\text{OCl})_2$ concentrations are given in $\mu\text{g g}^{-1}$ or mass percentage units, respectively, rather than following the practice for drinking water and wastewater treatment of expressing free available chlorine in parts per million (ppm) of chlorine equivalents, usually expressed as “ppm available chlorine”.

2.1.1. Preparation of powdered calcium hypochlorite

Powdered $\text{Ca}(\text{OCl})_2$ was freshly prepared by grinding commercially available granule product in a ceramic mortar in ambient conditions (eastern Norwegian indoors climate; room temperature between 20 and 25 °C). All powder was sieved through $\leq 45\ \mu\text{m}$, $\leq 75\ \mu\text{m}$, $\leq 125\ \mu\text{m}$, $\leq 150\ \mu\text{m}$, $\leq 180\ \mu\text{m}$, $\leq 250\ \mu\text{m}$, $355\ \mu\text{m}$ and $\leq 500\ \mu\text{m}$ test sieves (Merck, Darmstadt, DE) in descending order.

2.1.2. Preparation of solutions

Acetic acid buffer solutions (HOAc) of 0.25, 0.50, 1.0, 2.0 and 3.0 % concentrations (w/w) pH 4.3 ± 0.2 were prepared by diluting 100 % CH_3COOH with purified water, and pH was adjusted by the addition of NaOH pellets or NaOH solution 50 % (w/w) prepared from the pellets.

Starch solution 1.0 % (w/v) was prepared by suspending 1.0 g of soluble starch with 5 mL of purified water, followed by adding 95 mL of boiled purified water to the mixture. Sensitivity was tested immediately with KI and iodine solution and retested each time if the batch was more than one day old.

2.2. Quantification methods

2.2.1. Calcium hypochlorite content

Successively, 50 mL of purified water, 1.0 g of potassium iodide (KI) and 3 mL of glacial acetic acid were introduced into a flask. 100 mg of the solid sample (either granules or powdered variant) was transferred into the flask and titrated immediately with 0.1 M sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$), adding 2 mL of starch solution just prior to the equivalence point as an indicator. The titration was performed in triplicates with a standardized 25-mL burette (VWR chemicals, Radnor, PA, USA).

The concentration of $\text{Ca}(\text{OCl})_2$, w_1 , expressed as a mass fraction in %, is given by Eq. (2).

$$w_1 = \frac{V \times 3.575}{m_1} \times 100\% \quad (2)$$

where V is the volume of the $\text{Na}_2\text{S}_2\text{O}_3$ titration standard, in mL, m_1 is the mass of the granule or powder sample in milligram and 3.575 is the mass of $\text{Ca}(\text{OCl})_2$ in mg corresponding to 1.00 mL of the titration standard $c(\text{Na}_2\text{S}_2\text{O}_3) = 0.1\ \text{M}$.

2.2.2. Hypochlorous acid content

Successively, 10.00–25.00 g of the sample solution (depending on the theoretical concentration) and 0.5 g KI were introduced into a flask.

The solution was titrated immediately with 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$, adding 1 mL of starch solution just prior to the equivalence point as an indicator. In samples containing acetic acid buffers, 2 mL of glacial acetic acid was added at the end of the titration check for any remaining active chlorine species besides HOCl. The titration was performed with a standardized 25-mL burette (VWR chemicals, Radnor, PA, USA). The content was then calculated according to Eq. (3).

2.3. pH measurements

pH measurements were performed using an inoLab multi 9310 IDS pH-meter (Xylem Analytics, Letchworth, England) after calibration with calibration buffers pH 1.00, 4.00 and 7.00 (Merck, Darmstadt, DE).

2.4. Investigation of $\text{Ca}(\text{OCl})_2$ dissolution in acetic acid buffer

The dissolution of powdered $\text{Ca}(\text{OCl})_2$ in acetic acid buffer media was investigated in a multivariate mixed full-factorial experimental design. MODDE® version 5.0 (Umetrics, Sweden) was utilized for the design of experiments (DoE) and multivariate data analyses. The powder, equivalent to theoretical amounts of either 200 or 2000 $\mu\text{g g}^{-1}$ HOCl, was dissolved in 200 mL acetic acid buffer media 0.5 or 3.0 % (w/w). Powdered $\text{Ca}(\text{OCl})_2$ was investigated at six levels: particle size ranges $\leq 45\ \mu\text{m}$, 45–75 μm , 125–150 μm , 180–250 μm , 250–355 μm and 355–500 μm . Shaking time was 1 or 10 s on a digital vortex genie 2 230v (Scientific Industries, New York, USA) set at 2500 rpm to mix the samples. Aliquots (10.00–25.00 g) were removed from the surface of each sample and quantified immediately and compared to controls (fully dissolved samples). The dissolution of controls was examined visually against a black background. Granules were also tested and used as delivered by the manufacturer. The experiment was repeated two times ($n = 3$).

2.4.1. Data analysis

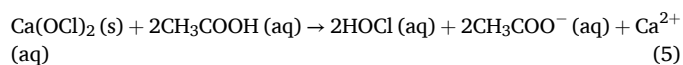
The concentration of HOCl, w_2 , expressed as a mass fraction in $\mu\text{g g}^{-1}$, given by Eq. (3):

$$w_2 = \frac{V \times 2623}{m_2} \quad (3)$$

where V is the volume of the $\text{Na}_2\text{S}_2\text{O}_3$ titration standard, in mL, m_2 is the mass of the test sample in grams and 2623 is the mass of HOCl in μg corresponding to 1.00 mL of the titration standard $c(\text{Na}_2\text{S}_2\text{O}_3) = 0.1\ \text{M}$. Experimental data was fitted to Eq. (3) to extract HOCl concentrations. The dissolution of powdered $\text{Ca}(\text{OCl})_2$ and subsequent conversion to HOCl at a given time point (1 or 10 s) was then calculated by dividing the quantified HOCl concentration of test samples with their respective controls, reported as a yield percentage of $\text{Ca}(\text{OCl})_2$ dissolved relative to control in Eq. (4):

$$\text{yield \%} = \frac{c_1}{c_2} \times 100\% \quad (4)$$

where c_1 is the concentration of HOCl after shaking at a given time point and c_2 is the concentration of the corresponding control sample. Eq. (3) is also applicable for the calculation of the active chlorine content, the general term consisting of OCl^- , HOCl and Cl_2 . However, for simplicity and better interpretation, we chose to calculate it as HOCl since this compound is the major product in the expected acid/base reaction to take place, shown in Eq. (5).



Note that the reaction takes place primarily between pH 4.0 and 5.6, at room temperature. The pK_a value of HOCl at 20–25 °C is between 7.53 and 7.58, and when the pH value is two units below pK_a , the HOCl to OCl^- content will be $\geq 99\%$ [49]. pH measurements were conducted on

each sample and confirmed to be between 4.26 and 5.56. pH below 4.0 is avoided as chlorine gas starts forming, as described by Wang et al. [15] in Fig. 1.

2.5. Homogeneity test of powdered high-assay calcium hypochlorite

The mass of $\text{Ca}(\text{OCl})_2$ powder and granules, equivalent to theoretical concentrations of 200–1000 $\mu\text{g g}^{-1}$ HOCl, was weighed on a Sartorius ENTRIS224i-1S analytical balance to 0.3 mg precision. Granules were used as-is and the particle size range of the powder was 125–150 μm . The solid was subsequently dissolved in acetic acid buffer 0.25–3.0 % (w/w) pH 4.3 ± 0.2 in a glass beaker at room temperature. In total, five different combinations of HOCl + HOAc were tested: 200 $\mu\text{g g}^{-1}$ HOCl + 0.25 % HOAc; 500 $\mu\text{g g}^{-1}$ HOCl + 1.0 % HOAc; 500 $\mu\text{g g}^{-1}$ HOCl + 2.0 % HOAc; 500 $\mu\text{g g}^{-1}$ HOCl + 3.0 % HOAc; 1000 $\mu\text{g g}^{-1}$ HOCl + 3.0 % HOAc. Three replicate samples were made for each combination ($n = 3$).

2.5.1. Data analysis

To normalize each data point, the experimental data was fitted into Eq. (2) instead of Eq. (3). The reproducibility is represented by the variation within the same group.

2.6. Measure of flow behavior

The powder flow of powdered $\text{Ca}(\text{OCl})_2$, particle size ranges 150–180 μm , 180–250 μm , 250–355 μm and 355–500 μm , was performed in accordance with European pharmacopoeia methods *Angle of repose* and *Compressibility index* and *Hausner ratio* (Ph. Eur. 11.5 20936, 07/2023). The angle of repose was investigated using a Pharma Test Type PTG powder characterization system (Hainburg, Germany) via the fixed funnel method. The compressibility index and Hausner ratio were determined with a STAV 2003 Jolting Volumeter (J. Engelsmann AG, Ludwigshafen, Germany) with a 250 mL cylinder (Funke Gerber, Berlin, Germany).

2.6.1. Technological characterization of the powders

The angle of repose was calculated based on Eq. (6), as shown below:

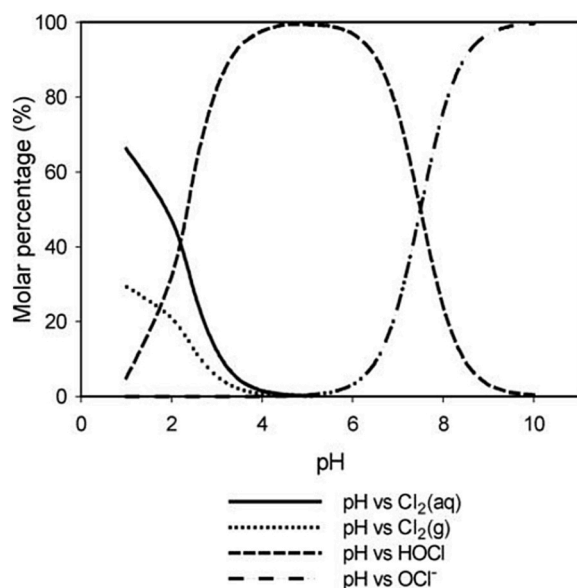


Fig. 1. The speciation of chlorine as a function of pH. Reproduced with permission from Wang et al. [15] under the Creative Commons Attribution License, Journal of Burns and Wounds; published by HMP Global, United States, 2007.

$$\text{Angle of repose} = \tan^{-1} \left(\frac{2h}{d} \right) \quad (6)$$

where h is the height of the powder, and the d is the width of the base of the cone. The Hausner ratio and compressibility index were calculated according to equations (7) and (8), respectively:

$$\text{Hausner ratio} = \frac{V_0}{V_f} \quad (7)$$

$$\text{Compressibility index} = 100 \left(\frac{V_0 - V_f}{V_0} \right) \quad (8)$$

where V_0 is the unsettled apparent volume, and V_f is the final tapped volume.

2.7. Stability studies

2.7.1. Short-term storage of $\text{Ca}(\text{OCl})_2$ granules vs powdered $\text{Ca}(\text{OCl})_2$

$\text{Ca}(\text{OCl})_2$ granules and powder were stored for 14 days at 25, 40 and 54 °C in VWR Incu-line IL 250R climate chambers (Radnor, PA, USA). All samples were stored in amber polyethylene (PET) bottles flushed with argon gas. The mass loss after storage was performed by comparing the mass of each sample at day 0 (baseline) with the mass at day 14, using a Sartorius Entris II analytical scale calibrated with 1.00 g and 100.00 g weights.

2.7.2. Powdered $\text{Ca}(\text{OCl})_2$ after long-term storage

Powdered $\text{Ca}(\text{OCl})_2$ was stored for a total of 24 months. Two storage conditions were investigated: ambient and dry conditions. Here, the ‘ambient condition’ is defined as samples stored in amber glass bottles at 25 °C in a VWR Incu-line IL 250R climate chamber. The ‘dry condition’ differs from the ‘ambient condition’ as samples are additionally stored in a desiccator with calcium chloride as a desiccant. All samples were stored for 12 months. The samples were then moved to a lab bench (23 ± 2 °C) and stored for another 12 months, for a total of 24 months storage. The samples were not flushed with argon gas prior to bottling.

2.7.3. Physical appearance of powdered $\text{Ca}(\text{OCl})_2$ after storage

The physical appearance of powdered $\text{Ca}(\text{OCl})_2$ was visually examined after two weeks storage at 25, 40 and 54 °C. It was also examined after 3, 6, 12 and 24 months of storage protected from light in a desiccator at room temperature (23 ± 2 °C) and in ambient conditions.

3. Results and discussion

3.1. HOCl conversion and dissolution study

The percentage (w/w) dissolved material of the commercial high-assay $\text{Ca}(\text{OCl})_2$ granules in acetic acid (HOAc) buffer at pH 4.3 ± 0.2 was not satisfactory (below 95 % conversion rate), as seen in Fig. 2A and B. The amount of $\text{Ca}(\text{OCl})_2$ to dissolve is expressed as the theoretical concentration of HOCl as either 200 or 2000 $\mu\text{g g}^{-1}$, which is equivalent to 40.6 mg and 406.0 mg high-assay $\text{Ca}(\text{OCl})_2$ (67.1 % w/w purity) per 100 mL acetic acid buffer, respectively. Two time points were chosen: 1 s to represent an almost “instantly dissolved” scenario, and 10 s as a maximum acceptable time for shaking. The results showed that the conversion rate was at best 39.3 ± 2.0 (see Fig. 2A) for the combination of $\text{Ca}(\text{OCl})_2$ granules equivalent to 200 $\mu\text{g g}^{-1}$ HOCl dissolved in 3.0 % HOAc buffer and shaken for 1 s, and 70.6 ± 1.9 (see Fig. 2B) for the combination of $\text{Ca}(\text{OCl})_2$ granules equivalent to 200 $\mu\text{g g}^{-1}$ HOCl dissolved in 3.0 % HOAc buffer and shaken for 10 s.

Commercial $\text{Ca}(\text{OCl})_2$ granules dissolved too slowly and were grinded into powders of six particle size ranges, ranging from ≤ 45 to 500 μm . For all samples shaken for 1 s, samples at size range 125–150 μm gave the highest and most consistent conversion from $\text{Ca}(\text{OCl})_2$ to HOCl

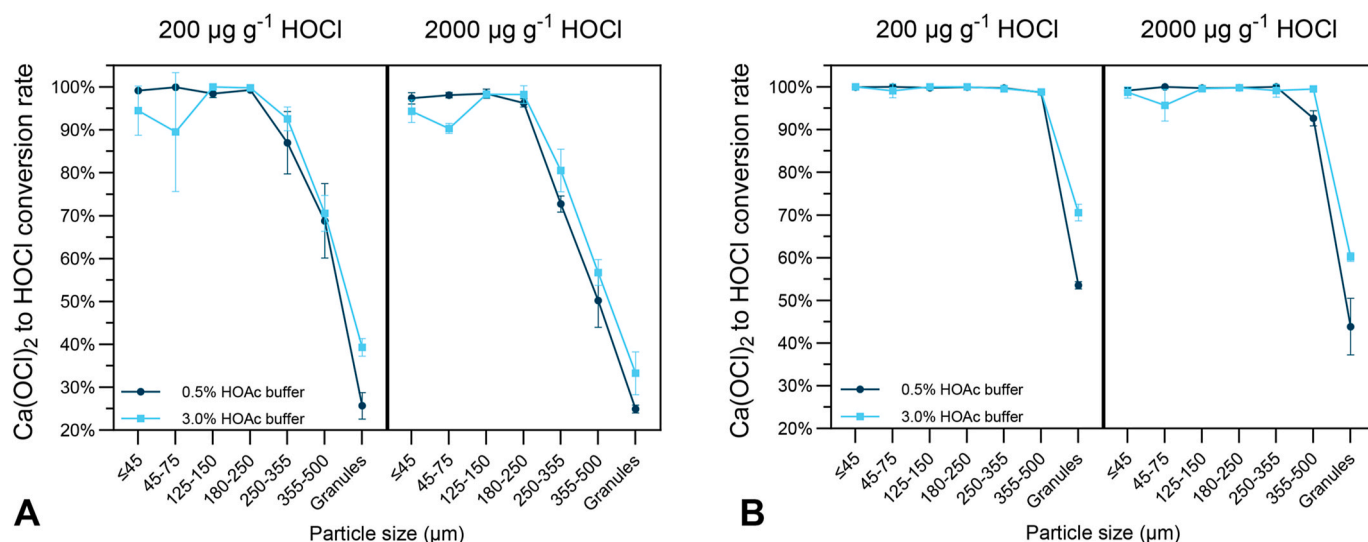


Fig. 2. A & B: The conversion rate of powdered calcium hypochlorite (particle sizes ≤ 45 – 500 μm) and granules to hypochlorous acid, equivalent to 200 or 2000 $\mu\text{g g}^{-1}$, after shaking for A) 1 s and B) 10 s in acetic acid buffers.

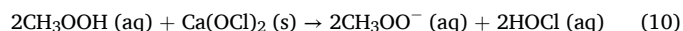
averaging at $98.8\% \pm 1.0$ across all combinations of buffer strength and amount of $\text{Ca}(\text{OCl})_2$ powder added. Combination $200 \mu\text{g g}^{-1}$ HOCl in 3.0 % HOAc buffer converted fully ($100.0\% \pm 0.0$) to HOCl, while combination $2000 \mu\text{g g}^{-1}$ HOCl in 3.0 % HOAc buffer converted 98.3 ± 0.8 . 1-second shaking for size range 180–250 μm was feasible with an average conversion of $98.4\% \pm 1.7$ $\text{Ca}(\text{OCl})_2$. 250–355 μm showed the highest conversion at $92.6\% \pm 2.8$ for the combination $200 \mu\text{g g}^{-1}$ HOCl in 3.0 % HOAc buffer, and the lowest was $72.7\% \pm 1.9$ for the combination $2000 \mu\text{g g}^{-1}$ HOCl in 0.5 % HOAc buffer, when samples were shaken for 1 s. Size range 355–500 μm , had a slower dissolution but followed the same trend; the highest conversion from $\text{Ca}(\text{OCl})_2$ was $70.5\% \pm 4.2$ for the combination $200 \mu\text{g g}^{-1}$ in 3.0 % HOAc buffer, and the lowest was $50.2\% \pm 6.2$ for the combination $2000 \mu\text{g g}^{-1}$ HOCl in 0.5 % HOAc buffer. These conversion rates are not considered satisfactory compared to the smaller particle sizes tested, specifically 125–150 and 180–250 μm , as the goal of shaking for 1 s was to test for ‘instant release’ of HOCl from the solid precursor $\text{Ca}(\text{OCl})_2$. Particle aggregation was observed for particle sizes $\leq 45 \mu\text{m}$ and 45–75 μm due to cohesive forces. When dissolved in 3.0 % (w/w) HOAc buffer, several of these samples aggregated in the solvent, before fully dissolving into a clear solution, which resulted in lower conversions of $\text{Ca}(\text{OCl})_2$ to HOCl and higher standard deviations, especially for samples shaken at only 1 s (Fig. 2A). When shaking time was adjusted from 1 to 10 s, the percentage of dissolved matter increased for all particle sizes (see Fig. 2B). Conversion percentages were above 99.4 % for size ranges ≤ 45 , 125–150, 180–250 and 250–355 μm and the standard deviations were below 0.9, regardless of the HOAc and HOCl concentration composition. Samples with particle size range 355–500 μm converted on average $97.4\% \pm 3.0$ of $\text{Ca}(\text{OCl})_2$ to HOCl, and the lowest amount dissolved was at $92.7\% \pm 1.8$ for the combination of $2000 \mu\text{g g}^{-1}$ HOCl in 0.5 % HOAc buffer.

As mentioned, the dissolution and conversion of high-assay $\text{Ca}(\text{OCl})_2$ granules (i.e., the original commercial granules) was deemed sub-optimal, and several steps were taken to optimize the dissolution rate. Wojtowicz [38] reports that the theoretical solubility of the $\text{Ca}(\text{OCl})_2$ compound to be 21 g per 100 mL water at 25 °C. Since the samples with the highest concentration equal less than 500 mg high-assay $\text{Ca}(\text{OCl})_2$ per 100 mL, the theoretical solubility of $\text{Ca}(\text{OCl})_2$ should not be the limiting factor here. However, $\text{Ca}(\text{OCl})_2$ granules are formulated to minimize moisture-induced decomposition and typically have particle sizes ranging from 0.30 to 2.38 mm (5–80 mesh) [40]. This delays the dissolution and thus the conversion of $\text{Ca}(\text{OCl})_2$ to HOCl. Several parameters influencing dissolution were therefore optimized using the

Noyes-Whitney equation (9). As evident in this equation, the greater the solubility of a compound, the greater is the compound’s dissolution rate:

$$\frac{dM}{dt} = kS(C_s - C) \quad (9)$$

where dM/dt is the mass rate of dissolution, k is the dissolution rate constant, S is the surface area of the exposed solid, C_s is the solubility of the solid at the particle surface and C is the concentration of the solid in a bulk solution at time t . According to this equation (9), as the particle size is reduced, the total effective surface area, S , is increased and thereby the dissolution rate is enhanced. Powder fineness is classified by the European Pharmacopoeia (Ph. Eur. 11.5 20935, 04/2019) into four groups: coarse ($>355 \mu\text{m}$), moderately fine (180–355 μm), fine (125–180 μm) and very fine ($\leq 125 \mu\text{m}$). $\text{Ca}(\text{OCl})_2$ granules were for this reason mechanically powdered to particle sizes between 45 and 500 μm . To additionally aid the dissolution rate of $\text{Ca}(\text{OCl})_2$, stirring the media by shaking was introduced to the experimental design, thus reducing the diffusion layer’s thickness. It was also important to control the pH value of the test samples since HOCl is the dominant chlorine species at pH 4.0–5.6 (see Fig. 1). Samples were kept within this pH interval and close to pH 4.3 ± 0.2 when possible, to avoid added significant decomposition errors to the dissolution study. Furthermore, HOCl is a strong oxidizer, which means a suitable solvent of $\text{Ca}(\text{OCl})_2$ is preferably an acidic buffer, has a pK_a value higher than 4.0 but lower than 5.6 and does not contain reducing functional groups [50]. This was the reason for dissolving $\text{Ca}(\text{OCl})_2$ in acetic acid buffer (HOAc buffer), as the pK_a value of acetic acid is 4.76 at 25 °C, and this compound cannot be oxidized by HOCl or $\text{Ca}(\text{OCl})_2$ [52]. The proposed acid-base reaction of $\text{Ca}(\text{OCl})_2$ in HOAc buffer at pH 4.3 ± 0.2 is shown below (Eq. (10)):



Adam et al. [23] reports that acetic acid buffers (0.352 M, i.e. 2.1 % w/w) increase the initial rate of HOCl decomposition by a factor of 1.3 compared to non-buffered systems, however their experiments were conducted at pH 7.1, where the decomposition rate is close to the maximum and the buffer effect of acetate is no longer present.

The particle aggregation observed within this study is consistent with literature, which reports that particle sizes falling below 100 μm become more cohesive [53]. In the present study, the cohesive forces are further enhanced by the high ionic strength in the acetic acid buffer, as the powder dissolution rate is determined by its solubility in the diffusion layer surrounding each dissolving particle. The same cohesive

tendencies in media were not observed for $\text{Ca}(\text{OCl})_2$ powder dissolved in the weaker HOAc buffer (0.5 % w/w), as depicted in Fig. 2A and B. Particle size ranges ≤ 45 and 45–75 μm were difficult to work with overall, other examples being the challenge of weighing the correct amount of powder for sampling, and aggregation formation in the powder containers due to cohesive and adhesive effects.

3.1.1. Multivariate evaluation

The following four factors were studied in the full factorial design: particle size, acetic acid buffer concentration, amount of $\text{Ca}(\text{OCl})_2$ powder added (expressed as the theoretical concentration of HOCl) and shaking time (shake). The response factor was the dissolution and subsequent conversion of solid $\text{Ca}(\text{OCl})_2$ to HOCl. The overall results are depicted in Fig. 2A and B and discussed in section 3.1. The multiple linear regression analysis (see Fig. 3) revealed that three out of four main effects were statistically significant: particle size ($****p < 0.000$), shaking time ($****p < 0.000$), and HOCl content ($***p < 0.001$). The response factor, i.e. the dissolution (or the conversion rate percentage) decreases with increasing particle size and HOCl concentration, whereas it increases with increased shaking time (see Fig. 4A–C). The effect of HOAc buffer concentration alone was not significant ($p > 0.05$), however the interaction effect with particle size was ($**p < 0.01$, Fig. 5A). All interaction plots from statistically significant interactions are shown in Fig. 5A–D. Interactions and non-linearity were mostly observed between particle size and the other factors. There was especially a strong interaction effect observed for shaking time and particle size ($****p < 0.000$, Figs. 3 and 5B). Furthermore, Fig. 5D depicts that shaking time interacts positively with the amount of $\text{Ca}(\text{OCl})_2$ powder added ($*p < 0.05$). In other words, by increasing the time of shaking, powder dissolved faster and gave better conversion rates even for high-concentration ($2000 \mu\text{g g}^{-1}$ HOCl) samples. The model obtained from the design was statistically significant ($****p < 0.000$) with a coefficient of determination, R^2 , of 0.74.

3.2. Homogeneity of powdered high-assay calcium hypochlorite

Reproducibility of high-assay $\text{Ca}(\text{OCl})_2$ granules and powder as an HOCl-precursor was investigated since the former is a commercial product with a concentration of about 70 % (w/w). A variable particle size range (0.30–2.38 mm [40]) in the granule product may also impact the reproducibility of each sampling. A low reproducibility would impact the potential use of high-assay $\text{Ca}(\text{OCl})_2$ for future product development. Five different combinations of $\text{Ca}(\text{OCl})_2$ powder or granules dissolved in HOAc buffer medium (here referred to as HOCl + HOAc) were investigated, and the variance between the two types of Ca

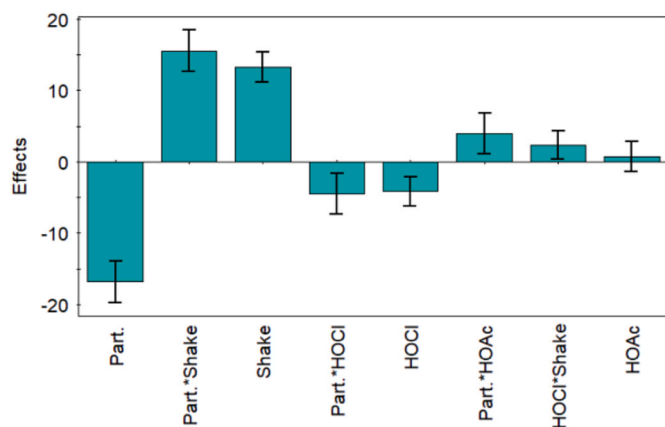


Fig. 3. Main and interaction effects on hypochlorous acid conversion rates. Part. = particle size; shaking = shaking time; HOCl = the theoretical hypochlorous acid concentration from powdered calcium hypochlorite; HOAc = acetic acid buffer.

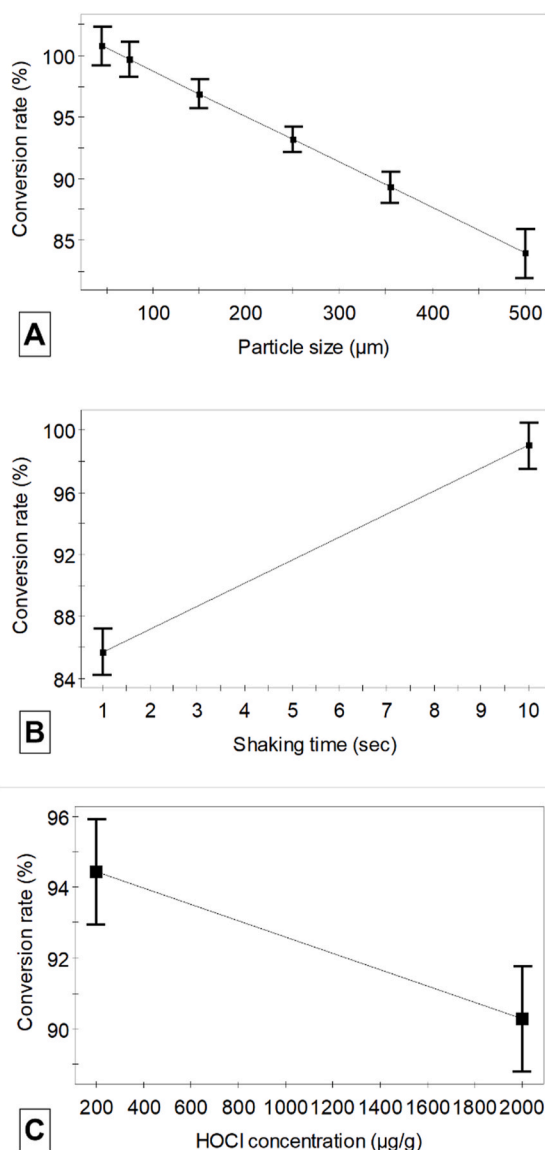


Fig. 4. A–C: Main effect plots for three significant factors: A) particle size, B) shaking time and C) powdered calcium hypochlorite given as theoretical concentrations of hypochlorous acid. For the sake of simplicity, the particle size is given as the size of their sieve mask rather than range. 45 = $\leq 45 \mu\text{m}$, 75 = 45–75 μm , 150 = 125–150 μm , 250 = 180–250 μm , 355 = 250–355 μm , 500 = 355–500 μm . The results are given as mean value \pm SD from three independent experiments.

(OCl) $_2$ is depicted in Fig. 6.

The results showed that the standard deviations were lower for every combination using powder compared to granules. As expected, the powder formulation has a higher reproducibility compared to granules as-is when dissolved in acetic acid buffer as a HOCl precursor.

3.3. Powder flow

Results on flow behavior of powdered $\text{Ca}(\text{OCl})_2$ are summarized in Table 1. Unsurprisingly, particle sizes 355–500 μm showed the best flow properties, as the flowability decreased for the descending particle sizes. Particle sizes below 150 μm was not tested as the sample powder did not flow due to adhesive/cohesive forces. In general, fine particles with very high surface-to-mass ratios are more adhesive/cohesive than coarse particles which are more influenced by gravitational forces [53]. Particles larger than 250 μm are usually relatively free flowing, however, it

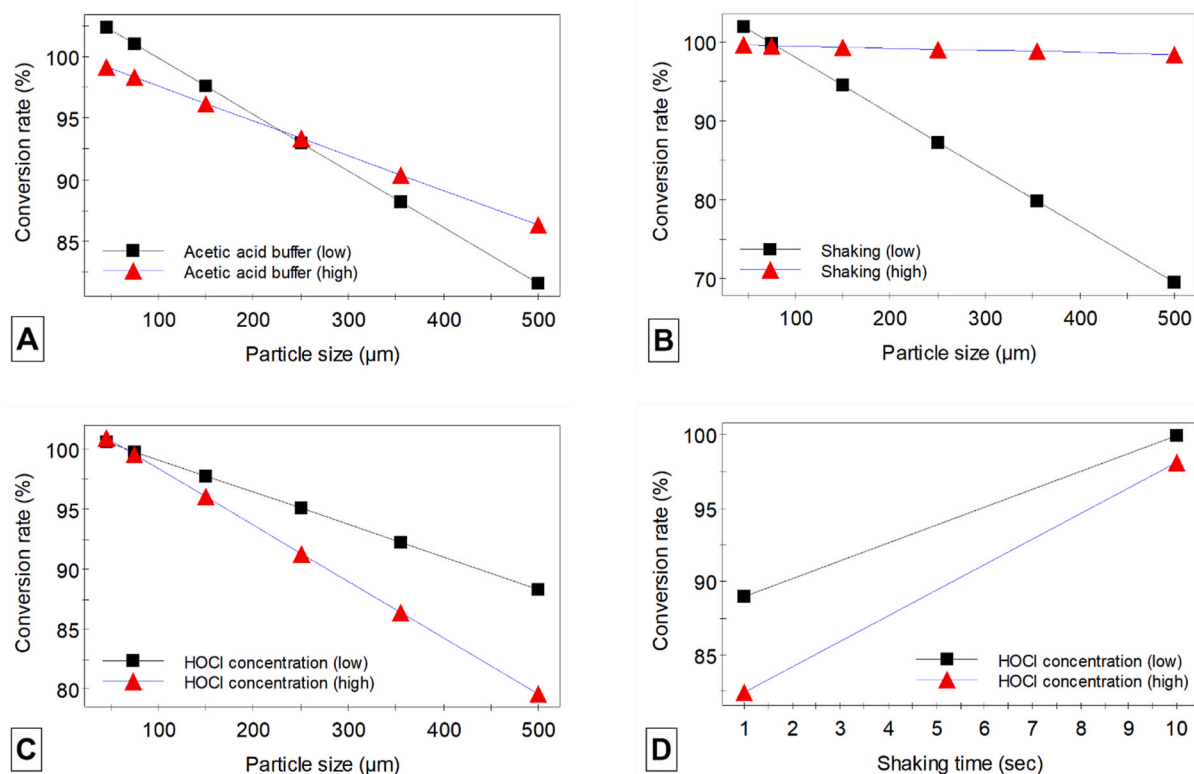


Fig. 5. A–D: Significant interaction effect plots for A) the effect of high- and low-level acetic acid buffer as a function of particle size, B) the effect of high- and low-level of shaking time as a function of particle size, C) the effect of high- and low-level concentrations of powdered calcium hypochlorite as a function of particle size, and D) the effect of high and low concentrations of powdered calcium hypochlorite as a function of shaking time. For the sake of simplicity, the particle size is given as the size of their sieve mask rather than range. 45 = ≤ 45 μm , 75 = 45–75 μm , 150 = 125–150 μm , 250 = 180–250 μm , 355 = 250–355 μm , 500 = 355–500 μm .

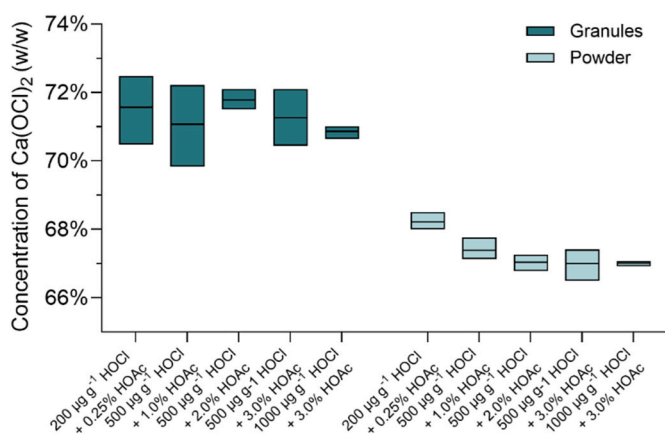


Fig. 6. Reproducibility investigation of using calcium hypochlorite granules versus powder as a hypochlorous acid precursor. Five different combinations of hypochlorous acid concentrations and acetic acid buffer concentrations (HOCl + HOAc) were tested. The results are given as mean value \pm SD from three independent experiments.

is important to find a reasonable particle size with regards to powder flow and dissolution in media (see section 3.1). Particle sizes that fall below 100 μm become more adhesive and cohesive, which was experimentally observed for powdered $\text{Ca}(\text{OCl})_2$ when dissolving it in acetic acid buffer (see section 3.1).

The shape of the powders was not investigated. Mechanical grinding in mortar and pestle was utilized in this study to powder the $\text{Ca}(\text{OCl})_2$ granules into smaller particle sizes. Spheres have minimum interparticle contact whereas a group of particle flakes or dendritic particles have a

high surface-to-volume ratio and thereby poorer flow properties. For future optimization of flow behavior, this aspect should be investigated.

3.4. Stability studies

3.4.1. Short-term

The stability of $\text{Ca}(\text{OCl})_2$ granules compared to the reformulated powdered variant, was investigated. The recovery percentage is displayed after 14 days of storage protected from light at different temperatures: 25, 40 and 54 $^{\circ}\text{C}$, shown in Fig. 7. 25 $^{\circ}\text{C}$ was chosen to represent ambient conditions; 40 $^{\circ}\text{C}$ to represent warmer climates and preliminary test accelerated ambient conditions; 54 $^{\circ}\text{C}$ to represent worst case scenario and test accelerated ambient conditions. According to ECHA's Guidance on the Biocidal Products Regulation, storage at 54 $^{\circ}\text{C}$ for 14 days may indicate 24 months storage as long as the compound is not heat sensitive [54]. The results showed that the granule formulation was not significantly more stable than powder ($p_{25^{\circ}\text{C}} = 0.19$, $p_{40^{\circ}\text{C}} > 0.99$, $p_{54^{\circ}\text{C}} = 0.84$) when stored in closed and dry compartments after 14 days. However, one thing to note is that grinding the granules to powder decomposes the $\text{Ca}(\text{OCl})_2$ content significantly ($***p < 0.0001$) from 71.31 $\% \pm 0.77$ to 67.33 $\% \pm 0.55$ (mean \pm SD). After the initial decomposition, the stability of powdered $\text{Ca}(\text{OCl})_2$ is not compromised as long as the storage condition is dry and at room temperature. Furthermore, temperature had a significant negative impact on the stability of $\text{Ca}(\text{OCl})_2$, whether as granules or powder, supporting previous findings by Gray and Halliburton [55] on thermal calcium hypochlorite decomposition. The recovery yield after 14 days for powder was 100.6 $\% \pm 2.0$ at 25 $^{\circ}\text{C}$, 96.0 $\% \pm 0.9$ at 40 $^{\circ}\text{C}$ and 71.8 $\% \pm 1.0$ at 54 $^{\circ}\text{C}$. Mass loss was respectively 0.0 $\%$, 0.2 $\%$ and 0.7 $\%$ at 25 $^{\circ}\text{C}$, 40 $^{\circ}\text{C}$ and 54 $^{\circ}\text{C}$. Based on these results, the mass loss may therefore be an indication of $\text{Ca}(\text{OCl})_2$ degradation. Bibby and Milestone [56] have

Table 1

Angle of repose, Hausner ratio and compressibility index of powdered $\text{Ca}(\text{OCl})_2$, particle size ranges 180–250 μm , 250–355 μm and 355–500 μm . Results are given as a mean value of three measurements \pm SD.

Particle size range (μm)	Flowability (seconds, per 100 g sample)	Angle of repose (degrees)	Hausner ratio	Compressibility index (%)	Flow property
150–180	Failed	Failed	–	–	Failed ^a
180–250	3.00 ± 0.04	33.1 ± 2.0	1.24 ± 0.00	19.1 ± 0.3	Moderately fine powder. Fair flow
250–355	2.54 ± 0.02	28.9 ± 0.5	1.17 ± 0.01	14.7 ± 0.7	Moderately fine powder. Good flow
355–500	2.46 ± 0.04	26.8 ± 0.0	1.12 ± 0.01	10.9 ± 0.3	Course particles. Good flow

^a The powder clogged in the funnel.

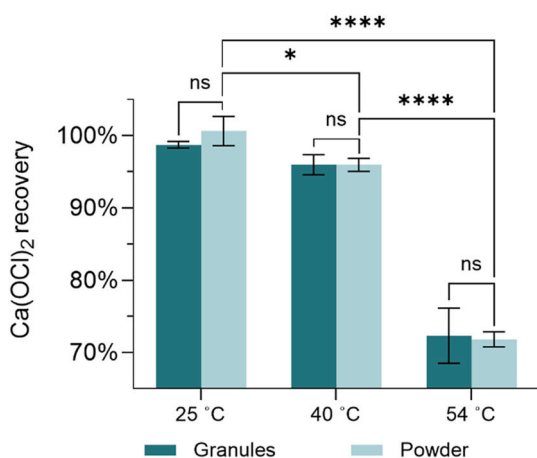


Fig. 7. Short-term recovery of calcium hypochlorite granules and powder at 25, 40 and 54 °C after 14 days of storage. The results are given as mean \pm SD from three formulation batches. Asterisks (*) denote significant differences, * $p < 0.05$, **** $p < 0.0001$, ns = non-significant.

reported that the $\text{Ca}(\text{OCl})_2$ compound decomposes at ca. 180 °C. Mass loss has previously been observed in high-grade bleaching powder around 50 °C, though the aforementioned authors believe this loss is dehydration, due to the hygroscopic nature of $\text{Ca}(\text{OCl})_2$ to readily absorb water from the atmosphere when finely ground. However, quantification and statistical tests indicate that $\text{Ca}(\text{OCl})_2$ significantly decomposes (**** $p < 0.0001$) at 54 °C compared to 25 °C, and that decomposition also occurs at 40 °C ($p < 0.02$). For this reason, accelerated stability testing is not compatible for high-assay $\text{Ca}(\text{OCl})_2$, especially above 50 °C. All samples were flushed with argon gas to avoid the introduction of external moisture to the experimental setup at the time of bottling.

3.4.2. Long-term

Based on the results from the short-term storage test, a long-term stability study at 23 ± 2 °C was performed using freshly powdered $\text{Ca}(\text{OCl})_2$ with particle size ≤ 150 μm . Ideally, the long-term stability tests should have been conducted for particle sizes ≥ 250 μm , ≤ 355 μm due to the experimental findings in flow behavior (see section 3.2), however this experiment was carried out before the flow of powdered $\text{Ca}(\text{OCl})_2$ was investigated. The short-term stability study (section 3.4.1) indicates that smaller particles were not significantly different in stability compared to larger particles when stored in closed and dry compartments. It is therefore reasonable to assume that the findings in the long-term stability study are applicable for particle sizes above 150 μm as well.

Two conditions for long-term storage were investigated, ambient (powder in brown glass vial) and dry (powder in brown glass vial in a desiccator with calcium chloride). Since $\text{Ca}(\text{OCl})_2$ is a hygroscopic solid as previously mentioned, absorption of moisture from the air could significantly impact the stability of powdered $\text{Ca}(\text{OCl})_2$. Recovery percentage of $\text{Ca}(\text{OCl})_2$ is displayed after 6, 12 and 24 months of storage, as

depicted in Fig. 8. $\text{Ca}(\text{OCl})_2$ is highly stable at room temperature and decomposes slowly when stored in tight and closed compartments (here defined as ambient conditions). After 6, 12 and 24 months, the recovery of $\text{Ca}(\text{OCl})_2$ was $98.7\% \pm 0.3$, $97.6\% \pm 0.4$ and $95.0\% \pm 0.7$, respectively. This means powdered $\text{Ca}(\text{OCl})_2$ had a total of 5.0 % decomposition after two years during ‘normal’ storage. When samples were stored in a desiccator to provide an extra dry environment, recovery percentage was $100.1\% \pm 0.4$ after 24 months. These results agree with the claim by Bibby and Milestone [56], where they argue that in the absence of free water, high-grade bleaching powder appeared to be stable indefinitely at room temperature. They also report that $\text{Ca}(\text{OCl})_2$ granules under normal storage conditions are slightly unstable and loses active chlorine content generally proportional to the free water content, which is around 4 % per year. Our results show that although there is significant loss in $\text{Ca}(\text{OCl})_2$ content when comparing ambient storage to dry at 12 months, with the same trend increasing after 24 months, a total of 5 % degradation of the active ingredient is still an acceptable shelf-life. The differences at 6 months storage between the two groups are non-significant ($p = 0.06$). Fig. 8 also shows a third condition where $\text{Ca}(\text{OCl})_2$ powder was stored in a pharma grade PET bottle, however, the data obtained is only from one single batch that was opened several times before and after assay (analyzed in triplicates, $n = 3$). It is included to illustrate how quickly $\text{Ca}(\text{OCl})_2$ decomposes in the presence of free water since $\text{Ca}(\text{OCl})_2$ rapidly absorbs water from the atmosphere, especially when finely ground. The loss of active substance after one year is $>7\%$.

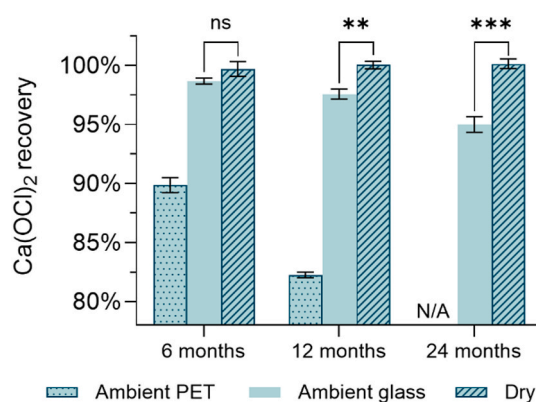


Fig. 8. Long-term recovery of powdered calcium hypochlorite after 6, 12 and 24 months of storage in two different storage conditions; ‘ambient glass’ = powder in brown glass vial and ‘dry’ = powder in brown glass vial in a desiccator, at 23 ± 2 °C. Results are shown as mean value \pm SD of three formulation batches. Asterisks (*) denote significant differences, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ns = non-significant. The figure also shows one batch of powdered calcium hypochlorite (results are given as mean value of three analysis \pm SD) stored in a PET bottle for 6 and 12 months. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4.3. Physical appearance

Powdered $\text{Ca}(\text{OCl})_2$ samples stored at 25 °C for 14 days had slightly caked (short-term storage samples) appearance. This was probably due to the moisture absorbed by $\text{Ca}(\text{OCl})_2$. The same was observed for samples stored at 40 °C. The caking tendency was significantly higher at 54 °C than at 40 °C, and the amber PET bottle was bleached at the bottom. There was less caking in the granules short-term storage samples.

After two years in dry and ambient storage at 23 ± 2 °C, the powdered $\text{Ca}(\text{OCl})_2$ samples were slightly caked and had to be lightly shaken to de-cake, especially the ambient condition samples. Samples were not discolored, and discoloration was not observed on the amber glass vial package material either.

4. Conclusion

This study successfully demonstrated a two-year stability of reformulated high-assay calcium hypochlorite as a finely ground powder under both ambient (≥ 95 % yield) and dry conditions (≥ 100 % yield) at room temperature. Temperatures above 40 °C and moisture should be avoided, as they negatively impact the stability of $\text{Ca}(\text{OCl})_2$. After optimization, powdered $\text{Ca}(\text{OCl})_2$ rapidly dissolved and converted to the major free chlorine species hypochlorous acid in an acetic acid buffer. Multivariate evaluation indicated that particle size and shaking time significantly affect dissolution. A 1-s shake, simulating instant release, with particles sized 180–250 μm resulted in ≥ 98 % conversion. Although not 100 %, this demonstrates a near-instant conversion to HOCl with minimal shaking while still exhibiting a fair powder flow. For complete conversion and optimal flow behavior, the ideal particle size is between 250 and 355 μm with 10 s of shaking.

In conclusion, this study is the first to demonstrate that stable HOCl products can be developed by dissolving the highly stable powdered $\text{Ca}(\text{OCl})_2$ in acetic acid buffer *in situ*. This novel formulation addresses the major stability issues observed in ready-to use HOCl formulations, particularly at high concentrations ($>200 \mu\text{g g}^{-1}$), by keeping chlorine separated from the liquid phase during storage.

The novelty of our formulation lies in several key innovations that address current limitations in chlorine-based antimicrobial delivery. Most significantly, the system provides single-use precision through exact dosing control, eliminating the need for tablet splitting or multiple units that characterize current NaDCC approaches. The formulation incorporates pH optimization through acetate buffering, which maintains the optimal pH range (4.3–5.6) for maximum HOCl release and antimicrobial activity without requiring the pH adjustments. The study has also experimentally proven a new method for formulating an *in-situ* stable product that combines two antimicrobial compounds—hypochlorous acid (HOCl) and acetic acid (HOAc)—stable at high concentrations. The system also demonstrates immediate activation with faster chlorine release at room temperature compared to NaDCC tablets, which require 30 s to 5 min for dissolution [57,58]. While our studies incorporated agitation to enhance dissolution and HOCl release, the inherent speed advantage remains evident. A critical innovation is also the elimination of cyanuric acid accumulation. Finally, the formulation enables ergonomic packaging innovations, such as flat bottles with small volumes, that optimize portability for field applications including military operations, emergency response, and remote healthcare settings, which potentially can provide rapid, stable wound treatment solutions when battlefield conditions demand immediate infection control. These findings facilitate the development of high-concentration and stable *in situ* formulations of HOCl, contributing to the global fight against biofilm infections and rising threat of antimicrobial resistance.

CRedit authorship contribution statement

Aina Kristin Pham: Writing – original draft, Visualization,

Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marianne Hiorth:** Writing – review & editing, Supervision, Resources, Methodology. **Pål Rongved:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Data availability statement

Data is available from the corresponding author upon reasonable request.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Professor Marianne Hiorth is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article. Aina K. Pham, the corresponding and lead author of this article is employed as an industrial PhD candidate and receives 50 % funding from SoftOx Solutions A/S, Fornebu, Norway. No conflict of interest applies to the rest of the authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jddst.2025.107292>.

Data availability

Data is available from the corresponding author upon reasonable request. The author does not have permission to share all data.

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