A QUANTITATIVE TITRATION MODEL FOR EVALUATING CALCIUM HYDROXIDE REMOVAL TECHNIQUES

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Introduction

Bacteria and their byproducts found within caries and the oral cavity invade the root canal system and are the etiology of pulp and periradicular pathosis (Lin L., Di Fiore, Lin J., & Rosenberg, 2006; Kakehashi, Stanley, & Fitzgerald, 1965). While innate defenses of the pulp such as outward flow of fluid, antibodies, and the obstructive odontoblast process combat these pathogens, destructive processes may ultimately consume the pulp (Nagaoka, Miyazaki, Lui, Iwamoto, Kitano, Kawagoe, 1995).

The goal of endodontic therapy is to eliminate microorganisms by cleaning, shaping, and obturation of the root canal system. Mechanical instrumentation of the root canal with saline alone has been shown to eliminate over 90% of bacteria from the root canal space (Siqueria, Lima, Magalhaes, Lopes, & de Uzeda, 1999). This high percentage exists despite complex tooth anatomy such as ovoid canals, fins, isthmuses, accessory canals, and C-shaped canals (Baisden, Kulild, & Weller, 1992; Melton, Krell, & Fuller, 1991; Mannocci, Peru, Sheriff, Cook, and Pitt Ford, 2005). However, it has been demonstrated that complete removal of all remnants of tissue, bacteria, and their byproducts is seldom possible (Bystrom & Sundqvist, 1985).

In order to address microbes that are housed in these various dentin architectures, irrigants such as ethanol, ethylene diaminetetraacetic acid (EDTA), sodium hypochlorite (NaOCl), chlorhexidine (CHX), and citric acid are routinely used as medicinal adjuncts. Other irrigants are used to mix with intracanal medicaments (ICMs) and to remove these dressings. Many studies have examined which type or concentration of irrigant is most effective. Buck, Cai, Eleazer, Staat, & Hurst (2001) found that alkaline solutions such as NaOCl, CHX, and ethanol were effective at detoxifying lipopolysaccharide. The ICM calcium hydroxide ($Ca(OH)_2$) was also effective.

Intracanal medicaments of a variety of materials have been used throughout the history of dentistry. Like endodontic irrigants, ICMs have been used for multiple reasons. Chong and Pitt Ford (1992) suggested an ICM may be used for reduction of bacteria, reduction of inflammatory mediators in periapical tissue, to render canals inert, to neutralize tissue debris, act as a barrier against leakage, and help to dry persistently wet canals. Without the use of an ICM, bacterial loads may rapidly increase (Bystrom, Claesson, & Sundqvist, 1985).

Some outcome studies have supported the use of ICMs. In a prospective clinical study, it was found there was a 10% greater healing rate in two step endodontic therapies using $Ca(OH)_2$ as an ICM; however, this difference was not statistically significant (Trope, Delano, & Orstavik, 1999). Research using a dog model with histological analysis determined that greater healing occurred in two visits (Katebzadeh, Hupp, & Trope, 1999). The necessity of using ICMs in infected root canals has been challenged recently. Penesis (2008) used mean periapical index scores to measure lesion reduction at one year in a randomized clinical trial. Calcium hydroxide mixed with CHX was used as an ICM. No difference was found in one versus two appointment treatment. In a systematic review of the literature, Ng, Mann, Rahbaran, Lewsey, & Gulabivala (2008) failed to identify the use of two appointments with ICMs as having a strong effect on the outcome of root canal treatment. They describe the use of ICMs as the main biological reason for multiple appointment endodontics. The debate for one versus two appointment endodontics may continue, but the practicing clinician may find circumstances in which another appointment is warranted—with the use of an ICM.

Calcium hydroxide has gained great favor as an ICM. The origin of the medicine is $CaCO_3$, or limestone. This can be heated to form calcium oxide, which combines with water to form $Ca(OH)_2$ (Fava & Sanders, 1999). Calcium hydroxide is used for many purposes, and in endodontics a great body of literature supports its use as an ICM. An *in vitro* study found that using $Ca(OH)_2$ within the canal system for 7 days eliminated all bacteria (Sjogren, Figdor, Spangberg, & Sundqvist, 1991). Hasselgren, Olsson, & Cvek (1998) demonstrated that $Ca(OH)_2$ may aid in the dissolution of necrotic tissue. Calcium hydroxide can stimulate formation of calcified tissue, and has been used in apexification as well as apexogenesis (Walia, Singh Chawla, & Gauba, 2001; Cvek, 1978).

Schilder (1967) describes the objectives of root canal treatment as cleaning, shaping, sterilization, and total obturation of the root canal system. These points are an effort to seal off the root canal system from the periodontal ligament and bone. Many obturation methods and materials have sought to accomplish this "seal," often relying on bonding or nonbonding sealers and core materials. Removal of debris and dental materials such as $Ca(OH)_2$ is a requirement of Schilder's original obturation objectives and a requirement for predictable use of any obturation system. Residual $Ca(OH)_2$ can contribute to clinical problems. One study used spectroscopy to measure the interaction of residual $Ca(OH)_2$ with ZOE sealers. It was observed that when even a small layer of $Ca(OH)_2$ was exposed to ZOE sealers *in vitro*, a rapid reduction of eugenolate O-H groups occurred within seconds. When compared to the standard spectroscopy graphs at different time points, this represented a flash set of the ZOE sealer and therefore interference with obturation (Margelos, Eliades, Verdelis, & Palaghias, 1997).

Many studies have attempted to measure removal of debris. Most use a direct visualization measurement technique—removing $Ca(OH)_2$, splitting the teeth, then photographs (through a microscope or camera) or micrographs to count pixels on a grid system where debris remains. Potential downfalls of this general approach include inaccuracies from splitting teeth, two-dimensional quantification on a curved, three-dimensional surface, inability to differentiate debris from $Ca(OH)_2$, cumulative unknown effects of reuse of the same samples using the Bramante technique, and difficulty in translating percent debris to clinical practice (Bramante, Berbert, & Borgese, 1987). Recently, micro CT studies have improved upon some aspects of this two-dimensional counting approach.

This study addresses the question: "How well do we remove the premixed calcium hydroxide paste Calasept® from the root canal system when it is used as an intracanal medicament?"

The approach utilized in this investigation seeks to eliminate many of the drawbacks of direct visualization by employing a quantitative chemical analysis to determine the amount of the unknown reactant $Ca(OH)_2$. In order to dissolve any remaining $Ca(OH)_2$, a glycerin solution will be used. Glycerin is a colorless, viscous solution with hygroscopic properties and therefore is a good moistener. Glycerin has a long history of being used to create paste mixes with $Ca(OH)_2$ and barium sulphate as well as other substances such parachlorophenol (Caliskan, Sen, & Ozinel, 1994; Fava and Saunders, 1999). Pilot studies were used to determine that a 60% : 40% glycerin : distilled water mixture was the best balance between dissolution and viscosity of the premixed proprietary paste Calasept®. The canal system in each tooth will be filled with a predetermined amount of Calasept® and different instrumentation techniques will be used to remove as much Calasept® as possible. Next, any $Ca(OH)_2$ left in the canal will be quantified in the following manner: 1. the 60% glycerin solution will be used to dissolve remaining $Ca(OH)_2$ in the tooth, 2. the alkaline mixture will then be transferred from the tooth to a tube where a titration with acid will be performed, and 3. comparison to a standard curve will allow for translation of moles of acid into mg of Calasept®.

Literature review has revealed very minimal use of quantitative chemical analysis using titration in endodontics. Cohen and Lasfargues (1988) used titration of $Ca(OH)_2$ and $Ca(CO)_3$ with HCL in order to determine the rate of carbonation. This microtitration model represents a novel approach to studying removal of $Ca(OH)_2$ in endodontics.

Literature Review

Mechanism of Calcium Hydroxide

$Ca(OH)_2$ as an intracanal medicament has been extensively studied and well established in clinical use. In 1920 Hermann introduced $Ca(OH)_2$ as an ICM. Calcium hydroxide properties include antimicrobial activity, tissue dissolution, resorption inhibition, and induction of hard tissue. In aqueous solution, $Ca(OH)_2$ diffuses into calcium and hydroxyl ions. Calcium hydroxide has a pH of 12.6. The large amount of hydroxyl ions liberated interferes with the bacterial cytoplasmic membrane integrity largely by interruption of transfer of nutrients and destruction of phospholipids from unsaturated fatty acids (Estrela, Sydney, Bammann, & Felippe, 1995). It has also been found that hydroxyl ions can create free radicals that can interfere not only with bacterial membranes but also bacterial DNA replication. The alkaline pH can alter enzyme activity and cellular metabolism. Also, $Ca(OH)_2$ acts as a physical barrier to bacterial proliferation that can raise the pH of dentin (Siqueira & Lopes, 1999). It has been demonstrated that calcium ions diffuse across the dentin from the canal to the external tooth surface (Tronstad, Barnett, Scwartzben, & Frasca, 1981). This diffusion of ions improves with smear layer removal (Foster, Kulild, & Weller, 1993). In one *in vitro* study of 12 teeth, holes were drilled in the cervical and apical regions at two different depths. pH soon increased in the inner dentin, peaking at 10.8 cervically and 9.7 apically. In outer dentin pH peaked in 2-3 weeks at 9.3 cervically and 9.0 apically (Nerwich, Figdor, & Messer, 1993). Heward and Sedley (2011) created simulated external resorption defects in roots of extracted human incisors and showed an increase in pH with $Ca(OH)_2$ and MTA over 4 weeks by measurement on the external root surface.

Calcium hydroxide has been shown to inactivate certain molecular virulence factors in both gram negative and gram positive bacteria. It also hydrolyzes the lipid moiety of bacterial LPS, resulting in the release of free hydroxyl fatty acids (Safavi & Nichols, 1993). Enzyme-linked immunosorbant assay determined that $Ca(OH)_2$ can detoxify lipotechioic acid from E. faecalis (Baik et al., 2008). Additionally, $Ca(OH)_2$ reduces TNF-alpha release from monocytes. In a DNA-DNA hybridization technique, $Ca(OH)_2$ as an ICM reduced pathogenic species associated with pulp necrosis (de Souza, Teles, Souto, Chaves, & Colombo, 2005). Use of calcium hydroxide as an ICM reduces postoperative pain in previously symptomatic teeth and decreases the amount of pain in retreatment cases (Yoldas, Topuz, Isci, & Oztunc, 2004). When dog premolar roots were filled with saline, endotoxin, or endotoxin plus $Ca(OH)_2$, then simply left open, histologically the endotoxin plus $Ca(OH)_2$ group was similar to the control group. This provides support for the effect of $Ca(OH)_2$ against endotoxin (Silva, Nelson-Filho, Leonardo, Rossi, & Pansani, 2002). However, Candida species as well as other microorganisms such as Olsenella uli may be resistant to $Ca(OH)_2$ (Waltimo, 1999; Rocas & Siqueria, 2010).

Importance of Calcium Hydroxide Removal

While $Ca(OH)_2$ is an effective antimicrobial ICM, its thorough removal before final obturation is imperative. $Ca(OH)_2$ may interfere with ZOE based sealer setting reactions. Roth sealer is one such commonly used ZOE based sealer, and is considered to be the gold standard other sealers are measured against. An *in vitro* study found that residual $Ca(OH)_2$ may interfere with sealer entrance into dentinal tubules and inhibit bonding of resin with dentin (Calt & Serper, 1999). In another study using methylene blue, leakage increased after $Ca(OH)_2$ ICM in the dye leakage portion of the experiment, but not fluid filtration (Kontakiotis, Wu, & Wesselink, 1997). Residual $Ca(OH)_2$ was also found to preferentially interact with eugenol in ZOE sealers, resulting in a flash set of sealer (Margelos et al., 1997). On the other hand, $Ca(OH)_2$ does not appear to interfere with the seal with Resilon® and Realseal® (Wang, Debelian, Teixeira, 2006).

Residual $Ca(OH)_2$ has been clearly shown to interfere with materials at the apex; in one *in vitro* study using dye leakage, it was determined that canals previously medicated with $Ca(OH)_2$ using an ICM resulted in greater leakage (S. Kim & Y. Kim, 2002). $Ca(OH)_2$ also interferes with white mineral trioxide aggregate (MTA), a material that has been used in endodontics for a variety of applications, such as apexification, perforation repair, obturation with open apices, obturation during retreatment, before and after root end surgery, and in dens in dente cases (Stefopoulos, Tsatsas, Kerezoudis, & Eliades, 2008; Whitworth, 2005; Bogen & Kuttler, 2009).

Some studies suggest residual $Ca(OH)_2$ is not a concern. Medication with $Ca(OH)_2$ improved marginal adaption of the MTA apical barrier (Bidar, Disfani, Gharagozloo, Khoynezhad, & Rouhani, 2010). Another investigation used extracted canines and premolars with straight roots to examine how pretreatment with $Ca(OH)_2$ affects apical leakage—results determined that the use of three different $Ca(OH)_2$ preparations as ICMs decreased dye leakage over the no ICM control group (Porkaew, Retief, Barfield, Lacefield, & Soong, 1990). An *in vitro* investigation demonstrated that ICM of $Ca(OH)_2$ did not interfere with methylene blue dye leakage (Holland, Alexandre, Murata, Dos Santos, & Dezan, 1995).

Calcium Hydroxide as a Premixed Paste

Over the years $Ca(OH)_2$ has been delivered to the root canal in multiple vehicles in an attempt to improve radiopacity, flow, consistency, and antibacterial action. Traditionally, $Ca(OH)_2$ powder has been mixed with a sterile solution such as water or saline and placed in the canal. Fava and Saunders (1999) classify $Ca(OH)_2$ preparations as aqueous, viscous, and oily. In aqueous preparations such as Calasept® and Ultracal®, the pH is high and release of ions is rapid. Viscous vehicles such as Calen® provide slower release for extended periods. Oily vehicles like Endoapex® and Vitapex® provide the slowest diffusion and the lowest solubility. Siqueria and Lopez suggested that because bacteria such as E. Faecalis can tolerate high pH (9-11) the delivery vehicle must not alter the pH significantly. Some mixtures of $Ca(OH)_2$, such as that with CMCP, have been shown to increase antimicrobial efficacy (Siqueria & Lopez, 1999). Using an agar diffusion model with various $Ca(OH)_2$ concentrations and delivery vehicles, one study found higher concentrations of $Ca(OH)_2$ had greater inhibition. Ultracal®, which uses a methylcellulose vehicle, was equally as effective at 35% $Ca(OH)_2$ as higher percentage $Ca(OH)_2$ pastes. This suggests the vehicle of delivery rather than the concentration of $Ca(OH)_2$ has important effects on the ability to remove $Ca(OH)_2$ paste (Blanscet, Tordik, & Goodell, 2008).

Calasept® is a premixed paste commonly used in the United States. According to Ghose, this formulation is made up of 52% $Ca(OH)_2$, 8% calcium chloride, 4% sodium bicarbonate, 8% potassium chloride, 0.35% sodium chloride, and 16% water (Ghose, Baghdady, & Hykmat, 1987). According to the material safety data sheet, Calasept® contains barium sulfate and has a pH of 12.4. The manufacturer's website states that 100 mg of Calasept contains 41.07 mg of $Ca(OH)_2$, 8.33 mg of barium sulfate, and 50.6 mg of sterile isotonic saline (J.S. Dental Manufacturing, Inc., 2011).

Ultrasonic Instrumentation

Richman (1957) introduced a method of canal debridement that included ultrasonics. Ultrasonic activation results in acoustic streaming, defined as the generation of time-independent, steady unidirectional circulation of fluid in the vicinity of a small vibrating object. Cunningham and Martin (1982) were among the first to assess the ability of ultrasonics to assist in debridement of the root canal system. The Endosonic Ultrasonic System was superior to conventional hand filing as determined by SEM. Ahmad, Roy, & Kamaruden (1992) described the action of acoustic streaming created by ultrasonics. Use of ultrasonics with small files, such as size #10-15 hand files, created eddies of greatest velocity and greatest displacement velocities. Contact with canal walls caused loss of this streaming phenomenon. Increased power setting on the ultrasonic unit increased efficacy. Jiang et al. (2011) demonstrated in an *in vitro* model that higher intensity resulted in a higher file amplitude and file oscillation, creating cleaner canals.

Results of Calcium Hydroxide Removal Studies

Recent studies have examined the effectiveness of modern methods of removing $Ca(OH)_2$ from the root canal. Lambrianidis, Margelos, & Beltes (1999) found that $Ca(OH)_2$ is incompletely removed with various irrigation methods. They used hand filing in combination with 3% NaOCl, 3% NaOCl + 17% EDTA, or normal saline. Results determined that 45% of the surface area was still covered with $Ca(OH)_2$. The same authors demonstrated regardless of whether sterile saline, CHX gel, or CHX solution was mixed with the $Ca(OH)_2$, residual $Ca(OH)_2$ remained (Lambrianidis, Margelos, & Beltes, 2006). Nandini, Velmurugan, & Kandaswamy (2006) found that various irrigants had different removal abilities; citric acid worked better than EDTA in removing Metapex®, a commercially available paste. Canals were significantly cleaner at the 1 mm level with 2.6% NaOCl than those cleaned using hand instrumentation alone.

Many studies have examined not only various irrigants but passive sonic and ultrasonic instrumentation. An early investigation found that sonically cleaned canals were not significantly cleaner than canals cleaned by hand alone, but it did increase ease of preparation (Tronstad et al., 1985). Torabinejad (1994) described (but did not test) a passive step back instrumentation technique; ultrasonics were used at 1 mm from working length and alternated with files. Then a #15 hand file was ultrasonically activated for 1-2 minutes. Lev, Reader, Beck, & Meyers (1987) found that the step back ultrasonic technique was more effective at the 1 and 3 mm levels for isthmuses of mandibular molars but not for canals. Stamos, Sadeghi, Haasch, & Gerstein (1987) determined that at 1 mm from the apex, ultrasonic instrumentation produced the cleanest canals—but there was no difference between hand and sonic instrumentation. Lastly, ultrasonic activation also improves use of 2% CHX in removing $Ca(OH)_2$ over irrigation alone (van der Sluis, Wu, & Wesselink, 2007).

Different time periods have been used for passive ultrasonic instrumentation (PUI). Balvedi, Versiani, Manna, and Biffi (2010) used 30 s of PUI for removing $Ca(OH)_2$ mixed with various carriers and found the PUI more effective than irrigation + hand file only. Another study used 5.25% NaOCl with 3 minutes of sonic and ultrasonic instrumentation and quantified 15.1% of residual debris left with sonic and 16.7% with ultrasonic instrumentation (Jensen, Walker, Hutter, & Nicoll, 1999). After hand instrumentation alone, 31.6% of canal walls had residual debris. Another study that utilized the 3 minute time period for PUI but was performed on teeth before extraction found that PUI created canals with significantly cleaner walls than irrigation with 5.25% NaOCl alone (Archer, Reader, Nist, Beck, & Meyers, 1992). Sabins, Johnson, and Hellstein (2003) found 19.6% of canal walls had residual debris remaining after 30 s or 1-minute sonic instrumentation and 15.4% rate with ultrasonic instrumentation, but hand instrumentation alone left 36.7% of walls covered with debris. This was in close agreement with Jensen et al. (1999); there was no difference between 30 and 60 s of PUI.

Root curvature also influences the effect of PUI. While the last two mentioned studies used curved molar roots, Crumpton, Goodell, and McClanahan (2005) utilized straight roots with rotary nickel titanium file preparation. They found 9.18% of residual debris on canal walls after rotary instrumentation alone and with no smear layer removal—far less than previous studies using curved roots. Straight roots help isolate an accurate measurement of residual $Ca(OH)_2$ because removal of debris is more predictable, especially when testing ultrasonics, which lose effectiveness when instruments contact a canal wall (Ahmad, Pitt Ford, & Crum, 1987). Another study examined routine irrigation, hydrodynamic irrigation, and PUI in straight and curved roots; PUI was more effective in straight canals, and hydrodynamic stimulation was more effective in curved canals (Amato, Vanoni-Heineken, Hecker, & Weiger, 2011).

Recently, removal of Calasept® was evaluated in extracted molar canals with more than 15 degrees of curvature (Kenee, Allemang, Johnson, Hellstein, & Nichol, 2006). Four groups were used: NaOCl rinse alone, NaOCl with EDTA rinse, NaOCl with a nickel titanium file rotated in the canal, and NaOCl with ultrasonic agitation. Rotary and ultrasonic techniques were found to be significantly better in $Ca(OH)_2$ when compared to irrigants/#35 K file only. A weakness in this study is that twelve molar teeth were reused several times to test the different categories. Cumulative effects of the EDTA, a chelating agent, may have skewed the results. It was not indicated that statistical tests took into account the reuse of the same specimens.

Computed tomography has also been used to examine removal of $Ca(OH)_2$. One study prepared 46 mandibular molars with root curvatures of 20 to 30° to study removal of Ultracal®. Groups included rotary instruments only and rotary after sonic or ultrasonic activation (for a total of 60 s of activation). Micro-CT was used to determine volume of $Ca(OH)_2$ remaining. Results indicated that rotary instrumentation plus PUI produced significantly better removal than rotary plus sonic activation (Wiseman et al., 2011). Nandini et al. (2006) used spiral CT to compare removal of $Ca(OH)_2$ powder in distilled water with the premixed $Ca(OH)_2$ paste Metapex®. Ten percent citric acid removed $Ca(OH)_2$ powder better than Metapex®, suggesting the vehicle in which $Ca(OH)_2$ is prepared affects removal.

Methods Used to Measure Calcium Hydroxide Removal

Effectiveness of $Ca(OH)_2$ removal from the root canal is affected by multiple factors. Anatomic factors such as tooth type, root curvature, and canal morphology may influence results. The packaging of $Ca(OH)_2$ affects interaction with instruments and irrigants like citric acid, EDTA, CHX, and NaOCl. Various PUI time periods have been also tested. It is possible to hold these factors constant, but one must choose a model to measure $Ca(OH)_2$ removal. The method used has a bearing on whether the data is parametric or nonparametric, the relevant statistical tests, and ultimately how the results can be applied to clinical practice.

General Inspection Method: Early studies in the evolution of instrumentation tactics used general inspection as a measurement technique. One such study utilized a stereomicroscope to examine both halves of the split tooth and overall cleanliness, smear layer removal, dentin chip removal, and tissue remnants were judged (Tronstad et al., 1985). Cunningham and Martin (1982) used anterior teeth to study $Ca(OH)_2$ removal. Teeth were split longitudinally and high and low power SEM was used to broadly compare removal methods. Teeth were paired with and without ultrasonic preparation. An observer determined which of the pair had the least debris and results were compared with a sign test.

Grading Systems: An improvement over general inspection of the dentin wall began with the use of grading systems. Various models have been used in which teeth are split in half and a grading system is devised. Ahmad et al. (1987) used polystyrene spheres to determine measurements of traverse displacement amplitude for acoustic streaming. The results were used in various $Ca(OH)_2$ removal methods *in vitro*. Teeth were split with a mallet and chisel, then SEM was performed at 200x, 500x, and 800x and representative sections were used to create a grading system. Three calibrated judges then assigned scores of 0, 1, 2, or 3.

Premade Groove: Some studies have qualitatively evaluated premade internal grooves that attempt to recreate internal anatomy variations. This type of data is usually ordinal or nominal data, and statistical tests therefore are nonparametric. One investigation created grooves in the internal dentin wall 2 mm by 0.2 mm by 0.5 mm and filled them with Ultracal®. Digital photography was performed at 40x through a microscope. A grading system was then devised to rank instrumentation methods for the removal of $Ca(OH)_2$ paste from the grooves (van der Sluis et al., 2007). Another study created four shallow depressions at different points along the root, including one groove positioned apically from the tip of the #25 file used for PUI. Teeth were reapproximated with a prefabricated jig. This method has demonstrated reproducibility in results. Digital images through a microscope were utilized, and grooves were judged as either "clean" or "not clean" (Jiang et al., 2011). An advantage of this technique is that the grooves created in the dentinal wall simulate irregularities seen in clinical situations. However, these types of studies often reuse the same tooth specimen for multiple measurements by reapproximating the tooth. Potential effects of reusing a specimen for multiple measurements are a concern—especially when a chelating agent such as EDTA was used. Another issue in the use of grading systems is that the area being evaluated may vary considerably in debris amount. Debris is not always distributed homogeneously, and an area being evaluated may give a higher or lower grade than an adjacent area.

Quantitative Evaluation Method: Evolution of $Ca(OH)_2$ removal studies has resulted in development of quantification methods that include microscopic, photographic, micrographic, and computed tomography. These methods usually involved determining what percentage or volume residual debris/$Ca(OH)_2$ is of the total area. Microscopic methods included subjective analysis through a microscope of remaining $Ca(OH)_2$ or assignment of a grade, and scanning electron microscopes have enabled the detection of minute amounts of debris. More recently, digital photographic images imported into software programs have been used. These quantitative methods have introduced the use of parametric data to debris removal studies.

Classic studies used creative techniques to quantify data. In 1987, Lev et al. decalcified samples and cut them in cross section with an autocut microtome and a carborundum knife at 1 and 3 mm from the apex. Histologic slides were projected on a screen and traced to determine percentage of remaining debris. Stamos et al. (1987) also made histologic sections and projected the slide to make tracings of remaining debris. Jensen used photomicrographs of split teeth; projections were made onto a screen to create a two by three foot image, and superimposition of a grid system allowed the evaluator to count squares that contained debris. Remaining debris was quantified (Jensen et al., 1999). Quantification of remaining tissue/debris has also been examined using a pre-extraction tooth model. One study compared step back versus step back + PUI *in vivo*, then immediately extracted the teeth. Histologic axial cross sections were then used to determine the percent of cleanliness (Archer et al., 1992).

Modern software has allowed for new types of measurement. Sabins et al. (2003) used a Nikon N90S digital camera to take an image of split teeth on which several different removal techniques were used, and the digital image was then transferred into Adobe Photoshop® 5.0 software and enlarged 100 times. Lines were superimposed at the 3 and 6 mm level. Debris was traced and total number of pixels was counted by using the program's "histogram" function. Percentage of debris of the root canal system could then be calculated. One study used a Nikon Coolpix 4500 to take images. The image was imported into Adobe Photoshop® 7.0 and enlarged 10x. The "lasso" and "histogram" functions were used to quantify debris removal (Crumpton et al., 2005). Kenee et al. (2006) also imported digital photographs into Photoshop® to perform a pixel count; degree of enlargement was not specified. Several drawbacks exist for quantification through counting pixels in a software program. High quality images enhance our ability to enlarge photos—10x to 100x as discussed above. Since the dentin wall is not flat but has a curvature of approximately 180°, a certain degree of error is "built into” this method. What that error is has never been tested.

Hypothesis

Removal of the ICM, Calasept®, is improved with the use of PUI or a Greater Taper file when compared to hand filing and irrigation alone.

Experimental Design and Methods

This study was approved by the Institutional Review Board at the University of Minnesota.

Selection and Preparation of Teeth

Type of teeth: Extracted teeth with straight canals including single canal maxillary, mandibular canines, and some mandibular premolars were collected from the VA Medical Center and local oral surgery offices and stored in normal saline with 0.2% sodium azide. Sodium azide was chosen as it has been shown to preserve the physical characteristics of teeth (Reeh, Messer, & Douglass, 1989).

Preparation of teeth: Teeth were decoronated and the root length standardized at 17.5 mm. A #10 K file was placed so it was just seen beyond the major foreman with 2.5x loupes (Surgitel®, Ann Arbor, MI). One mm was subtracted to determine working length; if needed, additional tooth structure was removed from the coronal aspect to standardize the WL at 17.5 mm.

Standardization of curvature: Teeth were radiographed from the buccal and lingual view with the file in place as noted. Kodak RVG 6000 digital radiography was used for all images throughout the experiment. The Pruett, Clement, and Carnes (1997) method was used to determine angulation of canals. Canals with greater than 15° curvature were excluded to standardize curvature.

Endodontic preparation: A #25 K flex-O hand file (Dentsply-Maillefer, Johnson City, TN) was used to establish a glide path (Patino, Biedma, Liabana, Cantatore, & Bahillo, 2005). Lexicon™ Gates-Glidden (GG) burs (Dentsply-Maillefer, Johnson City, TN) were used to coronally flare prior to use of nitinol files (Davis, Marshall, & Baumgartner, 2002). A #4 GG bur (1.1 mm) was used to a 5 mm depth, a #3 GG bur (0.9 mm) was used to a 7 mm depth, and a #2 (0.7 mm) GG bur used to 9 mm depth. The K3 file system (SybronEndo, Cuyahoga Falls, OH) was chosen for its minimal canal aberration or transportation and ability to stay centered in the canal (Schäfer, & Florek, 2003; Schafer & Schlingemann, 2003). The K3 system also has been shown to have a reduced remaining debris load than other systems (Kum, Kazemi, Cha, & Zhu, 2006). With K3 rotary files, the teeth were prepared using an alternating crown down method according to the manufacturer's recommendation. A Model AEU-20 Aseptico rotary handpiece motor (Woodinville, WA) was used for all preparation at an 8:1 ratio (300 RPM).

All preparations were done according to manufacturers' recommendations. Crown down began with the #60-0.06, then to the #50-0.04, and the preparation was finished at the #50-0.06. If the #50-0.06 did not go to length, the #50-0.04 was repeated. In some cases, the alternating crown down method was continued using a #45-0.06, a #40-0.04, and a #35-0.06. The canal was then sequentially enlarged to a #50-0.06. The canal taper was then enlarged to a 0.06 taper #50 for three reasons: to allow for a greater volume of glycerin for the titration method, to ensure large enough size so that all teeth were prepared to the same taper and apical size, and to reflect the natural canal anatomy (Hecker, Bartha, Löst, & Weiger, 2010). Irrigation with one ml of NaOCl 5.25% between files and recapitulation was performed between each file. The end of the Maxi-I-probe (Dentsply, Elgin, IL) was set at 2 mm from the apex (Abou-Rass, & Piccinino, 1982). A #20 file was pushed just slightly out the apex of the tooth to remove any apical debris and to allow for later Calasept® to be expressed out the end, ensuring a complete fill of calcium hydroxide. Thirty seconds of passive ultrasonic irrigation with 5.25% NaOCl was used to remove residual debris (Balvedi, Versiani, Manna, & Biffi, 2010; Sabins et al., 2003). A one minute soak with 17% EDTA was used to remove the smear layer (Crumpton et al., 2005). A final 3 ml rinse of 5.2% NaOCl was performed and canals dried with paper points. The teeth were transferred from their NaZ-filled microcentrifuge storage containers (USA Scientific, Acala, FL) to pre-labeled ice cube trays (with sample number). Teeth were rinsed externally with 2 ml of 5.25% NaOCl and within the canal with 1 ml of NaOCl to remove any NaZ. Teeth were then dried with paper points. Next, each tooth was labeled with its sample number using a small piece of masking tape.

Filling of canal with calcium hydroxide: Pilot studies were used to test several $Ca(OH)_2$ placement methods including a reverse file spin and lentulo file placement technique. Radiographs were used to judge placement. It was determined that no method could provide a homogenous fill 100% of the time, but a very repeatable density was obtained in the following manner. First, the Calasept® needle was inserted until binding. Firm pressure was be used to force Calasept® out the apex until visualized. The needle was then gently loosened and slowly reversed out of the canal system while firm pressure was applied to the plunger. A spatula was used to wipe excess Calasept® flush with the coronal end of the tooth.

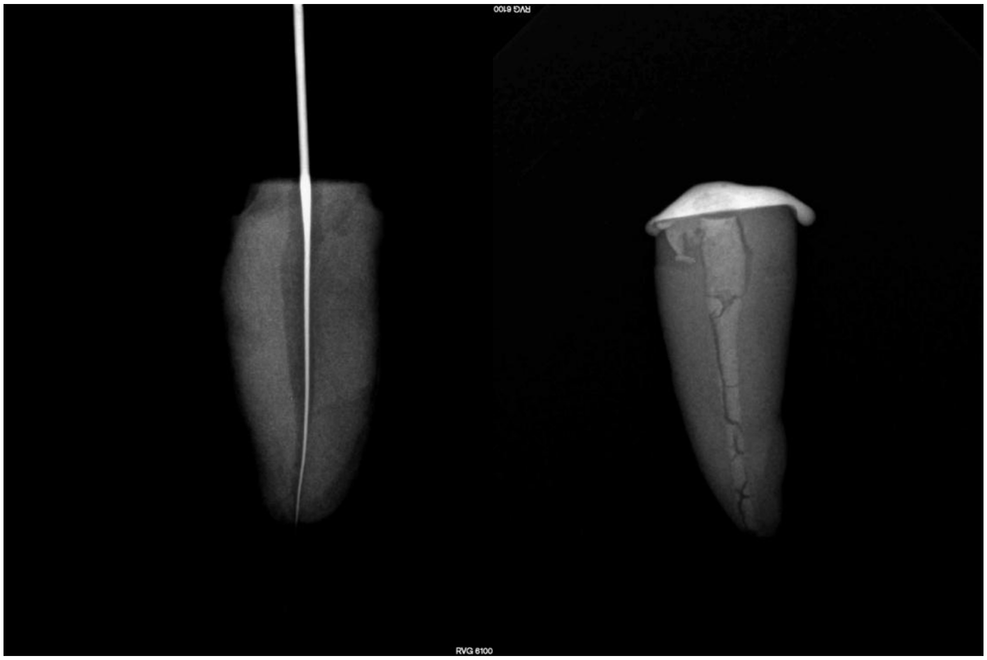


Figure 1. Sample 9 (left) pre-preparation and sample 16 (right) after Calasept® placement.

Radiographic confirmation of calcium hydroxide fill: A strong correlation exists between the radiographic appearance and the percent weight of $Ca(OH)_2$ delivered (Simcock & Hicks, 2006). Therefore, to verify complete filling of the canals, the teeth were radiographed. If the canal was judged to be incompletely filled, the tooth was reinjected with Calasept® and radiographed again. Calasept® was wiped off the external apical surface and fingernail varnish was applied in duplicate to the external surface of the entire root to prevent extrusion of glycerin in a later step.

Temporization of the teeth: Pilot studies were used to determine the best way to seal the coronal orifice. A very small sterile cotton pellet covered the canal orifice. Then Fuji IV resin modified glass ionomer (GC Corporation, Tokyo, Japan) was expressed over the pellet and cured with a Coltolux LED curing light (Coltene/Whaledent, Cuyahoga Falls, OH). Samples were placed into an incubator (Precision Scientific, Chennai, India) at 37° C for one week in a humid environment to simulate the clinical time period between appointments. After incubation, removal techniques were performed.

Calasept® removal groups: Four groups of removal techniques were used. For each group, a #30 K file and a #50 K file were used to remove dry Calasept®. Then, each of the following methods was performed:

1)
NaOCl 5.2% 3mL
EDTA 17% 3mL
NaOCl 5.2% 5mL
2)
NaOCl 5.2% 3mL
K3 #50-0.06 taper to WL
EDTA 17% 3mL
NaOCl 5.2% 5mL
3)
NaOCl 5.2% 3mL
Passive Ultrasonic Irrigation 30 seconds
EDTA 17% 3mL
NaOCl 5.2% 5mL
4)
NaOCl 5.2% 1.5mL
K3 #50-0.06 taper to WL
NaOCl 5.2% 1.5mL
Passive Ultrasonic Irrigation 30 seconds
EDTA 17% 3mL
NaOCl 5.2% 5mL

Negative control: No addition of Calasept® after initial instrumentation.

Positive Controls: Pilot studies were used to determine the optimal concentration and volume of glycerin. However, these parameters were not chosen to specifically dissolve the full canal-volume of $Ca(OH)_2$. A very $Ca(OH)_2$ saturated 100 microliter 60% glycerin solution—as determined through pilot studies—was chosen to serve as a positive control.

Glyerin Transfer: Removal of Remaining Calasept® from the Tooth

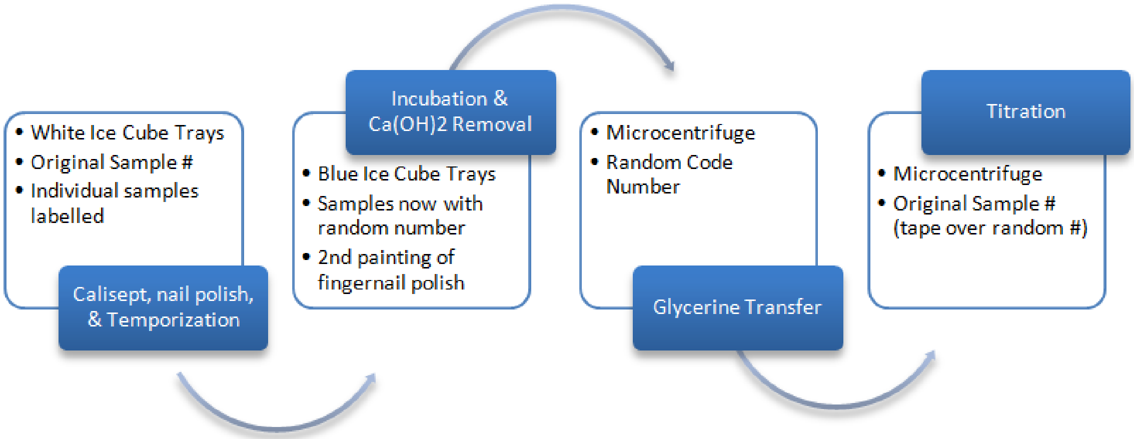
Teeth were removed from the incubator. Temporary restorations were gently removed with an excavator and the four experimental removal techniques applied. After the clinical removal techniques were completed (see pg 25), the remaining $Ca(OH)_2$ left in the canal was evaluated. A preparation of 60% glycerin : 40% distilled water at 40° Celsius was placed into the canal with a Ultradent® capillary tip (Ultradent® Products, Inc., South Jordon, Utah). The glycerin was removed using a narrow Ultradent® tip and a 10 ml syringe and the aliquot placed into a 1.5 ml microcentrifuge tube. Passive ultrasonic instrumentation for 10 s was performed with a #15 Zipperer (Roydent, Rochester Hills, MN) file at 2 mm from WL to help the remaining Calasept® dissolve between each aliquot. Aliquots were repeated until the level on the microcentrifuge tube reaches 1.1 mm—corresponding to 100 microliters. An endodontic ruler was used to measure and mark the 1.1 mm level on each 1.5 µl microcentrifuge tube (Fisher Scientific, Pittsburgh, PA). If the level did not quite reach 1.1 mm, glycerin was added to this point. Pilot studies were used to determine the least viscous glycerin percentage but best dissolving ability and temperature for optimal working characteristics. Pilot studies were also used to confirm that this percentage of glycerin could fully dissolve the available Calasept®.

Calibration of micropipettes: A single 20 microliter pipetman® micropipette (Gilson Inc., Middleton, WI) was used for all titrations. The tips were replaced when aliquots concentrations were changed. Extensive care was taken to ensure the tip never contacted the sides of either the storage beaker or the microcentrifuge tip. Delivery of each 10 microliter volume was performed swiftly with depression using the thumb. This ensured consistent technique.

Blinding of the operator: Using a random number table generated from Microsoft Excel (Microsoft Corporation, Redmond, Washington), each sample or control was given a random number. A second operator kept the "code," which matched the original sample number to the random number. Using Excel, the random numbers were arranged chronologically. The first twelve numbers were group 1, the next twelve were group 2, and so on. The teeth were placed into groups after filling with Calasept® and temporization, and before placement into the incubator for 1 week. Negative controls were given random numbers that were the same number of digits as the other samples; however, the negative controls were not placed into one of the four groups.

Before titration, the second operator covered up the random number with opaque tape and wrote the original sample number on the tape so that the random number could not be seen. Therefore, operator 1 did not know which group he was measuring (titrating). Operator 1 only saw the original sample number, which provided no information regarding the group. Immediately after each titration, the second operator took the data to "break the code." Thus, each sample had an equal method of being assigned to each group, and the operator was blinded in measurements both to samples and negative controls.

Figure 2: Preparation and blinding scheme.



Titration of the Mixture

A mixture composed of 40% sterile water and 60% glycerin (Humco Corporation, Texarkana, TX) was then titrated using 0.025, 0.05, 0.1, 0.5, 1, or 3 M HCL with 10 microliter aliquots using a micropipette. Each microcentrifuge tube was vortexed for 30 s and heated to 40 °C +/- 1° in a Hanau® low temperature water bath (Teledyne Water Pick, Fort Collins, CO). Before each titration began, the microcentrifuge tube was inspected visually to ensure there was no undissolved $Ca(OH)_2$. The optical density of the vial was inspected. Through pilot studies, it was discovered that the density as judged by the naked eye provided a good indicator of the pH start point and the change in pH in response to various molarities of HCL. A dense solution started 11.2 pH and over and would respond to higher concentrations such as 0.5 M. A clear solution often would measure in the pH of 10 range and the pH of the solution was reactive to smaller concentrations of HCL such as 0.1 and 0.2. Good laboratory hygiene practices were followed at each step of titration to ensure no contamination of samples occurred. The decision of which molarity HCL and which aliquot volume was judged by the operator according to experience during pilot studies. Also, pilot studies were used to develop an algorithm to determine which molarities were appropriate for titration. Usually titrations began with 10 microliters of 0.1 M. If the initial pH was higher, the operator may have used a higher molarity such as 0.2 M. If the initial pH of the mixture was lower (i.e. 11.0-11.4), the operator may have begun with a weaker concentration (i.e. 0.05 M HCL). Every attempt was made to equalize between samples the total volume of the final solution. pH measurements were recorded after each addition of HCL using a model HO4N-0001 semi-micro pH electrode (Lazar Research Laboratories, Los Angeles, CA) and a Model 60 pH meter (Lazar Research Laboratories, Los Angeles, CA). After each aliquot addition of acid to the microcentrifuge tube, the tube was vortexed for 10 s. Adequate time was given for each pH measurement—this was approximately 10-60 s for the meter to equalize.

Rigid laboratory practices were employed to make each measurement identical. The tip of the probe was inserted approximately 4 mm into the solution, and the holding tray was adjusted until the probe did not contact the walls of the microcentrifuge tube. The micro pH electrode and meter were calibrated with standard pH solutions (Omega Scientific, Tarzana, CA). While the mixture was in the basic range, calibration was performed with pH buffers 7 and 10. When the titration reached the acidic range, the pH meter was recalibrated with buffers 4 and 7. After each pH measurement, the tip of the electrode probe was thoroughly rinsed with distilled water and wiped with a Kim Wipe® (Kimberley-Clark Professional, Mississauga, Ontario) to ensure the probe did not contaminate the sample. Importantly, the microcentrifuge tube was vortexed for 10 s with a Vortex Genie Mixer (Scientific Products, Evanston, Ill.) after each addition of HCL. A single twenty microliter micropipette set at 10 microliters was used to deliver all aliquots. The micropipette was calibrated by delivering a specific amount of water to a microcentrifuge tube and weighing the tube. Then the weight was compared to the expected weight i.e. 10 microliters equaled 0.01g. Approximately every 20 pH readings, the pH meter and electrode was recalibrated.

pH of sample	Molarity Added (10 microliters)
>11.9	0.5
11.2 to 11.9	0.2
10.3 to 11.2	0.1
7 to 10.3	0.05

Table 1: An approximate algorithm for HCL addition.

Chemical reaction: Addition of HCL to $Ca(OH)_2$ is described by the following equation: $2 HCl + Ca(OH)_2 \rightarrow CaCl_2 + 2H2O$ (Cohen & Lasfargues, 1988).

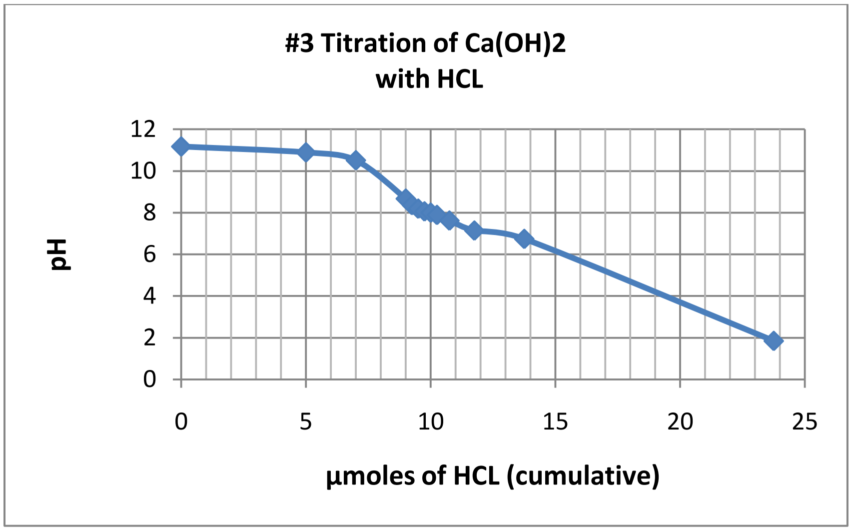
Standards

A standard curve is necessary to determine the weight of remaining $Ca(OH)_2$ in the instrumented canals. In order to develop a standard curve, thirty teeth were selected. Due to limitations in available canines, some single canal premolar teeth were also selected. The identical radiographic and preparation protocol was utilized for the standard teeth and for the samples. Premolar teeth used for the standard curve were randomly selected for $Ca(OH)_2$ levels by drawing standards out of a hat (i.e. standard A1 had an equal chance of being a canine or a premolar). The standard teeth were allowed to dry out for one day. Ten different standard weights were measured out from 0.0003 to 0.003 mg. Each weight was done in triplicate (Table 2). In the same manner as the samples, the glycerin/Calasept® mixtures were transferred to 1.5 ml microcentrifuge tubes. If the volume of solution transferred was below 100 µl, the solution was added to the 100 µl mark. Titration of each standard was performed in the exact manner as the samples. While preparing standards, it was found that the Calasept® mix within the manufacturer's tube was not always uniform. Some expressions of $Ca(OH)_2$ seemed to have more water content than others, but the operator attempted to ensure additions of Calasept® to the tubes were uniform in appearance.

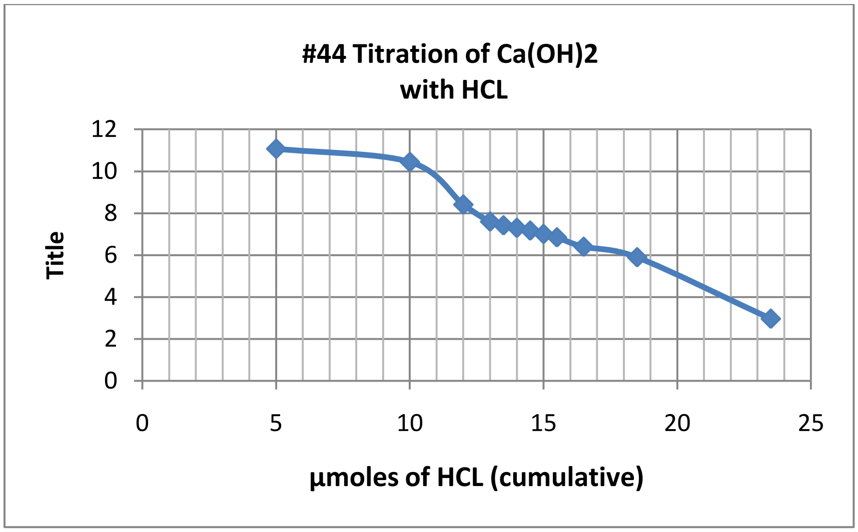
Determination of N: Using pilot study data, preliminary calculations were performed. When the sample size in each of the four groups was 12, a one-way analysis of variance was determined to have 80% power to detect at the 0.05 level a difference in means characterized by an effect size (variance between means divided by a common group variance) of 0.2483, assuming that the common standard deviation is 0.0004.

Titration curves: Each sample, standard, and positive and negative control was titrated in the same fashion. The same solutions of differing molarities were used as previously described. A total of 58 samples, 30 standards, 3 negative controls, and 3 positive controls were titrated. All laboratory conditions were held constant (See Table 2).

Figure 3: Representative titrations from groups 1 & 2.

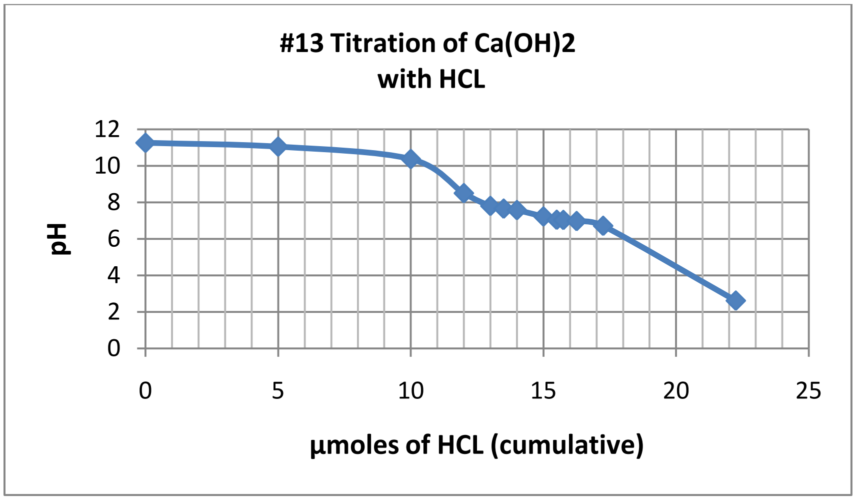


Sample 3, group 2

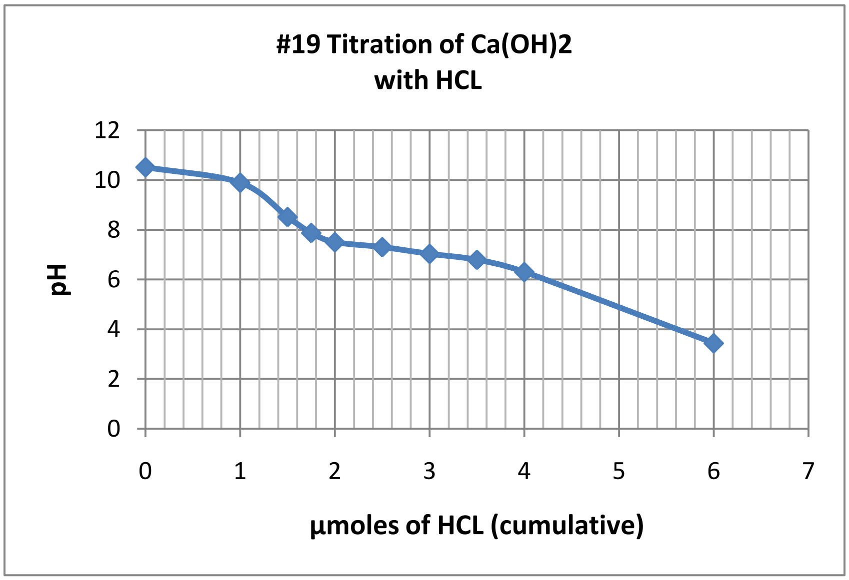


Sample 44, group 1

Figure 4: Representative titrations from groups 3 & 4.



Sample 13, group 3



Sample 19, group 4

Each standard was titrated to generate a curve. In order to take an accurate reading at pH=7 for each standard, linear regression was applied to each curve. This was accomplished by creating a new curve using data points around pH=7. The amount of data points used differed from 2 to 5, depending on the how many points were near pH=7. Most of the data points were between pH= 6 and 8, but in some cases points outside this range were used. Figure 5 is an example of standard F3 and its corresponding linear regression curve made using the original data but only three data points. Excel was used to apply a regression line to the data and a corresponding regression equation. To determine the cumulative µmoles at pH=7, the equation was first solved for x ($y = -1.55x + 15.265 \Rightarrow x = (y-15.265)/(-1.55)$). In the example of standard F3, "7" was substituted for y (pH=7). Therefore, $x=(7-15.265)/(-1.55)$. In this example x=cumulative µmoles of HCL=5.332258.

From each standard titration curve, two data points were used: the original weight of $Ca(OH)_2$ and the value of x. Appendix 1 demonstrates the weight and value of x (cumulative µmoles at pH=7) for each standard. From the last two columns a scatter plot was created*. A trendline was fitted with an associated equation. This important equation, $y=6.1098x$, was used to determine the original weight of $Ca(OH)_2$ in each experimental sample.* From this point on, this equation will be referred to as the standard equation.

An important point when making the standard line was that the y and x intercept were set to equal zero. This makes the assumption at zero grams of $Ca(OH)_2$ added to 100 µl of glycerin, the pH would not be above 7; therefore, no HCL would need to be added. This assumption is justified because in two of three negative controls, the pH was below 7 initially. In the third negative control, the pH was only slightly above 7.

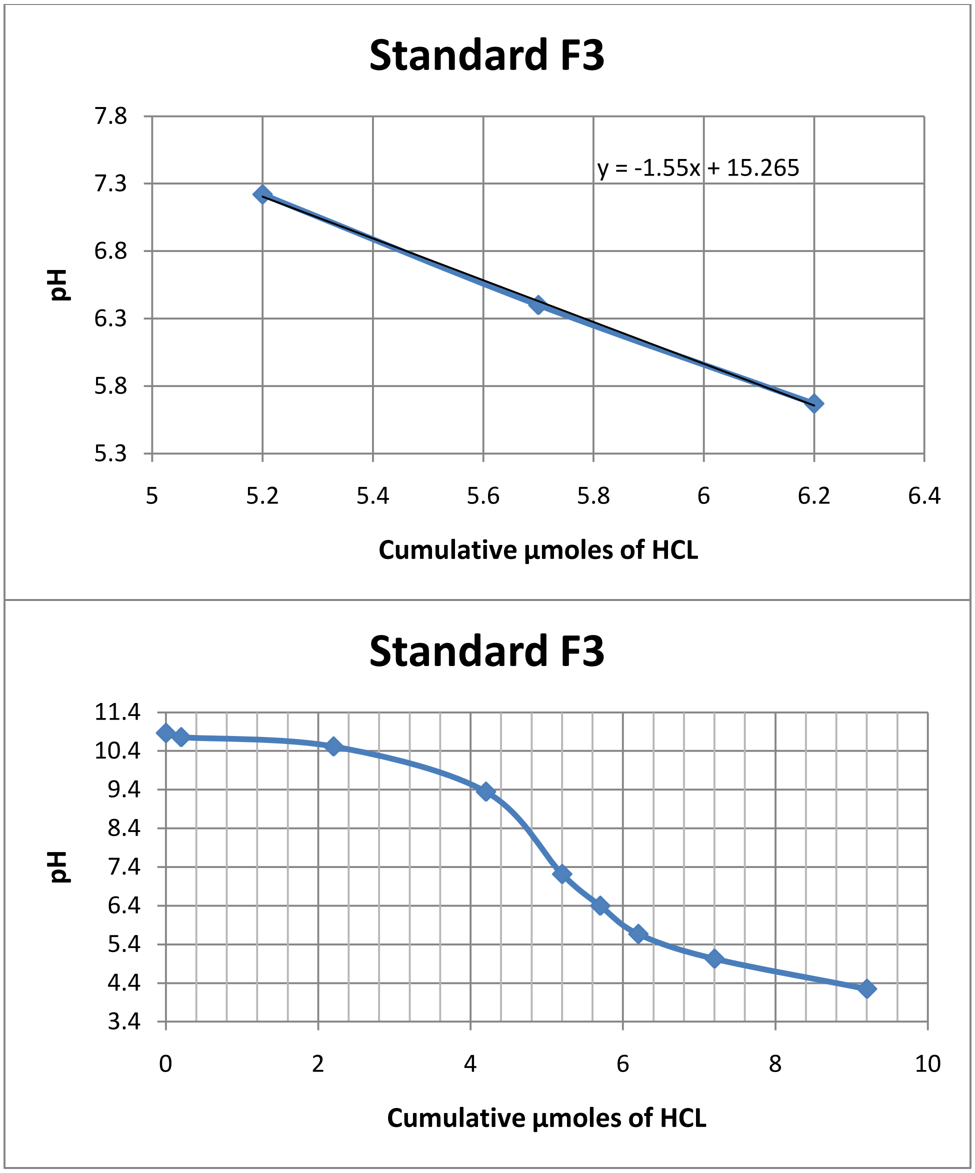


Figure 5. *Above*: An example of a standard curve titration. *Below*: Three data points taken from the above graph. Linear regression was applied and the resultant equation used to find cumulative micromoles at pH=7. In Standard F3, x=(7-15.265)/(-1.55)=5.332258.

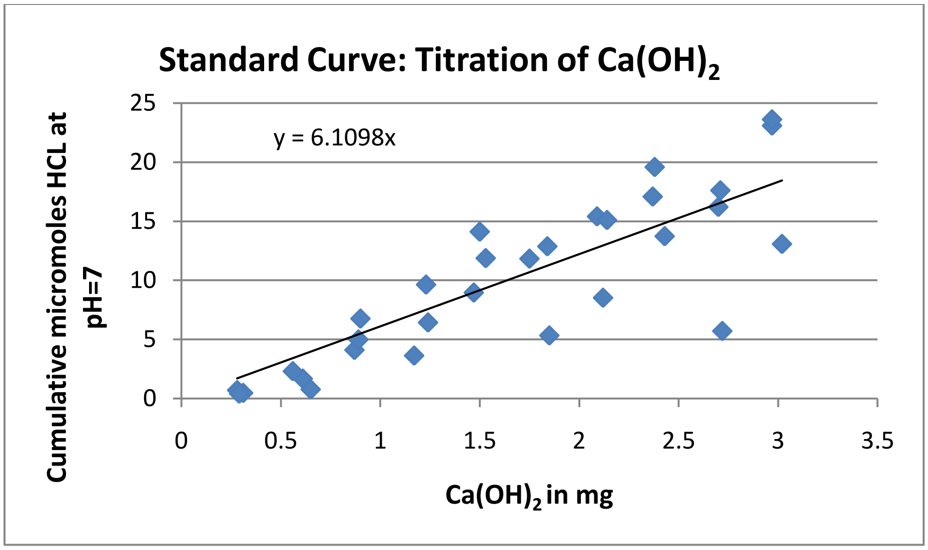


Figure 6: Graphical representation of the standard curve. The equation derived from the trendline is termed the "Standard Equation."

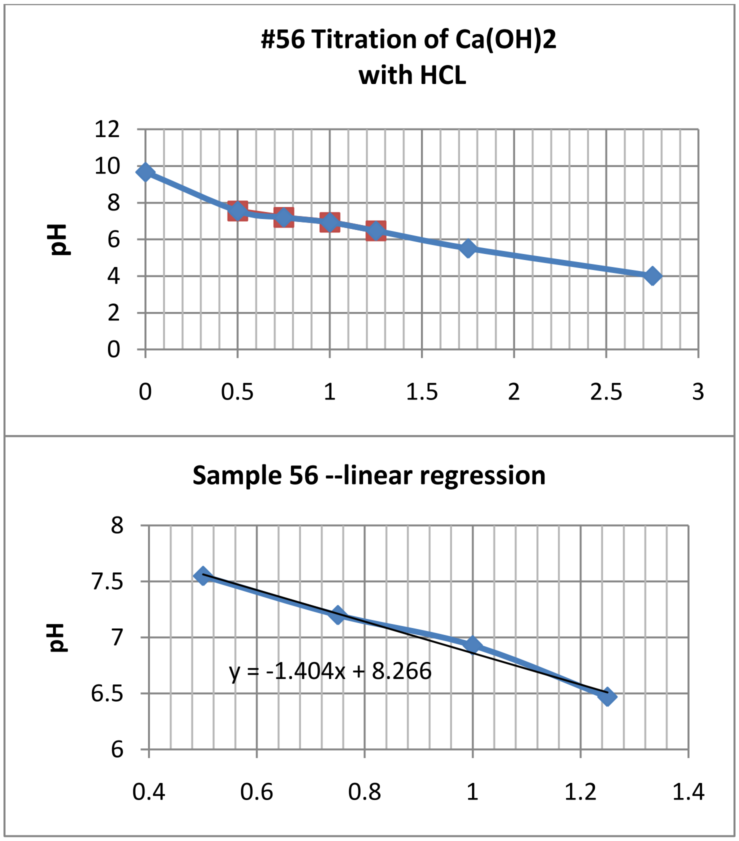


Figure 7. Sample of a titration curve and corresponding linear regression line.

Translation of Sample Data using the Standard Equation

After generating a standard scatter plot, standard line, and standard equation, it was then possible to determine the weights of Calasept® in the original samples. For each sample titration curve, a linear regression plot was made and associated equation was determined. This was done in identical fashion as the standard curves; every effort was made to use data points just above and below pH=7 to capture the steep area of the titration curve. From 2 to 5 data points were used, depending on how linear the points were aligned around pH=7. The cumulative µmoles at pH=7 was determined in the same fashion as the standards. The equation for each sample was solved for x when y=7 (pH=7). Then each sample had a single known datum point, x. Using the standard equation, $y=6.1098x$, the original weight of each sample of Calasept® was determined (see Table 2). With the random number table that blinded the operator, the code was broken and samples sorted into their respective groups.

Group	Sample	Cum µmo	x		Group	Sample	Cum µmo	x
1	35	10.77691	1.763872		3	34	0.516991	0.084617
1	42	2.632353	0.430841		3	41	164.1563	26.8677
1	16	4.631825	0.758098		3	18	2.478346	0.405635
1	44	15.03125	2.460187		3	49	0.400735	0.065589
1	32	2.102211	0.344072		3	13	15.93245	2.607687
1	25	16.14286	2.642125		3	47	0.319444	0.052284
1	54	27.56607	4.511779		3	55	0.290094	0.04748
1	6	25.94505	4.246466		3	22	0.642176	0.105106
1	37	2.629361	0.430351		3	58	2.65871	0.435155
1	1	1.812727	0.296692		3	43	0.27146	0.04443
1	33	0.313333	0.051284		3	48	1.185096	0.193966
1	5	2.4829	0.40638		3	20	0.388873	0.063647
2	9	7.533607	1.233037		4	23	0.399194	0.065337
2	52	4.333254	0.70923		4	50	0.284574	0.046577
2	51	1.449545	0.237249		4	21	0.377119	0.061724
2	3	12.71081	2.080396		4	26	0.229565	0.037573
2	29	0.956731	0.15659		4	39	1.012426	0.165705
2	53	1.530423	0.250487		4	24	0.456585	0.07473
2	36	1.02141	0.167176		4	17	0.276227	0.04521
2	45	1.368285	0.223949		4	12	4.258772	0.697039
2	46	1.727117	0.28268		4	56	0.901709	0.147584
2	8	1.5667	0.256424		4	19	3.078431	0.503851
2	4	1638.49	268.1741		4	7	0.705446	0.115461
2	40	1.005682	0.164601		4	30	5.947236	0.973393
Control	Grams	mg	X value		Control	Grams	mg	X value
N Con	57	0.010316	0.001688		P Con A	0.02955	29.55	233.8524
N Con	31	-0.00188	-0.00031		P Con B	0.03028	30.28	244.6577
N Con	28	-0.18757	-0.0307		P Con C	0.02992	29.92	233.7948

Table 2. Listed are the group by sample number, cumulative µmoles at pH=7, and calculated weight of Calasept® in mg using the standard equation (x=y/6.1098). The bottom columns represent the positive controls and negative controls with the same information.

Positive Controls

The positive controls consisted of three 1.5 ml microcentrifuge tubes. Calcium hydroxide was added to a certain weight. The tube was filled to the 1.1 mm mark (100µl) and titrated with HCL. The weight of $Ca(OH)_2$ placed in the tubes was determined by pilot studies that determined when 100 µl of glycerin was nearly fully saturated. The positive control maximizes the impact of the dependent variable on the independent variable. In this case, the dependent variable is the cumulative µmoles of HCL and the independent variable is the weight of $Ca(OH)_2$. A very large amount of Calasept® paste causes the use of a large amount of cumulative µmoles of HCL.

Negative Controls

A negative control is used to eliminate the impact of the dependent variable on the independent variable. In this study, teeth that were never filled with $Ca(OH)_2$ were used. The same amount of glycerin was added to these teeth and transferred to the microcentrifuge tubes. In fact, the negative controls were titrated during the blinding procedure so the operator did not know he was titrating a negative control. The results of the titrations indicated that the initial pH readings were 5.96, 6.97, and 7.17 for negative controls with sample numbers 28, 31, and 57 respectively. Applying the standard equation, the negative controls had original amounts of $Ca(OH)_2$ of 0.001688, -0.00031, and -0.0307 mg. These figures are very close to zero. Of course, the negative controls had zero Calasept® added, which confirmed that the titration method measured $Ca(OH)_2$ correctly. *When no Calasept® was present in negative controls, the titration indicated that no Calasept® was present*. Therefore, no background interference occurred in the measurements; the higher pH measurements of the samples were due to Calasept® remaining in the tooth after the removal techniques were applied.

A "Second" Standard Curve

Second Standard Curve: A second standard curve was calculated using only $Ca(OH)_2$ in microcentrifuge tubes and not placed in teeth. This was done to evaluate the effect of dentin on $Ca(OH)_2$ removal.

Second Standard Curve Procedure: In the second standard curve, Calasept® was added to empty microcentrifuge tubes. The microcentrifuge tube was placed on the measuring table with cover closed and upside down to ensure each measurement was performed the same. The tubes were wiped with a Kim wipe before being placed on an analytical scale (Ohaus Corporation, Pine Brook, NJ). After adding the Calasept®, the tube was reweighed to determine to amount. Eleven weight designations were made in triplicate (Appendix 1). Each triplicate sample was within 0.00003 g of the target weight. The standard weight range was determined through pilot studies. Next, 100 microliters of 60% glycerin at 40° C was added to each microcentrifuge tube and vortexed for 30 s to ensure full dissolution. A P200 pipetman® micropipette (Gilson Inc., Middleton, WI) was used to add 100 microliters of 60% glycerin. Note that the same original mix of glycerin and sterile water was used for the entire study to minimize error. A line was previously drawn at 1.1 mm from the bottom of the microcentrifuge tube to cross check that the volume was equal in all standards. Titrations were performed as noted previously. Since glycerin was added directly to microcentrifuge tubes rather than to teeth, there was no "glycerin transfer" step. The second standard curve demonstrates clearly that placement of $Ca(OH)_2$ within a tooth versus microcentrifuge tube greatly affects the results and thus only the first standard curve was used to evaluate results that can be extrapolated to clinical use.

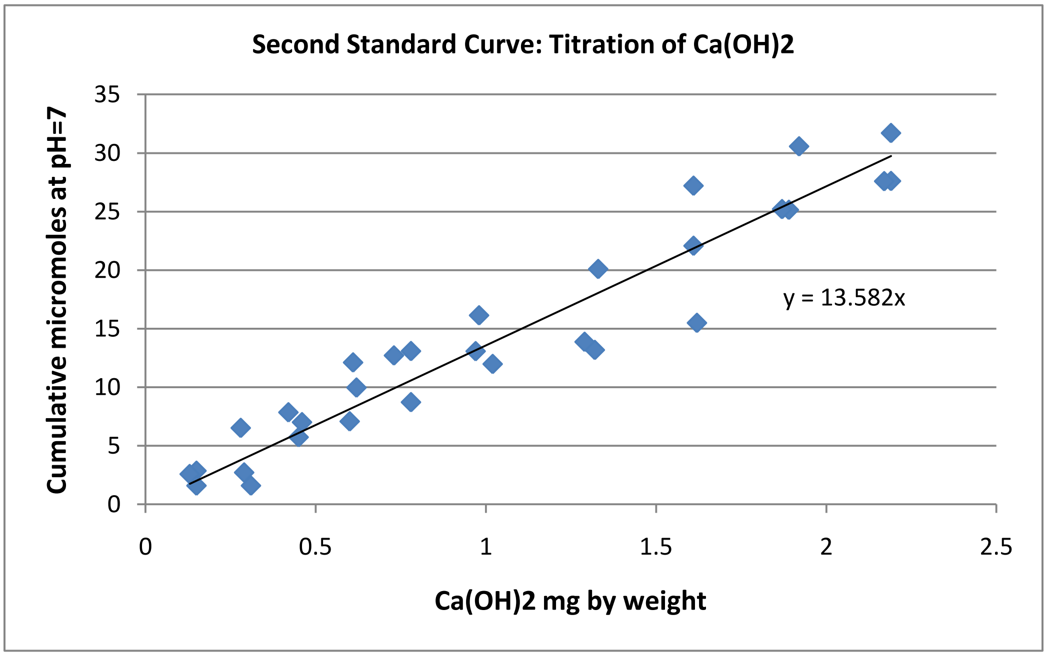


Figure 8. Second standard curve.

Outlier Samples: Two samples were not included in the data, samples #4 and #41. Both of these teeth were anatomical outliers. Examination of the radiographs of these teeth revealed that the pulp chambers were unusually large and significantly larger than other samples. Analysis of the data reflected that the corresponding mg of $Ca(OH)_2$ was quite large. These samples were not included in the data analysis.

Data Analysis

Sample Size Statement: When the sample size in each of the four groups was 12, a one-way analysis of variance had 80% power to detect at the 0.05 level a difference in means characterized by an effect size (variance between means divided by a common group variance) of 0.2483, assuming that the common standard deviation is 0.0004.

Statistical Analysis: Means and standard deviations of $Ca(OH)_2$ were calculated for each group. A one-way analysis of variance (ANOVA) was used to test for differences among the means of the four groups. If the ANOVA test was statistically significant, pairwise comparisons were made using a Tukey-Kramer multiple comparisons adjustment. P-values less than 0.05 were deemed statistically significant. SAS V9.1.3 (SAS Institute, Cary, NC) was used for the analysis.

Results

The groups differed significantly [F(3,42)=4.47, p=0.0082], indicating that there was a difference between the group means. The group 1 mean was significantly different than the means of groups 3 and 4, p=0.0291 and p=0.0104, respectively. No other comparisons were statistically significant.

Group	N	Mean (SD)
1	12	1.529 (1.59)
2	11	0.524 (0.61)
3	11	0.373 (0.75)
4	12	0.245 (0.31)

Table 3. Group means (SD) of $Ca(OH)_2$.

Groups	1	2	3	4
1	-	-	-	-
2	0.0711	-	-	-
3	0.0291	0.9825	-	-
4	0.0104	0.8962	0.9882	-

Table 4. P-values for pairwise comparisons.

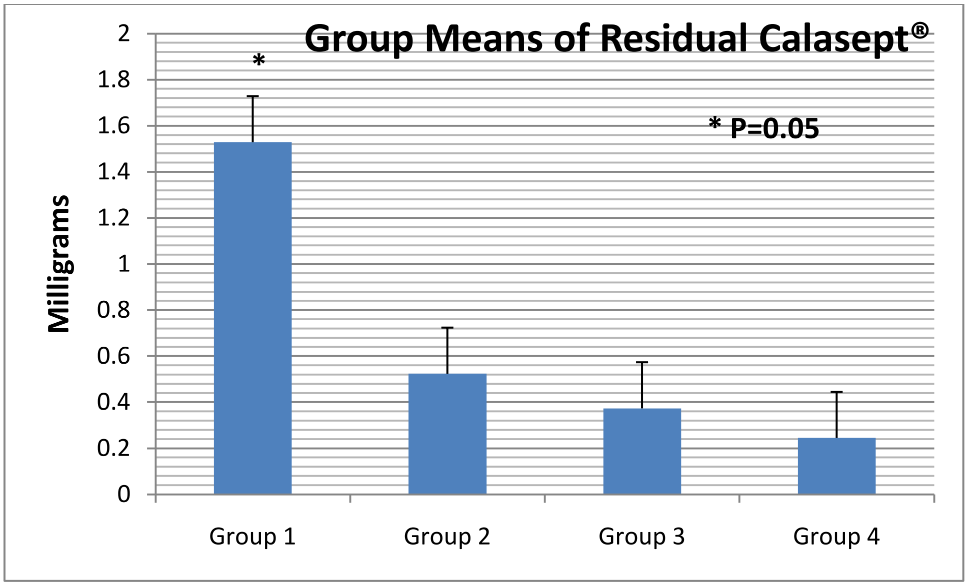


Figure 9. Bar graph of group means of residual Calasept® with standard error of the mean.

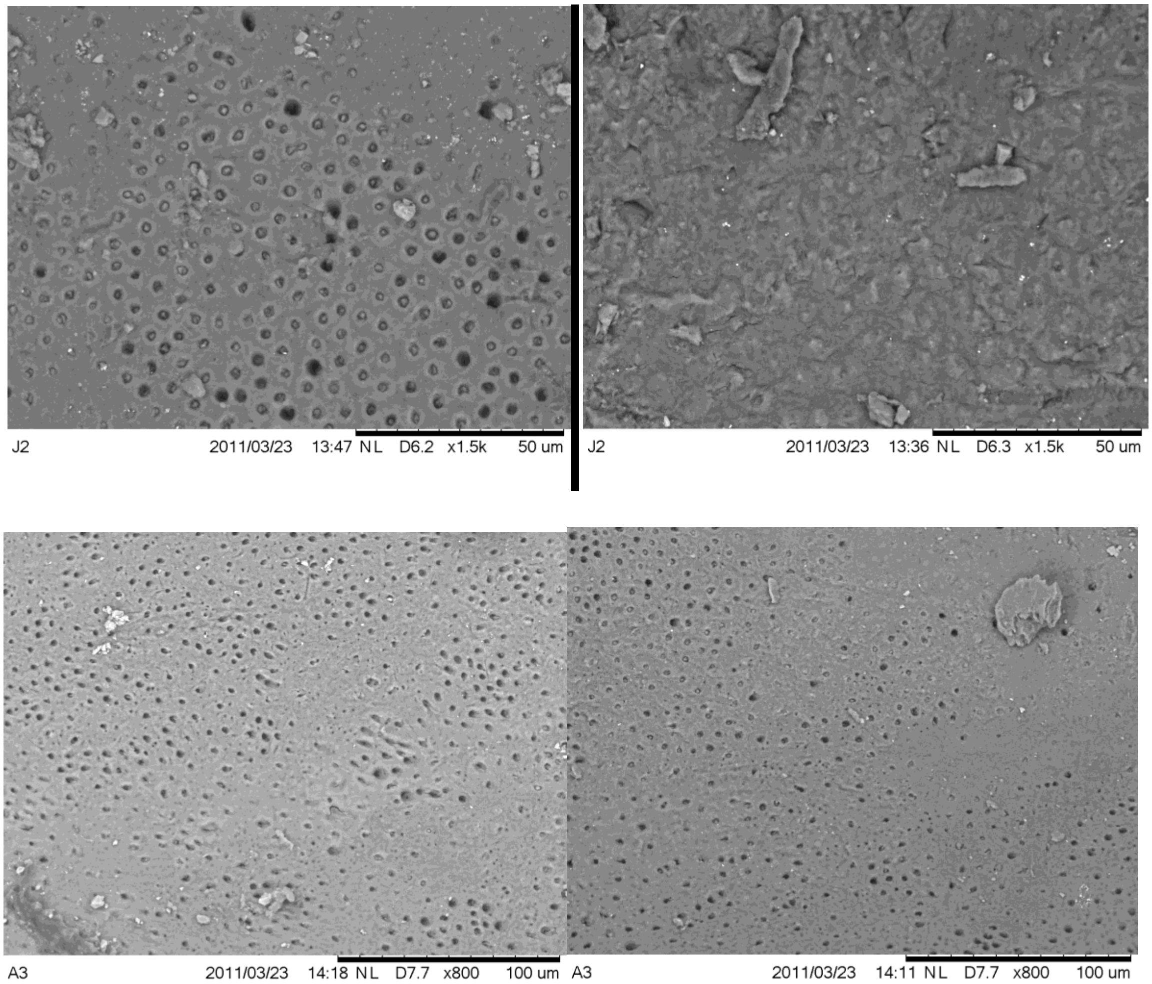


Figure 10. Scanning electron micrographs of standards A3 and J3 at various magnifications taken from the apical 1/3 after splitting teeth. Top left: J3 at 1500x, right: J3 at 1500x (different location), Bottom left: A3 at 800x, right: A3 at 800x (different location).

Verification that all Calasept® was removed from tooth: After the experiment, several representative teeth were split and SEM (Model TM3000 Table Top Microscope, Hitachi High-Technologies Corporation, Tokyo, Japan) was performed to visually access the tooth surface (Figure 10).

Discussion

The intracanal medicament calcium hydroxide has many indications in clinical endodontics. Ultimately, most usages of the drug require removal for predictable obturation. This study showed that no $Ca(OH)_2$ removal technique successfully removed all the $Ca(OH)_2$ from the canal system. This is in agreement with Lambrianidis et al. (1999) who found that with a hand file and various types of irrigant 25-40% of the walls remained covered with $Ca(OH)_2$.

One primary result of our study was that a combination of rotation of the final apical greater taper file (K3 0.04 #50) with ultrasonic activation for 30 s produced significantly better $Ca(OH)_2$ removal than a hand file with irrigation. Also, statistically significant was the 30 s use of an ultrasonically activated file which produced better $Ca(OH)_2$ removal than irrigation only groups. A trend existed that greater taper (group II) produced better removal than a #50 hand file plus irrigation (group I), and statistical significance was nearly achieved (p-value = 0.0711). These results are in disagreement with one study that found sonic activation of hand files produced canals of similar cleanliness as that of hand filing with irrigation (Tronstad et al., 1985).

Overall, the results of our study confirm a wealth of endodontic literature that finds passive ultrasonic instrumentation more effective than irrigation only techniques (Lev et al., 1987; Stamos et al., 1987; Jensen et al., 1999; Haidet & Reader, 1989; Archer

& Reader, 1992; Cunningham & Martin, 1982; Wiseman et al., 2011; and van der Sluis et al., 2007). Our study supported 30 s of PUI over hand filing and irrigation which is in agreement with other studies that found 30 s of PUI effective over other groups (Sabins et al., 2003). This is in contrast to other studies that used longer time periods for PUI, such as 3 minutes (Archer et al., 1992). The approach used in this study measures all the Calasept® remaining in the canal by giving a single number—milligrams of Calasept®. Whereas other studies used pixels counting, histologic cross sections, micro-CT, or other approaches, our method dissolved remaining $Ca(OH)_2$ and chemically quantified it. No other $Ca(OH)_2$ removal study has been able to express results in actual weight. Many randomly measured small areas of the canal and projected that information to an overall system. Close similarities in findings between a titration method and pixel-counting methods reciprocally confirmed both methods. Our method confirmed others regarding the effectiveness of ultrasonics.

Our study was similar in experimental group assignments to a recent investigation by Kenee et al. (2006), with one major difference; they did not have a greater taper rotary plus ultrasound group. Their study found both PUI and greater taper final apical file groups were more effective than a hand file plus irrigation technique alone. Our study agreed, as our ultrasonic (group III) was more effective than irrigation (group I) ($p=0.0291$).

One contrast with Kenee et al. (2006) is that our data showed a trend towards differences (p=0.0711) between the greater taper file (group II) and irrigation and hand filing (group I). It may be that both results are correct, since Kenee et al. (2006) expressed results in terms of percentage residual debris/Calasept® of the canal wall area, and our results were expressed in mg of Calasept®. They found debris/Calasept® levels were significantly lower in the Greater Taper group, but if a method that specifically measured Calasept® was applied, this threshold may no longer be statistically significant. A repeated measures ANOVA was not applied in their study, despite the fact that samples were reused multiple times. Another explanation might be that the glycerin transfer step in our study removed some Calasept® from dentinal tubules. If this is true, the "surface area" measurement is only part of the Calasept® measurement. If the glycerin/titration method is removing and measuring surface and deeper dentinal tubule Calasept®, any differences in remaining Calasept® on the dentinal surface becomes only a part of the overall measurement.

Examination of SEMs taken after our study revealed some debris mixed in with the glycerin (Figure 10). Perhaps the glycerin transfer failed to remove all the Calasept®. Or the glycerin transfer *did* remove all the Calasept®, and what was seen mixed in with the remaining glycerin was actually dentinal debris. As part of the transfer of $Ca(OH)_2$ into the microcentrifuge tubes, passive ultrasonic instrumentation was performed between each transfer. This was done to ensure the $Ca(OH)_2$ on the walls was incorporated into solution, and likely would have also incorporated more debris into the glycerin. One method to differentiate residual debris from Calasept® would be to radiolabel Calasept® and apply removal studies. Then, an autoradiographic analysis could be performed to determine "what we are actually looking at" after $Ca(OH)_2$ is removed. Allison, Weber, and Walton (1979) used such a technique with ^{45}Ca to prove that step back preparations had less leakage than serial preparation.

Recently, Wiseman et al. (2011) took serial micro-CT scans in order to determine if ultrasonic instrumentation was better than sonic instrumentation when both types were added to use of a greater taper type rotary file. Ultrasonic usage for three sets of 20 s was significantly better than sonic instrumentation. Results agree with the findings that ultrasonic is better than hand and irrigation alone. Our study found statistical difference and used 30 s of ultrasonic activation based on Sabins et al. (2003) while Wiseman et al. (2011) used 60 s of ultrasound (both at 2 mm from WL in decoronated teeth). Also, Ultracal® rather than Calasept® was the $Ca(OH)_2$ paste used. One potential limitation of their study is that micro-CT, while an incredible tool for studies in endodontics, may not be able to differentiate between debris and $Ca(OH)_2$. Results of their study may inadvertently incorporate debris into the data set. Another interesting point is that Wiseman et al. (2011) found no $Ca(OH)_2$ in their negative controls, while Kenee et al. (2006) found 0.615% debris. There may be a difference in sensitivity and ability to detect $Ca(OH)_2$ versus debris in a micro-CT and pixel-counting study. Our negative controls indicate that the pH change is due to the $Ca(OH)_2$; therefore we can confidently conclude differences between groups are also due to $Ca(OH)_2$ differences rather than dentin debris.

In an *in vitro* study, van der Sluis et al. (2007) found that irrigation with 50 ml of NaOCl and ultrasonic use was significantly better than irrigation alone when removing Ultracal® from premade grooves. Our data confirmed their findings. One difference between our study design and theirs was that we used a #50 hand file at WL before irrigation. Our study found that ultrasonics improved removal over hand filing + irrigation, not only over irrigation only as a group. Van der Sluis et al. (2007) also used single rooted mandibular premolars, while others used molars (Wiseman et al., 2011; Sabins et al., 2003). In our study, the majority of samples were canines with some intermixed single canal premolars. The canine tooth is wide buccal-lingually (Figure 1), while premolars and canines usually have smaller, more circular canals. Use of a greater taper file did not achieve statistical significance in removal over hand filing and irrigation (group I). One explanation for the finding is the wide buccal-lingual nature of the root canals. A K3 FAF alone may not have been enough to remove Calasept® from buccal and lingual irregularities, while the use of acoustic streaming was needed in these broad canals.

While no difference was found between our groups III and IV, the latter group with both ultrasound and a K3 0.06 file had the best removal. Our results using canine teeth may be more applicable to other teeth with irregular canals such as the distal canals of mandibular molars or the palatal canals of maxillary molars. Despite the fact that more clinical time is needed, optimal removal of $Ca(OH)_2$ in these situations may warrant use of both a greater taper rotary and PUI.

Since this technique can measure removal of $Ca(OH)_2$ from irregularities, future studies should use the titration methodologies in various canals types. An exciting new technology is the self adjusting file, which adapts to various canal shapes and is specially designed for use in irregular canals (Metzger et al., 2010). To circumvent the challenge of standardizing preparations, a device that accurately measures the amount of $Ca(OH)_2$ dispensed could be used when filling the canal, and our titration model to measure $Ca(OH)_2$ removal could be applied. The amount of $Ca(OH)_2$ retrieved versus amount placed could be calculated; this method could even be performed *in vivo*. Another future study would be to apply our titration technique to curved roots. Some $Ca(OH)_2$ removal studies have utilized root curvature, and it would be interesting to directly compare a pixel-counting method with the titration model.

A thought-provoking finding in this investigation occurred due to an error in study design. The author first created a standard curve using known Calasept® amounts in microcentrifuge tubes (termed the "second standard curve"). This was incorrect because standards, like the experimental samples, needed to be performed in extracted teeth; this was then performed. A comparison could be achieved between a standard curve generated in teeth versus tubes (Figure 6 versus Figure 7). More Calasept® was recovered from the tubes, but some factor was preventing full recovery of material from teeth. Perhaps the microirregularites in the dentin wall made recovery worse. Alternatively, diffusion of the Ca+ and OH- ions into dentinal tubules could have been responsible for our inability to fully remove the Calasept. This provides support that we are only removing a percentage of ICM we place, because the remainder diffuses throughout the tooth as designed.

A limitation of the study is one inherent to the use of very small amounts of liquid. While the largest available canals were used, each aliquot of glycerin transfer was perhaps only 10-20 microliters. Despite diligence in transferring the glycerin solution, some glycerin could have been imperfectly transferred, resulting in lost solution containing $Ca(OH)_2$. Also, during the passive ultrasonic instrumentation step (as part of the glycerin transfer, not $Ca(OH)_2$ removal), some vapor from the PUI may have been lost due to decoronation. This might have resulted in some lost glycerin fluid containing $Ca(OH)_2$. One might also say that in the glycerin transfer not all the $Ca(OH)_2$ dissolved. Efforts made to eliminate error included use of 10 aliquots in the transfer, PUI between aliquots, pilot studies that were aimed at determining correct glycerin percentages, and observation of the clarity of the solution. Often, later aliquots in the glycerin transfer were completely clear while earlier aliquots were white in color. Another difference between this and previous studies is in how the data are expressed.

This investigation found a raw number that represented remaining Calasept®, but no location of that material. An advantage of pixel-counting type studies is that they can quantify debris in certain regions of the root.

Another critique of this investigation is within the titrations. Because of the small amount of glycerin being titrated, the volume of mixture after the addition of HCL varied from 130 to 240 microliters. This variation in volume was due to challenges in titrating a broad range of $Ca(OH)_2$ that was represented in the samples. As a counterpoint to this critique, it can be said that at pH=7, there was less variation in cumulative volume of HCL added (usually 20-60 microliters). Any HCL added after pH=7 did not add meaningful data, but did allow for a complete titration curve. The number of data points available to make a titration curve varied—subsequently, the number of data points to make a linear regression line varied as well. Despite best efforts to gauge which concentration of HCL to add in each aliquot (and therefore keep end volumes approximately equal), these issues may have caused some curves to be more accurate than others.

A substantial body of literature justifies the use of $Ca(OH)_2$, but predictable obturation requires removal. Various techniques have studied removal, nearly all of which are a variation of counting areas and expressing the result as a percentage of the canal wall (or histologic section) remaining covered with $Ca(OH)_2$. The percentage is then used in statistical comparison, and recommendations for certain techniques are justified. Given how $Ca(OH)_2$ removal studies are saturated with this technique, drawbacks of the general model are rarely discussed. Overall, when a quantification pixel-counting method has been used, the canals have often been circular. When premade grooves were created in dentin walls to simulate irregularities, these studies had other drawbacks such as reuse of specimens, multiple reapproximations of specimens, and lack of parametric data. Regardless of the technique, splitting of the teeth before quantification was mandatory. Another challenge in interpretation of these studies is in the difficulty in translating "percent remaining debris of calcium hydroxide" to clinical practice.

We desired to improve upon limitations of previous models by introduction of a novel method that allowed for quantitative chemical measurement in large, somewhat irregular canals. Agreement in results of other studies has verified this technique. In modern endodontics, great emphasis is being placed on a higher level of evidence. By applying this novel titration *in vivo,* endodontics may be able to better understand exactly how well $Ca(OH)_2$ removal is being performed in the mouth.

Conclusions

1. The novel glycerin transfer and micro titration technique described in this study is a sensitive and accurate model that can be employed in future $Ca(OH)_2$ removal studies. The model circumvents drawbacks of previous studies including area measurements on three dimensional surfaces, effects of reuse of samples, effects of splitting teeth, and inability to directly measure amount of calcium hydroxide.

2. No removal method fully removed Calasept® from the root canal system.

3. The addition of passive ultrasonic instrumentation to a hand file and irrigation resulted in significantly better $Ca(OH)_2$ removal over hand filing and irrigation alone.

4. The 30 s time frame, rather than longer PUI times, was adequate to achieve statistically better $Ca(OH)_2$ removal.

5. Use of 30 s of passive ultrasonic instrumentation in combination with a K3 0.06 taper rotary file after hand filing and irrigation provided the lowest group mean of Calasept®, and may be most effective in very irregularly shaped canals.

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Appendix I.

Standard specimens with respective weights in grams (left middle) and milligrams (right middle) and cumulative micromoles at pH=7.

TOOTH	WEIGHT	Mg	µmoles
a1	0.00031	0.31	0.466682
a2	0.00028	0.28	0.70592
a3	0.00029	0.29	0.410099
b1	0.00061	0.61	1.675192
b2	0.00056	0.56	2.306818
b3	0.00065	0.65	0.768775
c1	0.0009	0.9	6.759696
c2	0.00087	0.87	4.104956
c3	0.00089	0.89	4.987356
d1	0.00123	1.23	9.637615
d2	0.00117	1.17	3.629442
d3	0.00124	1.24	6.430928
e1	0.00153	1.53	11.87791
e2	0.0015	1.5	14.11789
e3	0.00147	1.47	8.96129
f1	0.00175	1.75	11.83333
f2	0.00184	1.84	12.8758
f3	0.00185	1.85	5.332258
g1	0.00209	2.09	15.40984
g2	0.00212	2.12	8.525
g3	0.00214	2.14	15.10189
h1	0.00238	2.38	19.58756
h2	0.00237	2.37	17.08889
h3	0.00243	2.43	13.73379
i1	0.00272	2.72	5.709302
i2	0.0027	2.7	16.21135
i3	0.00271	2.71	17.60976
j1	0.00297	2.97	23.09556
j2	0.00297	2.97	23.6198
j3	0.00302	3.02	13.07781

Appendix II.

A "second" standard curve. These standards, while not used in data analysis, were used to make a standard curve. Titrations were performed in tubes rather than teeth.

Standard	Grams	mg	µmoles
2a	0.00015	0.15	2.872024
2b	0.00013	0.13	2.589273
2c	0.00015	0.15	1.604167
3a	0.00031	0.31	1.604167
3b	0.00029	0.29	2.72508
3c	0.00028	0.28	6.531965
4a	0.00046	0.46	7.019822
4b	0.00045	0.45	5.743468
4c	0.00042	0.42	7.863128
5a	0.00061	0.61	12.12228
5b	0.0006	0.6	7.091503
5c	0.00062	0.62	9.976754
6a	0.00073	0.73	12.71756
6b	0.00078	0.78	8.729026
6c	0.00078	0.78	13.0936
7a	0.00098	0.98	16.14678
7b	0.00097	0.97	13.08324
7c	0.00102	1.02	11.99044
8a	0.00132	1.32	13.18905
8b	0.00129	1.29	13.88438
8c	0.00133	1.33	20.10061
9a	0.00161	1.61	27.21519
9b	0.00162	1.62	15.50127
9c	0.00161	1.61	22.08586
10a	0.00189	1.89	25.15714
10b	0.00192	1.92	30.57806
10c	0.00187	1.87	25.21717
11a	0.00219	2.19	27.61638
11b	0.00217	2.17	27.60047
11c	0.00219	2.19	31.71593

Appendix III.

Titration data for Samples. Columns from left to right: pH readings, concentration of HCL added, volume of HCL added, moles of HCL added, cumulative moles of HCL added, cumulative micromoles of HCL added, and pH (listed again).

Sample 1						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.72	-	-	-	0.00000000	0	8.72
7.52	0.025	0.00001	0.00000025	0.00000025	0.25	7.52
6.83	0.025	0.00001	0.00000025	0.00000050	0.5	6.83
6.31	0.025	0.00001	0.00000025	0.00000075	0.75	6.31
5.26	0.05	0.00001	0.00000050	0.00000125	1.25	5.26
2.91	0.1	0.00001	0.00000100	0.00000225	2.25	2.91
2.08	0.2	0.00001	0.00000200	0.00000425	4.25	2.08

Sample 3						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.17	-	-	-	0.00000000	0	11.17
10.89	0.5	0.00001	0.00000500	0.00000500	5	10.89
10.51	0.2	0.00001	0.00000200	0.00000700	7	10.51
8.67	0.2	0.00001	0.00000200	0.00000900	9	8.67
8.36	0.025	0.00001	0.00000025	0.00000925	9.25	8.36
8.19	0.025	0.00001	0.00000025	0.00000950	9.5	8.19
8.06	0.025	0.00001	0.00000025	0.00000975	9.75	8.06
8	0.025	0.00001	0.00000025	0.00001000	10	8
7.89	0.025	0.00001	0.00000025	0.00001025	10.25	7.89
7.62	0.05	0.00001	0.00000050	0.00001075	10.75	7.62
7.14	0.1	0.00001	0.00000100	0.00001175	11.75	7.14
6.74	0.2	0.00001	0.00000200	0.00001375	13.75	6.74
1.85	1	0.00001	0.00001000	0.00002375	23.75	1.85

Sample 4						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.52	-	-	-	0.00000000	0	11.52
11.45	0.5	0.00001	0.00000500	0.00000500	5	11.45
11.36	0.5	0.00001	0.00000500	0.00001000	10	11.36
11.15	0.5	0.00001	0.00000500	0.00001500	15	11.15
8.97	1	0.00001	0.00001000	0.00002500	25	8.97
8.23	0.1	0.00001	0.00000100	0.00002600	26	8.23
7.97	0.1	0.00001	0.00000100	0.00002700	27	7.97
7.34	0.2	0.00001	0.00000200	0.00002900	29	7.34
6.95	0.2	0.00001	0.00000200	0.00003100	31	6.95
5.01	0.5	0.00001	0.00000500	0.00003600	36	5.01

Sample 5						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.63	-	-	-	0.00000000	0	10.63
3.32	0.5	0.00001	0.00000500	0.00000500	5	3.32
1.33	0.5	0.00001	0.00000500	0.00001000	10	1.33
1.09	0.5	0.00001	0.00000500	0.00001500	15	1.09

Sample 6						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.54	-	-	-	0.00000000	0	11.54
11.46	0.5	0.00001	0.00000500	0.00000500	5	11.46
11.13	1	0.00001	0.00001000	0.00001500	15	11.13
10.5	0.5	0.00001	0.00000500	0.00002000	20	10.5
8.84	0.2	0.00001	0.00000200	0.00002200	22	8.84
7.36	0.2	0.00001	0.00000200	0.00002400	24	7.36
7.36	0.1	0.00001	0.00000100	0.00002500	25	7.36
7.08	0.1	0.00001	0.00000100	0.00002600	26	7.08
6.03	0.2	0.00001	0.00000200	0.00002800	28	6.03
2.16	0.5	0.00001	0.00000500	0.00003300	33	2.16

Sample 7						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.67	-	-	-	0.00000000	0	8.67
8.3	0.025	0.00001	0.00000025	0.00000025	0.25	8.3
7.37	0.025	0.00001	0.00000025	0.00000050	0.5	7.37
7	0.025	0.00001	0.00000025	0.00000075	0.75	7
6.36	0.025	0.00001	0.00000025	0.00000100	1	6.36
1.66	0.5	0.00001	0.00000500	0.00000600	6	1.66
1.3	0.5	0.00001	0.00000500	0.00001100	11	1.3

Sample 8						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.2	-	-	-	0.00000000	0	10.2
6.57	0.2	0.00001	0.00000200	0.00000200	2	6.57
6.31	0.025	0.00001	0.00000025	0.00000225	2.25	6.31
6.07	0.025	0.00001	0.00000025	0.00000250	2.5	6.07
4.93	0.05	0.00001	0.00000050	0.00000300	3	4.93
3.04	0.1	0.00001	0.00000100	0.00000400	4	3.04
1.94	0.2	0.00001	0.00000200	0.00000600	6	1.94

Sample 9						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.12	-	-	-	0.00000000	0	11.12
10.16	0.5	0.00001	0.00000500	0.00000500	5	10.16
7.25	0.2	0.00001	0.00000200	0.00000700	7	7.25
7.2	0.025	0.00001	0.00000025	0.00000725	7.25	7.2
6.88	0.05	0.00001	0.00000050	0.00000775	7.75	6.88
6.61	0.05	0.00001	0.00000050	0.00000825	8.25	6.61
6.3	0.1	0.00001	0.00000100	0.00000925	9.25	6.3
5.44	0.2	0.00001	0.00000200	0.00001125	11.25	5.44
1.96	0.5	0.00001	0.00000500	0.00001625	16.25	1.96

Sample 12						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.67	-	-	-	0.00000000	0	10.67
10.43	0.1	0.00001	0.00000100	0.00000100	1	10.43
7.71	0.2	0.00001	0.00000200	0.00000300	3	7.71
7.4	0.025	0.00001	0.00000025	0.00000325	3.25	7.4
7.33	0.025	0.00001	0.00000025	0.00000350	3.5	7.33
7.25	0.025	0.00001	0.00000025	0.00000375	3.75	7.25
7.13	0.025	0.00001	0.00000025	0.00000400	4	7.13
6.99	0.025	0.00001	0.00000025	0.00000425	4.25	6.99
6.25	0.05	0.00001	0.00000050	0.00000475	4.75	6.25
1.87	0.5	0.00001	0.00000500	0.00000975	9.75	1.87

Sample 13						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.27	-	-	-	0.00000000	0	11.27
11.06	0.5	0.00001	0.00000500	0.00000500	5	11.06
10.38	0.5	0.00001	0.00000500	0.00001000	10	10.38
8.51	0.2	0.00001	0.00000200	0.00001200	12	8.51
7.81	0.1	0.00001	0.00000100	0.00001300	13	7.81
7.66	0.05	0.00001	0.00000050	0.00001350	13.5	7.66
7.58	0.05	0.00001	0.00000050	0.00001400	14	7.58
7.23	0.1	0.00001	0.00000100	0.00001500	15	7.23
7.05	0.05	0.00001	0.00000050	0.00001550	15.5	7.05
7.04	0.025	0.00001	0.00000025	0.00001575	15.75	7.04
6.98	0.05	0.00001	0.00000050	0.00001625	16.25	6.98
6.72	0.1	0.00001	0.00000100	0.00001725	17.25	6.72
2.62	0.5	0.00001	0.00000500	0.00002225	22.25	2.62

Sample 16						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.78	-	-	-	0.00000000	0	10.78
10.33	0.2	0.00001	0.00000200	0.00000200	2	10.33
7.22	0.2	0.00001	0.00000200	0.00000400	4	7.22
7.21	0.025	0.00001	0.00000025	0.00000425	4.25	7.21
7	0.05	0.00001	0.00000050	0.00000475	4.75	7
6.47	0.1	0.00001	0.00000100	0.00000575	5.75	6.47
4.48	0.2	0.00001	0.00000200	0.00000775	7.75	4.48
1.67	0.5	0.00001	0.00000500	0.00001275	12.75	1.67

Sample 17						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.26	-	-	-	0.00000000	0	8.26
6.98	0.025	0.00001	0.00000025	0.00000025	0.25	6.98
6.1	0.025	0.00001	0.00000025	0.00000050	0.5	6.1
5.57	0.025	0.00001	0.00000025	0.00000075	0.75	5.57
4.16	0.05	0.00001	0.00000050	0.00000125	1.25	4.16
2.47	0.1	0.00001	0.00000100	0.00000225	2.25	2.47
1.87	0.2	0.00001	0.00000200	0.00000425	4.25	1.87

Sample 18						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.12	-	-	-	0.00000000	0	10.12
8.43	0.1	0.00001	0.00000100	0.00000100	1	8.43
7.52	0.05	0.00001	0.00000050	0.00000150	1.5	7.52
7.33	0.025	0.00001	0.00000025	0.00000175	1.75	7.33
7.25	0.025	0.00001	0.00000025	0.00000200	2	7.25
7.13	0.025	0.00001	0.00000025	0.00000225	2.25	7.13
7.1	0.025	0.00001	0.00000025	0.00000250	2.5	7.1
6.77	0.025	0.00001	0.00000025	0.00000275	2.75	6.77
5.83	0.05	0.00001	0.00000050	0.00000325	3.25	5.83
3.15	0.2	0.00001	0.00000200	0.00000525	5.25	3.15

Sample 19						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.51	-	-	-	0.00000000	0	10.51
9.89	0.1	0.00001	0.00000100	0.00000100	1	9.89
8.51	0.05	0.00001	0.00000050	0.00000150	1.5	8.51
7.87	0.025	0.00001	0.00000025	0.00000175	1.75	7.87
7.5	0.025	0.00001	0.00000025	0.00000200	2	7.5
7.3	0.05	0.00001	0.00000050	0.00000250	2.5	7.3
7.03	0.05	0.00001	0.00000050	0.00000300	3	7.03
6.79	0.05	0.00001	0.00000050	0.00000350	3.5	6.79
6.3	0.05	0.00001	0.00000050	0.00000400	4	6.3
3.44	0.2	0.00001	0.00000200	0.00000600	6	3.44

Sample 20						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.6	-	-	-	0.00000000	0	8.6
7.26	0.025	0.00001	0.00000025	0.00000025	0.25	7.26
6.82	0.025	0.00001	0.00000025	0.00000050	0.5	6.82
6.24	0.025	0.00001	0.00000025	0.00000075	0.75	6.24
5.36	0.05	0.00001	0.00000050	0.00000125	1.25	5.36
4.54	0.05	0.00001	0.00000050	0.00000175	1.75	4.54
2.55	0.2	0.00001	0.00000200	0.00000375	3.75	2.55

Sample 21						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.48	-	-	-	0.00000000	0	8.48
7.3	0.025	0.00001	0.00000025	0.00000025	0.25	7.3
6.71	0.025	0.00001	0.00000025	0.00000050	0.5	6.71
5.6	0.025	0.00001	0.00000025	0.00000075	0.75	5.6
5.3	0.025	0.00001	0.00000025	0.00000100	1	5.3
4.61	0.05	0.00001	0.00000050	0.00000150	1.5	4.61
2.77	0.2	0.00001	0.00000200	0.00000350	3.5	2.77

Sample 22						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.87	-	-	-	0.00000000	0	8.87
7.79	0.025	0.00001	0.00000025	0.00000025	0.25	7.79
7.11	0.025	0.00001	0.00000025	0.00000050	0.5	7.11
7.08	0.025	0.00001	0.00000025	0.00000075	0.75	7.08
6.26	0.025	0.00001	0.00000025	0.00000100	1	6.26
5.3	0.05	0.00001	0.00000050	0.00000150	1.5	5.3
2.66	0.2	0.00001	0.00000200	0.00000350	3.5	2.66

Sample 23						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.39	-	-	-	0.00000000	0	8.39
7.31	0.025	0.00001	0.00000025	0.00000025	0.25	7.31
6.87	0.025	0.00001	0.00000025	0.00000050	0.5	6.87
6.07	0.025	0.00001	0.00000025	0.00000075	0.75	6.07
5.35	0.05	0.00001	0.00000050	0.00000125	1.25	5.35
3.78	0.1	0.00001	0.00000100	0.00000225	2.25	3.78
2.45	0.2	0.00001	0.00000200	0.00000425	4.25	2.45

Sample 24						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.49	-	-	-	0.00000000	0	8.49
7.53	0.025	0.00001	0.00000025	0.00000025	0.25	7.53
6.99	0.025	0.00001	0.00000025	0.00000050	0.5	6.99
6.11	0.025	0.00001	0.00000025	0.00000075	0.75	6.11
5.28	0.05	0.00001	0.00000050	0.00000125	1.25	5.28
2.63	0.2	0.00001	0.00000200	0.00000325	3.25	2.63
1.9	0.5	0.00001	0.00000500	0.00000825	8.25	1.9

Sample 25						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.44	-	-	-	0.00000000	0	11.44
11.26	0.5	0.00001	0.00000500	0.00000500	5	11.26
10.57	0.5	0.00001	0.00000500	0.00001000	10	10.57
7.45	0.5	0.00001	0.00000500	0.00001500	15	7.45
7.4	0.025	0.00001	0.00000025	0.00001525	15.25	7.4
7.1	0.05	0.00001	0.00000050	0.00001575	15.75	7.1
6.96	0.05	0.00001	0.00000050	0.00001625	16.25	6.96
6.77	0.05	0.00001	0.00000050	0.00001675	16.75	6.77
6.47	0.1	0.00001	0.00000100	0.00001775	17.75	6.47
2.34	0.5	0.00001	0.00000500	0.00002275	22.75	2.34

Sample 26						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.4	-	-	-	0.00000000	0	8.4
6.92	0.025	0.00001	0.00000025	0.00000025	0.25	6.92
5.3	0.025	0.00001	0.00000025	0.00000050	0.5	5.3
3.62	0.05	0.00001	0.00000050	0.00000100	1	3.62
2.58	0.1	0.00001	0.00000100	0.00000200	2	2.58
1.86	0.5	0.00001	0.00000500	0.00000700	7	1.86

Sample 28						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
5.96	-	-	-	0.00000000	0	5.96
3.96	0.025	0.00001	0.00000025	0.00000025	0.25	3.96
2.81	0.025	0.00001	0.00000025	0.00000050	0.5	2.81
2.58	0.05	0.00001	0.00000050	0.00000100	1	2.58
2.33	0.1	0.00001	0.00000100	0.00000200	2	2.33
2.06	0.2	0.00001	0.00000200	0.00000400	4	2.06

Sample 29						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.57	-	-	-	0.00000000	0	9.57
7.54	0.05	0.00001	0.00000050	0.00000050	0.5	7.54
7.09	0.025	0.00001	0.00000025	0.00000075	0.75	7.09
7.01	0.025	0.00001	0.00000025	0.00000100	1	7.01
6.86	0.025	0.00001	0.00000025	0.00000125	1.25	6.86
6.62	0.025	0.00001	0.00000025	0.00000150	1.5	6.62
5.49	0.05	0.00001	0.00000050	0.00000200	2	5.49
3.81	0.1	0.00001	0.00000100	0.00000300	3	3.81

Sample 30						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.91	-	-	-	0.00000000	0	10.91
10.78	0.1	0.00001	0.00000100	0.00000100	1	10.78
10.03	0.2	0.00001	0.00000200	0.00000300	3	10.03
8.06	0.1	0.00001	0.00000100	0.00000400	4	8.06
7.57	0.05	0.00001	0.00000050	0.00000450	4.5	7.57
7.38	0.05	0.00001	0.00000050	0.00000500	5	7.38
7.19	0.05	0.00001	0.00000050	0.00000550	5.5	7.19
6.97	0.05	0.00001	0.00000050	0.00000600	6	6.97
5.84	0.1	0.00001	0.00000100	0.00000700	7	5.84
3.44	0.2	0.00001	0.00000200	0.00000900	9	3.44

Sample 31						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
6.97	-	-	-	0.00000000	0	6.97
2.98	0.025	0.00001	0.00000025	0.00000025	0.25	2.98
2.59	0.05	0.00001	0.00000050	0.00000075	0.75	2.59
2.29	0.1	0.00001	0.00000100	0.00000175	1.75	2.29
2.09	0.2	0.00001	0.00000200	0.00000375	3.75	2.09
1.7	0.5	0.00001	0.00000500	0.00000875	8.75	1.7

Sample 31						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
6.97	-	-	-	0.00000000	0	6.97
2.98	0.025	0.00001	0.00000025	0.00000025	0.25	2.98
2.59	0.05	0.00001	0.00000050	0.00000075	0.75	2.59
2.29	0.1	0.00001	0.00000100	0.00000175	1.75	2.29
2.09	0.2	0.00001	0.00000200	0.00000375	3.75	2.09
1.7	0.5	0.00001	0.00000500	0.00000875	8.75	1.7

Sample 32						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.47	-	-	-	0.00000000	0	9.47
7.5	0.1	0.00001	0.00000100	0.00000100	1	7.5
7.41	0.025	0.00001	0.00000025	0.00000125	1.25	7.41
7.1	0.05	0.00001	0.00000050	0.00000175	1.75	7.1
6.91	0.05	0.00001	0.00000050	0.00000225	2.25	6.91
6.75	0.05	0.00001	0.00000050	0.00000275	2.75	6.75
5.85	0.1	0.00001	0.00000100	0.00000375	3.75	5.85
4.25	0.2	0.00001	0.00000200	0.00000575	5.75	4.25

Sample 33						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.59	-	-	-	0.00000000	0	8.59
7.17	0.025	0.00001	0.00000025	0.00000025	0.25	7.17
6.48	0.025	0.00001	0.00000025	0.00000050	0.5	6.48
5.67	0.025	0.00001	0.00000025	0.00000075	0.75	5.67
3.64	0.1	0.00001	0.00000100	0.00000175	1.75	3.64
2.3	0.2	0.00001	0.00000200	0.00000375	3.75	2.3
1.79	0.5	0.00001	0.00000500	0.00000875	8.75	1.79

Sample 34						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.68	-	-	-	0.00000000	0	8.68
7.55	0.025	0.00001	0.00000025	0.00000025	0.25	7.55
7.09	0.025	0.00001	0.00000025	0.00000050	0.5	7.09
6.47	0.025	0.00001	0.00000025	0.00000075	0.75	6.47
5.62	0.025	0.00001	0.00000025	0.00000100	1	5.62
3.81	0.1	0.00001	0.00000100	0.00000200	2	3.81
2.4	0.2	0.00001	0.00000200	0.00000400	4	2.4

Sample 35						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.28	-	-	-	0.00000000	0	11.28
11	0.5	0.00001	0.00000500	0.00000500	5	11
7.39	0.5	0.00001	0.00000500	0.00001000	10	7.39
7.32	0.025	0.00001	0.00000025	0.00001025	10.25	7.32
7.02	0.05	0.00001	0.00000050	0.00001075	10.75	7.02
6.86	0.025	0.00001	0.00000025	0.00001100	11	6.86
6.71	0.05	0.00001	0.00000050	0.00001150	11.5	6.71
5.45	0.2	0.00001	0.00000200	0.00001350	13.5	5.45
2.01	0.5	0.00001	0.00000500	0.00001850	18.5	2.01

Sample 36						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.3	-	-	-	0.00000000	0	9.3
7.57	0.05	0.00001	0.00000050	0.00000050	0.5	7.57
7.2	0.025	0.00001	0.00000025	0.00000075	0.75	7.2
7.04	0.025	0.00001	0.00000025	0.00000100	1	7.04
6.81	0.025	0.00001	0.00000025	0.00000125	1.25	6.81
5.97	0.05	0.00001	0.00000050	0.00000175	1.75	5.97
2.69	0.2	0.00001	0.00000200	0.00000375	3.75	2.69

Sample 37						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.26	-	-	-	0.00000000		10.26
9.05	0.1	0.00001	0.00000100	0.00000100	1	9.05
7.4	0.05	0.00001	0.00000050	0.00000150	1.5	7.4
7.3	0.025	0.00001	0.00000025	0.00000175	1.75	7.3
7.22	0.025	0.00001	0.00000025	0.00000200	2	7.22
7.05	0.05	0.00001	0.00000050	0.00000250	2.5	7.05
7.01	0.025	0.00001	0.00000025	0.00000275	2.75	7.01
6.82	0.025	0.00001	0.00000025	0.00000300	3	6.82
6.43	0.05	0.00001	0.00000050	0.00000350	3.5	6.43
4.04	0.2	0.00001	0.00000200	0.00000550	5.5	4.04

Sample 39						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.63	-	-	-	0.00000000		9.63
7.71	0.05	0.00001	0.00000050	0.00000050	0.5	7.71
7.2	0.025	0.00001	0.00000025	0.00000075	0.75	7.2
6.95	0.025	0.00001	0.00000025	0.00000100	1	6.95
6.49	0.05	0.00001	0.00000050	0.00000150	1.5	6.49
4.83	0.1	0.00001	0.00000100	0.00000250	2.5	4.83
2.5	0.2	0.00001	0.00000200	0.00000450	4.5	2.5

Sample 40						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.41	-	-	-	0.00000000	0	9.41
7.01	0.1	0.00001	0.00000100	0.00000100	1	7.01
6.57	0.025	0.00001	0.00000025	0.00000125	1.25	6.57
6.4	0.025	0.00001	0.00000025	0.00000150	1.5	6.4
5.3	0.05	0.00001	0.00000050	0.00000200	2	5.3
3.93	0.1	0.00001	0.00000100	0.00000300	3	3.93
2.3	0.2	0.00001	0.00000200	0.00000500	5	2.3

Sample 41						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.8	-	-	-	0.00000000	0	11.8
11.86	1	0.00001	0.00001000	0.00001000	10	11.86
11.76	1	0.00001	0.00001000	0.00002000	20	11.76
11.85	3	0.00001	0.00003000	0.00005000	50	11.85
11.66	3	0.00001	0.00003000	0.00008000	80	11.66
11.6	1	0.00001	0.00001000	0.00009000	90	11.6
11.58	1	0.00001	0.00001000	0.00010000	100	11.58
11.1	3	0.00001	0.00003000	0.00013000	130	11.1
10.85	1	0.00001	0.00001000	0.00014000	140	10.85
9.15	1	0.00001	0.00001000	0.00015000	150	9.15
7.74	0.5	0.00001	0.00000500	0.00015500	155	7.74
7.71	0.2	0.00001	0.00000200	0.00015700	157	7.71
7.16	0.5	0.00001	0.00000500	0.00016200	162	7.16
6.75	0.5	0.00001	0.00000500	0.00016700	167	6.75
1.1	3	0.00001	0.00003000	0.00019700	197	1.1

Sample 42						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.86	-	-	-	0.00000000		9.86
8.21	0.05	0.00001	0.00000050	0.00000050	0.5	8.21
7.99	0.025	0.00001	0.00000025	0.00000075	0.75	7.99
7.63	0.05	0.00001	0.00000050	0.00000125	1.25	7.63
7.45	0.05	0.00001	0.00000050	0.00000175	1.75	7.45
7.19	0.05	0.00001	0.00000050	0.00000225	2.25	7.19
6.95	0.05	0.00001	0.00000050	0.00000275	2.75	6.95
6.68	0.05	0.00001	0.00000050	0.00000325	3.25	6.68
5.25	0.1	0.00001	0.00000100	0.00000425	4.25	5.25

Sample 43						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.68	-	-	-	0.00000000		8.68
7.1	0.025	0.00001	0.00000025	0.00000025	0.25	7.1
6.05	0.025	0.00001	0.00000025	0.00000050	0.5	6.05
5.08	0.025	0.00001	0.00000025	0.00000075	0.75	5.08
4.17	0.025	0.00001	0.00000025	0.00000100	1	4.17
3.36	0.025	0.00001	0.00000025	0.00000125	1.25	3.36
2.97	0.025	0.00001	0.00000025	0.00000150	1.5	2.97

Sample 44						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
	-	-	-			
11.34					0	11.34
11.07	0.5	0.00001	0.00000500	0.00000500	5	11.07
10.44	0.5	0.00001	0.00000500	0.00001000	10	10.44
8.41	0.2	0.00001	0.00000200	0.00001200	12	8.41
7.6	0.1	0.00001	0.00000100	0.00001300	13	7.6
7.42	0.05	0.00001	0.00000050	0.00001350	13.5	7.42
7.3	0.05	0.00001	0.00000050	0.00001400	14	7.3
7.17	0.05	0.00001	0.00000050	0.00001450	14.5	7.17
7.01	0.05	0.00001	0.00000050	0.00001500	15	7.01
6.85	0.05	0.00001	0.00000050	0.00001550	15.5	6.85
6.4	0.1	0.00001	0.00000100	0.00001650	16.5	6.4
5.9	0.2	0.00001	0.00000200	0.00001850	18.5	5.9
2.97	0.5	0.00001	0.00000500	0.00002350	23.5	2.97

Sample 45						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	pH	micromoles
9.28	-	-	-			
7.47	0.05	0.00001	0.00000050	0.00000050	7.47	0.5
7.44	0.025	0.00001	0.00000025	0.00000075	7.44	0.75
7.05	0.05	0.00001	0.00000050	0.00000125	7.05	1.25
6.93	0.025	0.00001	0.00000025	0.00000150	6.93	1.5
6.15	0.05	0.00001	0.00000050	0.00000200	6.15	2
4.6	0.1	0.00001	0.00000100	0.00000300	4.6	3
2.41	0.2	0.00001	0.00000200	0.00000500	2.41	5

Sample 46						
pH	[HCl]	HCl (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.31				0.00000000	0	10.31
9.82	0.05	0.00001	0.00000050	0.00000050	0.5	9.82
7.85	0.05	0.00001	0.00000050	0.00000100	1	7.85
7.36	0.025	0.00001	0.00000025	0.00000125	1.25	7.36
7.15	0.025	0.00001	0.00000025	0.00000150	1.5	7.15
7.1	0.025	0.00001	0.00000025	0.00000175	1.75	7.1
6.9	0.025	0.00001	0.00000025	0.00000200	2	6.9
6.15	0.05	0.00001	0.00000050	0.00000250	2.5	6.15
4.54	0.1	0.00001	0.00000100	0.00000350	3.5	4.54
1.88	0.5	0.00001	0.00000500	0.00000850	8.5	1.88

Sample 47						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.44				0.00000000	0	8.44
7.2	0.025	0.00001	0.00000025	0.00000025	0.25	7.2
6.48	0.025	0.00001	0.00000025	0.00000050	0.5	6.48
5.32	0.025	0.00001	0.00000025	0.00000075	0.75	5.32
4.87	0.025	0.00001	0.00000025	0.00000100	1	4.87
3.62	0.05	0.00001	0.0000005	0.00000150	1.5	3.62
2.49	0.1	0.00001	0.000001	0.00000250	2.5	2.49

Sample 48						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.01				0.00000000	0	9.01
7.95	0.025	0.00001	0.00000025	0.00000025	0.25	7.95
7.66	0.025	0.00001	0.00000025	0.00000050	0.5	7.66
7.37	0.025	0.00001	0.00000025	0.00000075	0.75	7.37
7.1	0.025	0.00001	0.00000025	0.00000100	1	7.1
7.03	0.025	0.00001	0.00000025	0.00000125	1.25	7.03
6.7	0.025	0.00001	0.00000025	0.00000150	1.5	6.7
5.52	0.05	0.00001	0.0000005	0.00000200	2	5.52
3.5	0.1	0.00001	0.000001	0.00000300	3	3.5

Sample 49						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.81				0.00000000	0	8.81
7.41	0.025	0.00001	0.00000025	0.00000025	0.25	7.41
6.73	0.025	0.00001	0.00000025	0.00000050	0.5	6.73
5.76	0.025	0.00001	0.00000025	0.00000075	0.75	5.76
5.14	0.025	0.00001	0.00000025	0.00000100	1	5.14
3.99	0.05	0.00001	0.0000005	0.00000150	1.5	3.99
2.66	0.1	0.00001	0.000001	0.00000250	2.5	2.66

Sample 50						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.36				0.00000000	0	8.36
7.13	0.025	0.00001	0.00000025	0.00000025	0.25	7.13
6.19	0.025	0.00001	0.00000025	0.00000050	0.5	6.19
5.16	0.025	0.00001	0.00000025	0.00000075	0.75	5.16
4.39	0.025	0.00001	0.00000025	0.00000100	1	4.39
3.69	0.025	0.00001	0.00000025	0.00000125	1.25	3.69
2.44	0.1	0.00001	0.000001	0.00000225	2.25	2.44

Sample 51						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.15				0.00000000	0	9.15
7.58	0.05	0.00001	0.0000005	0.00000050	0.5	7.58
7.43	0.025	0.00001	0.00000025	0.00000075	0.75	7.43
7.25	0.025	0.00001	0.00000025	0.00000100	1	7.25
7.11	0.025	0.00001	0.00000025	0.00000125	1.25	7.11
7.01	0.025	0.00001	0.00000025	0.00000150	1.5	7.01
6.78	0.025	0.00001	0.00000025	0.00000175	1.75	6.78
6.11	0.05	0.00001	0.0000005	0.00000225	2.25	6.11
4.59	0.1	0.00001	0.000001	0.00000325	3.25	4.59
2.29	0.2	0.00001	0.000002	0.00000525	5.25	2.29

Sample 52						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.1				0.00000000	0	11.1
10.69	0.2	0.00001	0.000002	0.00000200	2	10.69
7.26	0.2	0.00001	0.000002	0.00000400	4	7.26
7.02	0.025	0.00001	0.00000025	0.00000425	4.25	7.02
6.97	0.025	0.00001	0.00000025	0.00000450	4.5	6.97
6.4	0.05	0.00001	0.0000005	0.00000500	5	6.4
4.75	0.2	0.00001	0.000002	0.00000700	7	4.75
1.8	0.5	0.00001	0.000005	0.00001200	12	1.8

Sample 53						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
10.35				0.00000000	0	10.35
9.18	0.05	0.00001	0.0000005	0.00000050	0.5	9.18
7.4	0.05	0.00001	0.0000005	0.00000100	1	7.4
7.21	0.025	0.00001	0.00000025	0.00000125	1.25	7.21
7.03	0.025	0.00001	0.00000025	0.00000150	1.5	7.03
6.83	0.025	0.00001	0.00000025	0.00000175	1.75	6.83
5.93	0.05	0.00001	0.0000005	0.00000225	2.25	5.93
4.35	0.1	0.00001	0.000001	0.00000325	3.25	4.35
1.71	0.5	0.00001	0.000005	0.00000825	8.25	1.71

Sample 54						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
11.65				0.00000000	0	11.65
11.57	0.5	0.00001	0.000005	0.00000500	5	11.57
11.2	1	0.00001	0.00001	0.00001500	15	11.2
10.34	0.5	0.00001	0.000005	0.00002000	20	10.34
8.43	0.2	0.00001	0.000002	0.00002200	22	8.43
8.06	0.1	0.00001	0.000001	0.00002300	23	8.06
7.96	0.05	0.00001	0.0000005	0.00002350	23.5	7.96
7.78	0.1	0.00001	0.000001	0.00002450	24.5	7.78
7.12	0.2	0.00001	0.000002	0.00002650	26.5	7.12
7.04	0.1	0.00001	0.000001	0.00002750	27.5	7.04
6.83	0.1	0.00001	0.000001	0.00002850	28.5	6.83
3.1	0.2	0.00001	0.000002	0.00003050	30.5	3.1

Sample 55						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
8.52				0.00000000	0	8.52
7.17	0.025	0.00001	0.00000025	0.00000025	0.25	7.17
6.11	0.025	0.00001	0.00000025	0.00000050	0.5	6.11
5.01	0.025	0.00001	0.00000025	0.00000075	0.75	5.01
4.15	0.025	0.00001	0.00000025	0.00000100	1	4.15
3.08	0.05	0.00001	0.0000005	0.00000150	1.5	3.08
2.35	0.1	0.00001	0.000001	0.00000250	2.5	2.35

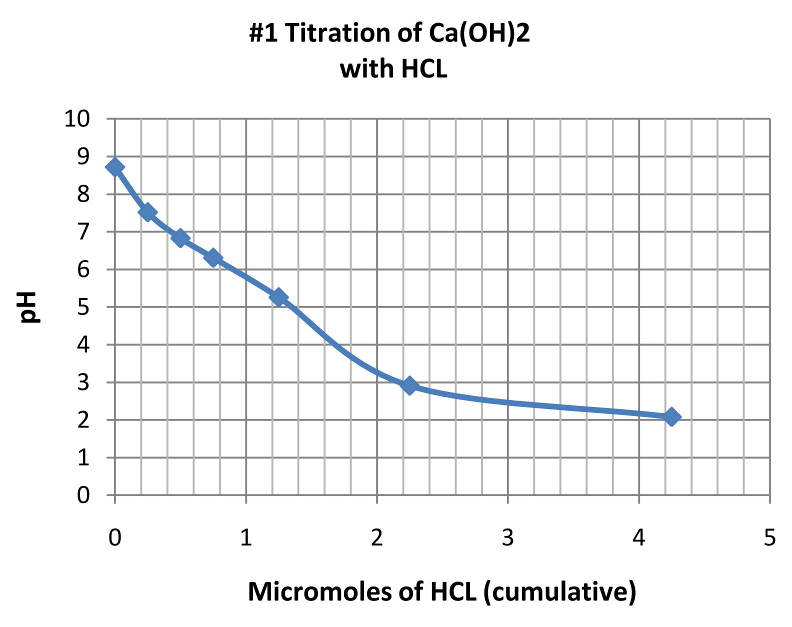
Sample 56						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.67				0.00000000	0	9.67
7.55	0.05	0.00001	0.0000005	0.00000050	0.5	7.55
7.2	0.025	0.00001	0.00000025	0.00000075	0.75	7.2
6.93	0.025	0.00001	0.00000025	0.00000100	1	6.93
6.47	0.025	0.00001	0.00000025	0.00000125	1.25	6.47
5.51	0.05	0.00001	0.0000005	0.00000175	1.75	5.51
4.01	0.1	0.00001	0.000001	0.00000275	2.75	4.01

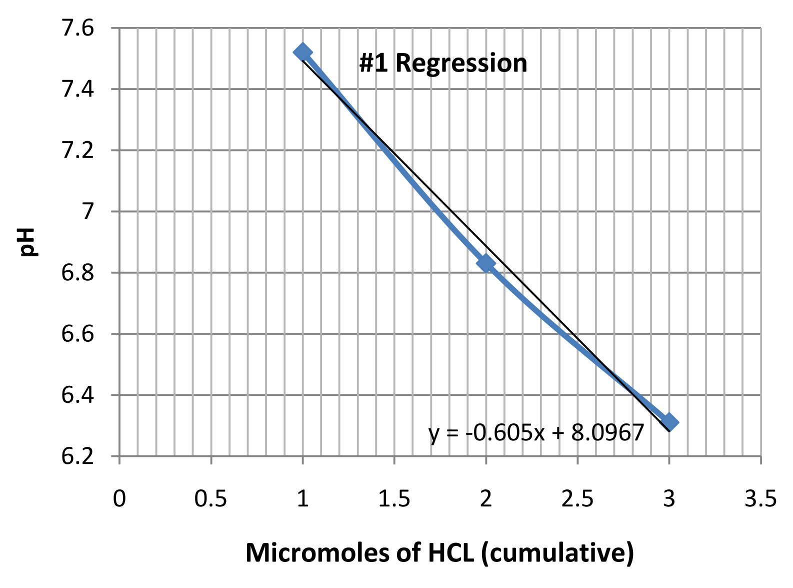
Sample 57						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
7.17				0.00000000	0	7.17
3.05	0.025	0.00001	0.00000025	0.00000025	0.25	3.05
2.46	0.025	0.00001	0.00000025	0.00000050	0.5	2.46
2.34	0.025	0.00001	0.00000025	0.00000075	0.75	2.34
2.24	0.05	0.00001	0.0000005	0.00000125	1.25	2.24
2	0.1	0.00001	0.000001	0.00000225	2.25	2
1.71	0.2	0.00001	0.000002	0.00000425	4.25	1.71

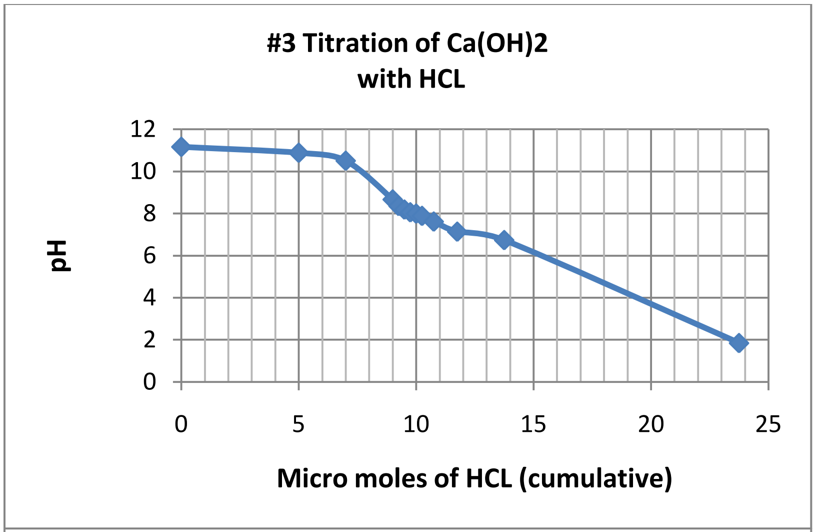
Sample 58						
pH	[HCl]	HCL (L)	HCl Added (m	HCl Cumulative (	Micromol	pH
9.47				0.00000000	0	9.47
8.13	0.05	0.00001	0.0000005	0.00000050	0.5	8.13
7.86	0.025	0.00001	0.00000025	0.00000075	0.75	7.86
7.58	0.05	0.00001	0.0000005	0.00000125	1.25	7.58
7.29	0.05	0.00001	0.0000005	0.00000175	1.75	7.29
7.11	0.05	0.00001	0.0000005	0.00000225	2.25	7.11
6.98	0.05	0.00001	0.0000005	0.00000275	2.75	6.98
6.52	0.05	0.00001	0.0000005	0.00000325	3.25	6.52
5.67	0.1	0.00001	0.000001	0.00000425	4.25	5.67
2.02	0.5	0.00001	0.000005	0.00000925	9.25	2.02

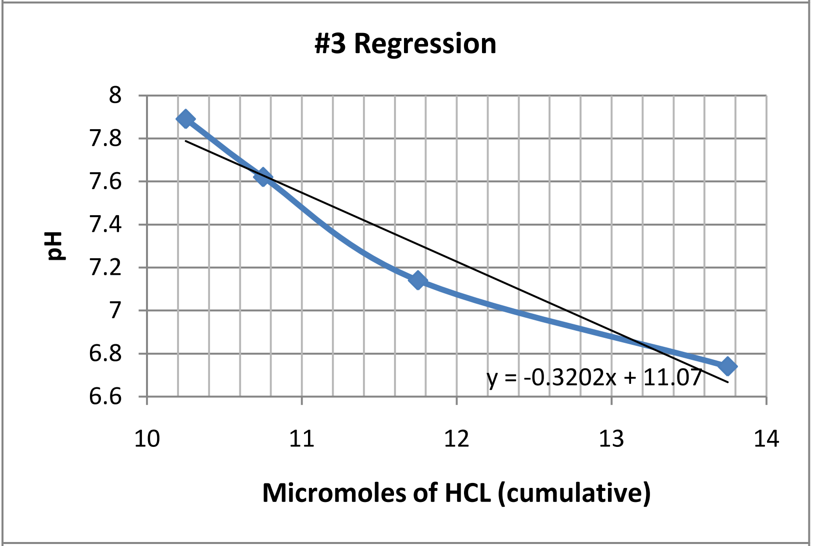
Appendix IV.

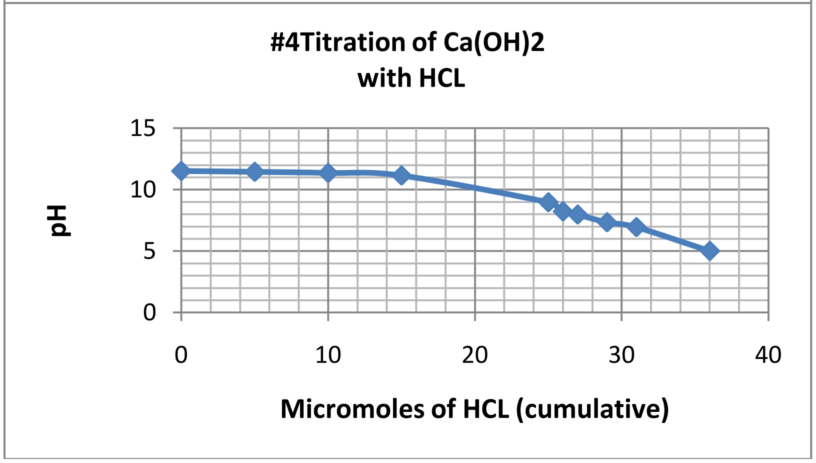
Titration graphs and linear regression graphs for samples.

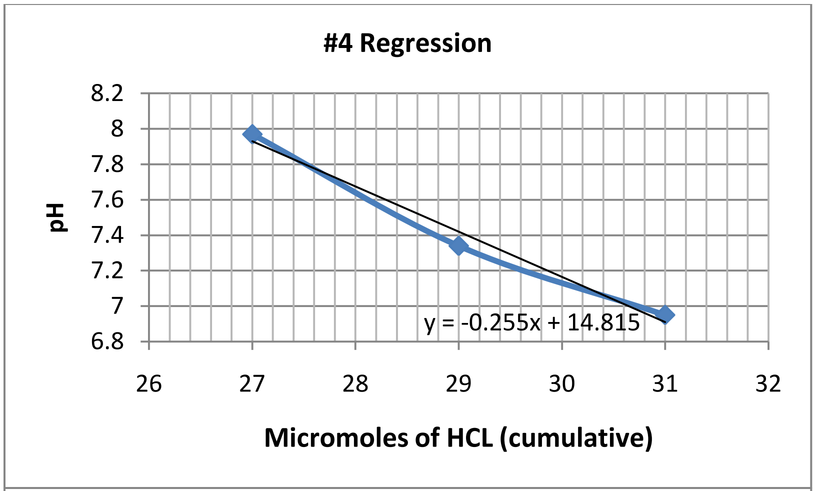




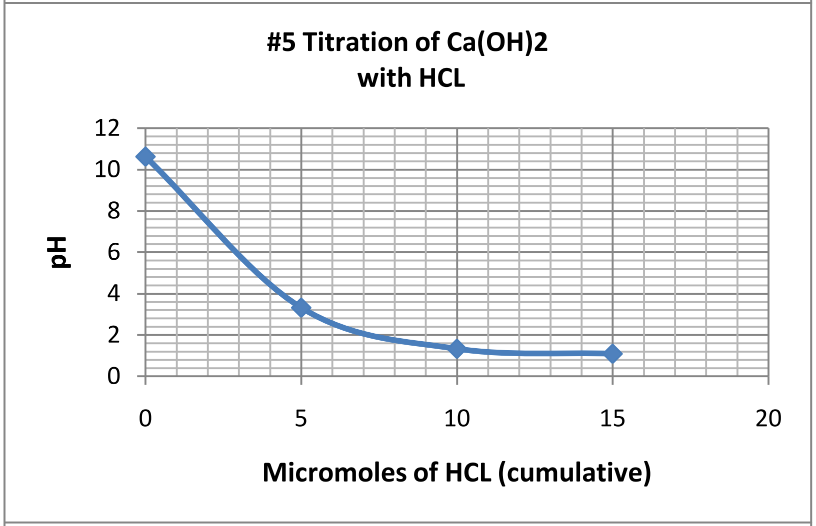

#3 Titration of Ca(OH)2
with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
20
25
Micro moles of HCL (cumulative)


#3 Regression
pH
8
7.8
7.6
7.4
7.2
7
6.8
6.6
10
11
12
13
14
y = -0.3202x + 11.07
Micromoles of HCL (cumulative)

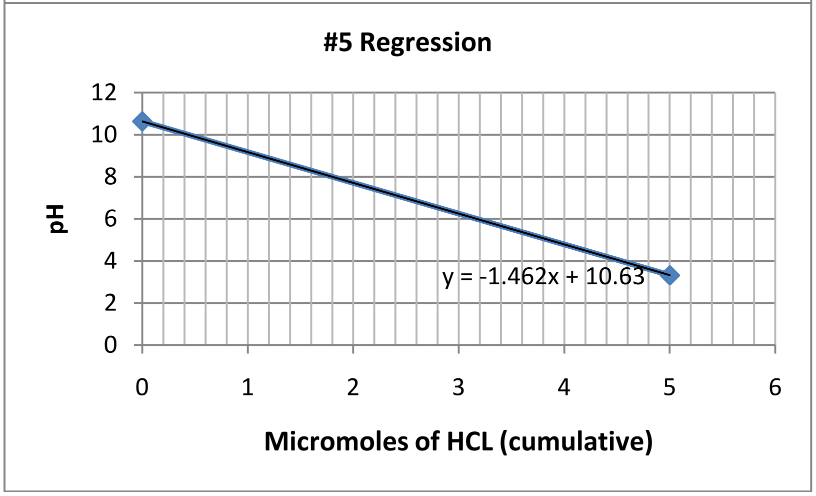

#4Titration of Ca(OH)2
with HCL
pH
15
10
5
0
0
10
20
30
40
Micromoles of HCL (cumulative)



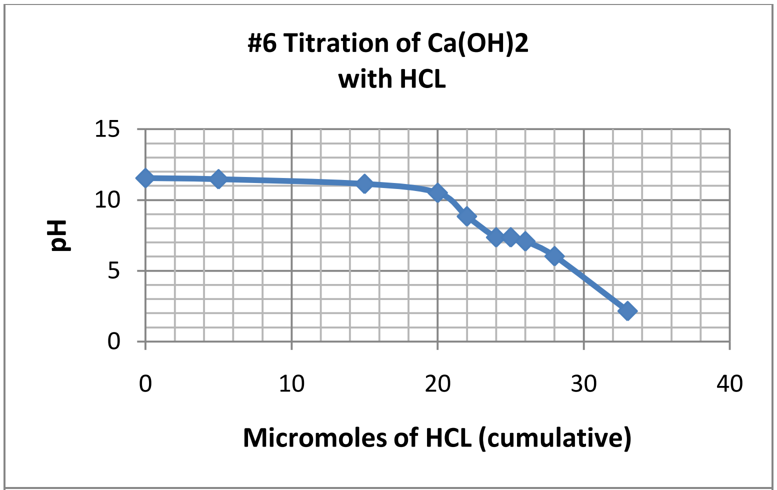
#4 Regression

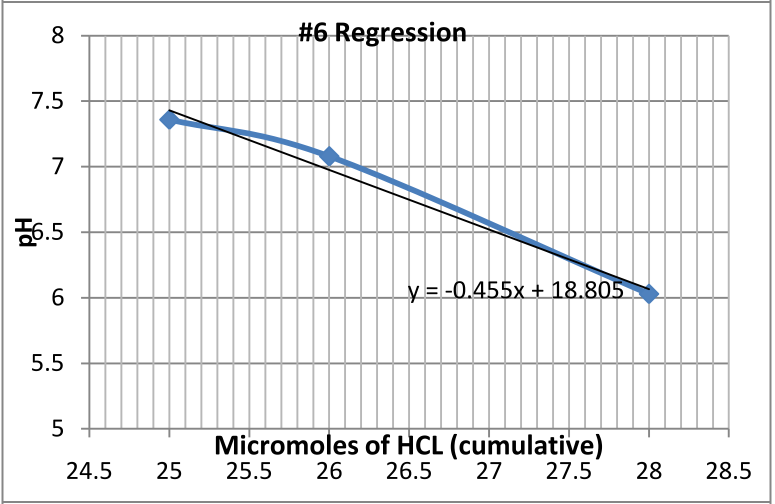


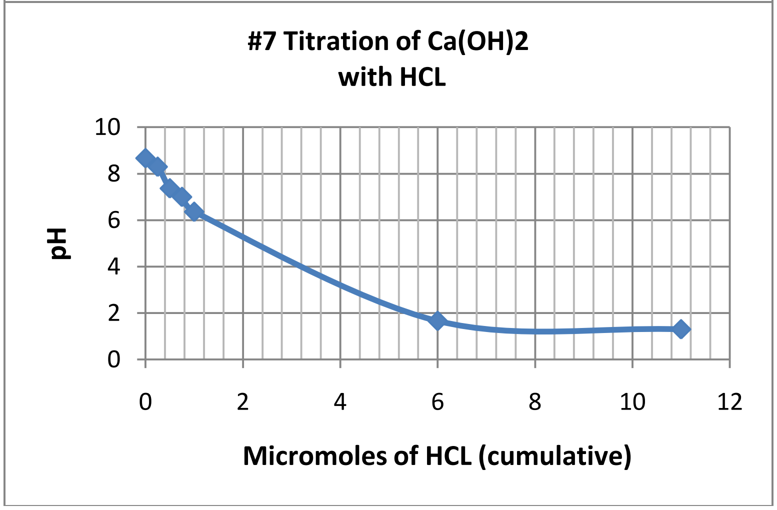
#5 Titration of Ca(OH)2 with HCL

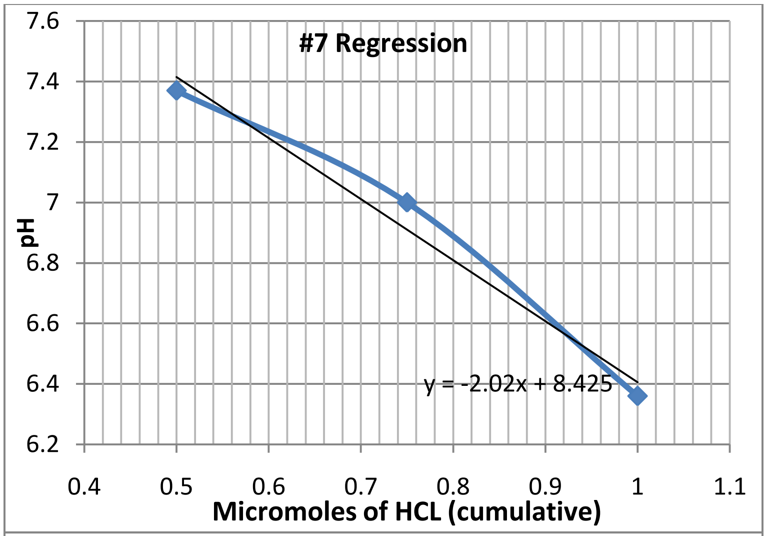


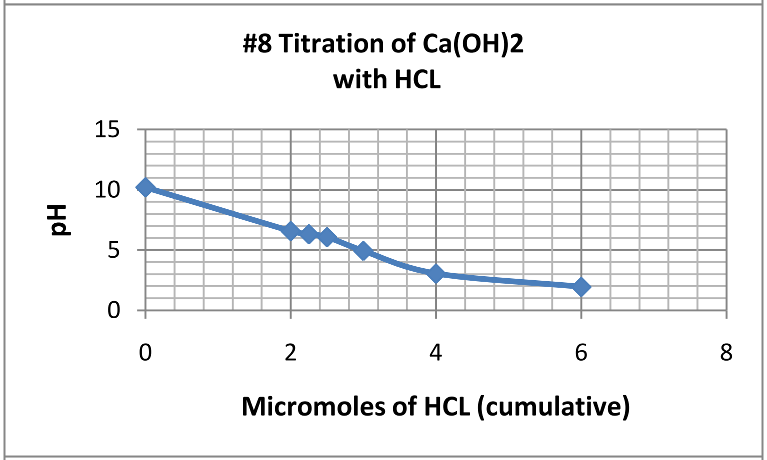
#5 Regression

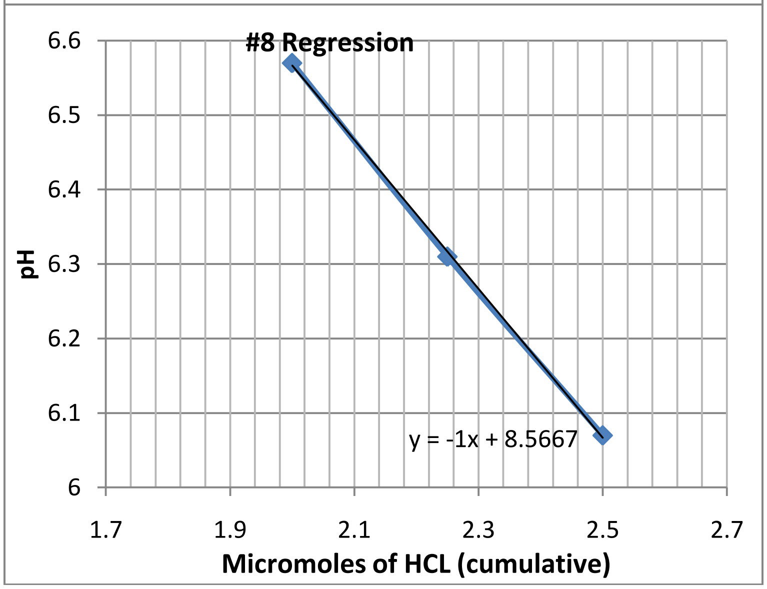

#6 Titration of Ca(OH)2 with HCL

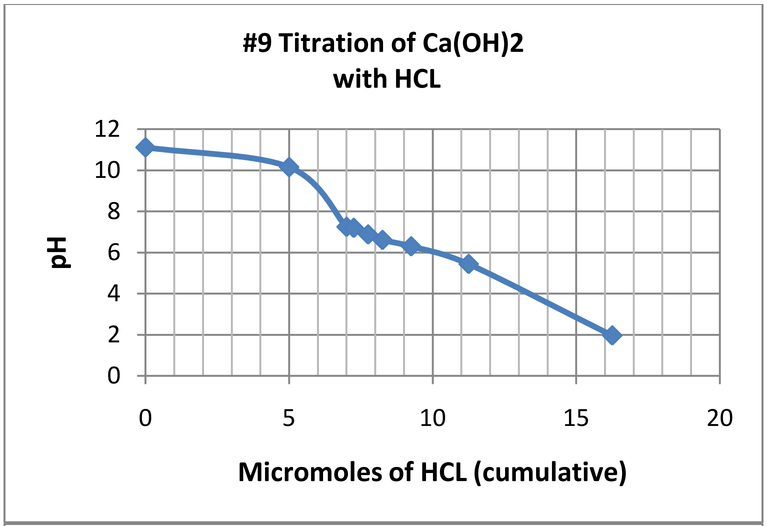

#6 Regression

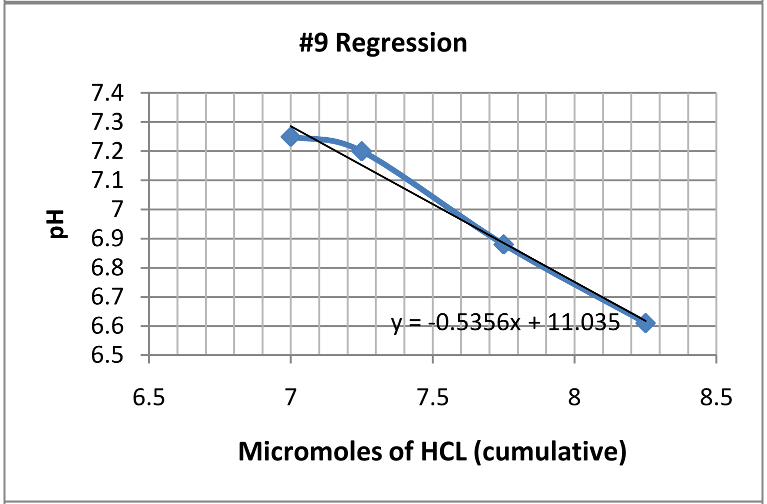

#7 Titration of Ca(OH)2 with HCL

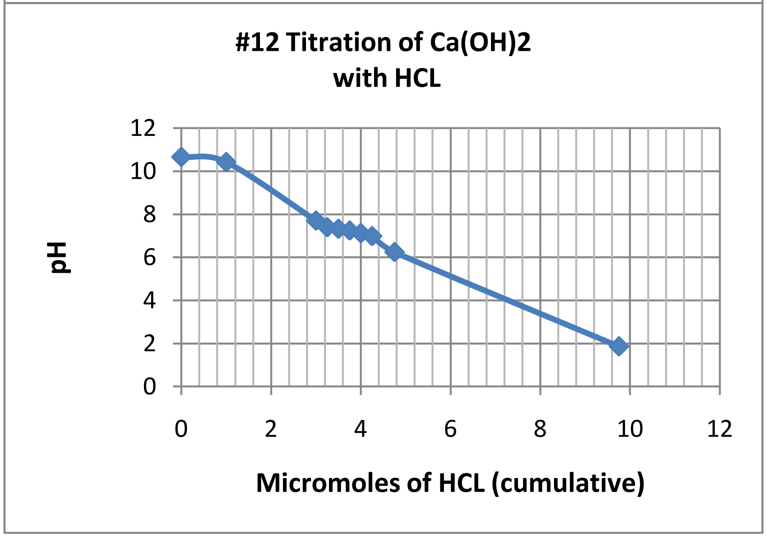

#7 Regression
pH
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
y = -2.02x + 8.425
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
Micromoles of HCL (cumulative)

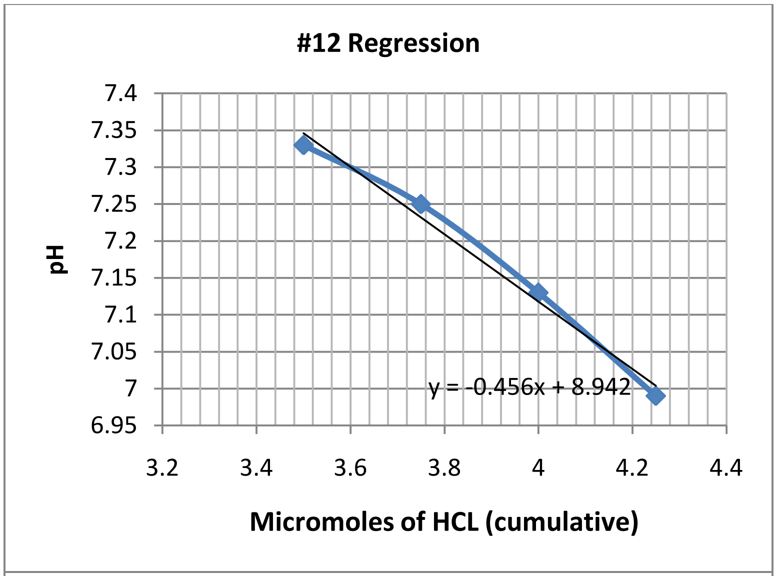

#8 Titration of Ca(OH)2
with HCL
pH
15
10
5
0
0
2
4
6
8
Micromoles of HCL (cumulative)

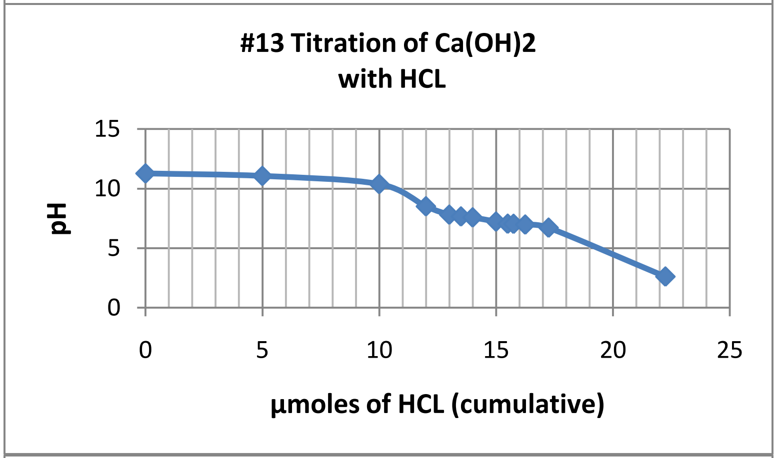

#8 Regression
pH
6.6
6.5
6.4
6.3
6.2
6.1
6
y = -1x + 8.5667
1.7
1.9
2.1
2.3
2.5
2.7
Micromoles of HCL (cumulative)

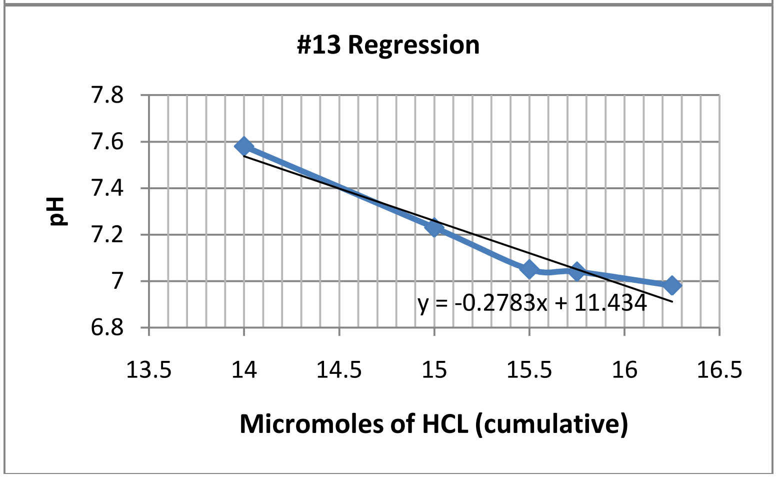

#9 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
20
Micromoles of HCL (cumulative)

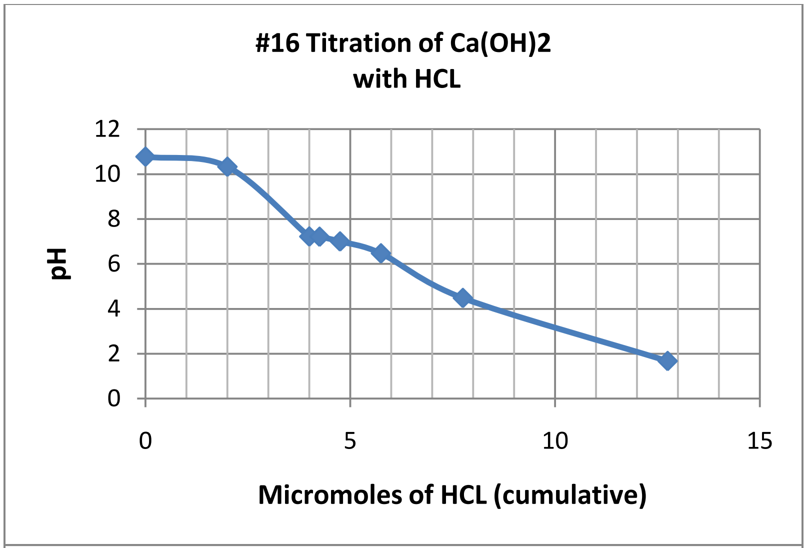

#9 Regression
pH
7.4
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
6.5
7
7.5
8
8.5
y = -0.5356x + 11.035
Micromoles of HCL (cumulative)

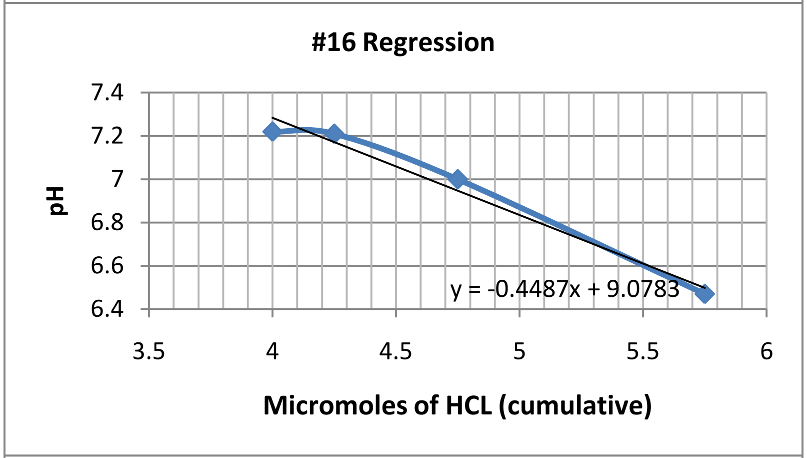

#12 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
12
Micromoles of HCL (cumulative)

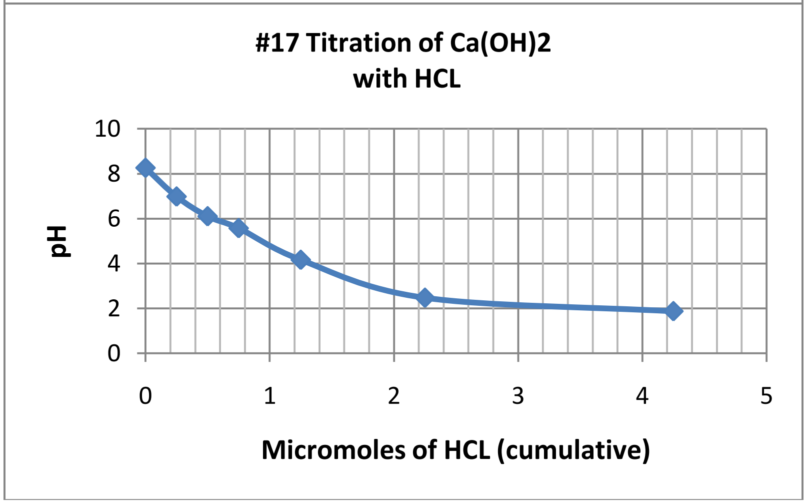

#12 Regression
pH
7.4
7.35
7.3
7.25
7.2
7.15
7.1
7.05
7
6.95
3.2
3.4
3.6
3.8
4
4.2
4.4
y = -0.456x + 8.942
Micromoles of HCL (cumulative)

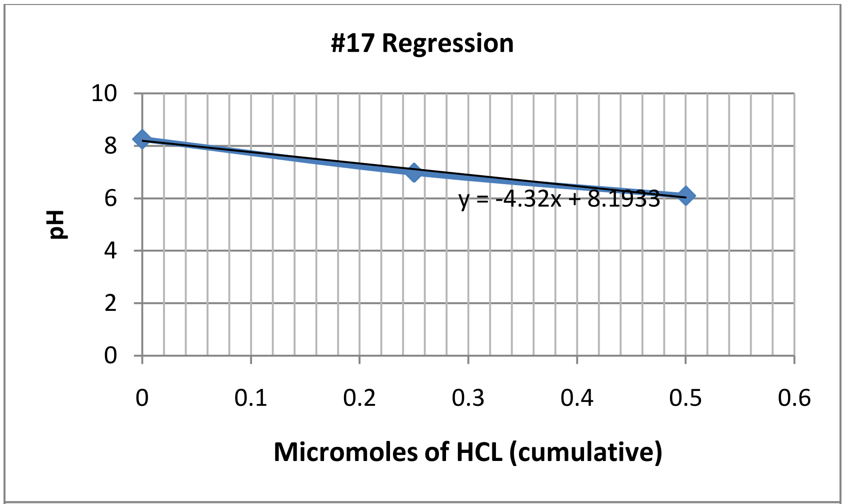

#13 Titration of Ca(OH)2 with HCL
pH
15
10
5
0
0
5
10
15
20
25
µmoles of HCL (cumulative)


#13 Regression
pH
7.8
7.6
7.4
7.2
7
6.8
13.5
14
14.5
15
15.5
16
16.5
y = -0.2783x + 11.434
Micromoles of HCL (cumulative)

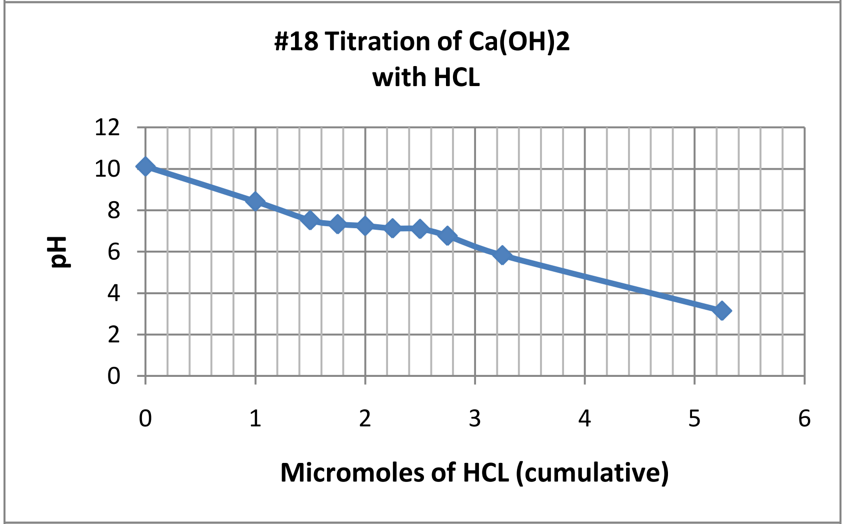

#16 Titration of Ca(OH)2 with HCL


#16 Regression

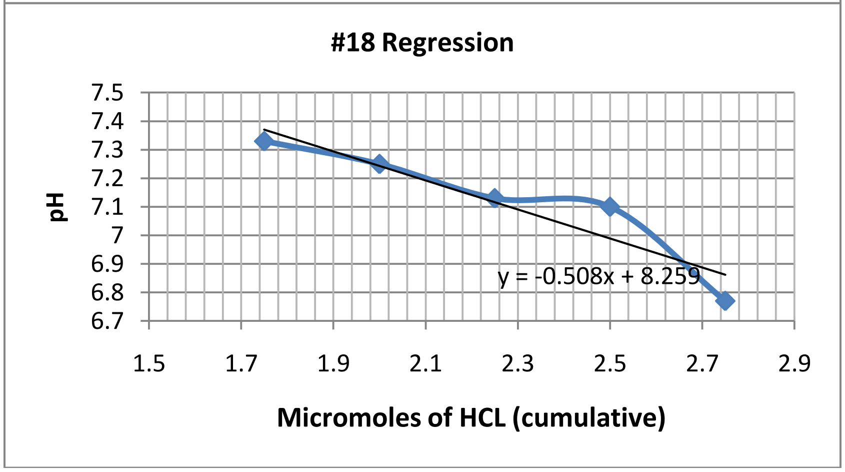

#17 Titration of Ca(OH)2 with HCL



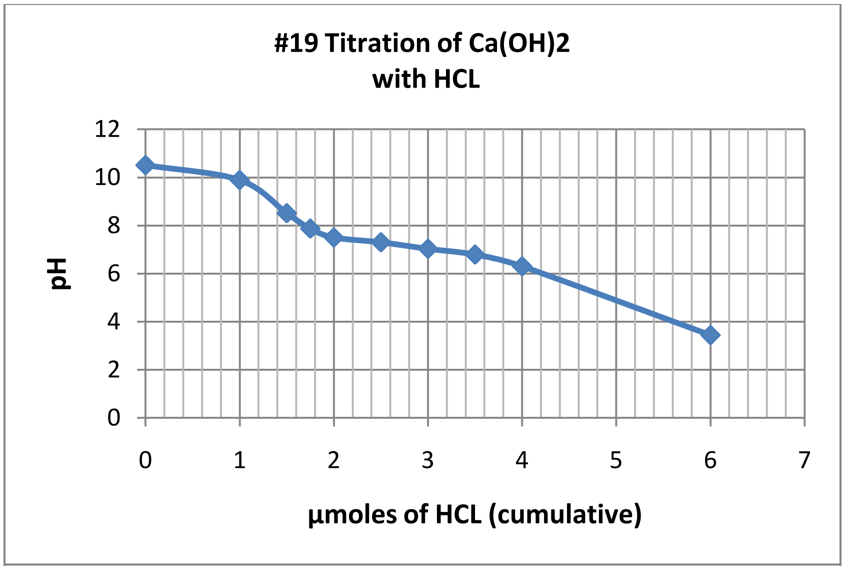
#17 Regression

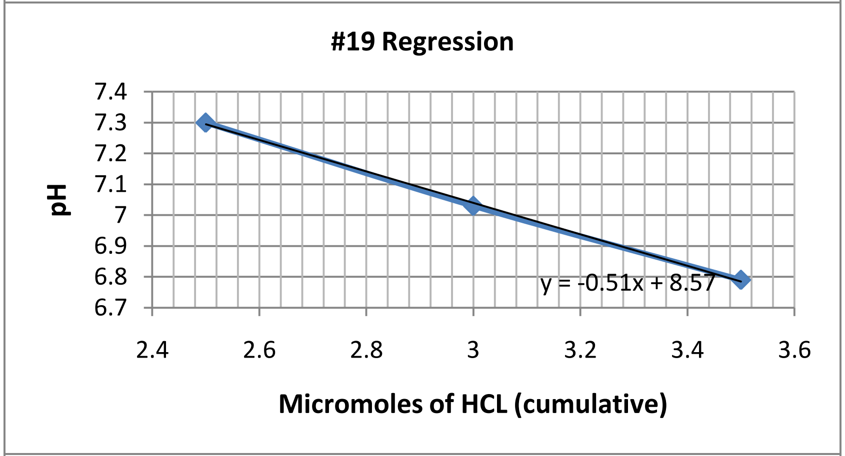


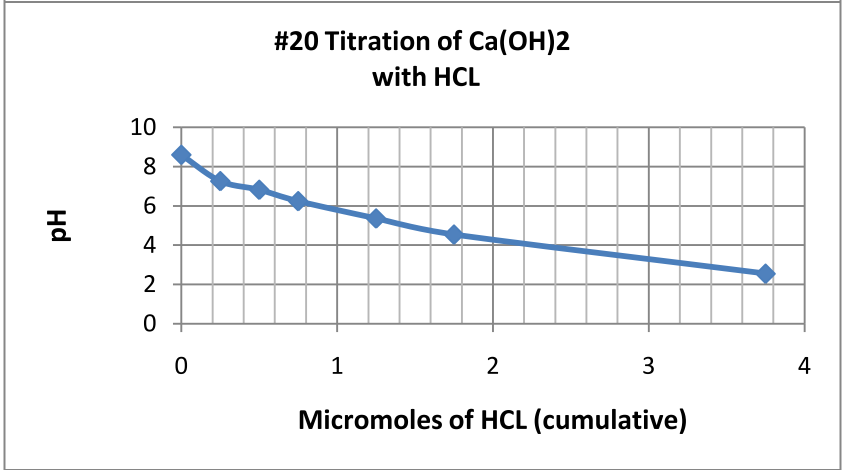
#18 Titration of Ca(OH)2 with HCL

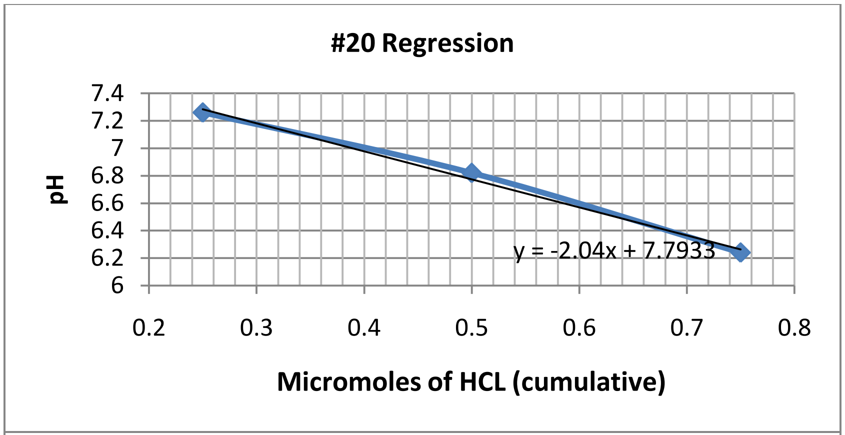


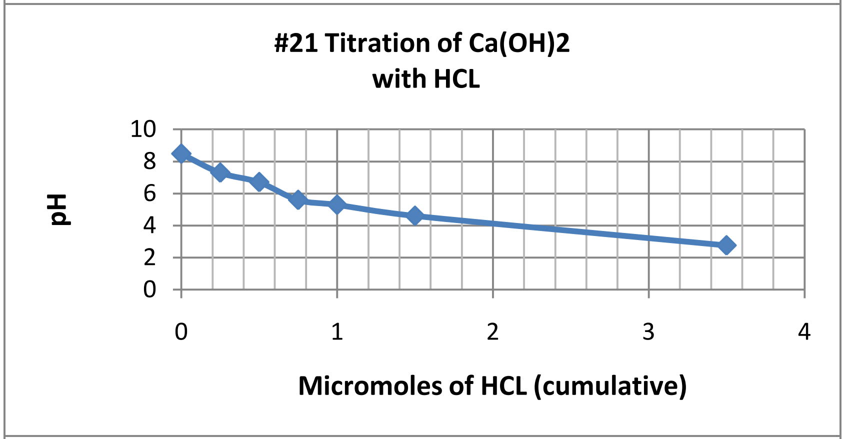
#18 Regression

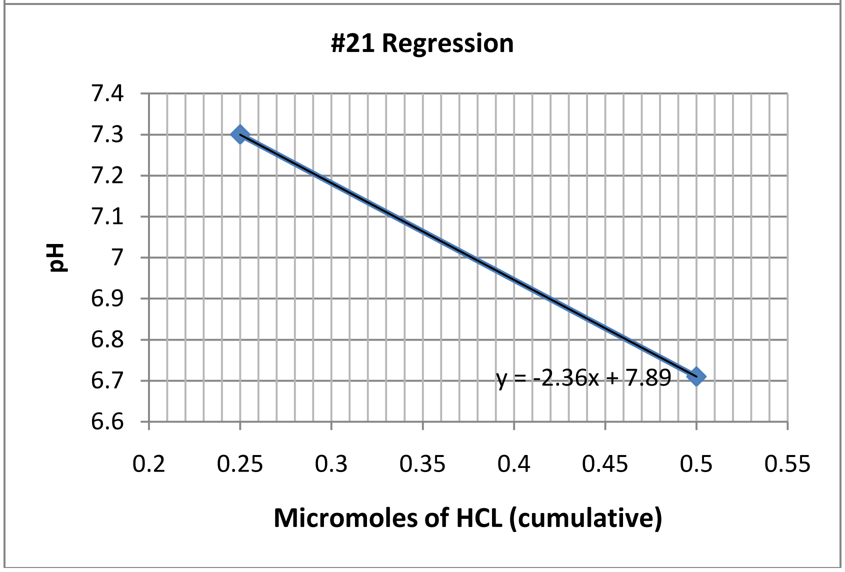

#19 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
µmoles of HCL (cumulative)

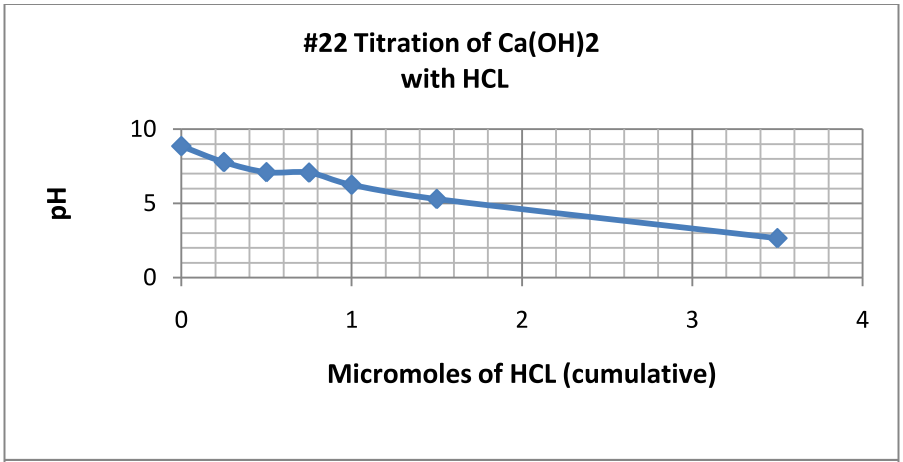

#19 Regression
pH
7.4
7.3
7.2
7.1
7
6.9
6.8
6.7
2.4
2.6
2.8
3
3.2
3.4
3.6
y = -0.51x + 8.57
Micromoles of HCL (cumulative)

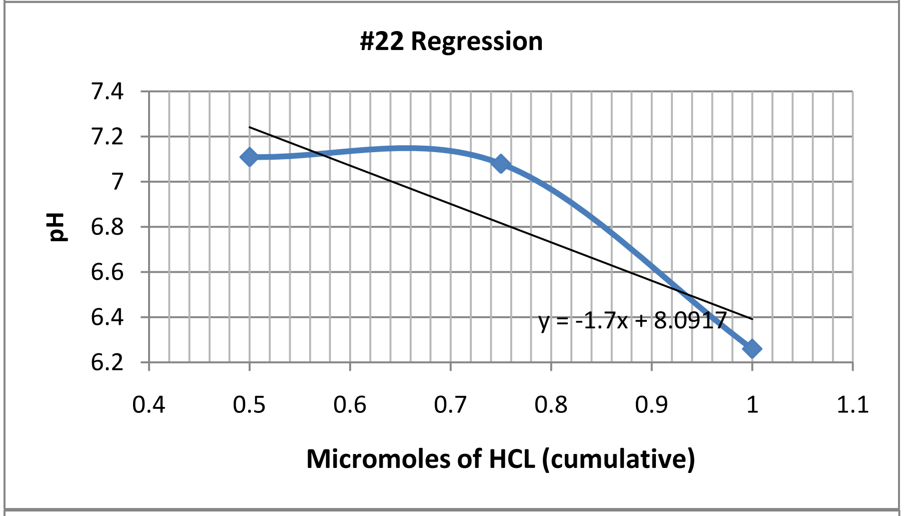

#20 Titration of Ca(OH)2 with HCL
pH
10
8
6
4
2
0
0
1
2
3
4
Micromoles of HCL (cumulative)

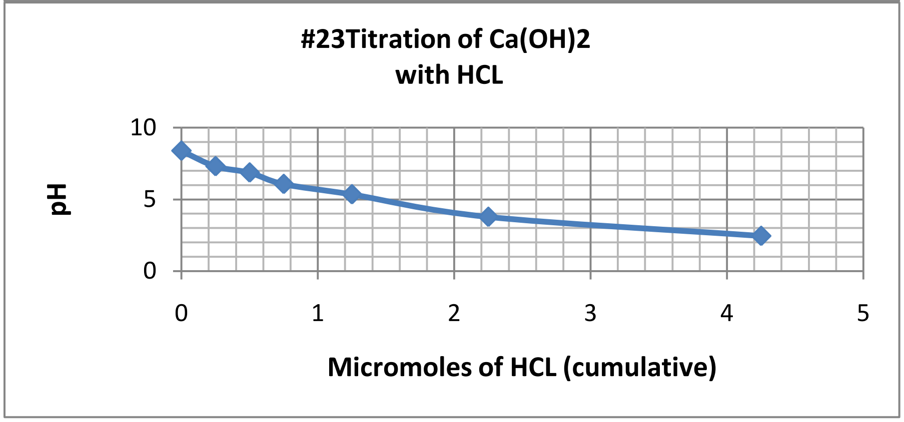

#20 Regression
pH
7.4
7.2
7
6.8
6.6
6.4
6.2
6
y = -2.04x + 7.7933
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Micromoles of HCL (cumulative)

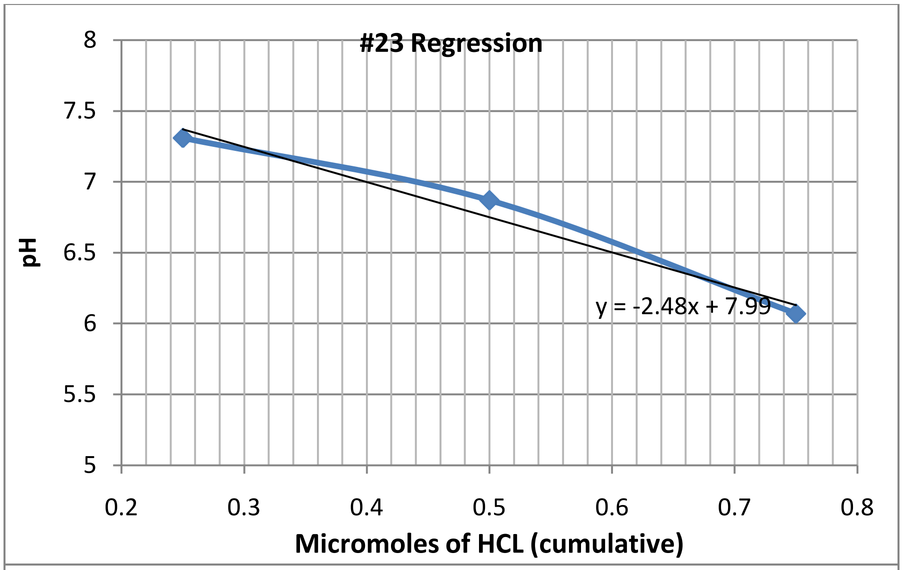

#21 Titration of Ca(OH)2
with HCL
pH
10
8
6
4
2
0
0
1
2
3
4
Micromoles of HCL (cumulative)

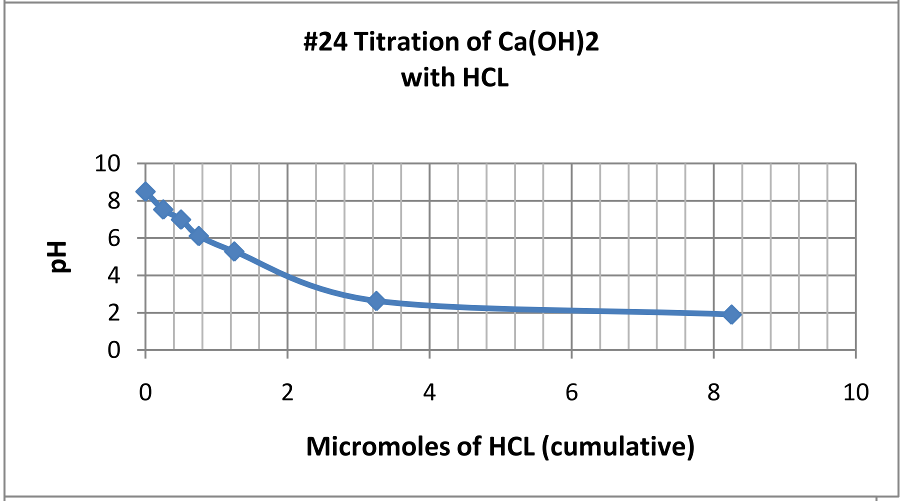

#21 Regression
pH
7.4
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
y = -2.36x + 7.89
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
Micromoles of HCL (cumulative)

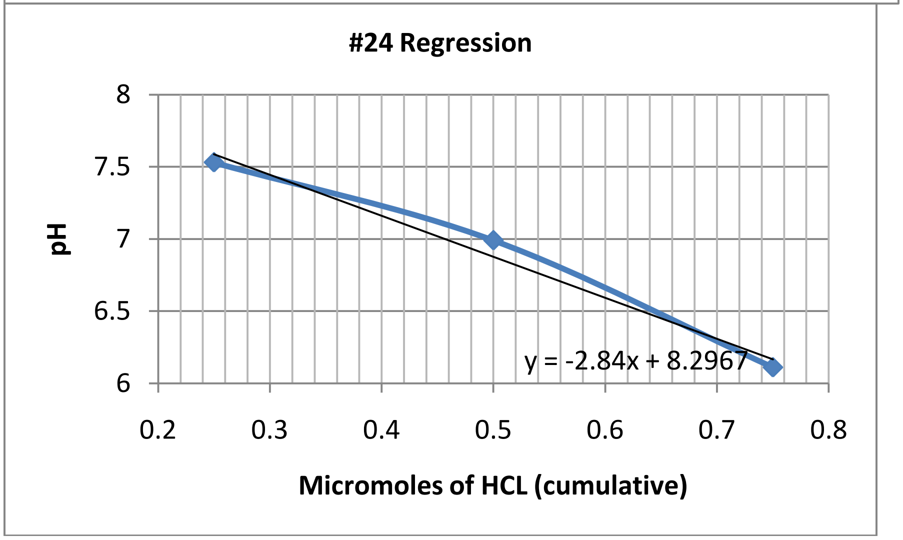

#22 Titration of Ca(OH)2
with HCL
10
5
0
pH
0
1
2
3
4
Micromoles of HCL (cumulative)

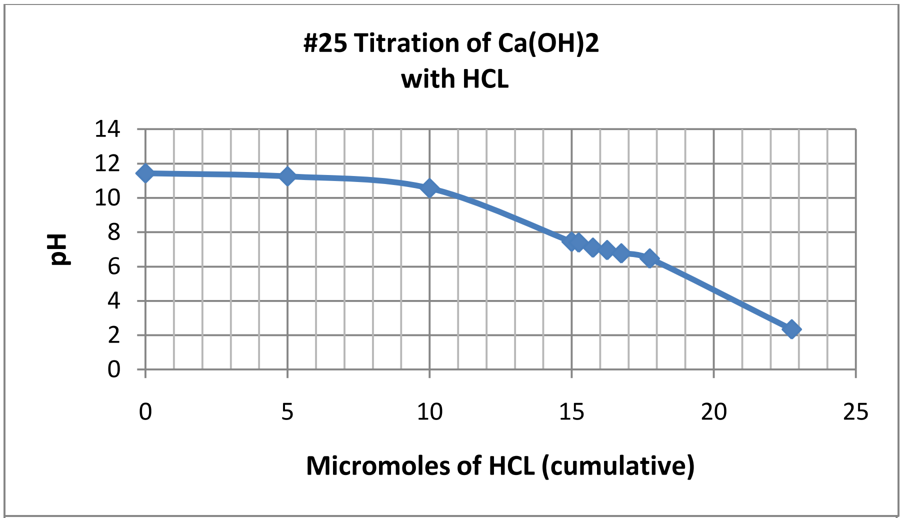

#22 Regression
7.4
7.2
7
6.8
6.6
6.4
6.2
pH
y = -1.7x + 8.0917
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
Micromoles of HCL (cumulative)


#23Titration of Ca(OH)2
with HCL
10
5
0
pH
0
1
2
3
4
5
Micromoles of HCL (cumulative)

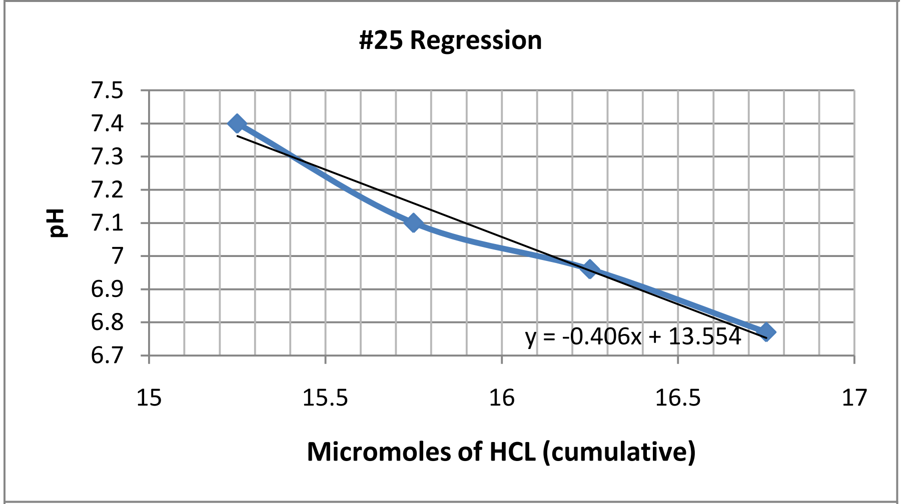

#23 Regression
pH
8
7.5
7
6.5
6
5.5
5
0.2
0.3
0.4
0.5
0.6
0.7
0.8
y = -2.48x + 7.99
Micromoles of HCL (cumulative)


#24 Titration of Ca(OH)2
with HCL
pH
10
8
6
4
2
0
0
2
4
6
8
10
Micromoles of HCL (cumulative)

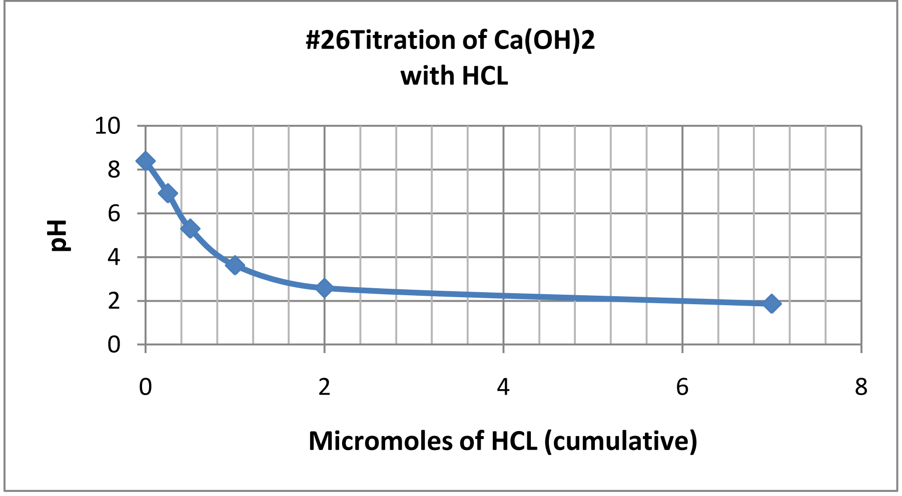

#24 Regression
pH
8
7.5
7
6.5
6
0.2
0.3
0.4
0.5
0.6
0.7
0.8
y = -2.84x + 8.2967
Micromoles of HCL (cumulative)



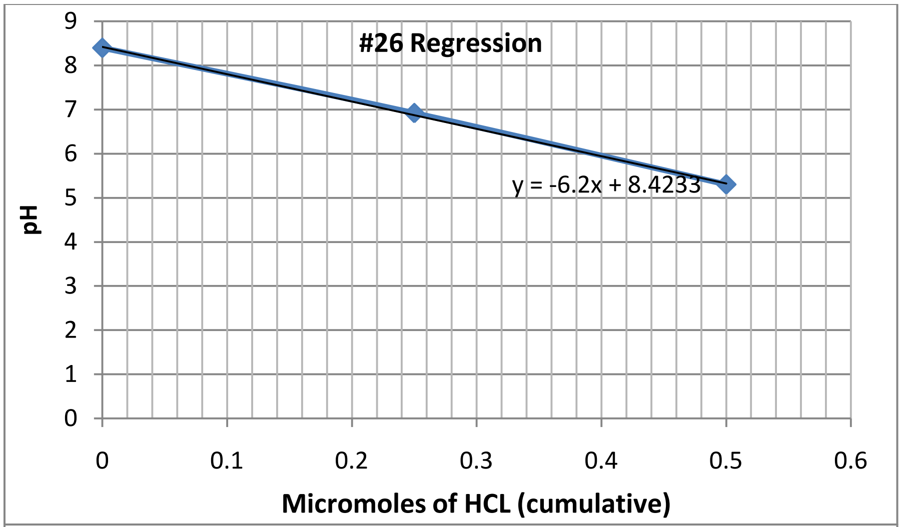
#25 Titration of Ca(OH)2 with HCL

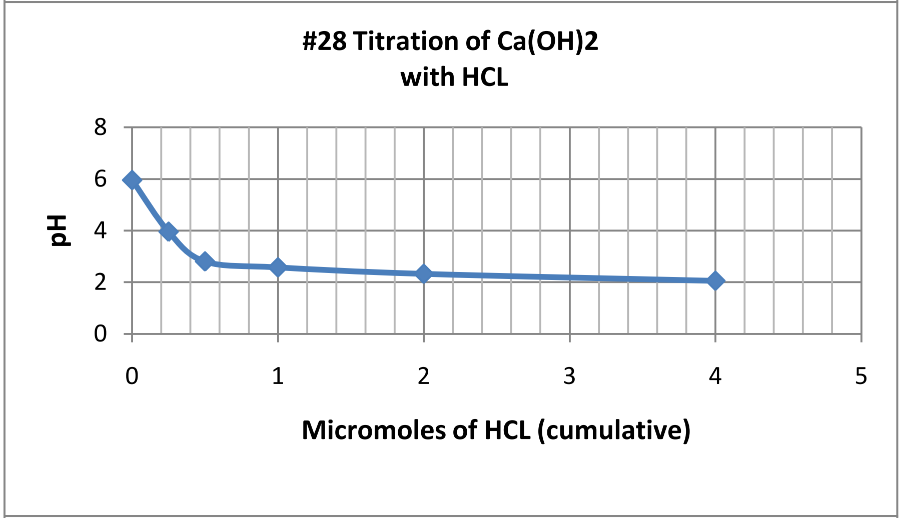


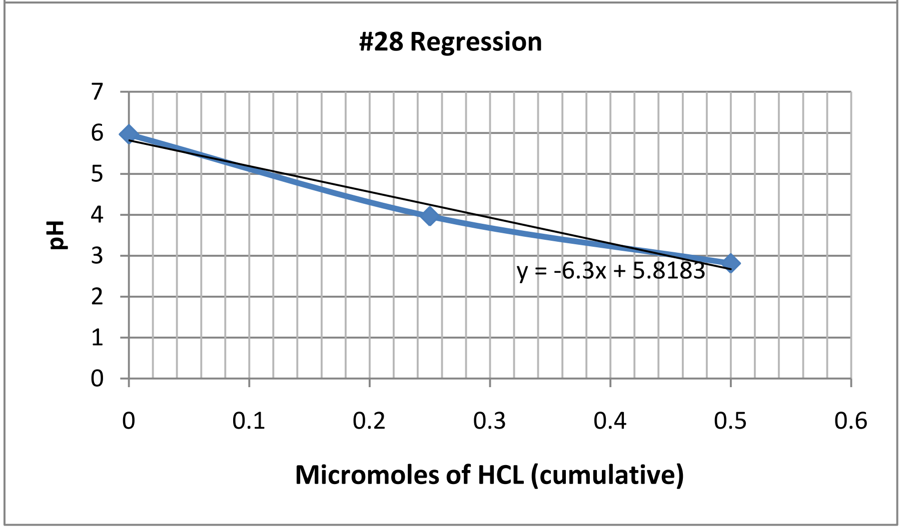
#25 Regression

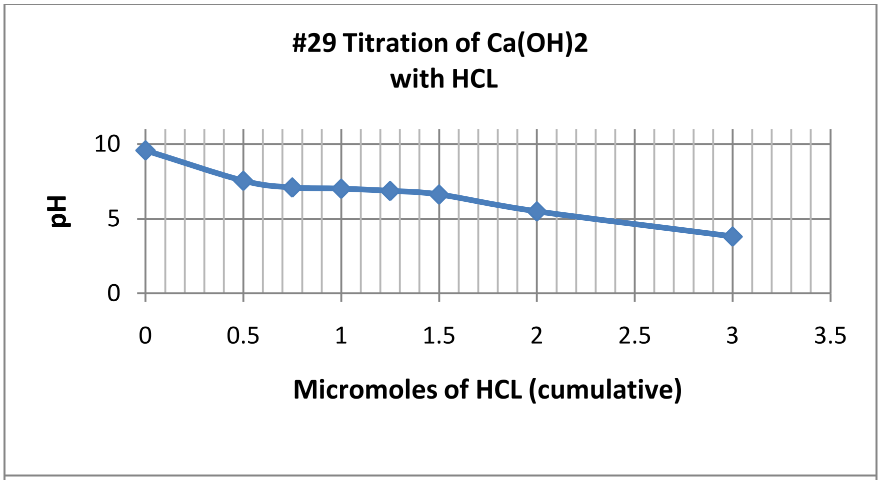


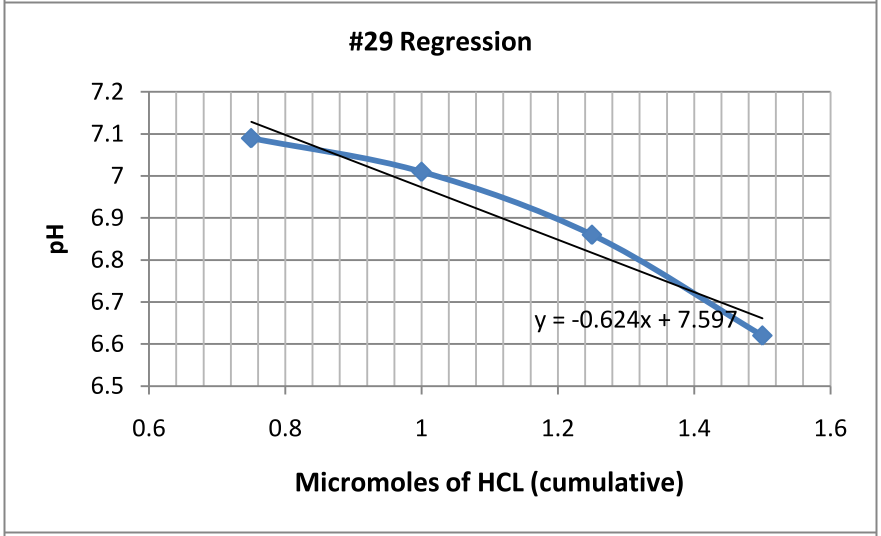
#26Titration of Ca(OH)2 with HCL

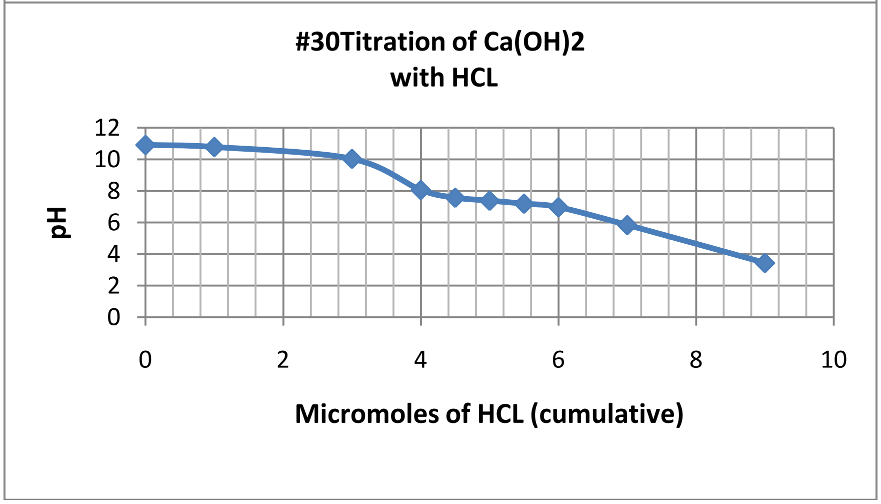

#26 Regression
pH
9
8
7
6
5
4
3
2
1
0
y = -6.2x + 8.4233
0
0.1
0.2
0.3
0.4
0.5
0.6
Micromoles of HCL (cumulative)

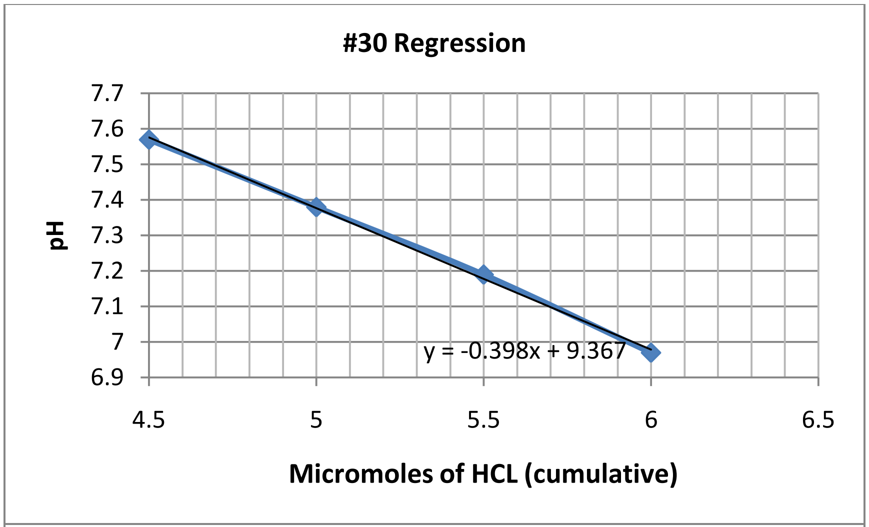

#28 Titration of Ca(OH)2
with HCL
pH
8
6
4
2
0
0
1
2
3
4
5
Micromoles of HCL (cumulative)


#28 Regression
pH
7
6
5
4
3
2
1
0
y = -6.3x + 5.8183
0
0.1
0.2
0.3
0.4
0.5
0.6
Micromoles of HCL (cumulative)

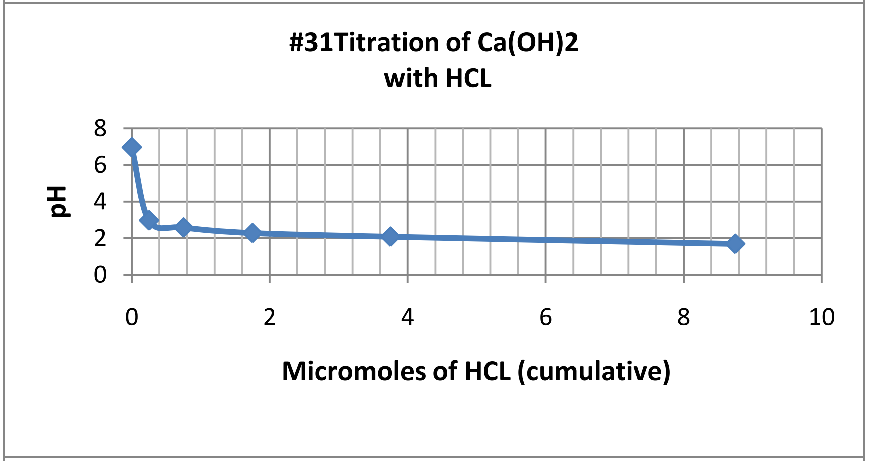

#29 Titration of Ca(OH)2
with HCL
pH
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
Micromoles of HCL (cumulative)


#29 Regression
pH
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
y = -0.624x + 7.597
0.6
0.8
1
1.2
1.4
1.6
Micromoles of HCL (cumulative)

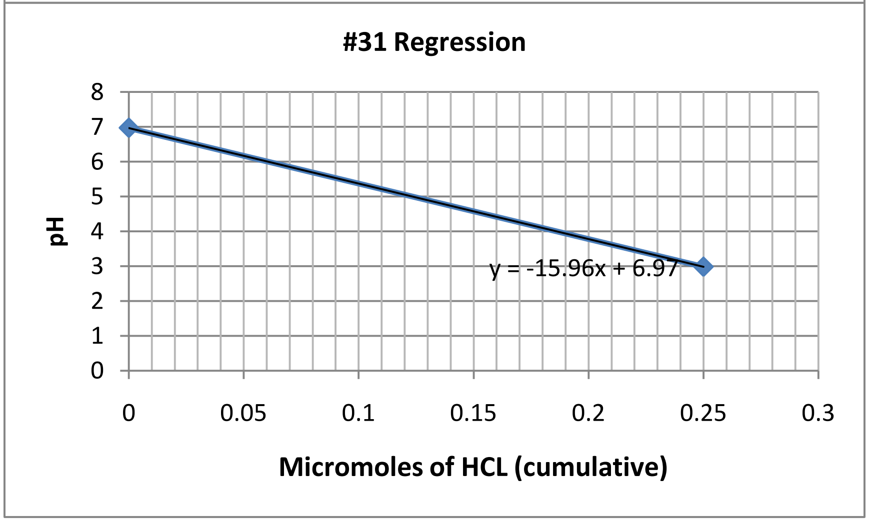

#30Titration of Ca(OH)2
with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
Micromoles of HCL (cumulative)



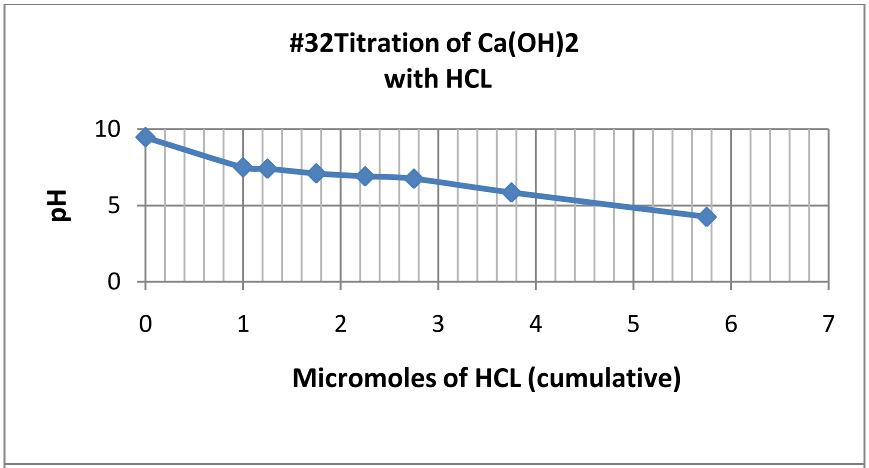
#30 Regression

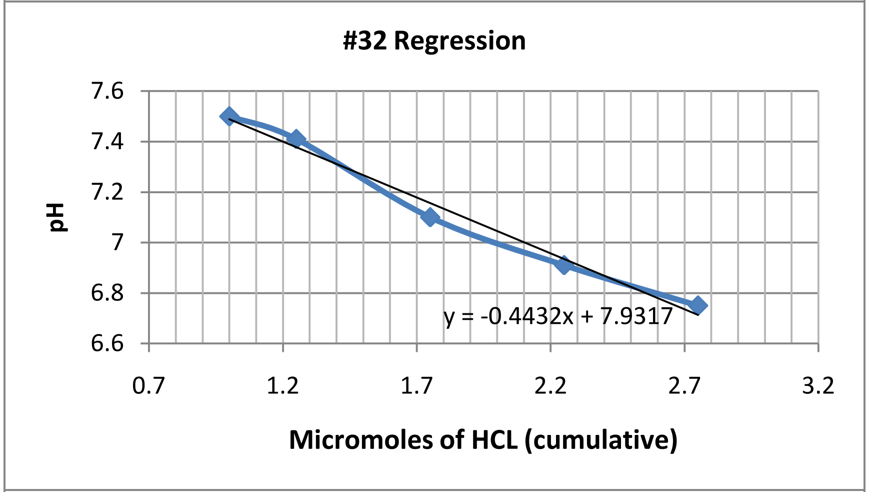


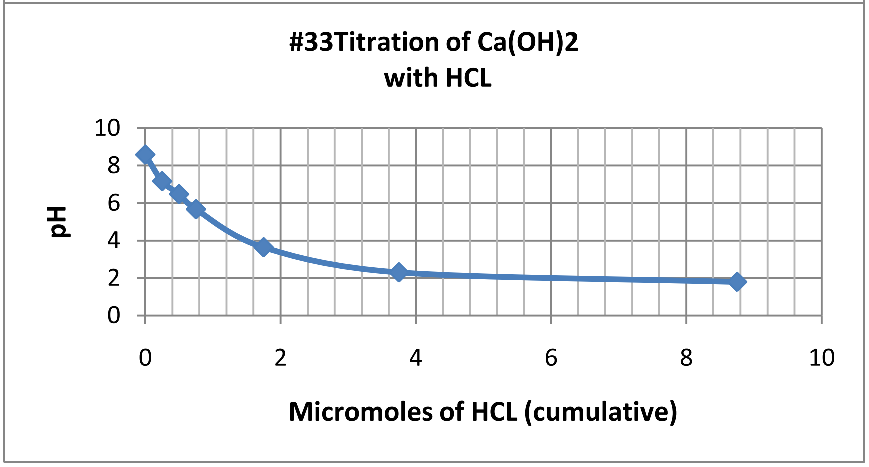
#31Titration of Ca(OH)2 with HCL

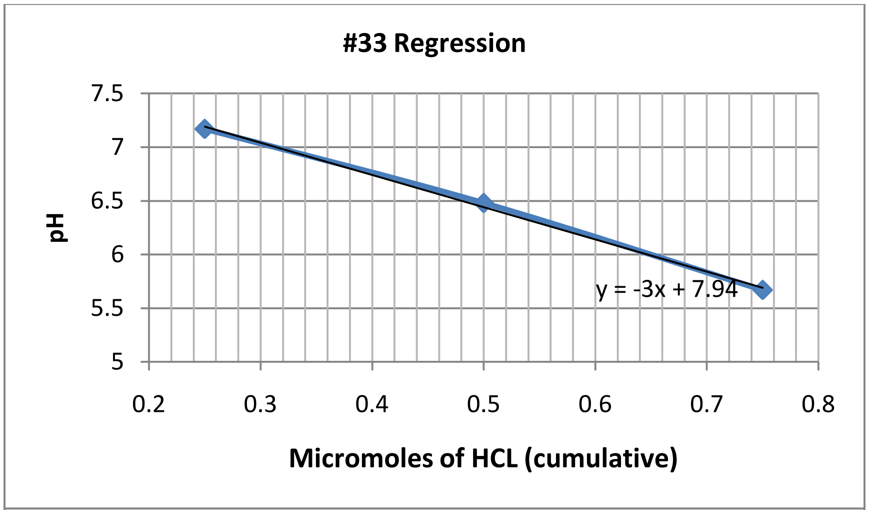


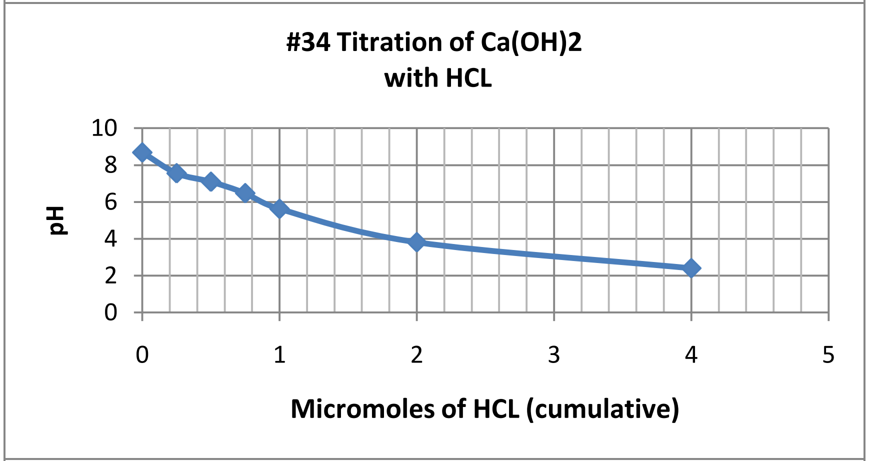
#31 Regression

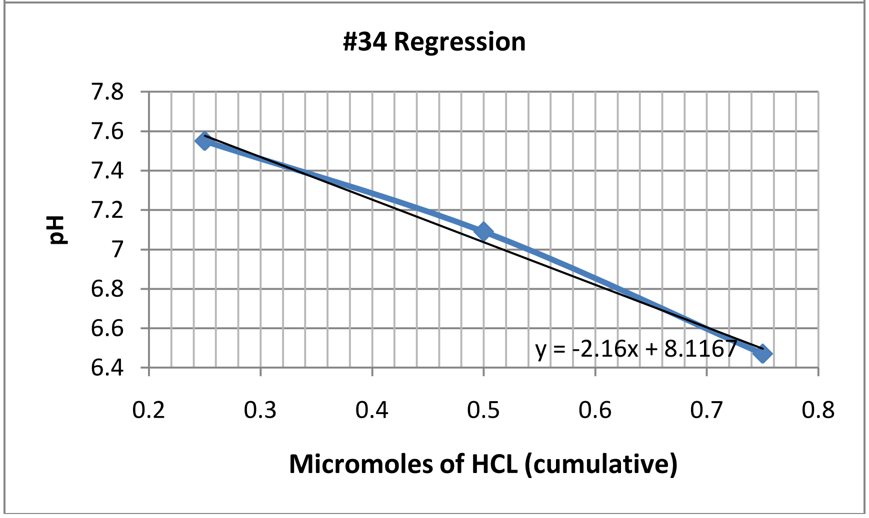

#32Titration of Ca(OH)2 with HCL
10
5
0
pH
0
1
2
3
4
5
6
7
Micromoles of HCL (cumulative)

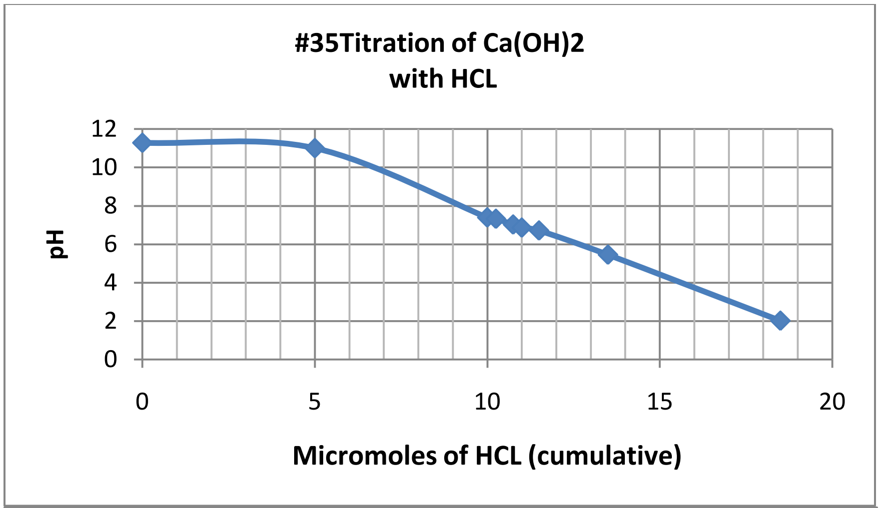

#32 Regression
7.6
7.4
7.2
7
6.8
6.6
pH
y = -0.4432x + 7.9317
0.7
1.2
1.7
2.2
2.7
3.2
Micromoles of HCL (cumulative)

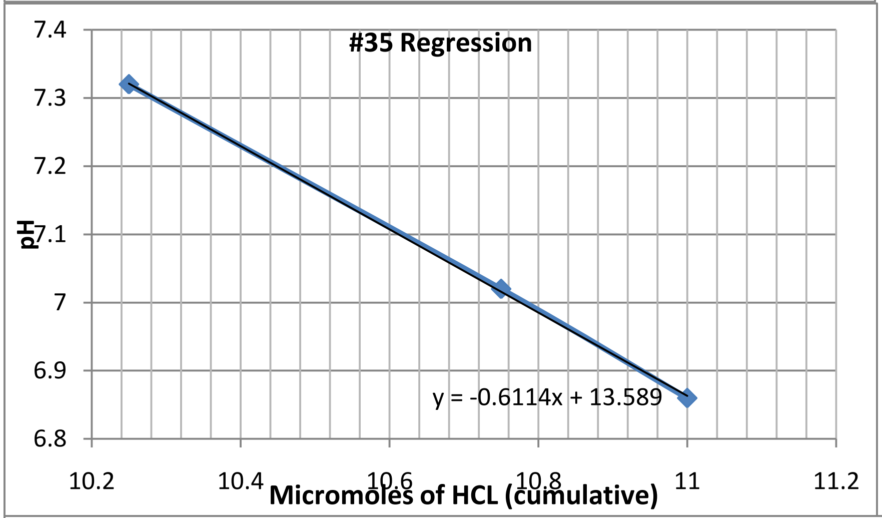

#33Titration of Ca(OH)2 with HCL
10
8
6
4
2
0
pH
0
2
4
6
8
10
Micromoles of HCL (cumulative)

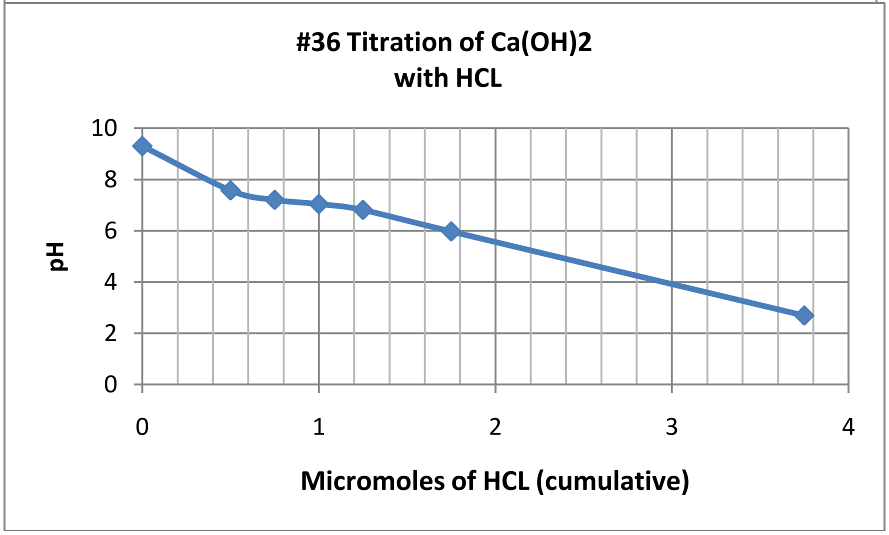

#33 Regression
pH
7.5
7
6.5
6
5.5
5
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Micromoles of HCL (cumulative)
y = -3x + 7.94

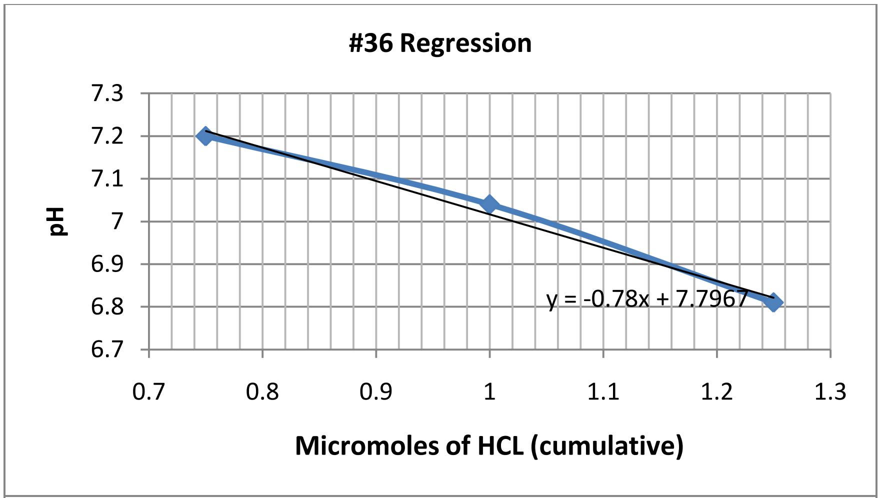

#34 Titration of Ca(OH)2 with HCL
pH
10
8
6
4
2
0
0
1
2
3
4
5
Micromoles of HCL (cumulative)

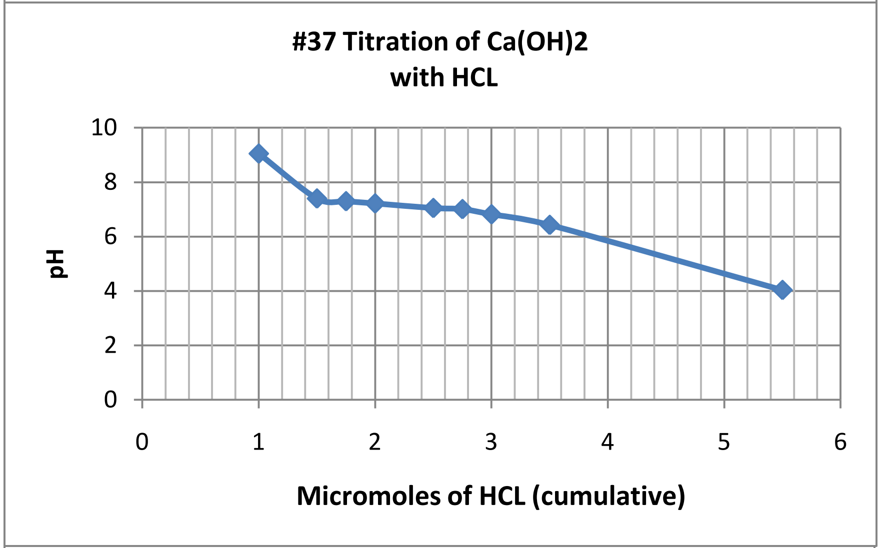

#34 Regression
pH
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Micromoles of HCL (cumulative)
y = -2.16x + 8.1167

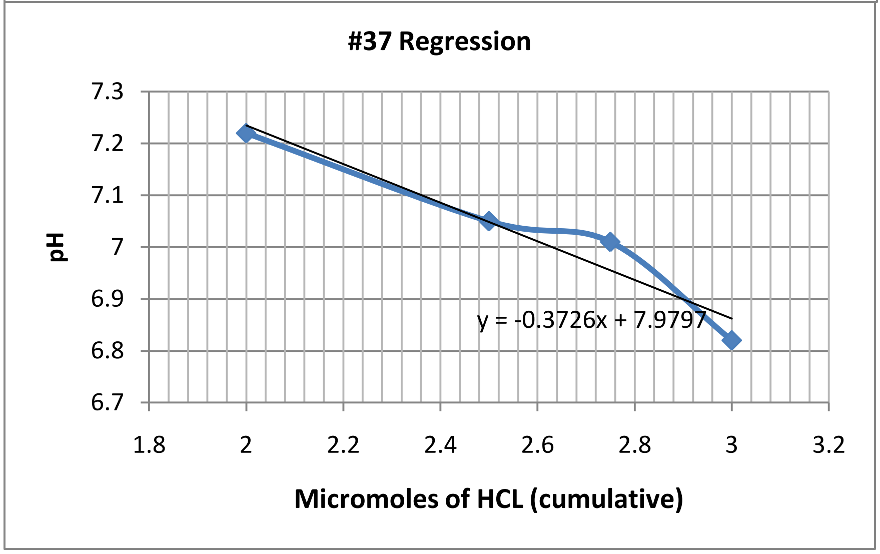

#35Titration of Ca(OH)2
with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
20
Micromoles of HCL (cumulative)

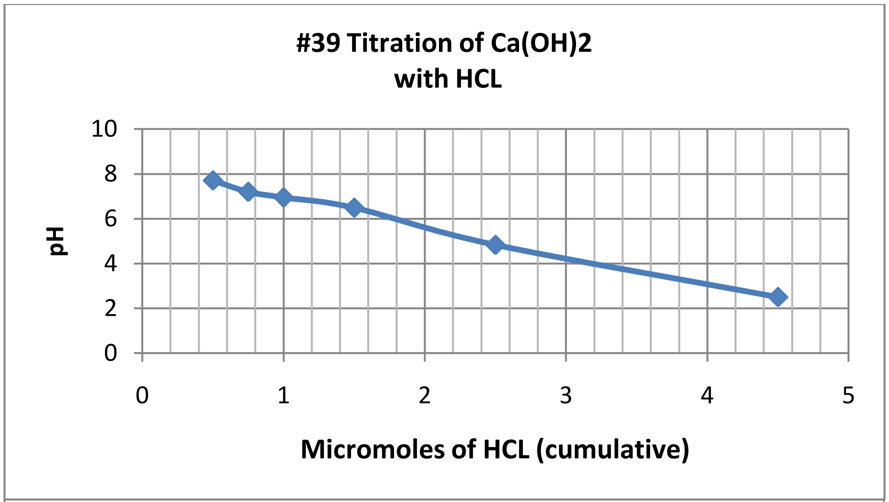

#35 Regression
pH
7.4
7.3
7.2
7.1
7
6.9
6.8
y = -0.6114x + 13.589
10.2
10.4
10.6
10.8
11
11.2
Micromoles of HCL (cumulative)

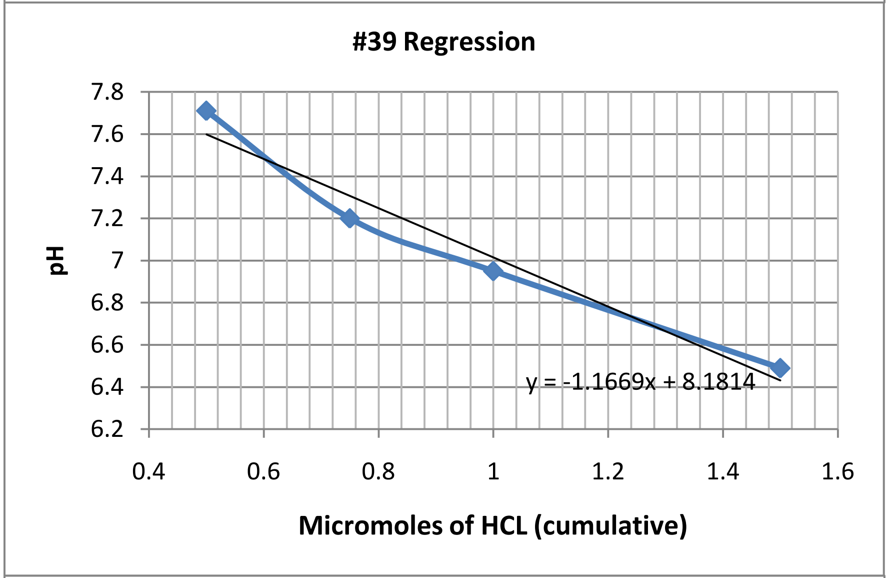

#36 Titration of Ca(OH)2
with HCL
pH
10
8
6
4
2
0
0
1
2
3
4
Micromoles of HCL (cumulative)

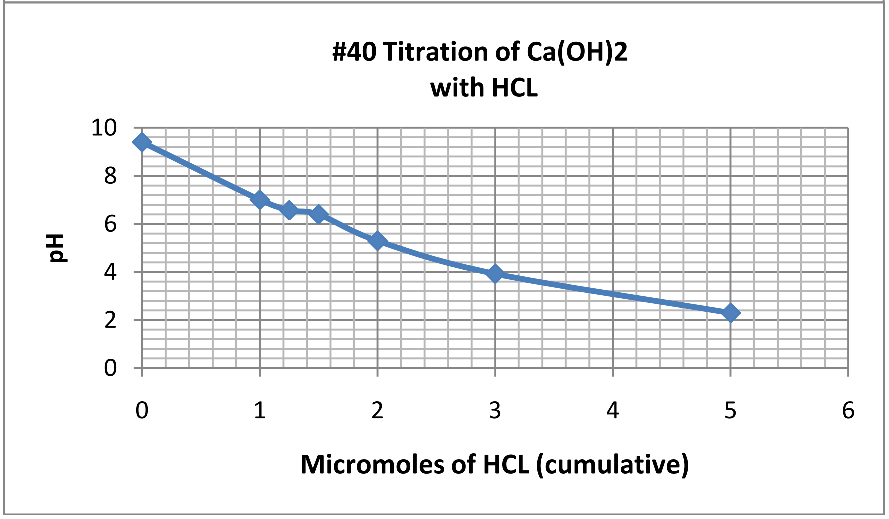

#36 Regression
pH
7.3
7.2
7.1
7
6.9
6.8
6.7
y = -0.78x + 7.7967
0.7
0.8
0.9
1
1.1
1.2
1.3
Micromoles of HCL (cumulative)

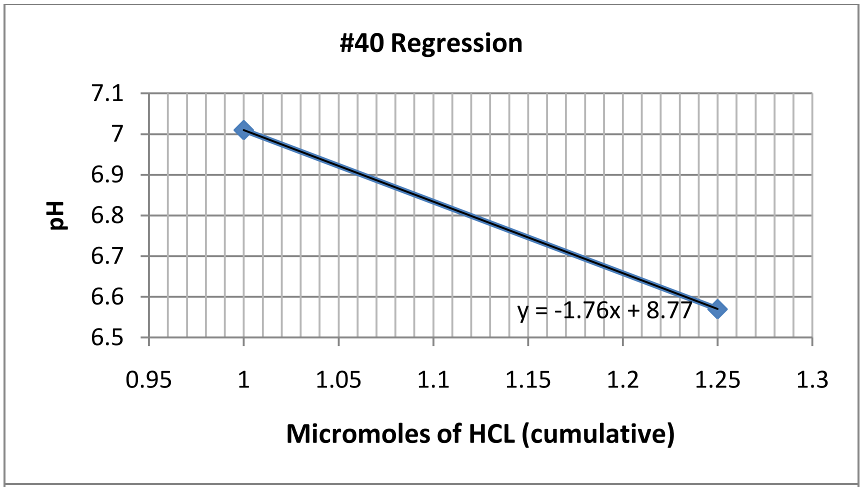

#37 Titration of Ca(OH)2
with HCL
pH
10
8
6
4
2
0
0
1
2
3
4
5
6
Micromoles of HCL (cumulative)

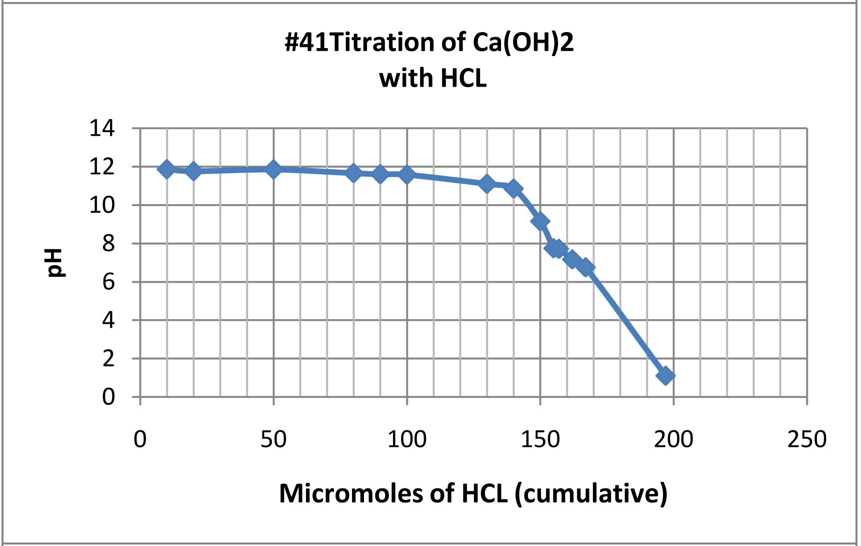

#37 Regression
pH
7.3
7.2
7.1
7
6.9
6.8
6.7
y = -0.3726x + 7.9797
1.8
2
2.2
2.4
2.6
2.8
3
3.2
Micromoles of HCL (cumulative)

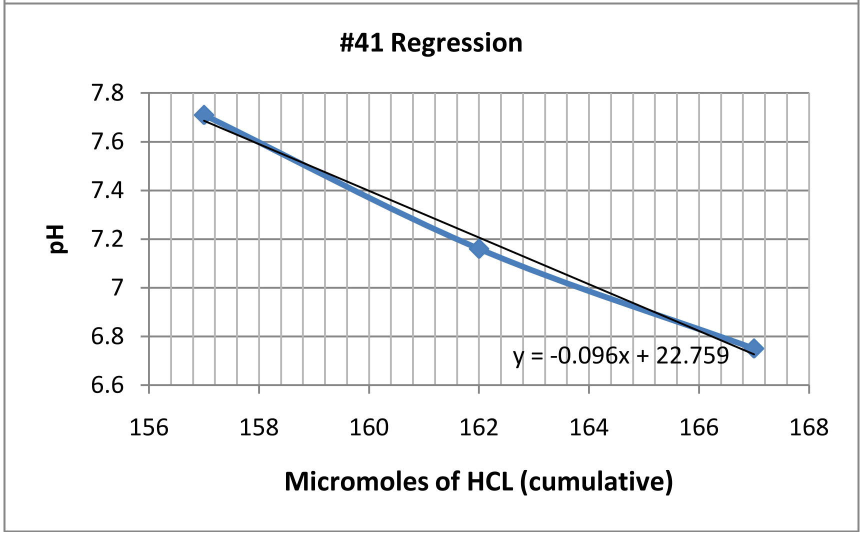

#39 Titration of Ca(OH)2
with HCL
pH
10
8
6
4
2
0
0
1
2
3
4
5
Micromoles of HCL (cumulative)

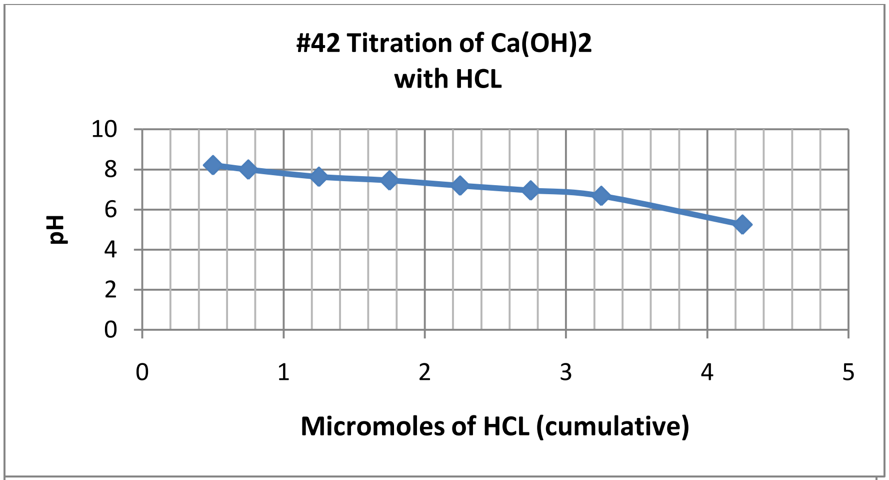

#39 Regression
pH
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
y = -1.1669x + 8.1814
0.4
0.6
0.8
1
1.2
1.4
1.6
Micromoles of HCL (cumulative)

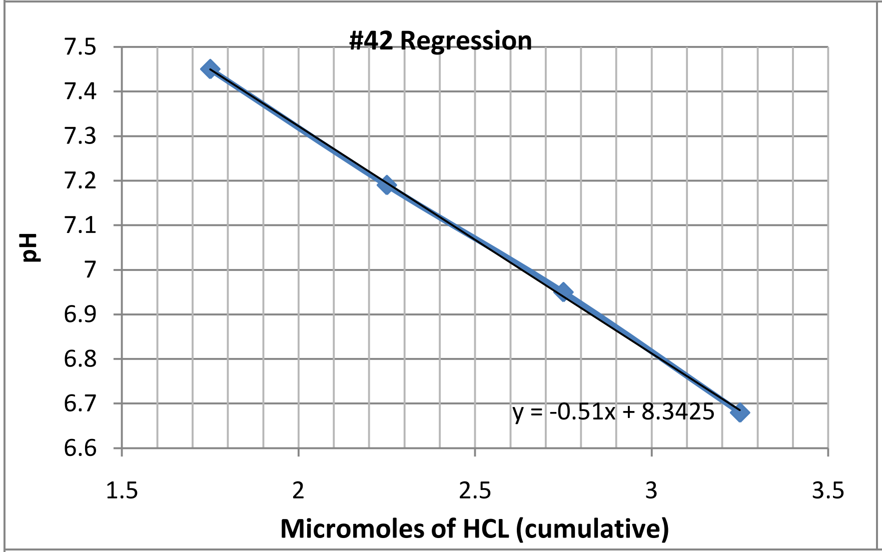

#40 Titration of Ca(OH)2
with HCL
pH
10
8
6
4
2
0
0
1
2
3
4
5
6
Micromoles of HCL (cumulative)

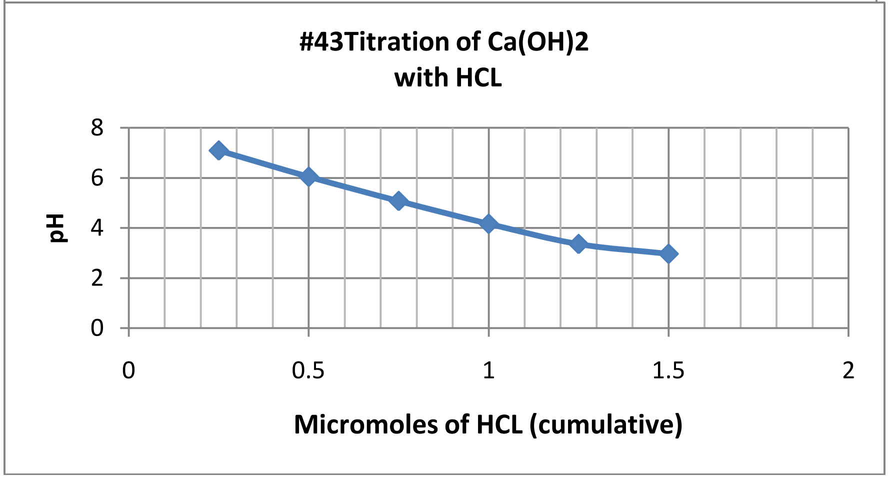

#40 Regression
pH
7.1
7
6.9
6.8
6.7
6.6
6.5
y = -1.76x + 8.77
0.95
1
1.05
1.1
1.15
1.2
1.25
1.3
Micromoles of HCL (cumulative)

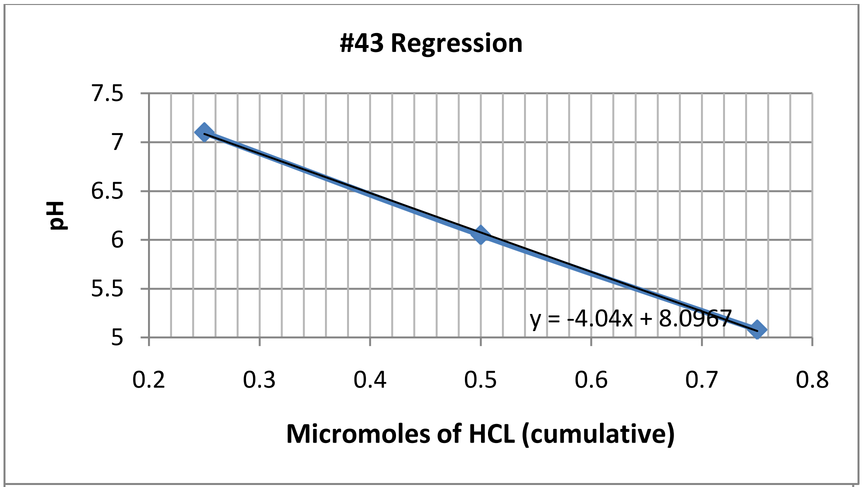

#41Titration of Ca(OH)2
with HCL
pH
14
12
10
8
6
4
2
0
0
50
100
150
200
250
Micromoles of HCL (cumulative)

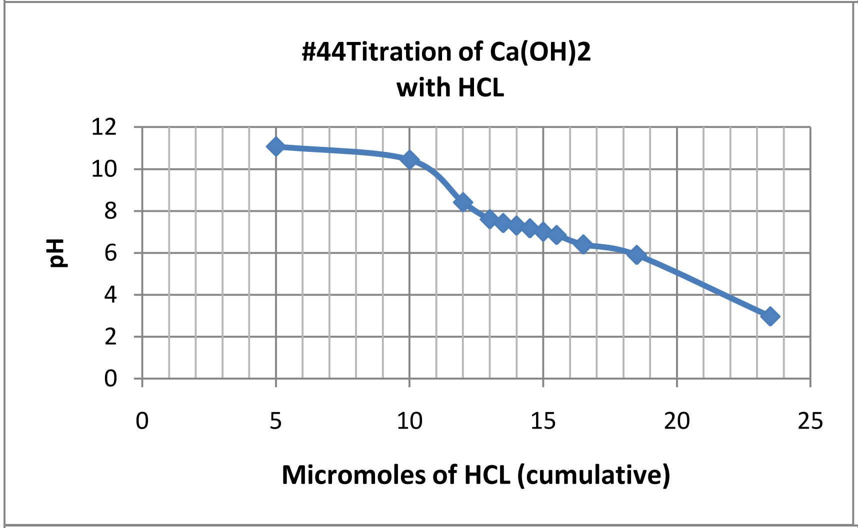

#41 Regression
pH
7.8
7.6
7.4
7.2
7
6.8
6.6
y = -0.096x + 22.759
156
158
160
162
164
166
168
Micromoles of HCL (cumulative)

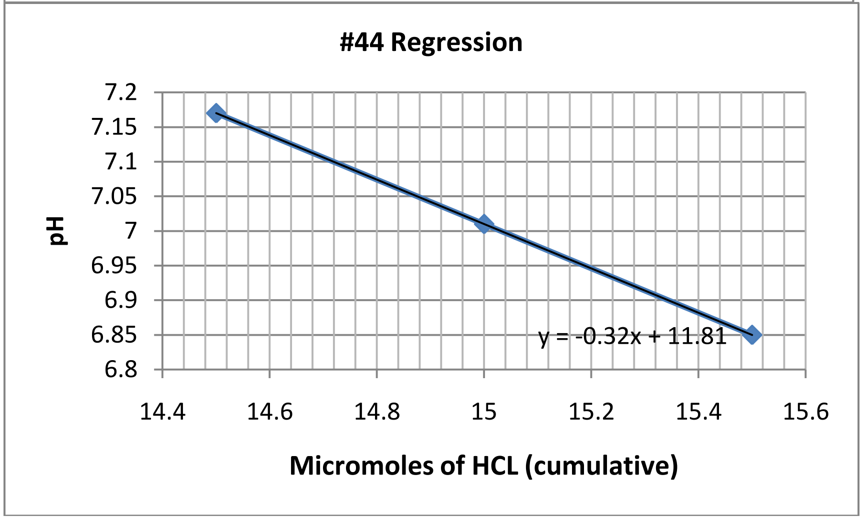

#42 Titration of Ca(OH)2 with HCL

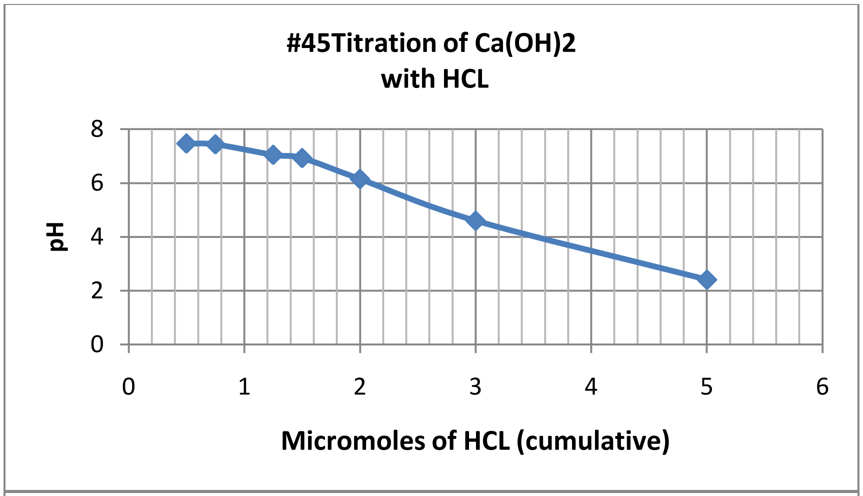

#42 Regression


#43Titration of Ca(OH)2 with HCL

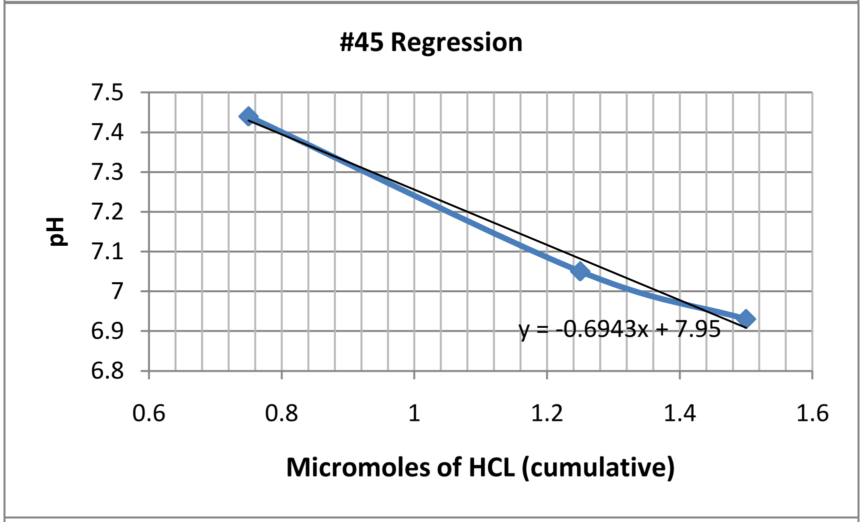

#43 Regression
pH
7.5
7
6.5
6
5.5
5
y = -4.04x + 8.0967
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Micromoles of HCL (cumulative)


#44Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
20
25
Micromoles of HCL (cumulative)

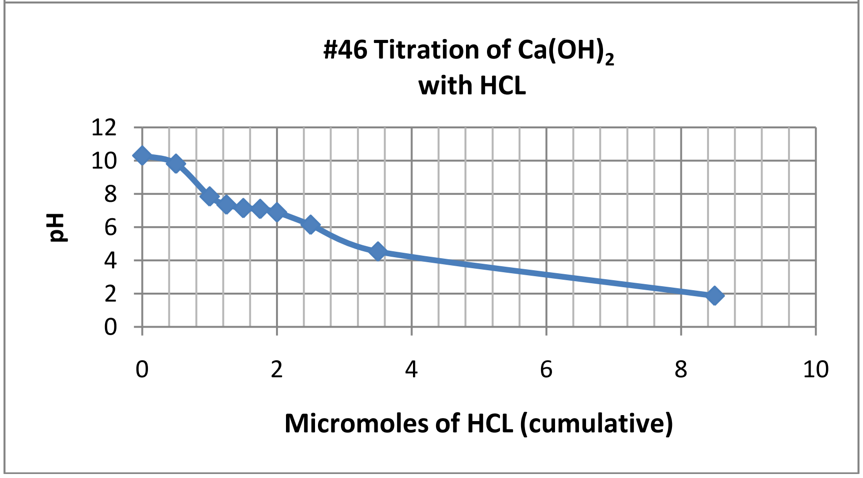

#44 Regression
pH
7.2
7.15
7.1
7.05
7
6.95
6.9
6.85
6.8
y = -0.32x + 11.81
14.4
14.6
14.8
15
15.2
15.4
15.6
Micromoles of HCL (cumulative)



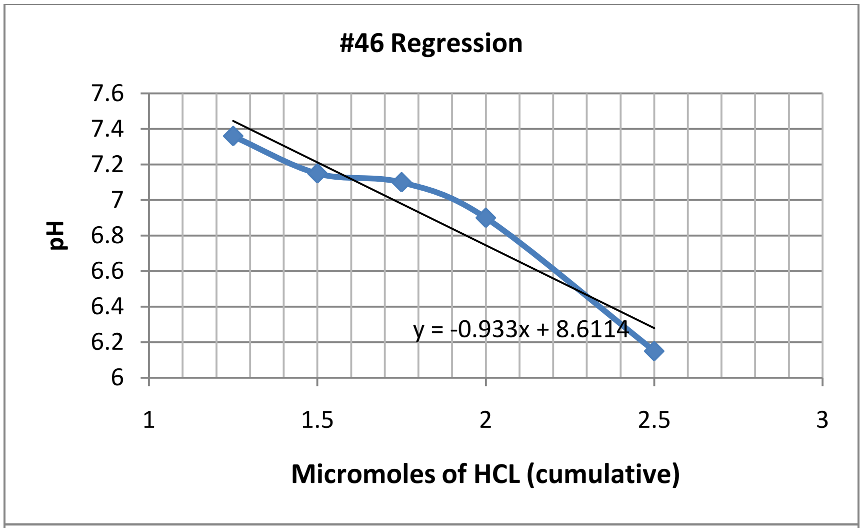
#45Titration of Ca(OH)2 with HCL

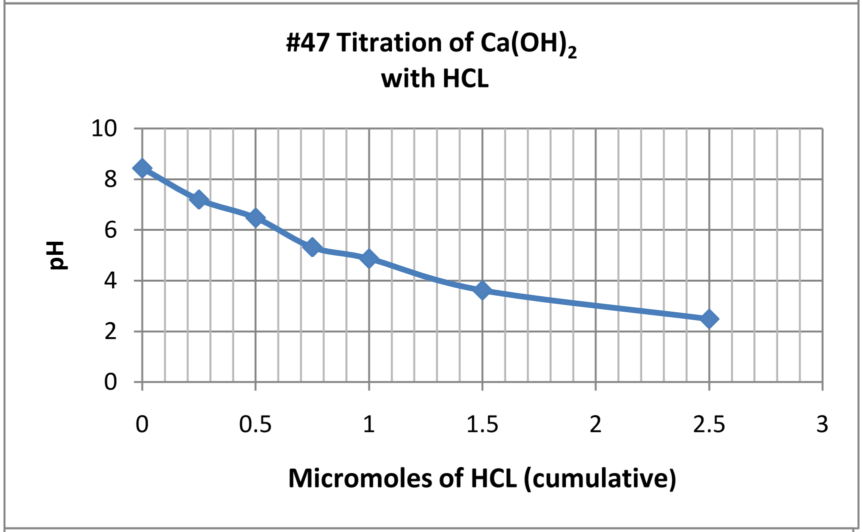


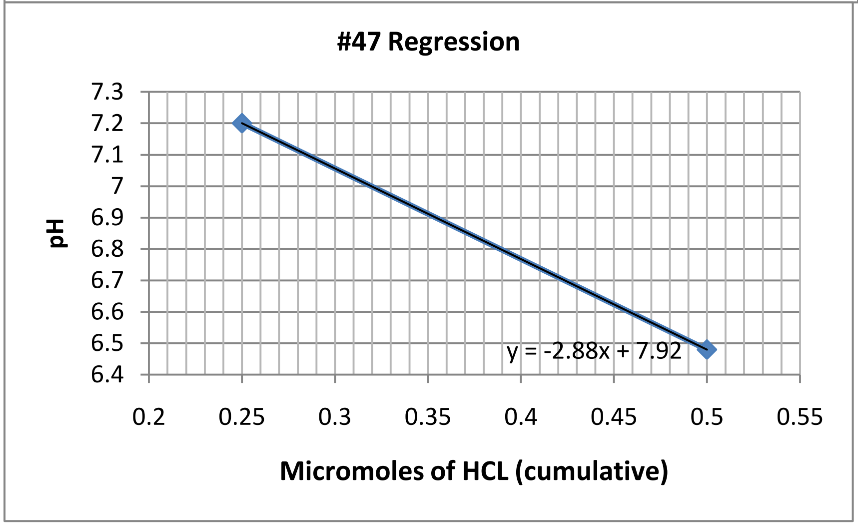
#45 Regression

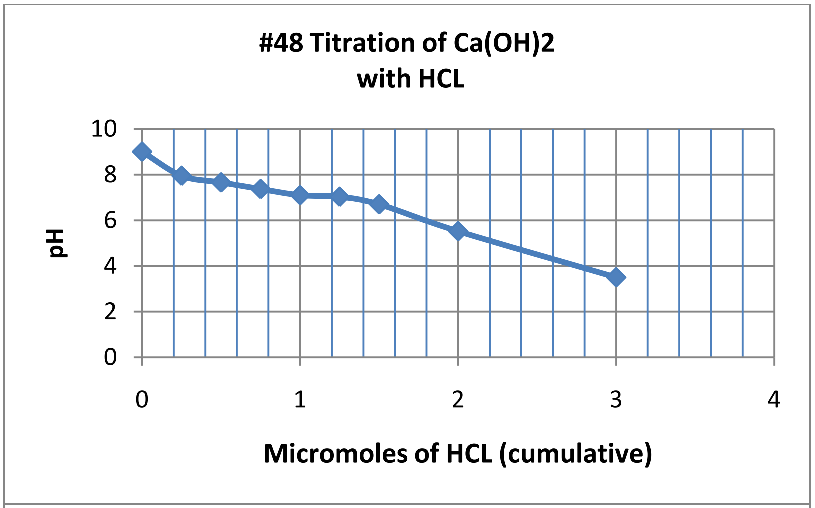


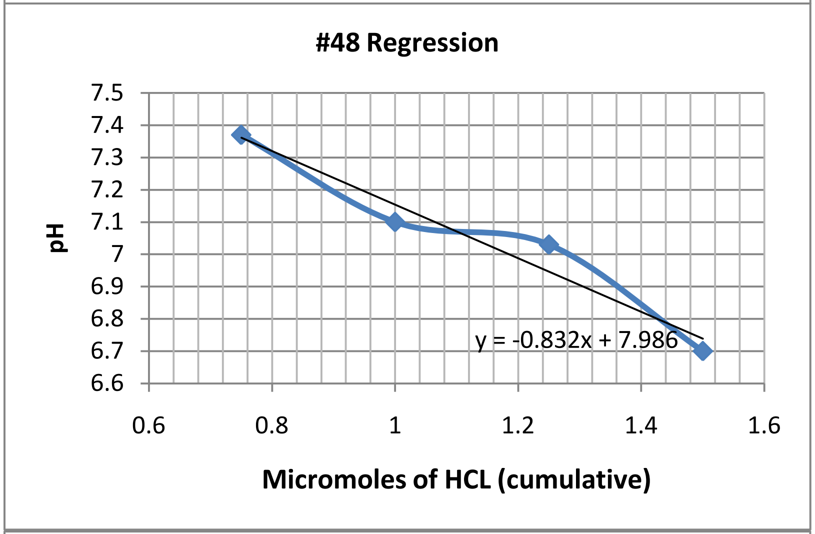
#46 Titration of Ca(OH)$_2$ with HCL

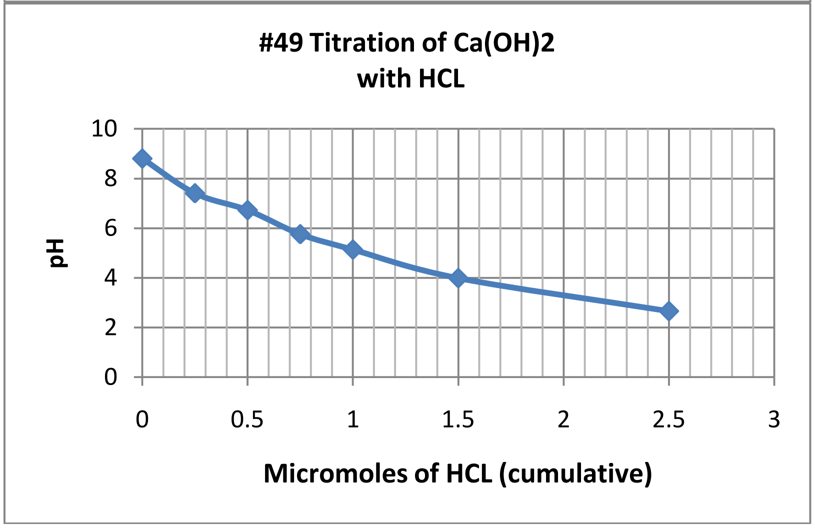

#46 Regression
pH
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
y = -0.933x + 8.6114
1
1.5
2
2.5
3
Micromoles of HCL (cumulative)

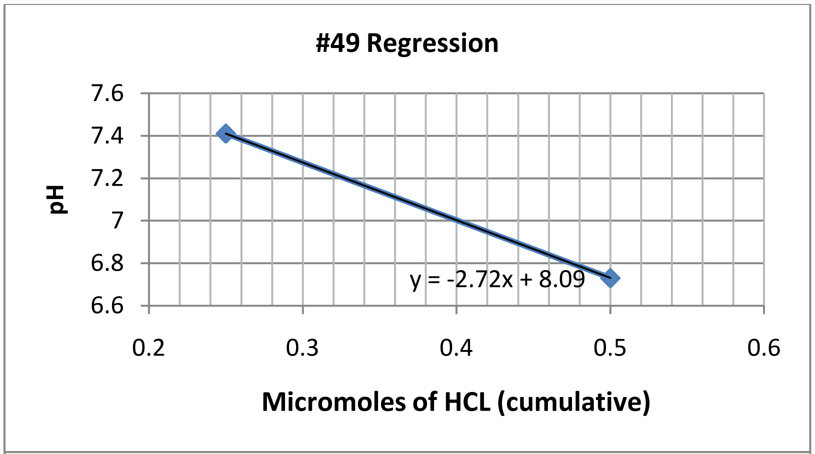

#47 Titration of Ca(OH)2 with HCL
pH
10
8
6
4
2
0
0
0.5
1
1.5
2
2.5
3
Micromoles of HCL (cumulative)

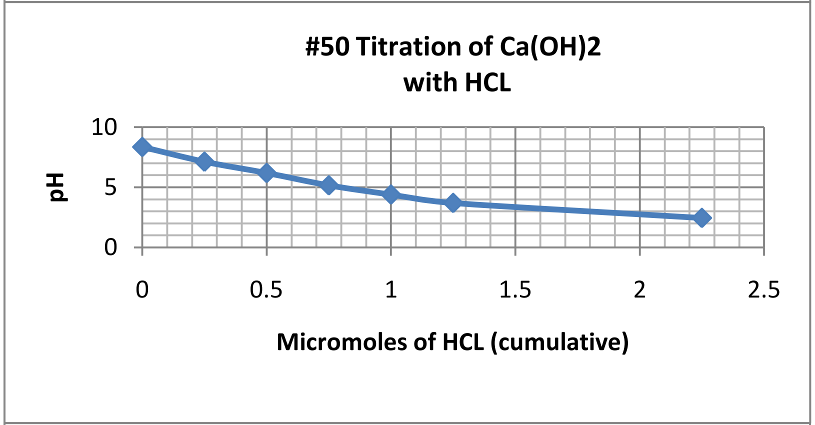

#47 Regression
pH
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
6.4
y = -2.88x + 7.92
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
Micromoles of HCL (cumulative)

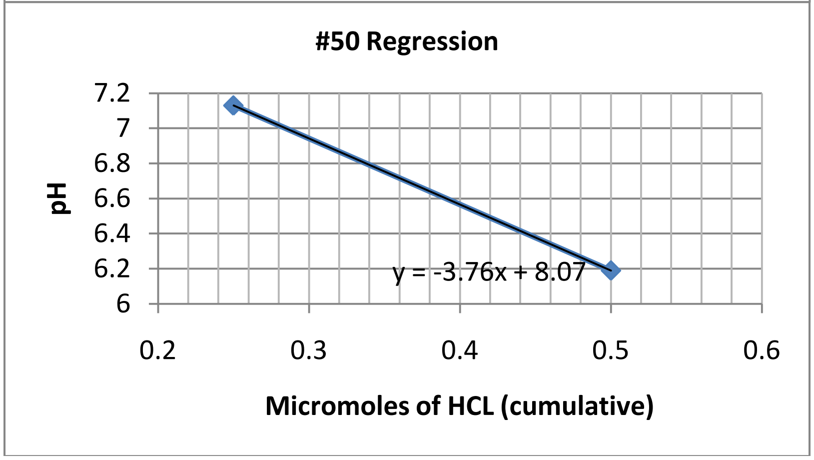

#48 Titration of Ca(OH)2 with HCL

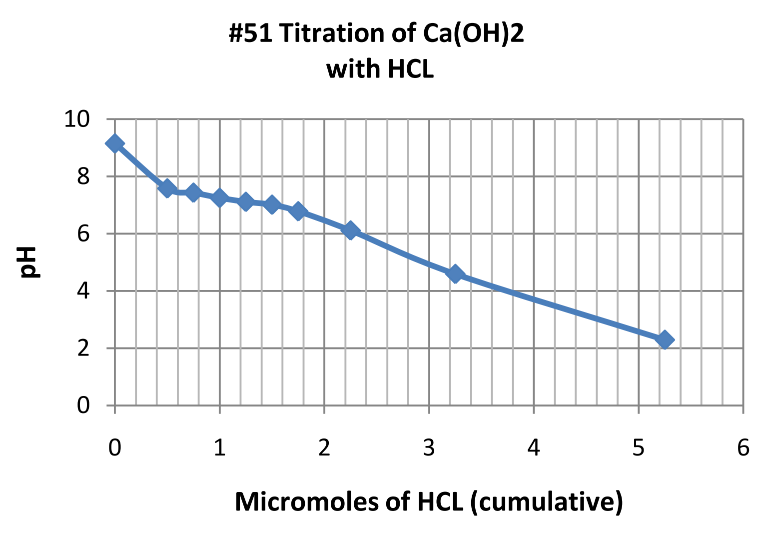

#48 Regression


#49 Titration of Ca(OH)2 with HCL

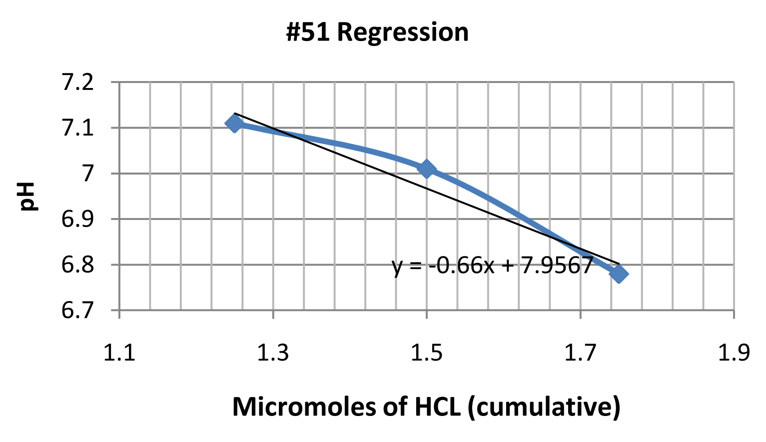

#49 Regression
pH
7.6
7.4
7.2
7
6.8
6.6
y = -2.72x + 8.09
0.2
0.3
0.4
0.5
0.6
Micromoles of HCL (cumulative)


#50 Titration of Ca(OH)2 with HCL
pH
10
5
0
0
0.5
1
1.5
2
2.5
Micromoles of HCL (cumulative)

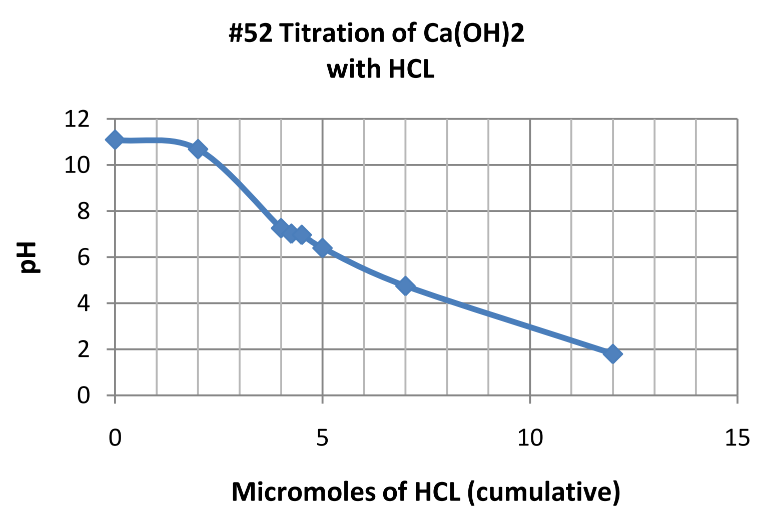

#50 Regression
pH
7.2
7
6.8
6.6
6.4
6.2
6
y = -3.76x + 8.07
0.2
0.3
0.4
0.5
0.6
Micromoles of HCL (cumulative)



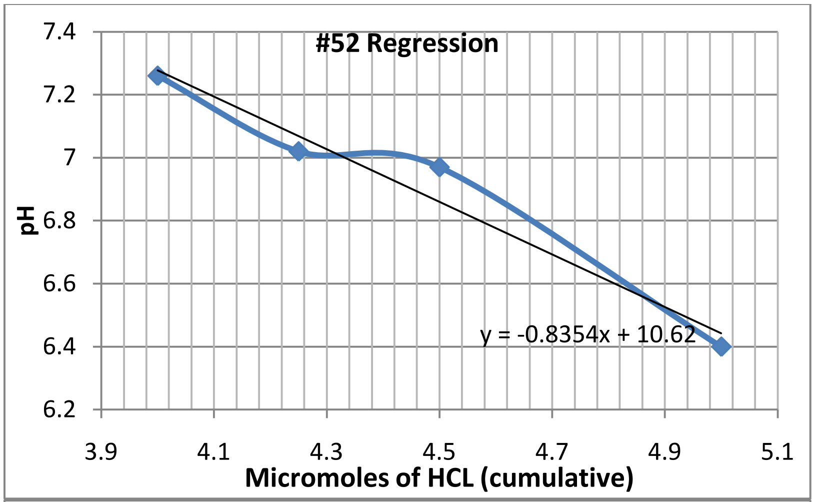
#51 Titration of Ca(OH)2 with HCL

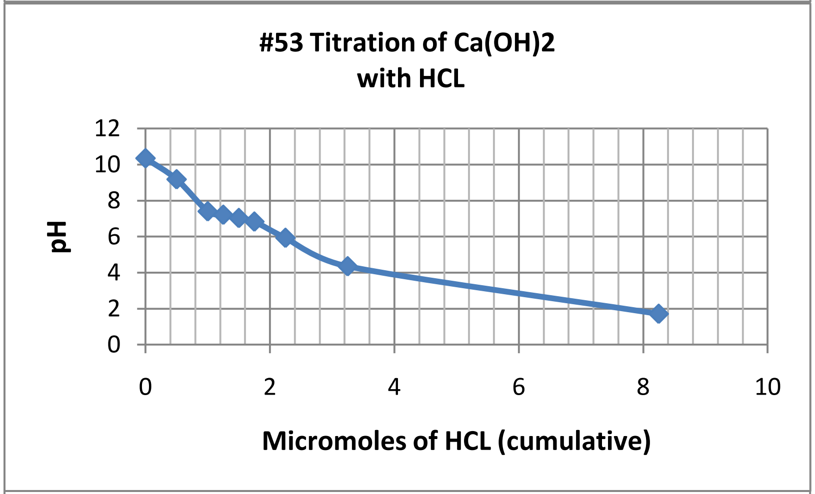


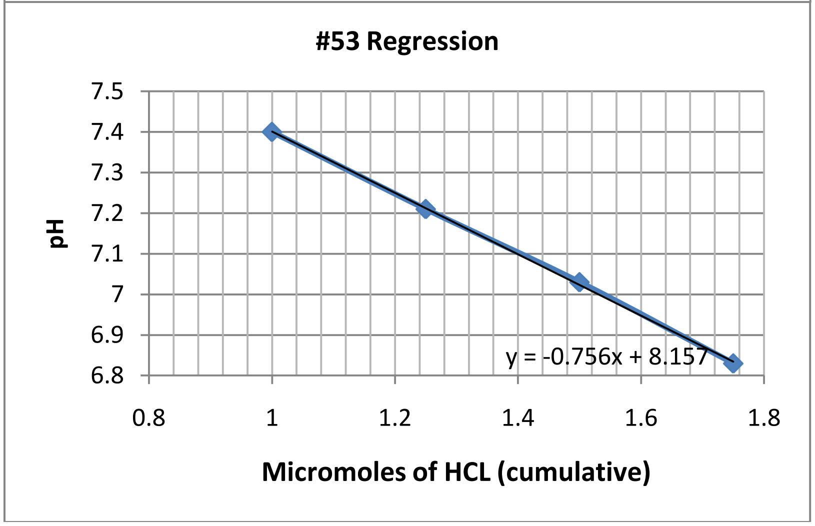
#51 Regression

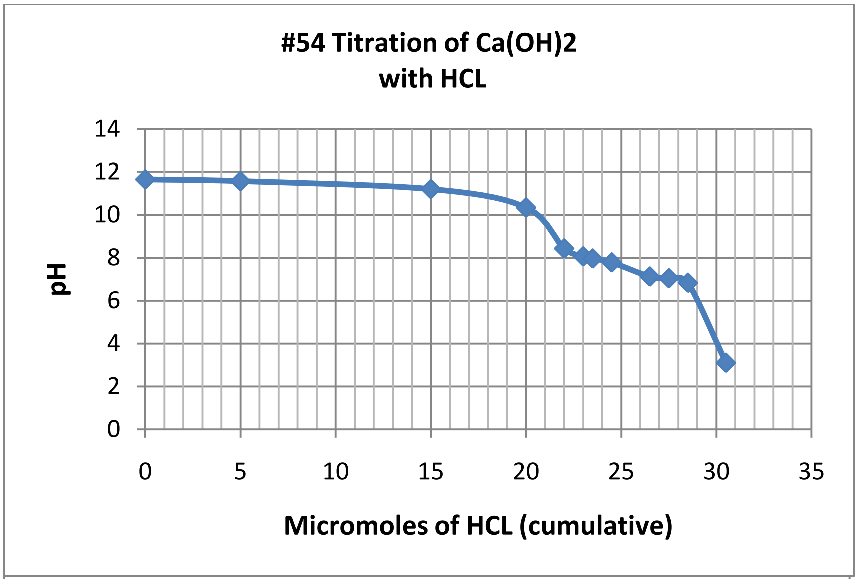


#52 Titration of Ca(OH)2 with HCL

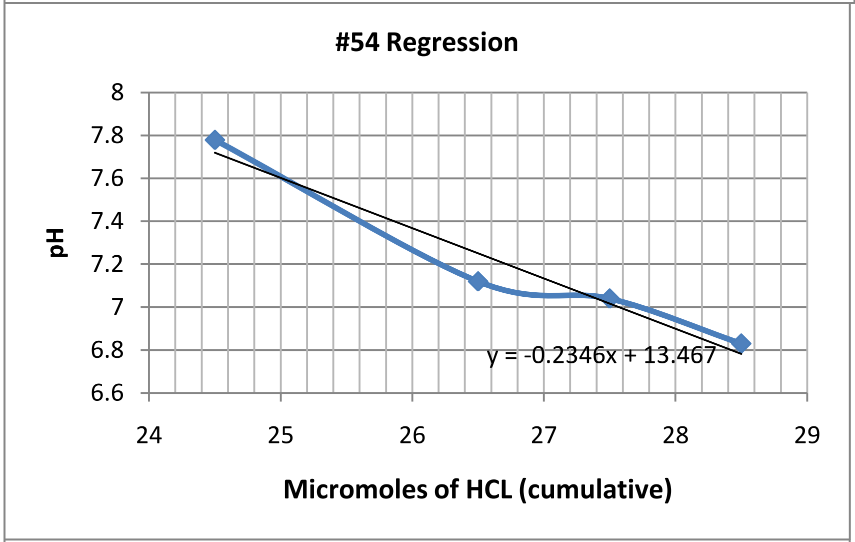

#52 Regression
pH
7.4
7.2
7
6.8
6.6
6.4
6.2
3.9
4.1
4.3
4.5
4.7
4.9
5.1
y = -0.8354x + 10.62
Micromoles of HCL (cumulative)


#53 Titration of Ca(OH)2
with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
Micromoles of HCL (cumulative)

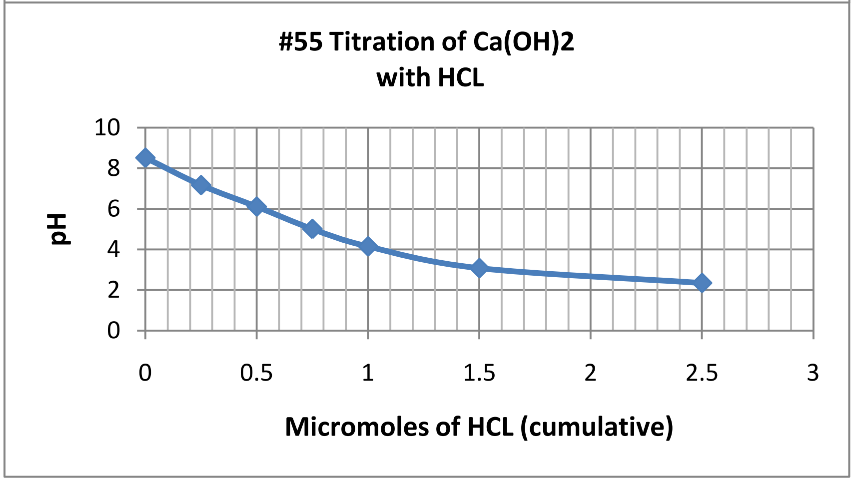

#53 Regression
pH
7.5
7.4
7.3
7.2
7.1
7
6.9
6.8
0.8
1
1.2
1.4
1.6
1.8
y = -0.756x + 8.157
Micromoles of HCL (cumulative)



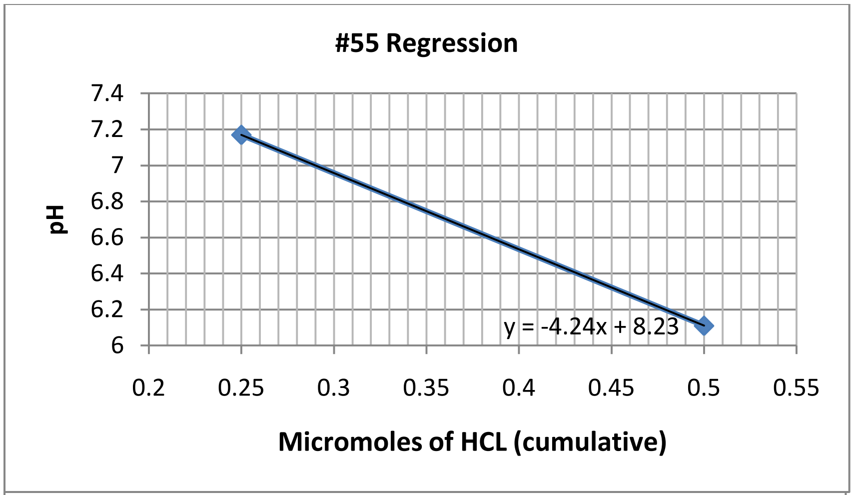
#54 Titration of Ca(OH)2 with HCL

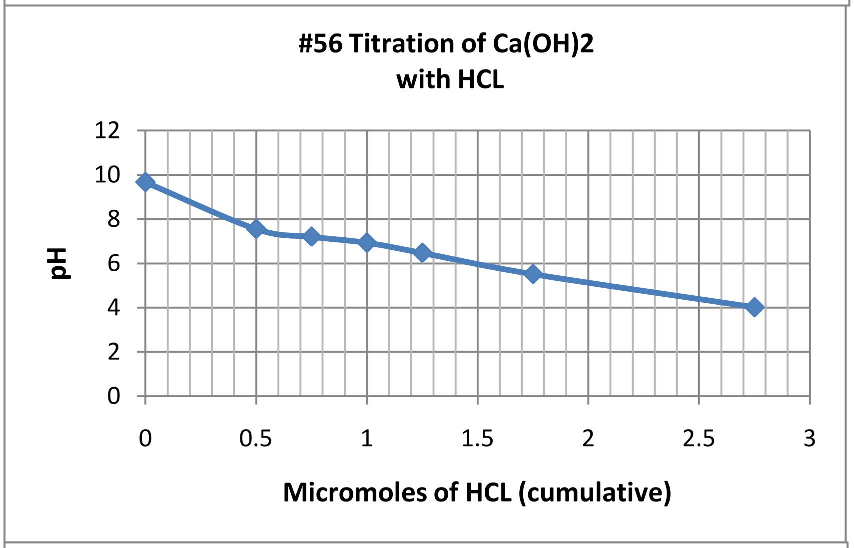


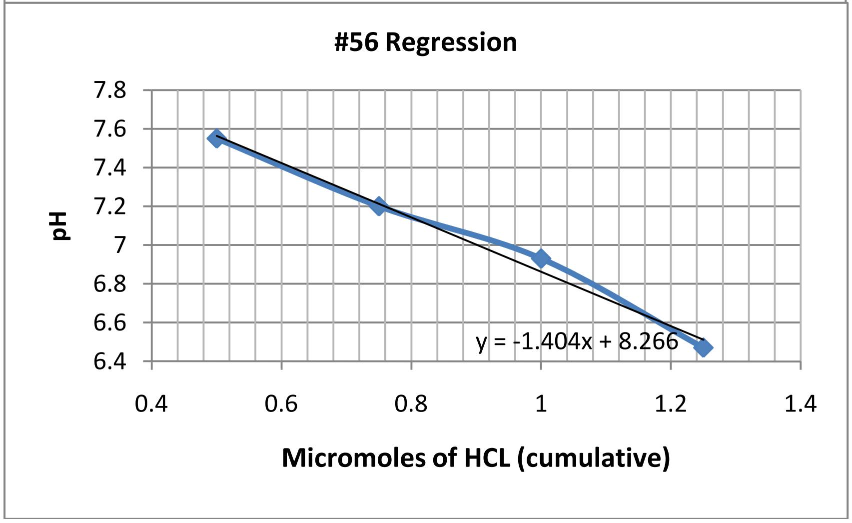
#54 Regression

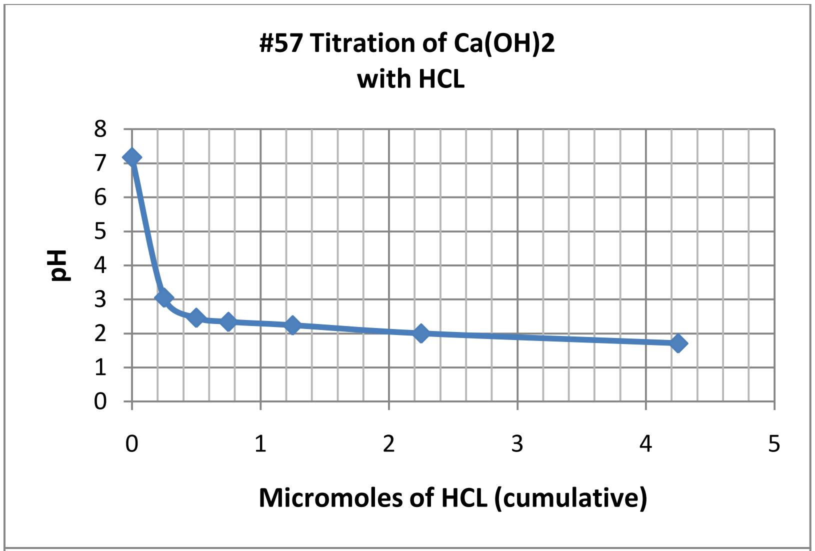


#55 Titration of Ca(OH)2 with HCL

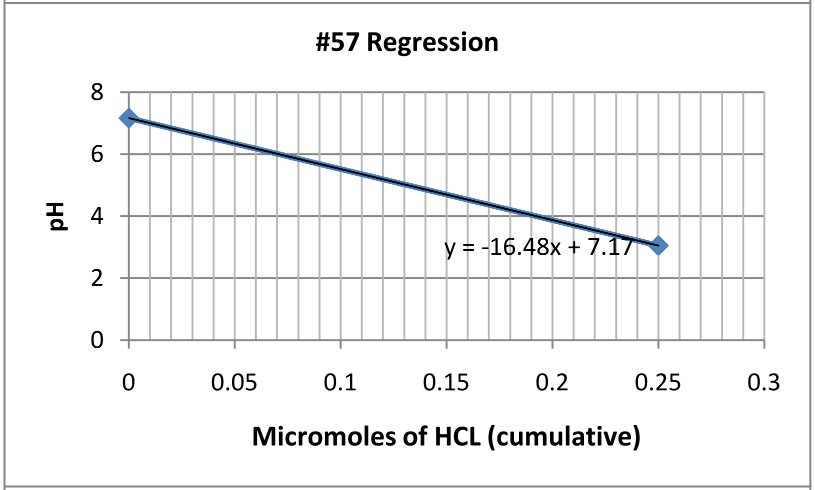

#55 Regression
pH
7.4
7.2
7
6.8
6.6
6.4
6.2
6
y = -4.24x + 8.23
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
Micromoles of HCL (cumulative)


#56 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
0.5
1
1.5
2
2.5
3
Micromoles of HCL (cumulative)

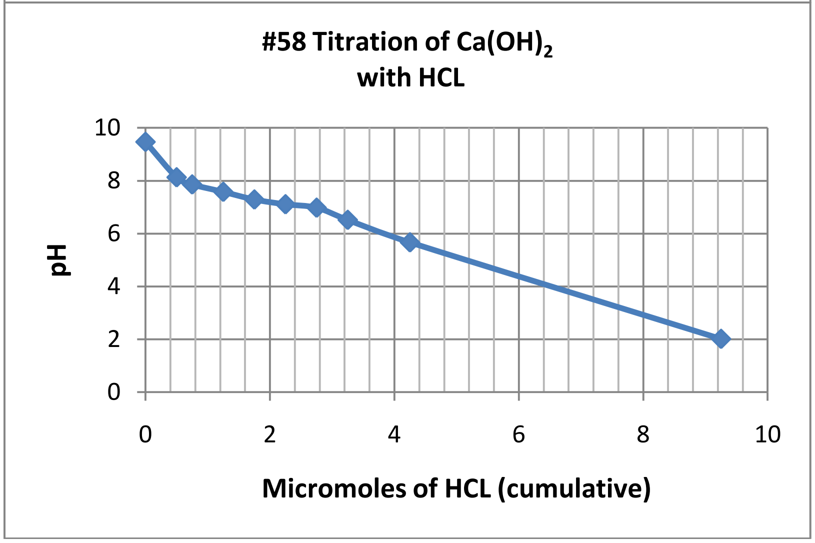

#56 Regression
pH
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
y = -1.404x + 8.266
0.4
0.6
0.8
1
1.2
1.4
Micromoles of HCL (cumulative)



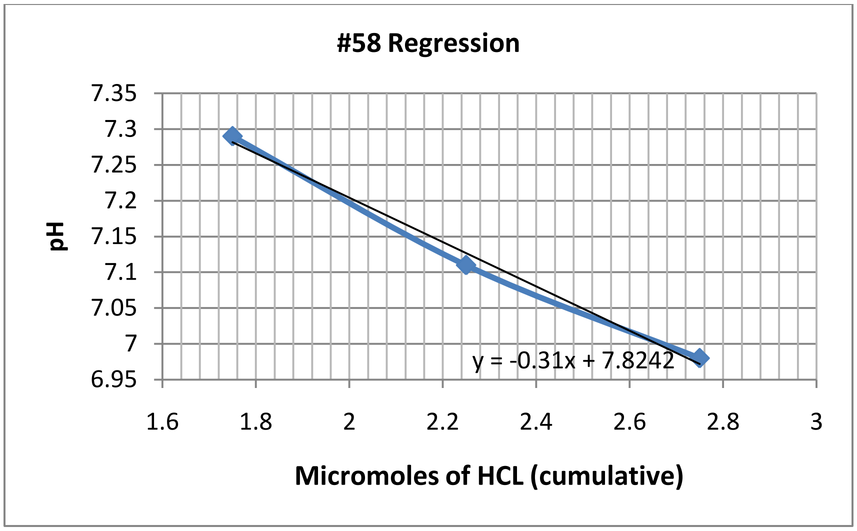
#57 Titration of Ca(OH)2 with HCL



#57 Regression



#58 Titration of Ca(OH)$_2$ with HCL


#58 Regression
pH
7.35
7.3
7.25
7.2
7.15
7.1
7.05
7
6.95
y = -0.31x + 7.8242
1.6
1.8
2
2.2
2.4
2.6
2.8
3
Micromoles of HCL (cumulative)

Appendix V.

Titration data for Standards. Columns from left to right: pH readings, concentration of HCL added, volume of HCL added, moles of HCL added, cumulative moles of HCL added, cumulative micromoles of HCL added, and pH (listed again).

Standard A1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
8.89				0	0	8.89
7.55	0.025	0.00001	0.00000025	0.00000025	0.25	7.55
6.78	0.025	0.00001	0.00000025	0.0000005	0.5	6.78
6.45	0.025	0.00001	0.00000025	0.00000075	0.75	6.45
5.41	0.025	0.00001	0.00000025	0.000001	1	5.41
4.84	0.05	0.00001	0.0000005	0.0000015	1.5	4.84

Standard A2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.1				0	0	10.1
8.37	0.025	0.00001	0.00000025	0.00000025	0.25	8.37
7.38	0.025	0.00001	0.00000025	0.0000005	0.5	7.38
6.88	0.025	0.00001	0.00000025	0.00000075	0.75	6.88
6.51	0.025	0.00001	0.00000025	0.000001	1	6.51
5.98	0.05	0.00001	0.0000005	0.0000015	1.5	5.98
5.58	0.05	0.00001	0.0000005	0.000002	2	5.58
4.98	0.05	0.00001	0.0000005	0.0000025	2.5	4.98

Standard A3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
8.84				0	0	8.84
7.45	0.025	0.00001	0.00000025	0.00000025	0.25	7.45
6.8	0.025	0.00001	0.00000025	0.0000005	0.5	6.8
5.93	0.025	0.00001	0.00000025	0.00000075	0.75	5.93
5.35	0.025	0.00001	0.00000025	0.000001	1	5.35
4.64	0.05	0.00001	0.0000005	0.0000015	1.5	4.64

Standard B1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.9				0	0	10.9
8.55	0.1	0.00001	0.000001	0.000001	1	8.55
7.3	0.05	0.00001	0.0000005	0.0000015	1.5	7.3
6.83	0.025	0.00001	0.00000025	0.00000175	1.75	6.83
6.52	0.025	0.00001	0.00000025	0.000002	2	6.52
6.03	0.05	0.00001	0.0000005	0.0000025	2.5	6.03
5.44	0.05	0.00001	0.0000005	0.000003	3	5.44
4.31	0.1	0.00001	0.000001	0.000004	4	4.31

Standard B2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.02				0	0	11.02
10.56	0.1	0.00001	0.000001	0.000001	1	10.56
7.57	0.1	0.00001	0.000001	0.000002	2	7.57
7.04	0.025	0.00001	0.00000025	0.00000225	2.25	7.04
6.69	0.025	0.00001	0.00000025	0.0000025	2.5	6.69
6.16	0.05	0.00001	0.0000005	0.000003	3	6.16
5.33	0.1	0.00001	0.000001	0.000004	4	5.33
4.58	0.1	0.00001	0.000001	0.000005	5	4.58

Standard B3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
9.03				0	0	9.03
7.22	0.05	0.00001	0.0000005	0.0000005	0.5	7.22
7.06	0.025	0.00001	0.00000025	0.00000075	0.75	7.06
6.83	0.025	0.00001	0.00000025	0.000001	1	6.83
6.47	0.025	0.00001	0.00000025	0.00000125	1.25	6.47
6.25	0.025	0.00001	0.00000025	0.0000015	1.5	6.25
5.55	0.05	0.00001	0.0000005	0.000002	2	5.55
5.15	0.05	0.00001	0.0000005	0.0000025	2.5	5.15

Standard C1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.67				0	0	11.67
9.96	0.5	0.00001	0.000005	0.000005	5	9.96
7.84	0.1	0.00001	0.000001	0.000006	6	7.84
7.45	0.025	0.00001	0.00000025	0.00000625	6.25	7.45
7.2	0.025	0.00001	0.00000025	0.0000065	6.5	7.2
7.1	0.025	0.00001	0.00000025	0.00000675	6.75	7.1
6.66	0.025	0.00001	0.00000025	0.000007	7	6.66
6.01	0.1	0.00001	0.000001	0.000008	8	6.01
5.11	0.1	0.00001	0.000001	0.000009	9	5.11

Standard C2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.25				0	0	11.25
10.7	0.2	0.00001	0.000002	0.000002	2	10.7
6.92	0.2	0.00001	0.000002	0.000004	4	6.92
6.64	0.025	0.00001	0.00000025	0.00000425	4.25	6.64
6.17	0.05	0.00001	0.0000005	0.00000475	4.75	6.17
5.36	0.1	0.00001	0.000001	0.00000575	5.75	5.36
4.39	0.1	0.00001	0.000001	0.00000675	6.75	4.39

Standard C3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.57				0	0	11.57
11.44	0.2	0.00001	0.000002	0.000002	2	11.44
10.5	0.2	0.00001	0.000002	0.000004	4	10.5
7.02	0.1	0.00001	0.000001	0.000005	5	7.02
6.46	0.025	0.00001	0.00000025	0.00000525	5.25	6.46
6.15	0.025	0.00001	0.00000025	0.0000055	5.5	6.15
5.45	0.05	0.00001	0.0000005	0.000006	6	5.45
4.52	0.1	0.00001	0.000001	0.000007	7	4.52

Standard D1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.94				0	0	10.94
10.84	0.2	0.00001	0.000002	0.000002	2	10.84
10.74	0.2	0.00001	0.000002	0.000004	4	10.74
10.58	0.2	0.00001	0.000002	0.000006	6	10.58
10.03	0.2	0.00001	0.000002	0.000008	8	10.03
7.76	0.1	0.00001	0.000001	0.000009	9	7.76
7.02	0.05	0.00001	0.0000005	0.0000095	9.5	7.02
6.67	0.05	0.00001	0.0000005	0.00001	10	6.67
5.94	0.1	0.00001	0.000001	0.000011	11	5.94
5.53	0.1	0.00001	0.000001	0.000012	12	5.53
3.87	0.2	0.00001	0.000002	0.000014	14	3.87

Standard D2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	Micromole
10.66				0	0	10.66
10.28	0.2	0.00001	0.000002	0.000002	2	10.28
6.06	0.2	0.00001	0.000002	0.000004	4	6.06
4.44	0.1	0.00001	0.000001	0.000005	5	4.44
3.45	0.1	0.00001	0.000001	0.000006	6	3.45
1.94	0.1	0.00001	0.000001	0.000007	7	1.94

Standard D3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.72				0	0	10.72
10.67	0.2	0.00001	0.000002	0.000002	2	10.67
10.4	0.2	0.00001	0.000002	0.000004	4	10.4
9.4	0.1	0.00001	0.000001	0.000005	5	9.4
8.37	0.05	0.00001	0.0000005	0.0000055	5.5	8.37
7.43	0.05	0.00001	0.0000005	0.000006	6	7.43
6.91	0.05	0.00001	0.0000005	0.0000065	6.5	6.91
6.46	0.05	0.00001	0.0000005	0.000007	7	6.46
6.11	0.1	0.00001	0.000001	0.000008	8	6.11
5.7	0.1	0.00001	0.000001	0.000009	9	5.7

Standard E1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.96				0	0	10.96
10.88	0.2	0.00001	0.000002	0.000002	2	10.88
10.85	0.2	0.00001	0.000002	0.000004	4	10.85
10.84	0.2	0.00001	0.000002	0.000006	6	10.84
10.65	0.2	0.00001	0.000002	0.000008	8	10.65
10.09	0.2	0.00001	0.000002	0.00001	10	10.09
8.51	0.1	0.00001	0.000001	0.000011	11	8.51
6.79	0.1	0.00001	0.000001	0.000012	12	6.79
5.95	0.1	0.00001	0.000001	0.000013	13	5.95
4.58	0.2	0.00001	0.000002	0.000015	15	4.58

Standard E2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.05				0	0	11.05
10.92	0.5	0.00001	0.000005	0.000005	5	10.92
10.76	0.2	0.00001	0.000002	0.000007	7	10.76
10.67	0.2	0.00001	0.000002	0.000009	9	10.67
10.44	0.2	0.00001	0.000002	0.000011	11	10.44
8.42	0.2	0.00001	0.000002	0.000013	13	8.42
7.01	0.1	0.00001	0.000001	0.000014	14	7.01
6.62	0.05	0.00001	0.0000005	0.0000145	14.5	6.62
5.6	0.1	0.00001	0.000001	0.0000155	15.5	5.6
4.95	0.2	0.00001	0.000002	0.0000175	17.5	4.95

Standard E3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.96				0	0	10.96
10.64	0.5	0.00001	0.000005	0.000005	5	10.64
10.29	0.2	0.00001	0.000002	0.000007	7	10.29
9.1	0.1	0.00001	0.000001	0.000008	8	9.1
7.71	0.05	0.00001	0.0000005	0.0000085	8.5	7.71
6.95	0.05	0.00001	0.0000005	0.000009	9	6.95
6.16	0.05	0.00001	0.0000005	0.0000095	9.5	6.16
5.46	0.1	0.00001	0.000001	0.0000105	10.5	5.46
4.33	0.2	0.00001	0.000002	0.0000125	12.5	4.33

Standard F1

pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.06				0	0	11.06
11	0.05	0.00001	0.0000005	0.0000005	0.5	11
10.96	0.1	0.00001	0.000001	0.0000015	1.5	10.96
10.9	0.2	0.00001	0.000002	0.0000035	3.5	10.9
10.87	0.2	0.00001	0.000002	0.0000055	5.5	10.87
10.74	0.2	0.00001	0.000002	0.0000075	7.5	10.74
10.4	0.2	0.00001	0.000002	0.0000095	9.5	10.4
8.34	0.1	0.00001	0.000001	0.0000105	10.5	8.34
7.8	0.05	0.00001	0.0000005	0.000011	11	7.8
7.24	0.05	0.00001	0.0000005	0.0000115	11.5	7.24
6.84	0.05	0.00001	0.0000005	0.000012	12	6.84
6.32	0.1	0.00001	0.000001	0.000013	13	6.32
5.5	0.1	0.00001	0.000001	0.000014	14	5.5
4.42	0.2	0.00001	0.000002	0.000016	16	4.42

Standard F2

pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.93				0	0	10.93
10.9	0.2	0.00001	0.000002	0.000002	2	10.9
10.85	0.2	0.00001	0.000002	0.000004	4	10.85
10.77	0.2	0.00001	0.000002	0.000006	6	10.77
10.63	0.2	0.00001	0.000002	0.000008	8	10.63
10.23	0.2	0.00001	0.000002	0.00001	10	10.23
9.41	0.1	0.00001	0.000001	0.000011	11	9.41
8	0.1	0.00001	0.000001	0.000012	12	8
7.25	0.05	0.00001	0.0000005	0.0000125	12.5	7.25
6.92	0.05	0.00001	0.0000005	0.000013	13	6.92
5.83	0.1	0.00001	0.000001	0.000014	14	5.83
4.97	0.2	0.00001	0.000002	0.000016	16	4.97

Standard F3

pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.87				0	0	10.87
10.76	0.02	0.00001	0.0000002	0.0000002	0.2	10.76
10.52	0.2	0.00001	0.000002	0.0000022	2.2	10.52
9.35	0.2	0.00001	0.000002	0.0000042	4.2	9.35
7.22	0.1	0.00001	0.000001	0.0000052	5.2	7.22
6.4	0.05	0.00001	0.0000005	0.0000057	5.7	6.4
5.67	0.05	0.00001	0.0000005	0.0000062	6.2	5.67
5.03	0.1	0.00001	0.000001	0.0000072	7.2	5.03
4.25	0.2	0.00001	0.000002	0.0000092	9.2	4.25

Standard G1

pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.52				0	0	11.52
11.5	0.2	0.00001	0.000002	0.000002	2	11.5
11.46	0.5	0.00001	0.000005	0.000007	7	11.46
10.55	0.5	0.00001	0.000005	0.000012	12	10.55
8.95	0.1	0.00001	0.000001	0.000013	13	8.95
7.81	0.1	0.00001	0.000001	0.000014	14	7.81
7.45	0.05	0.00001	0.0000005	0.0000145	14.5	7.45
7.17	0.05	0.00001	0.0000005	0.000015	15	7.17
6.98	0.05	0.00001	0.0000005	0.0000155	15.5	6.98
6.5	0.1	0.00001	0.000001	0.0000165	16.5	6.5
6	0.2	0.00001	0.000002	0.0000185	18.5	6

Standard G2

pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.85				0	0	11.85
11.23	0.5	0.00001	0.000005	0.000005	5	11.23
5.23	0.5	0.00001	0.000005	0.00001	10	5.23
4.16	0.2	0.00001	0.000002	0.000012	12	4.16
3.24	0.2	0.00001	0.000002	0.000014	14	3.24
2.15	0.2	0.00001	0.000002	0.000016	16	2.15

Standard G3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.94				0	0	11.94
11.75	0.5	0.00001	0.000005	0.000005	5	11.75
11.7	0.2	0.00001	0.000002	0.000007	7	11.7
11.48	0.2	0.00001	0.000002	0.000009	9	11.48
11.03	0.2	0.00001	0.000002	0.000011	11	11.03
8.45	0.2	0.00001	0.000002	0.000013	13	8.45
7.64	0.1	0.00001	0.000001	0.000014	14	7.64
7.03	0.1	0.00001	0.000001	0.000015	15	7.03
6.85	0.05	0.00001	0.0000005	0.0000155	15.5	6.85
6.41	0.1	0.00001	0.000001	0.0000165	16.5	6.41
5.95	0.2	0.00001	0.000002	0.0000185	18.5	5.95

Standard H1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.8				0	0	11.8
11.78	0.5	0.00001	0.000005	0.000005	5	11.78
11.75	0.2	0.00001	0.000002	0.000007	7	11.75
11.7	0.2	0.00001	0.000002	0.000009	9	11.7
11.65	0.2	0.00001	0.000002	0.000011	11	11.65
11.54	0.2	0.00001	0.000002	0.000013	13	11.54
11.16	0.2	0.00001	0.000002	0.000015	15	11.16
8.92	0.2	0.00001	0.000002	0.000017	17	8.92
7.75	0.1	0.00001	0.000001	0.000018	18	7.75
7.46	0.1	0.00001	0.000001	0.000019	19	7.46
6.6	0.1	0.00001	0.000001	0.00002	20	6.6
5.55	0.2	0.00001	0.000002	0.000022	22	5.55

Standard H2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.75				0	0	11.75
11.54	0.5	0.00001	0.000005	0.000005	5	11.54
11.4	0.2		0.000002	0.000007	7	11.4
10.7	0.2	0.00001	0.000002	0.000009	9	10.7
9.18	0.2	0.00001	0.000002	0.000011	11	9.18
8.7	0.1	0.00001	0.000001	0.000012	12	8.7
8.12	0.1	0.00001	0.000001	0.000013	13	8.12
7.8	0.1	0.00001	0.000001	0.000014	14	7.8
7.5	0.1	0.00001	0.000001	0.000015	15	7.5
7.25	0.1	0.00001	0.000001	0.000016	16	7.25
7.01	0.1	0.00001	0.000001	0.000017	17	7.01
6.8	0.1	0.00001	0.000001	0.000018	18	6.8
6.4	0.2	0.00001	0.000002	0.00002	20	6.4

Standard H3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.9				0	0	11.9
11.72	0.5	0.00001	0.000005	0.000005	5	11.72
11.12	0.5	0.00001	0.000005	0.00001	10	11.12
9.02	0.2	0.00001	0.000002	0.000012	12	9.02
7.56	0.1	0.00001	0.000001	0.000013	13	7.56
6.75	0.1	0.00001	0.000001	0.000014	14	6.75
6.11	0.1	0.00001	0.000001	0.000015	15	6.11
5.3	0.2	0.00001	0.000002	0.000017	17	5.3

Standard I1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
10.88				0	0	10.88
10.66	0.2	0.00001	0.000002	0.000002	2	10.66
9.94	0.2	0.00001	0.000002	0.000004	4	9.94
6.5	0.2	0.00001	0.000002	0.000006	6	6.5
5.06	0.2	0.00001	0.000002	0.000008	8	5.06
4.18	0.2	0.00001	0.000002	0.00001	10	4.18

Standard I2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.24				0	0	11.24
11.17	0.2	0.00001	0.000002	0.000002	2	11.17
11.16	0.2	0.00001	0.000002	0.000004	4	11.16
10.87	0.5	0.00001	0.000005	0.000009	9	10.87
10.57	0.2	0.00001	0.000002	0.000011	11	10.57
8.71	0.2	0.00001	0.000002	0.000013	13	8.71
7.4	0.2	0.00001	0.000002	0.000015	15	7.4
7.02	0.1	0.00001	0.000001	0.000016	16	7.02
6.99	0.05	0.00001	0.0000005	0.0000165	16.5	6.99
6.54	0.1	0.00001	0.000001	0.0000175	17.5	6.54
5.95	0.2	0.00001	0.000002	0.0000195	19.5	5.95

Standard I3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.3				0	0	11.3
11.16	0.5	0.00001	0.000005	0.000005	5	11.16
10.97	0.5	0.00001	0.000005	0.00001	10	10.97
10.72	0.2	0.00001	0.000002	0.000012	12	10.72
10.3	0.2	0.00001	0.000002	0.000014	14	10.3
8	0.2	0.00001	0.000002	0.000016	16	8
7.28	0.1	0.00001	0.000001	0.000017	17	7.28
6.78	0.1	0.00001	0.000001	0.000018	18	6.78
6.46	0.1	0.00001	0.000001	0.000019	19	6.46
5.87	0.2	0.00001	0.000002	0.000021	21	5.87

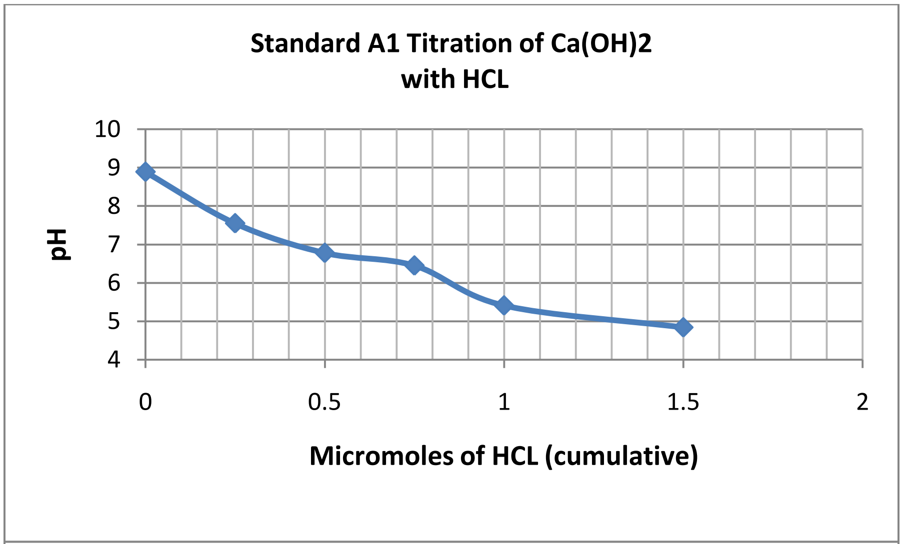
Standard J1						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.38				0	0	11.38
11.25	0.5	0.00001	0.000005	0.000005	5	11.25
11.3	0.5	0.00001	0.000005	0.00001	10	11.3
11.05	0.5	0.00001	0.000005	0.000015	15	11.05
10.76	0.2	0.00001	0.000002	0.000017	17	10.76
10.45	0.2	0.00001	0.000002	0.000019	19	10.45
8.3	0.2	0.00001	0.000002	0.000021	21	8.3
7.5	0.1	0.00001	0.000001	0.000022	22	7.5
7.03	0.1	0.00001	0.000001	0.000023	23	7.03
6.6	0.1	0.00001	0.000001	0.000024	24	6.6
5.97	0.2	0.00001	0.000002	0.000026	26	5.97

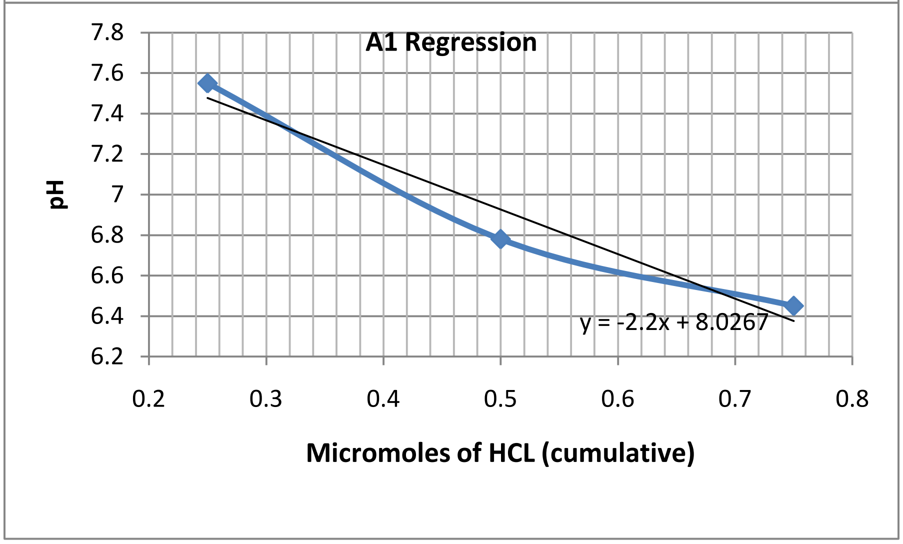
Standard J2						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.59				0	0	11.59
11.54	0.5	0.00001	0.000005	0.000005	5	11.54
11.4	0.5	0.00001	0.000005	0.00001	10	11.4
11.1	0.5	0.00001	0.000005	0.000015	15	11.1
10.97	0.2	0.00001	0.000002	0.000017	17	10.97
10.7	0.2	0.00001	0.000002	0.000019	19	10.7
8.9	0.2	0.00001	0.000002	0.000021	21	8.9
7.9	0.1	0.00001	0.000001	0.000022	22	7.9
7.15	0.1	0.00001	0.000001	0.000023	23	7.15
6.89	0.1	0.00001	0.000001	0.000024	24	6.89
5.74	0.2	0.00001	0.000002	0.000026	26	5.74

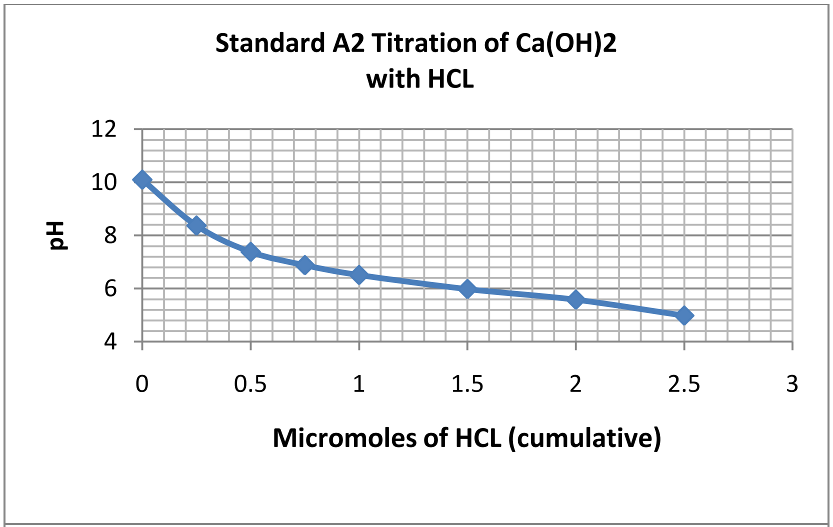
Standard J3						
pH	[HCl]	HCl (L)	HCl Added (	HCl Cumulat	Micromol	pH
11.55				0	0	11.55
11.38	0.5	0.00001	0.000005	0.000005	5	11.38
10.16	0.5	0.00001	0.000005	0.00001	10	10.16
7.53	0.2	0.00001	0.000002	0.000012	12	7.53
7.05	0.1	0.00001	0.000001	0.000013	13	7.05
6.85	0.05	0.00001	0.0000005	0.0000135	13.5	6.85
6.21	0.1	0.00001	0.000001	0.0000145	14.5	6.21
5.82	0.2	0.00001	0.000002	0.0000165	16.5	5.82

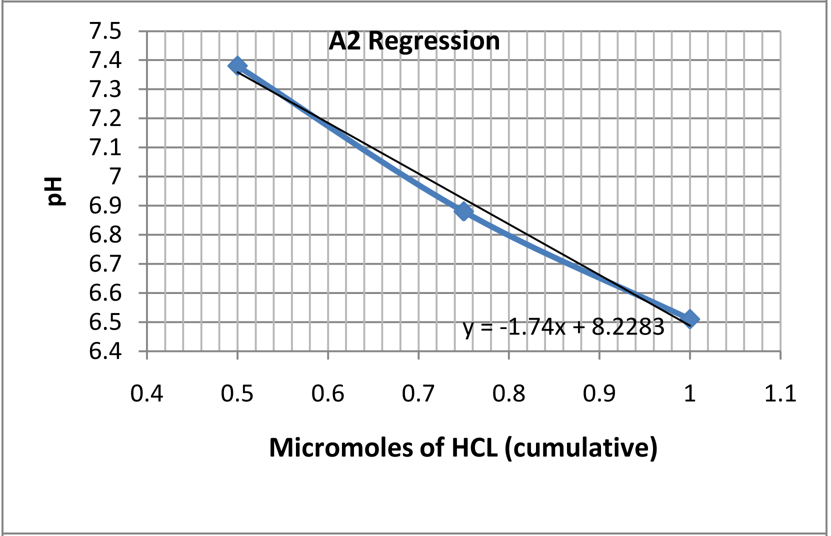
Appendix VI.

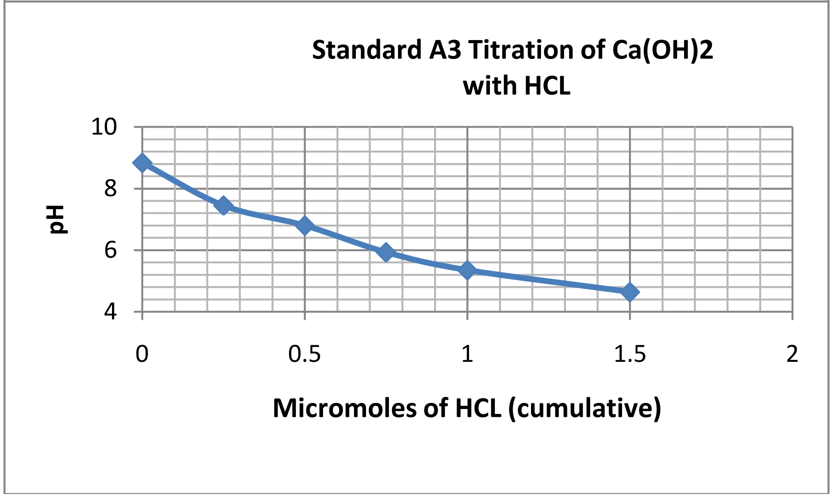
Titration graphs and linear regression graphs for the actual standard curve.

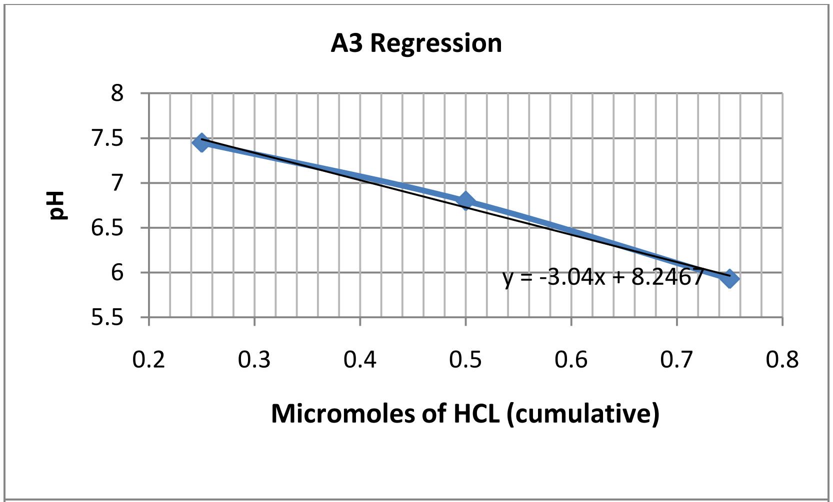


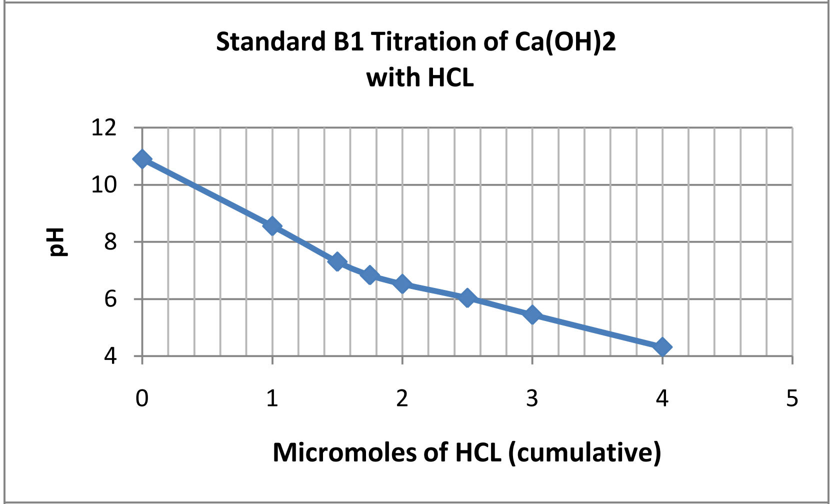


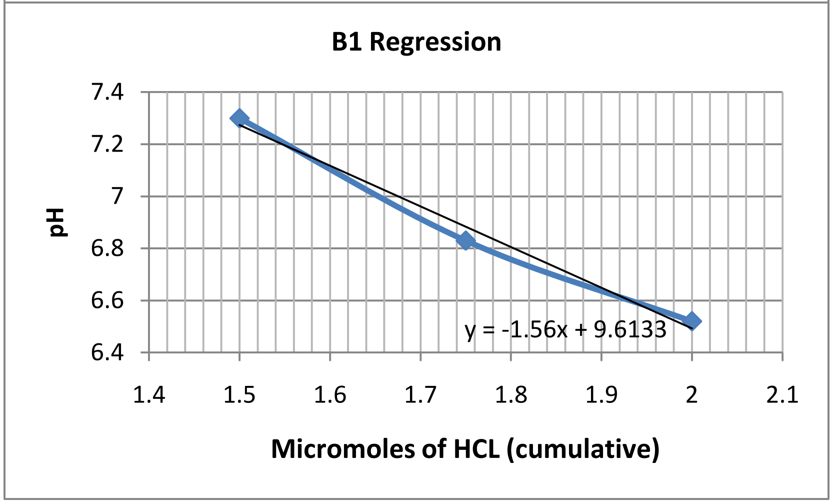

Standard A2 Titration of Ca(OH)2 with HCL
12
10
8
6
4
pH
0
0.5
1
1.5
2
2.5
3
Micromoles of HCL (cumulative)

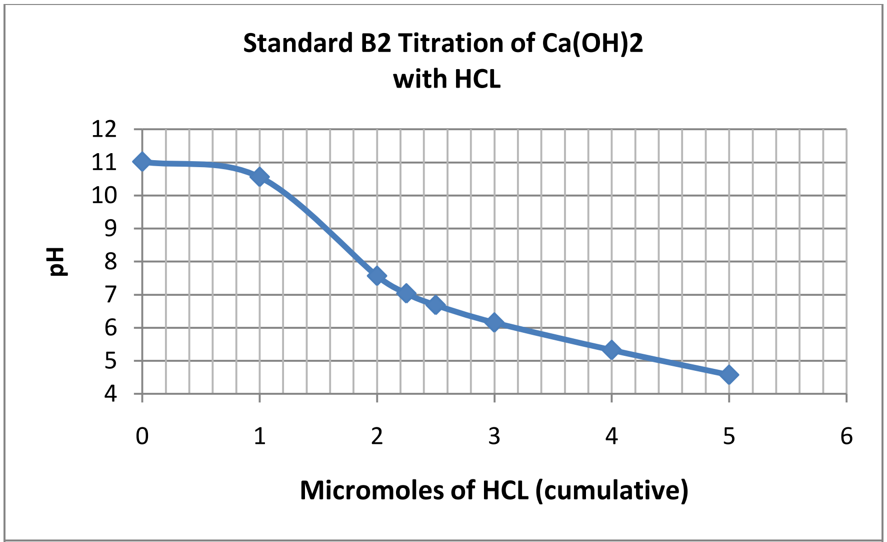

A2 Regression
7.5
7.4
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
6.4
pH
y = -1.74x + 8.2283
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
Micromoles of HCL (cumulative)


Standard A3 Titration of Ca(OH)2 with HCL
10
8
6
4
pH
0
0.5
1
1.5
2
Micromoles of HCL (cumulative)

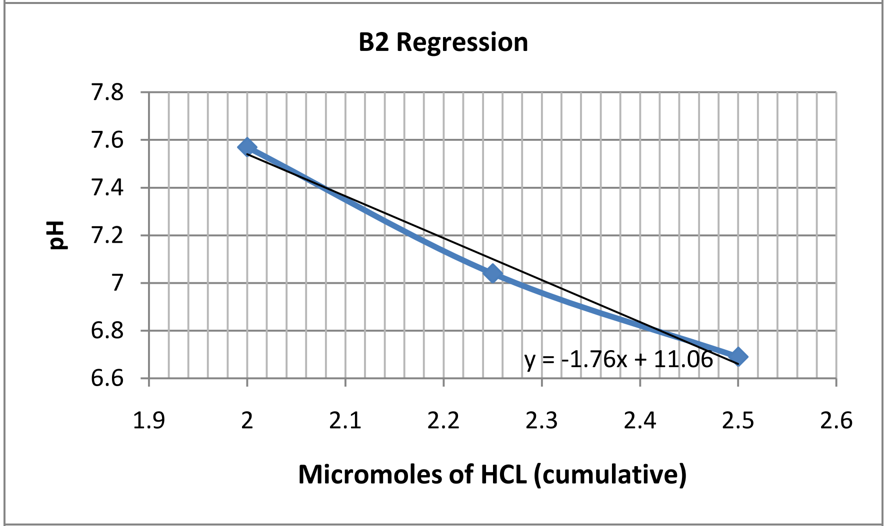

A3 Regression
pH
8
7.5
7
6.5
6
5.5
0.2
0.3
0.4
0.5
0.6
0.7
0.8
y = -3.04x + 8.2467
Micromoles of HCL (cumulative)


Standard B1 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
0
1
2
3
4
5
Micromoles of HCL (cumulative)

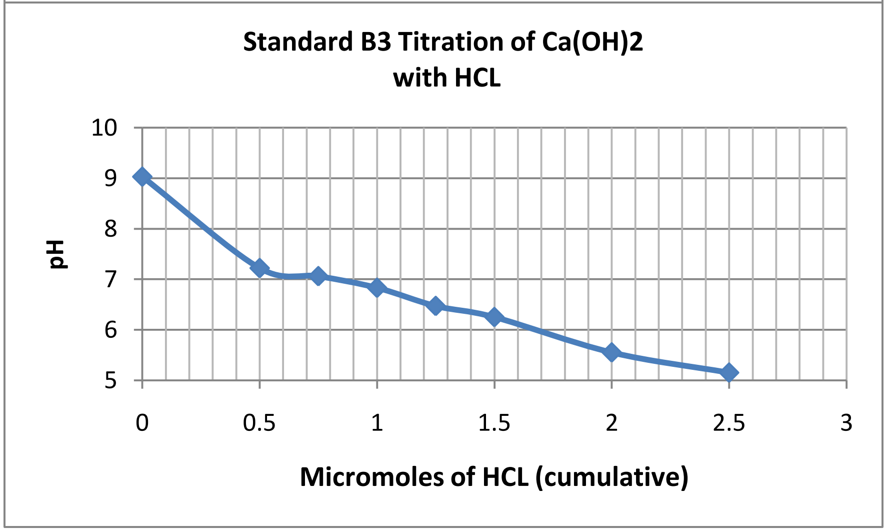

B1 Regression
pH
7.4
7.2
7
6.8
6.6
6.4
1.4
1.5
1.6
1.7
1.8
1.9
2
2.1
y = -1.56x + 9.6133
Micromoles of HCL (cumulative)



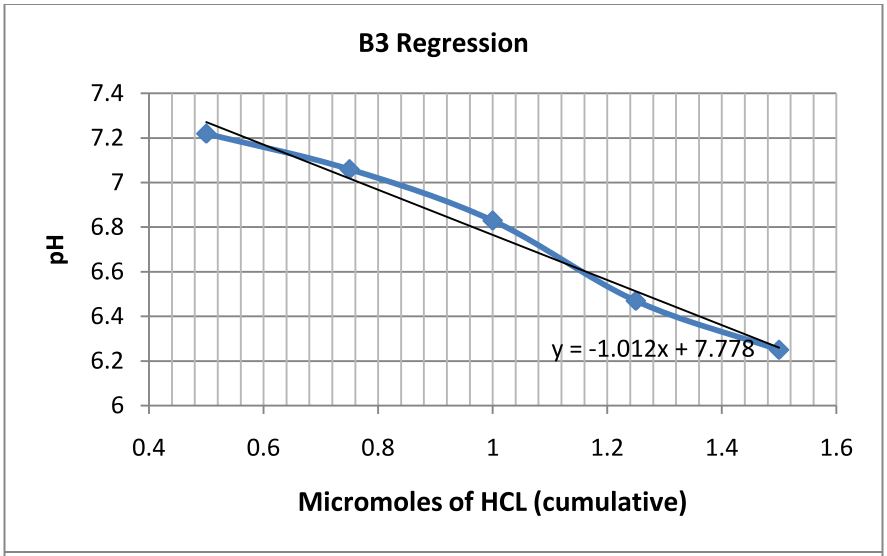
Standard B2 Titration of Ca(OH)2 with HCL

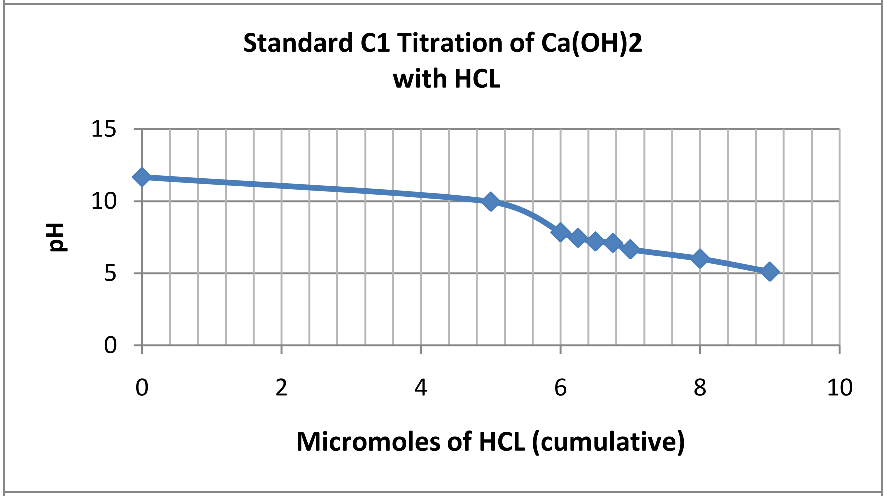


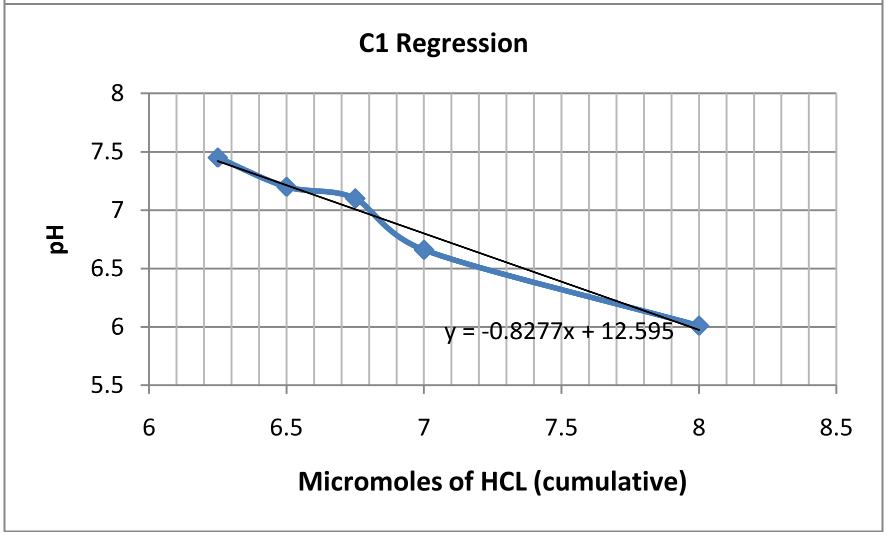
B2 Regression

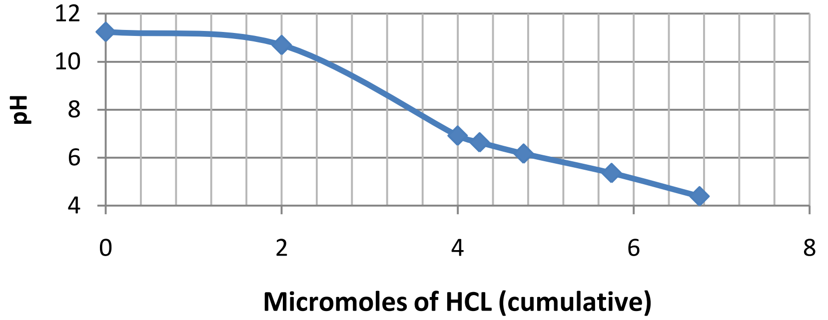


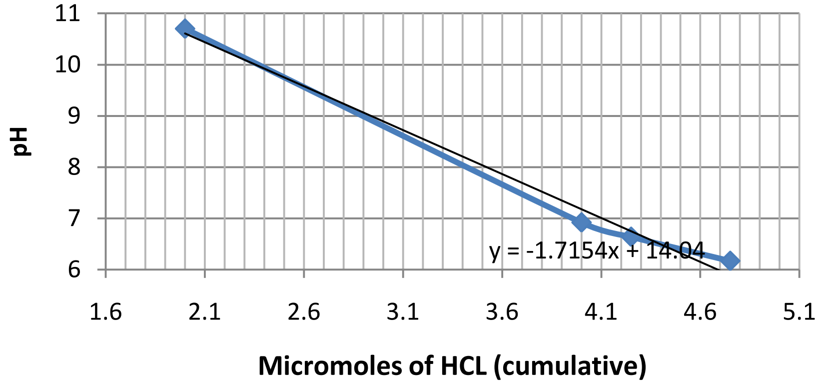
Standard B3 Titration of Ca(OH)2 with HCL

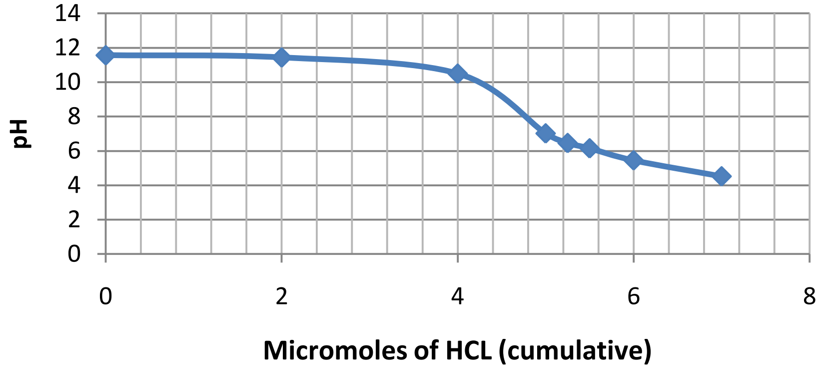

B3 Regression
pH
7.4
7.2
7
6.8
6.6
6.4
6.2
6
0.4
0.6
0.8
1
1.2
1.4
1.6
y = -1.012x + 7.778
Micromoles of HCL (cumulative)

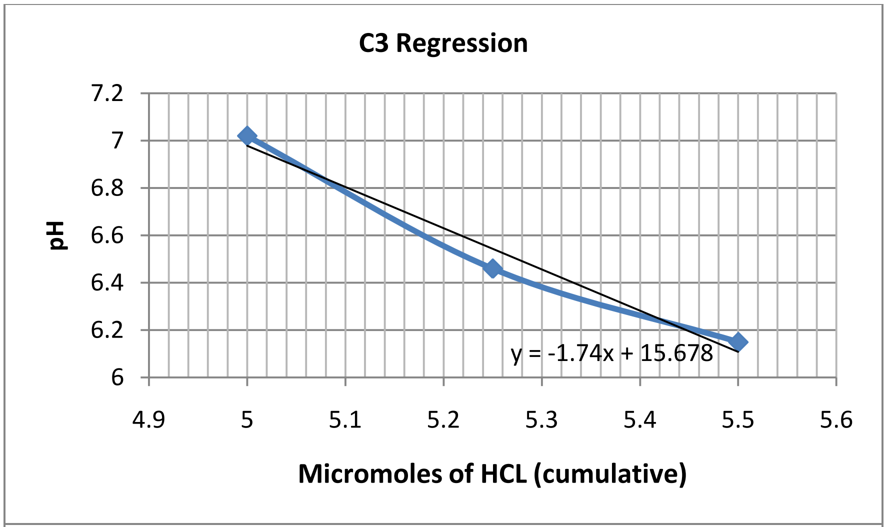

Standard C1 Titration of Ca(OH)2 with HCL
pH
15
10
5
0
0
2
4
6
8
10
Micromoles of HCL (cumulative)

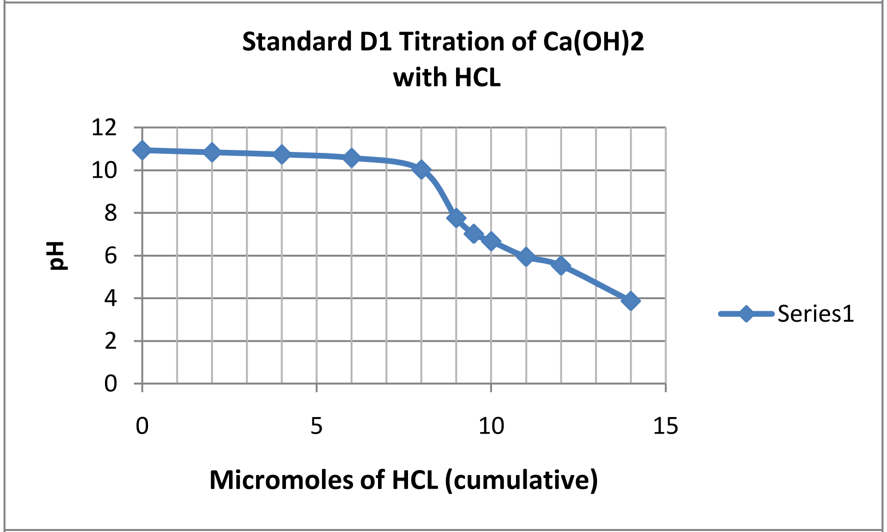

C1 Regression
pH
8
7.5
7
6.5
6
5.5
6
6.5
7
7.5
8
8.5
y = -0.8277x + 12.595
Micromoles of HCL (cumulative)

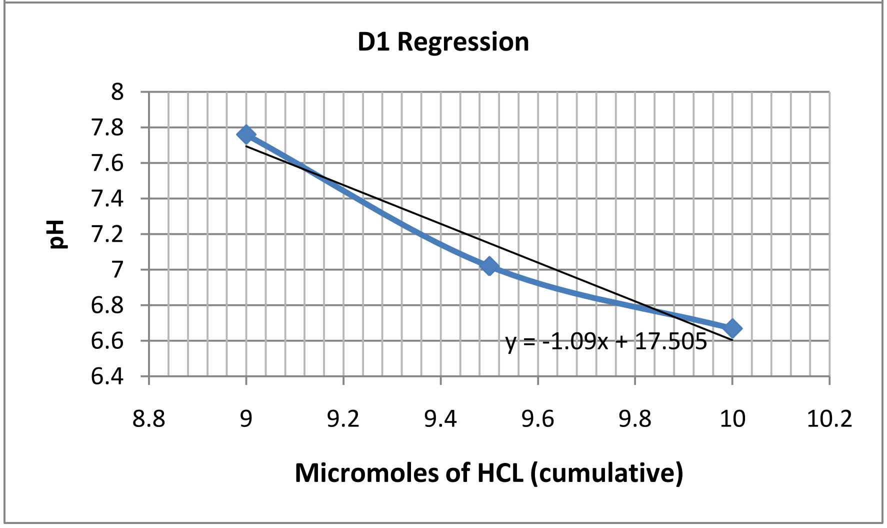

Standard C2 Titration of Ca(OH)2 with HCL

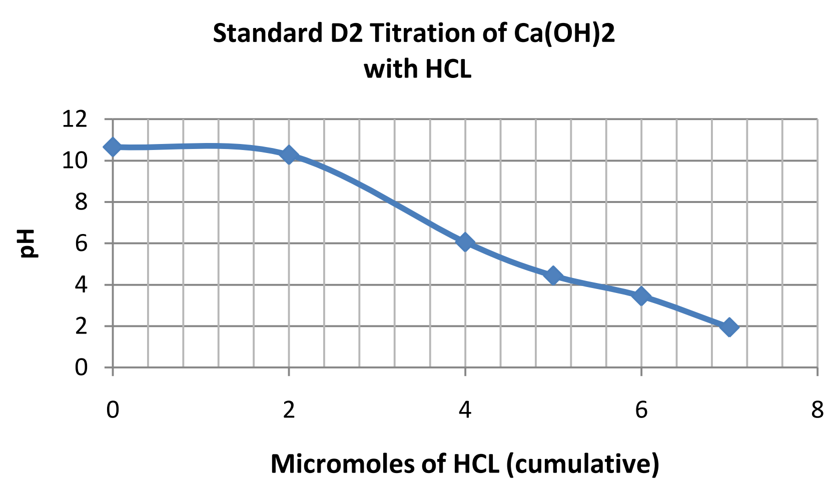

C2 Regression

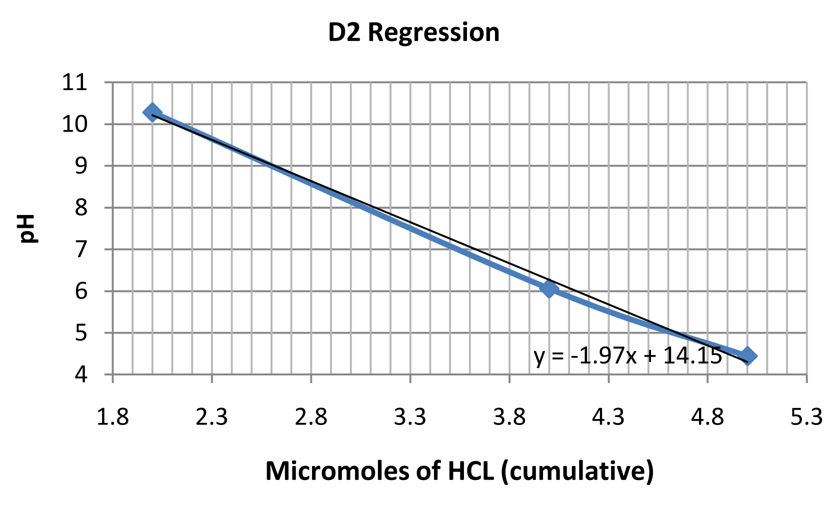

Standard C3 Titration of Ca(OH)2 with HCL

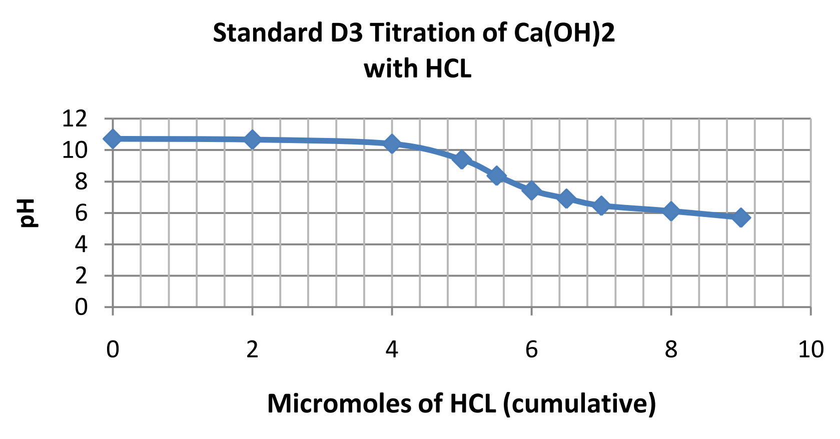

C3 Regression
pH
7.2
7
6.8
6.6
6.4
6.2
6
y = -1.74x + 15.678
4.9
5
5.1
5.2
5.3
5.4
5.5
5.6
Micromoles of HCL (cumulative)

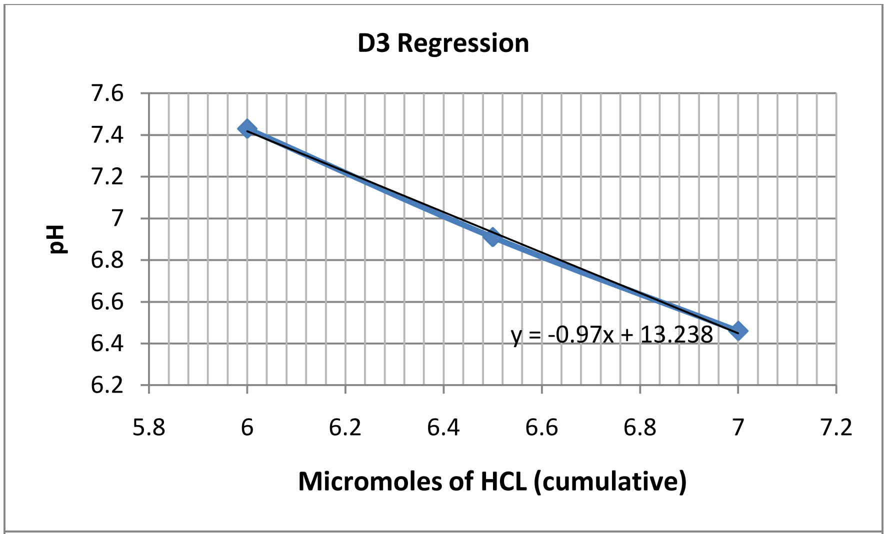

Standard D1 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
Micromoles of HCL (cumulative)
Series1

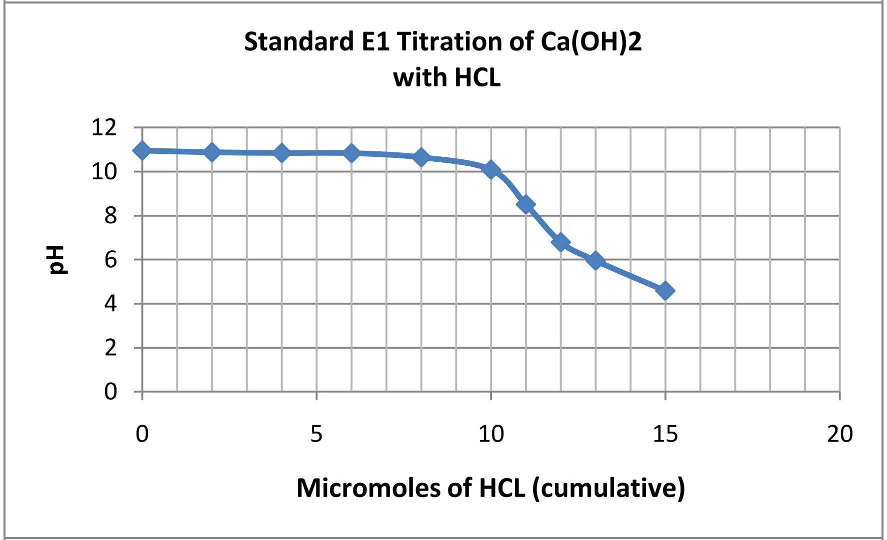

D1 Regression
pH
8
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
y = -1.09x + 17.505
8.8
9
9.2
9.4
9.6
9.8
10
10.2
Micromoles of HCL (cumulative)

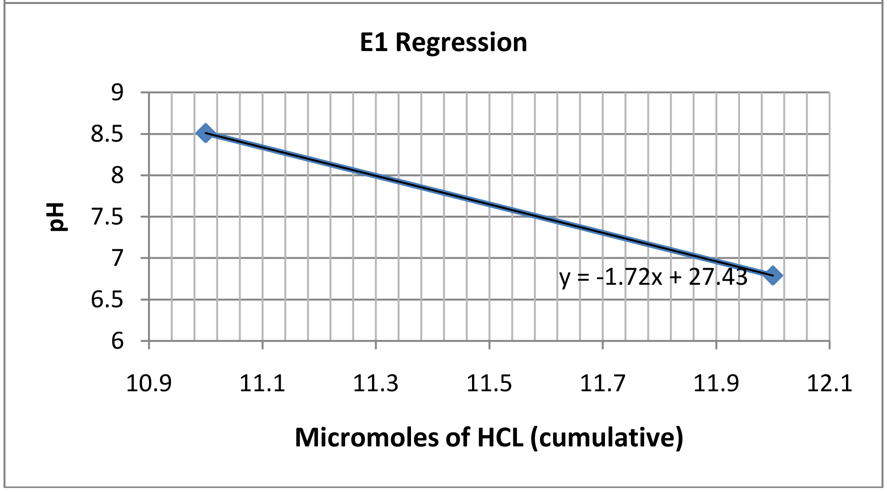

Standard D2 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
Micromoles of HCL (cumulative)

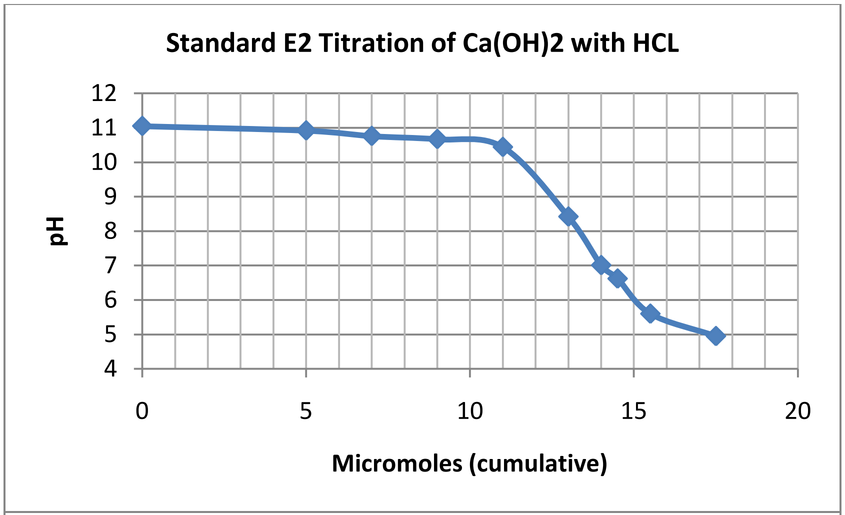

D2 Regression
pH
11
10
9
8
7
6
5
4
y = -1.97x + 14.15
1.8
2.3
2.8
3.3
3.8
4.3
4.8
5.3
Micromoles of HCL (cumulative)

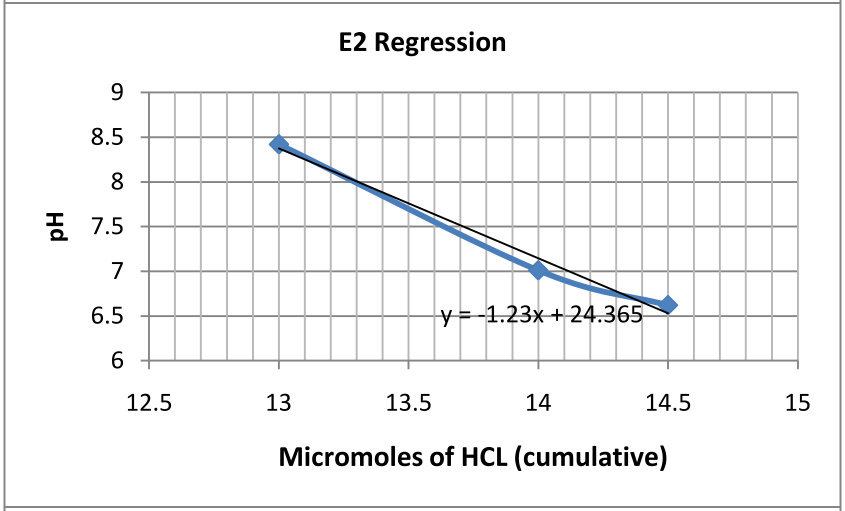

Standard D3 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
Micromoles of HCL (cumulative)

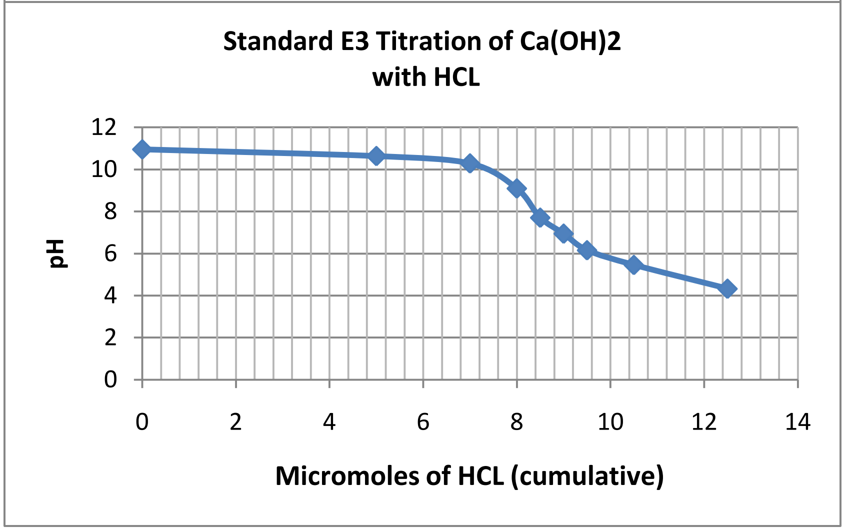

D3 Regression
pH
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
5.8
6
6.2
6.4
6.6
6.8
7
7.2
y = -0.97x + 13.238
Micromoles of HCL (cumulative)

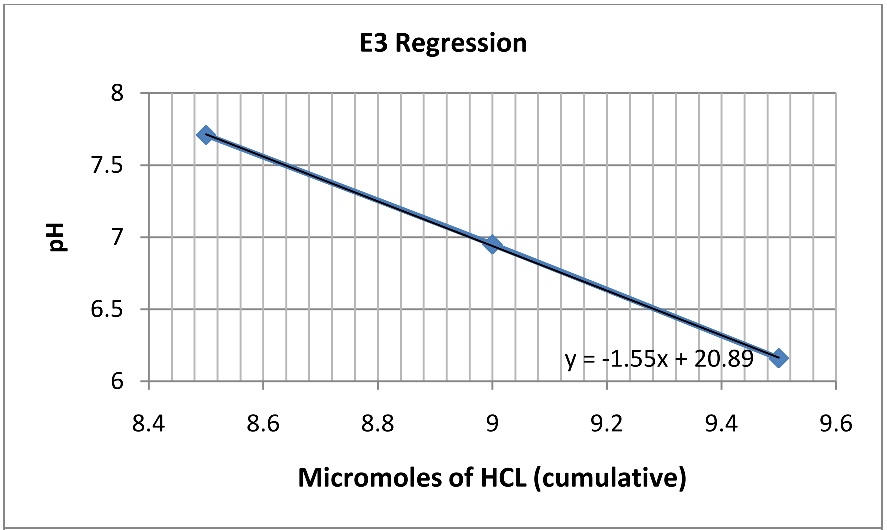

Standard E1 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
20
Micromoles of HCL (cumulative)

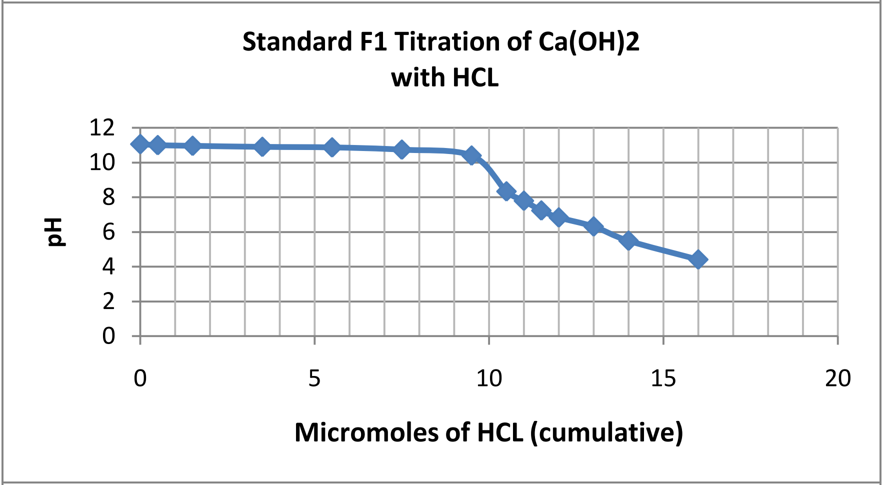

E1 Regression
pH
9
8.5
8
7.5
7
6.5
6
10.9
11.1
11.3
11.5
11.7
11.9
12.1
y = -1.72x + 27.43
Micromoles of HCL (cumulative)

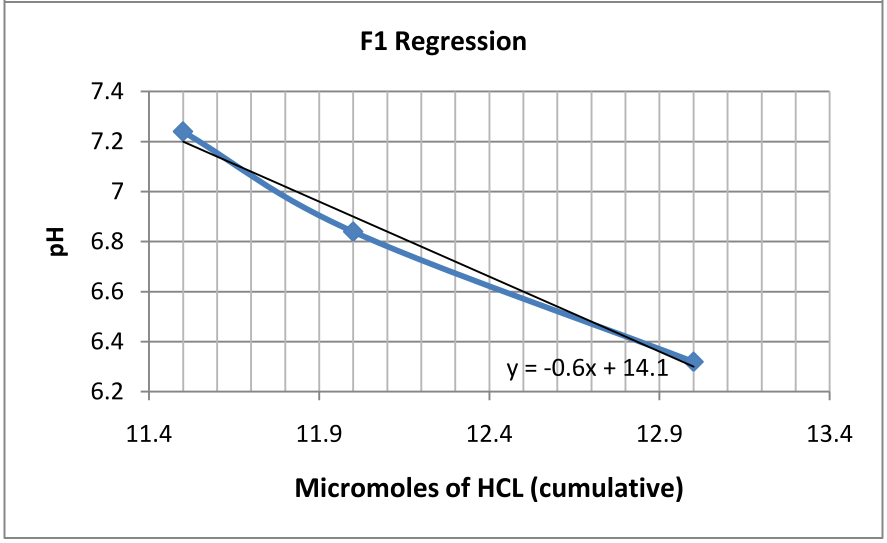

Standard E2 Titration of Ca(OH)2 with HCL

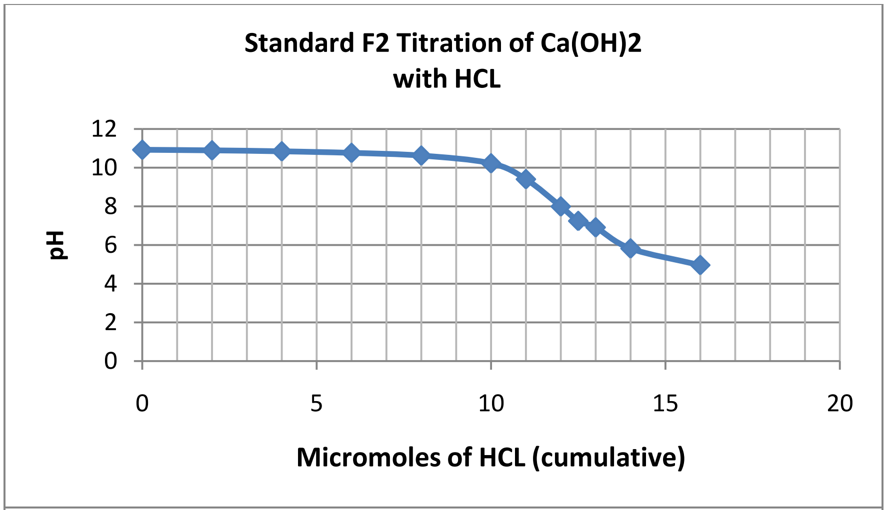

E2 Regression

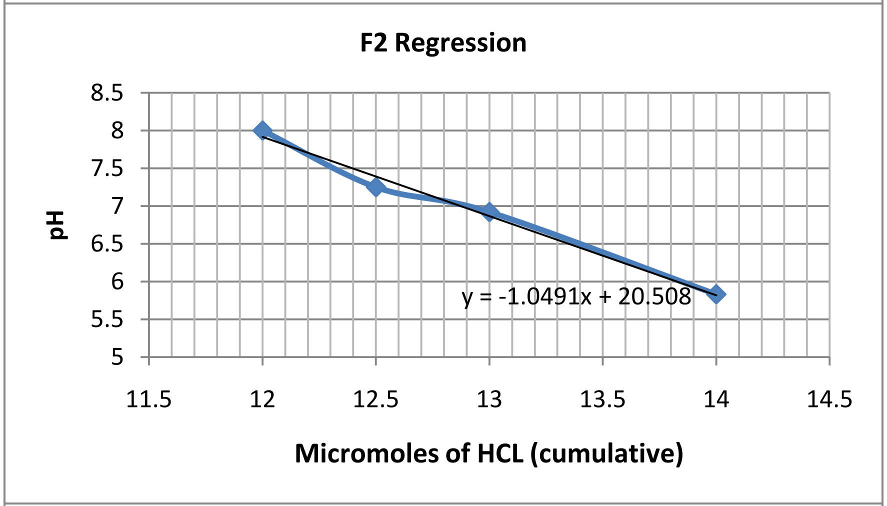

Standard E3 Titration of Ca(OH)2 with HCL

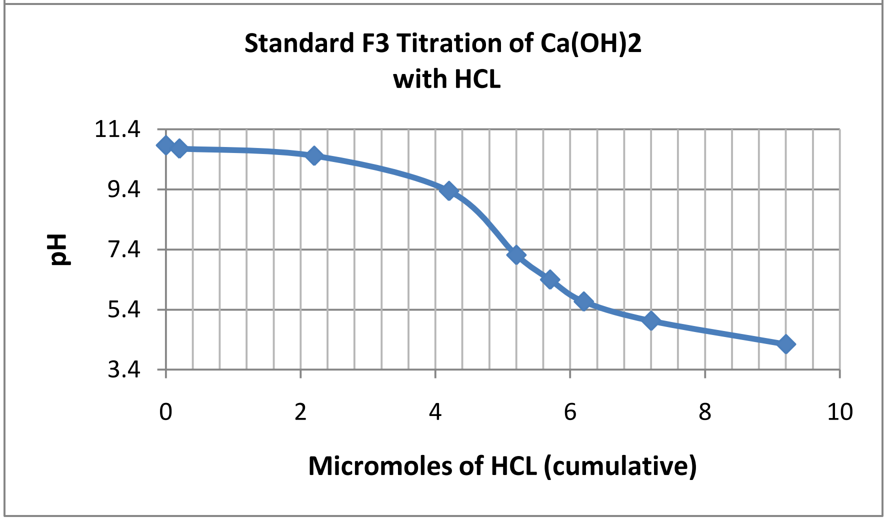

E3 Regression

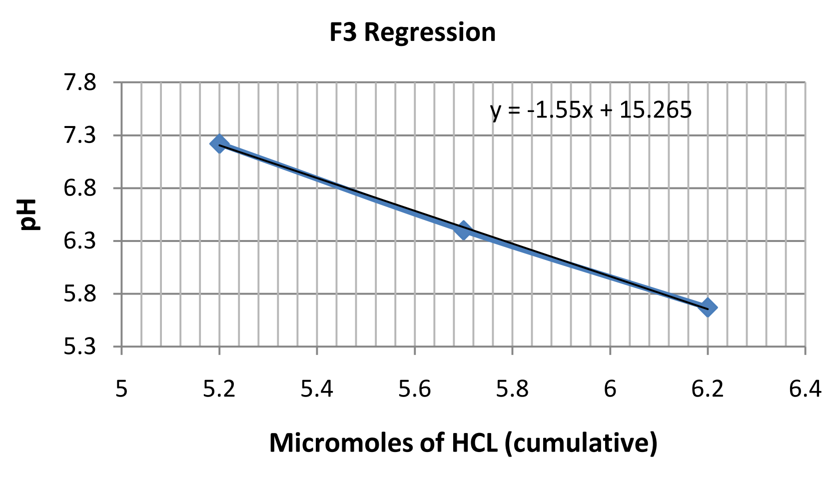

Standard F1 Titration of Ca(OH)2 with HCL

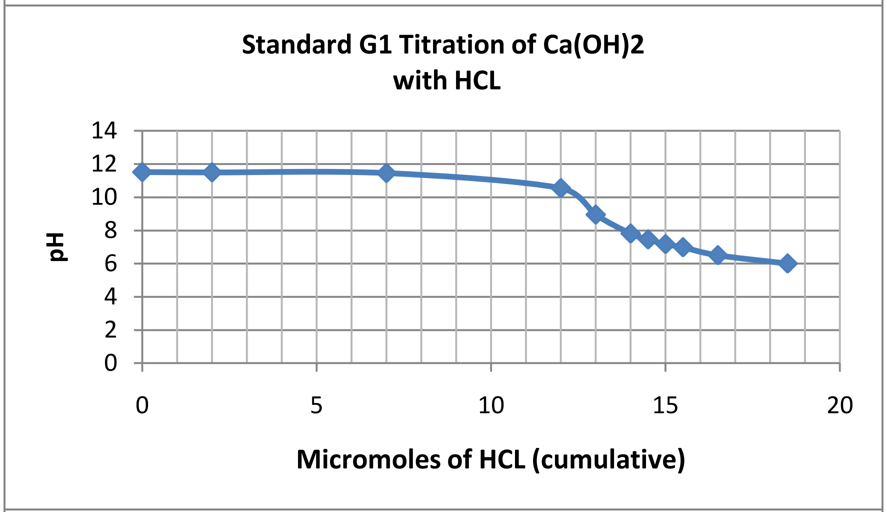

F1 Regression

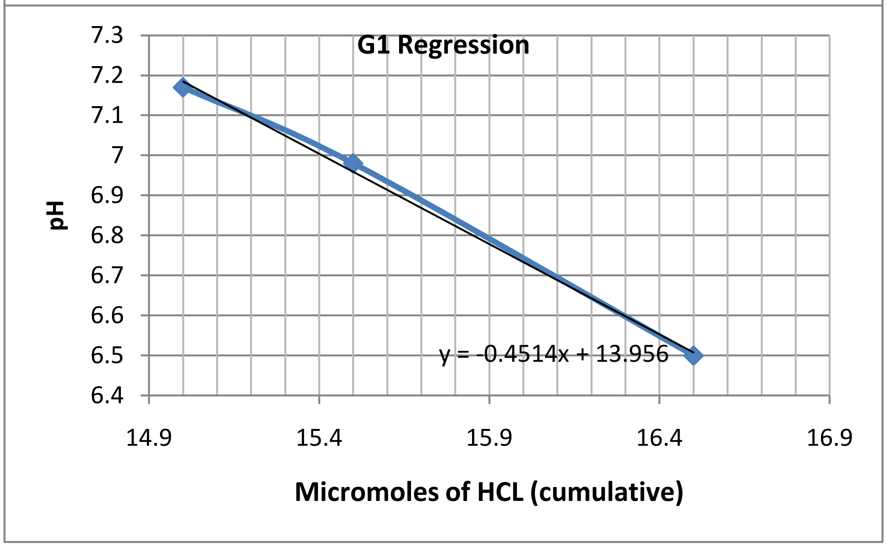

Standard F2 Titration of Ca(OH)2 with HCL

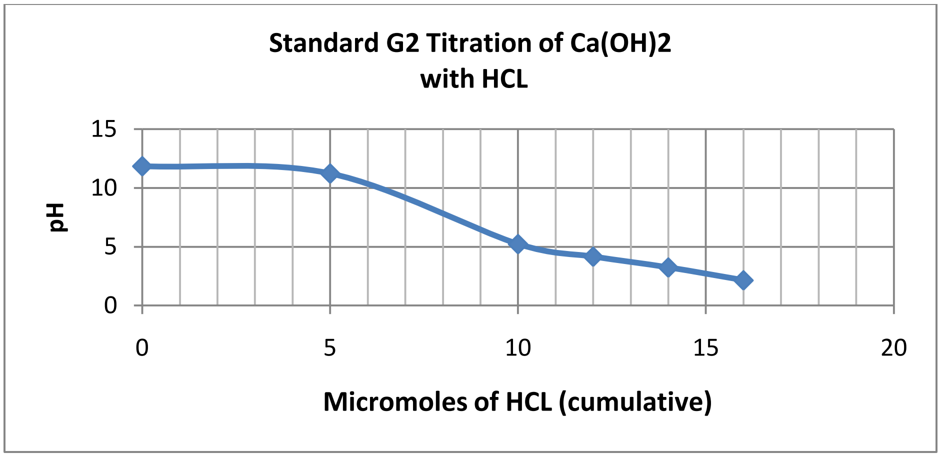

F2 Regression

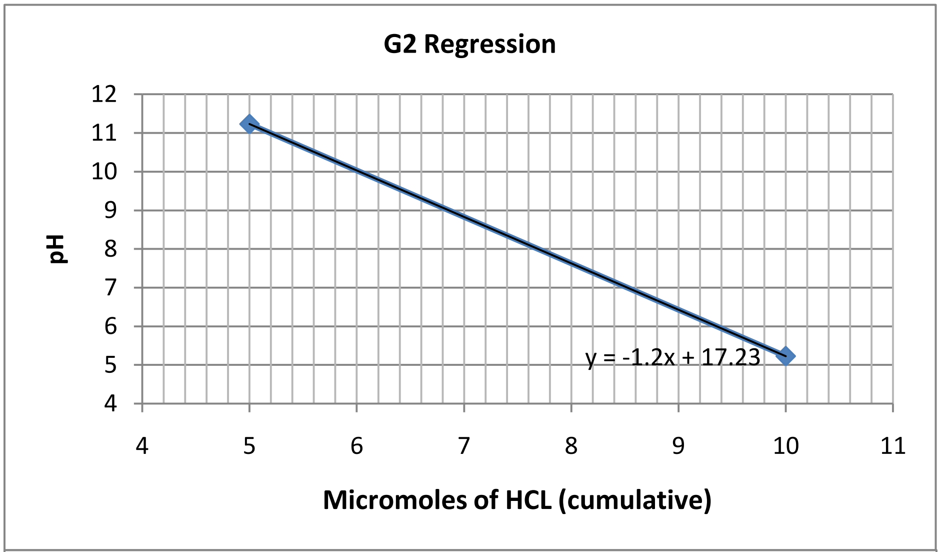

Standard F3 Titration of Ca(OH)2 with HCL

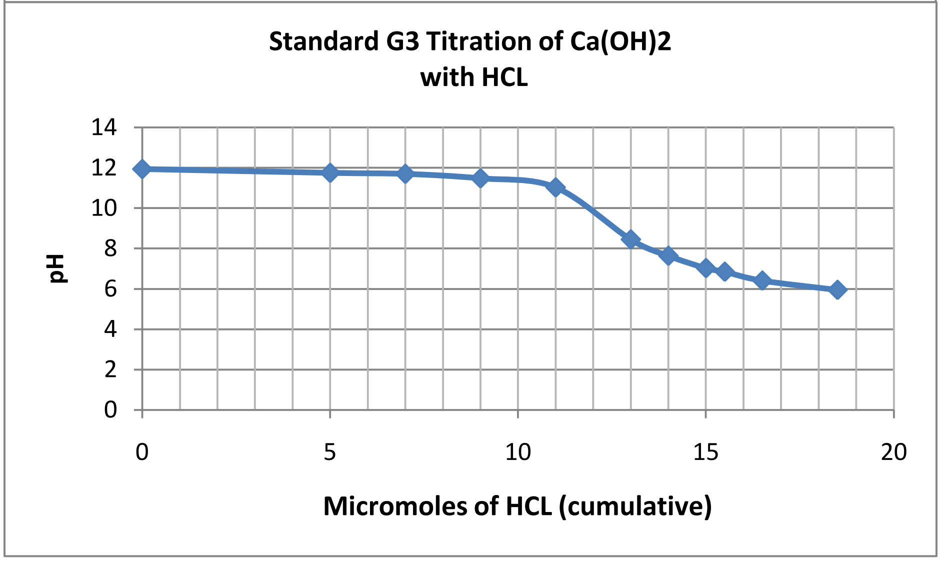

F3 Regression
y = -1.55x + 15.265
pH
7.8
7.3
6.8
6.3
5.8
5.3
5
5.2
5.4
5.6
5.8
6
6.2
6.4
Micromoles of HCL (cumulative)

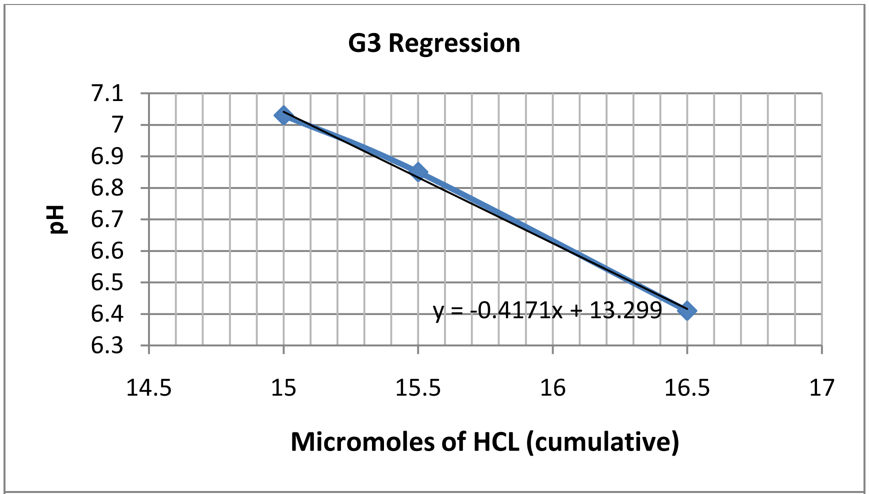

Standard G1 Titration of Ca(OH)2 with HCL
pH
14
12
10
8
6
4
2
0
0
5
10
15
20
Micromoles of HCL (cumulative)

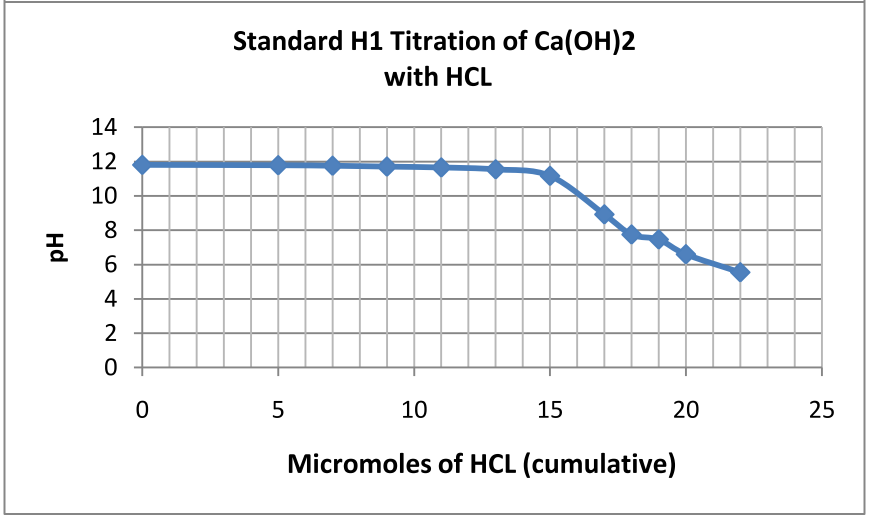

G1 Regression
y = -0.4514x + 13.956
pH
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
6.4
14.9
15.4
15.9
16.4
16.9
Micromoles of HCL (cumulative)

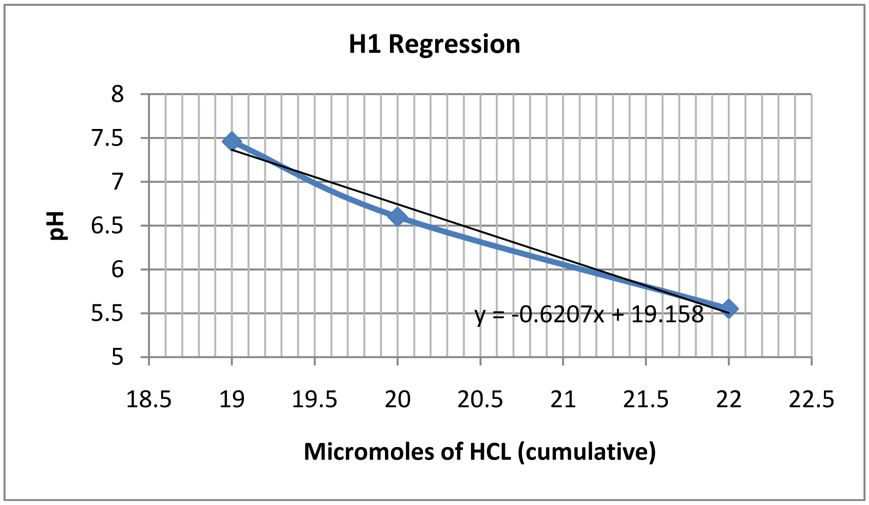

Standard G2 Titration of Ca(OH)2 with HCL
pH
15
10
5
0
0
5
10
15
20
Micromoles of HCL (cumulative)

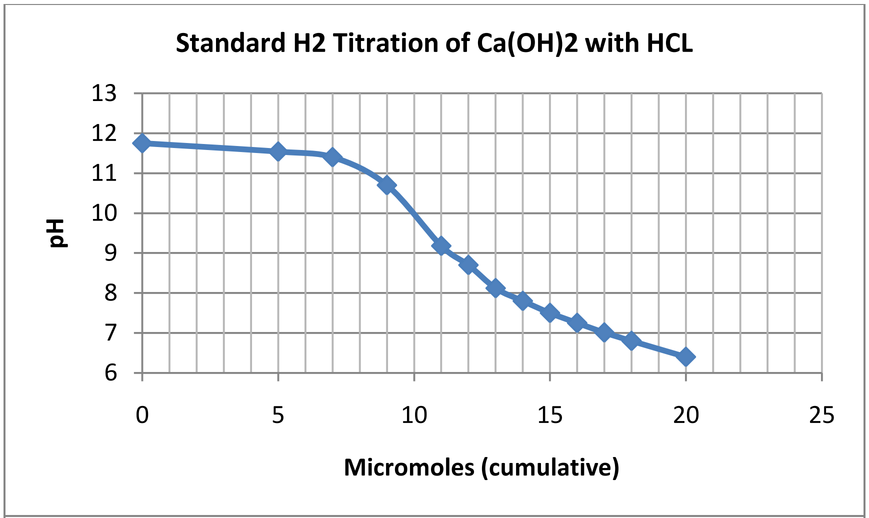

G2 Regression
pH
12
11
10
9
8
7
6
5
4
4
5
6
7
8
9
10
11
y = -1.2x + 17.23
Micromoles of HCL (cumulative)

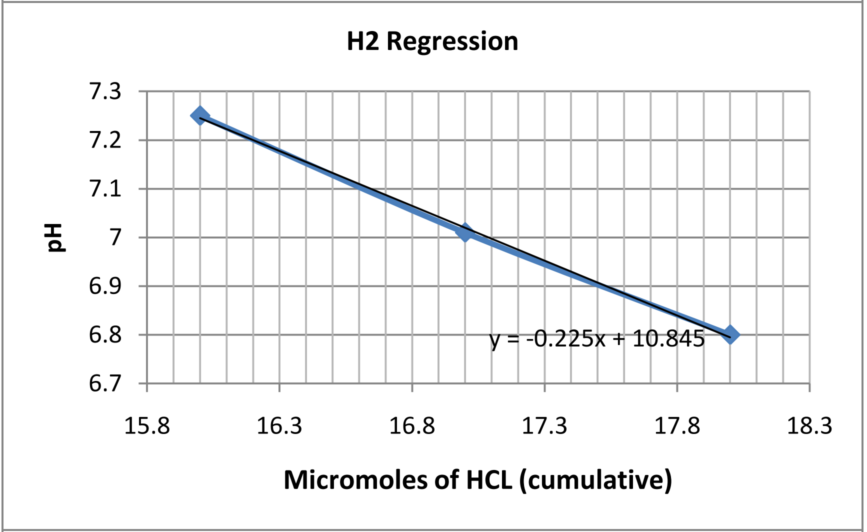

Standard G3 Titration of Ca(OH)2 with HCL
pH
14
12
10
8
6
4
2
0
0
5
10
15
20
Micromoles of HCL (cumulative)

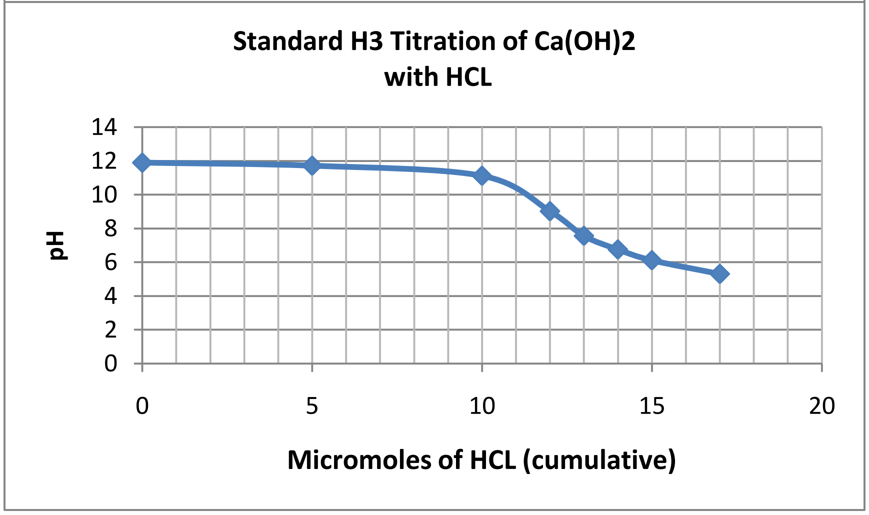

G3 Regression
7.1
7
6.9
6.8
6.7
6.6
6.5
6.4
6.3
pH
y = -0.4171x + 13.299
14.5
15
15.5
16
16.5
17
Micromoles of HCL (cumulative)

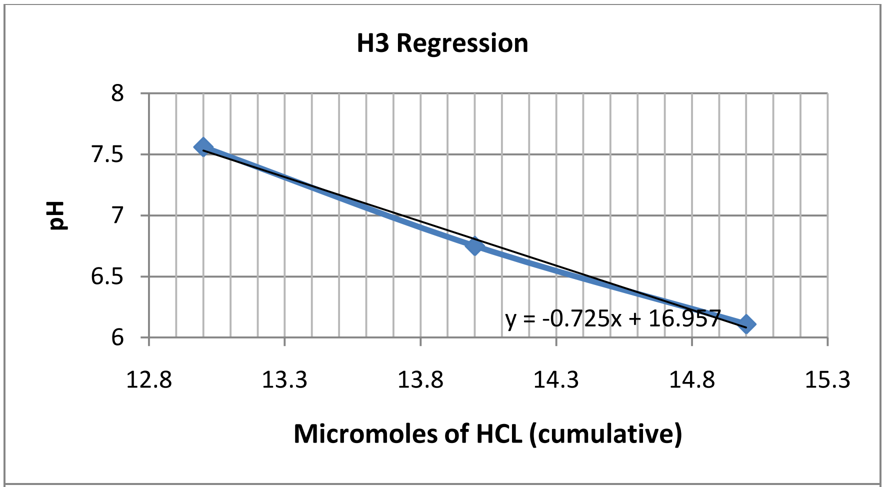

Standard H1 Titration of Ca(OH)2 with HCL
14
12
10
8
6
4
2
0
pH
0
5
10
15
20
25
Micromoles of HCL (cumulative)

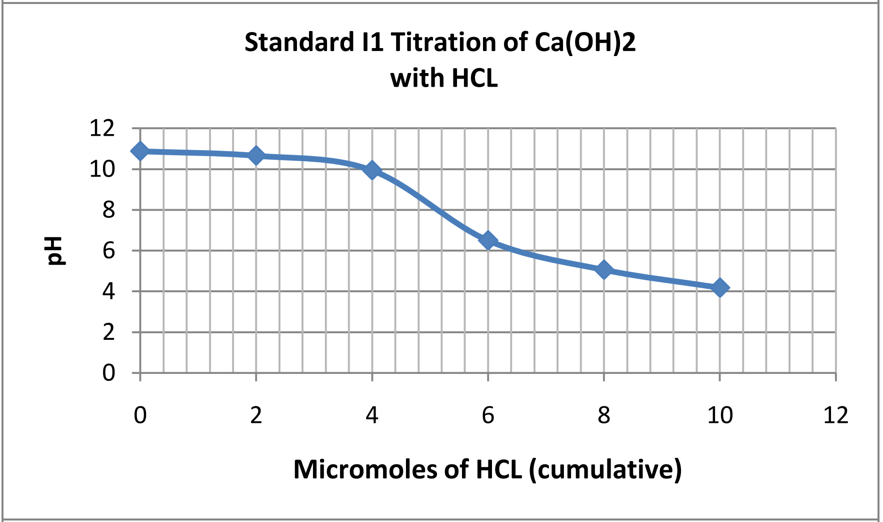

H1 Regression
8
7.5
7
6.5
6
5.5
5
pH
y = -0.6207x + 19.158
18.5
19
19.5
20
20.5
21
21.5
22
22.5
Micromoles of HCL (cumulative)

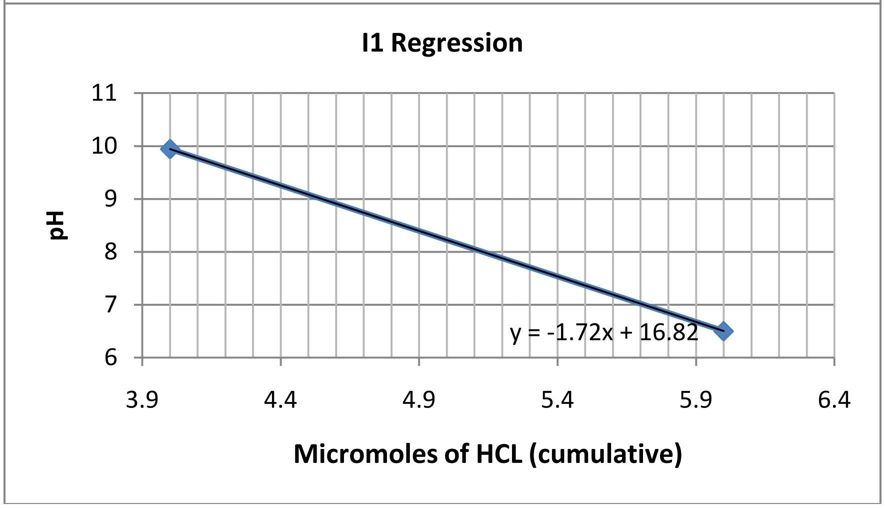

Standard H2 Titration of Ca(OH)2 with HCL

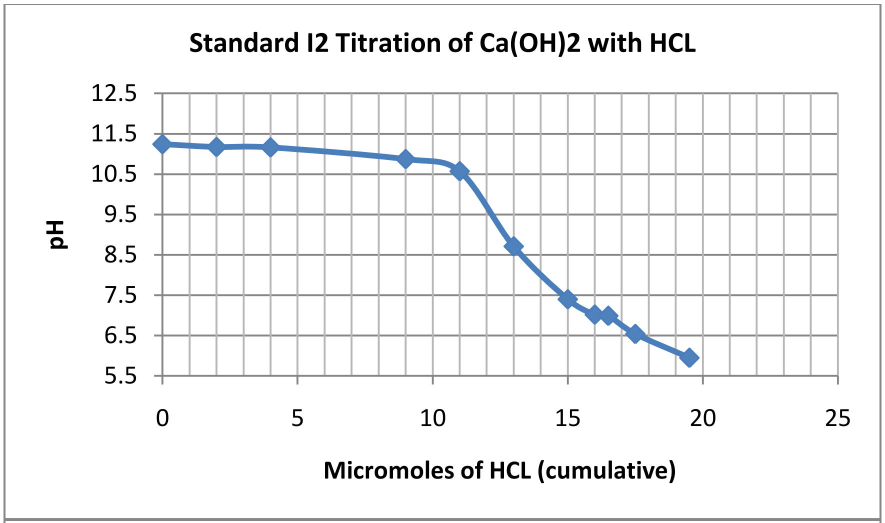

H2 Regression

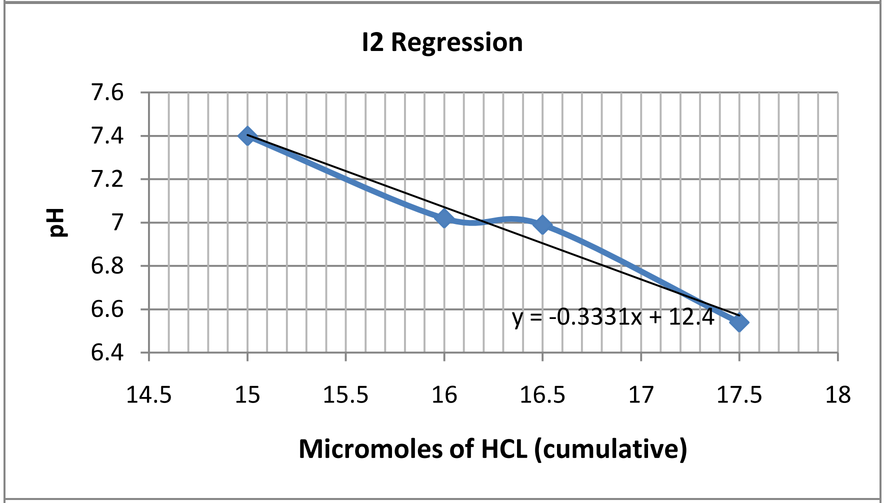

Standard H3 Titration of Ca(OH)2 with HCL

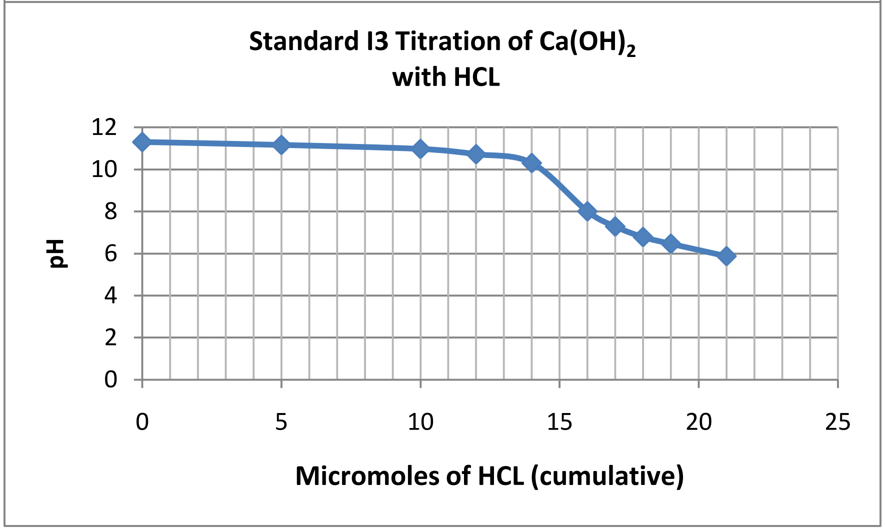

H3 Regression
pH
8
7.5
7
6.5
6
12.8
13.3
13.8
14.3
14.8
15.3
y = -0.725x + 16.957
Micromoles of HCL (cumulative)


Standard I1 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
12
Micromoles of HCL (cumulative)

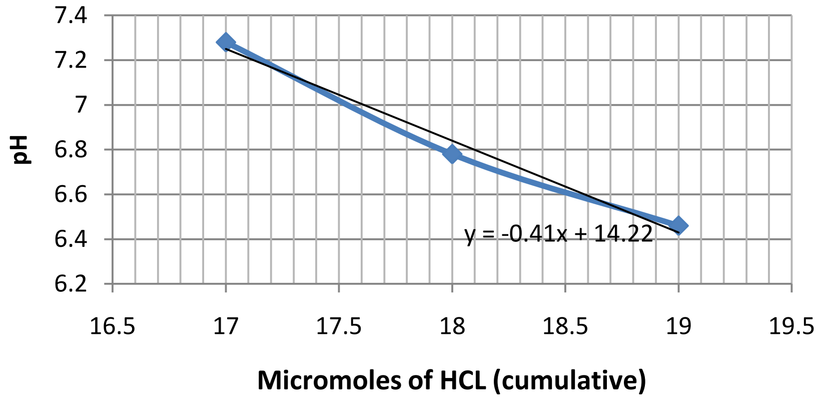

I1 Regression
pH
11
10
9
8
7
6
3.9
4.4
4.9
5.4
5.9
6.4
y = -1.72x + 16.82
Micromoles of HCL (cumulative)


Standard I2 Titration of Ca(OH)2 with HCL
pH
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
0
5
10
15
20
25
Micromoles of HCL (cumulative)

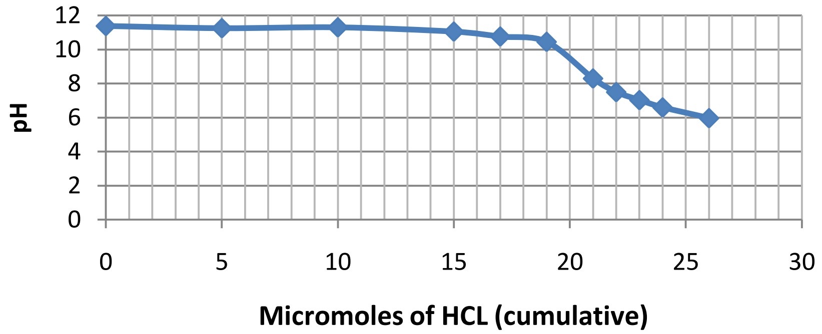

I2 Regression
pH
7.6
7.4
7.2
7
6.8
6.6
6.4
y = -0.3331x + 12.4
14.5
15
15.5
16
16.5
17
17.5
18
Micromoles of HCL (cumulative)


Standard I3 Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
20
25
Micromoles of HCL (cumulative)

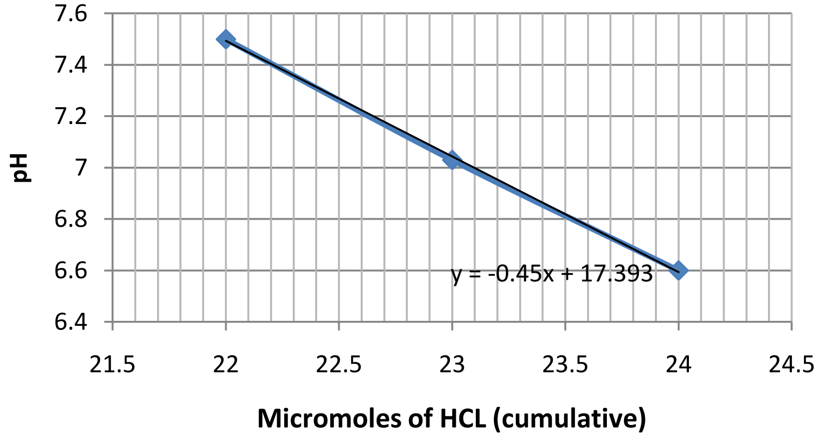
I3 Regression

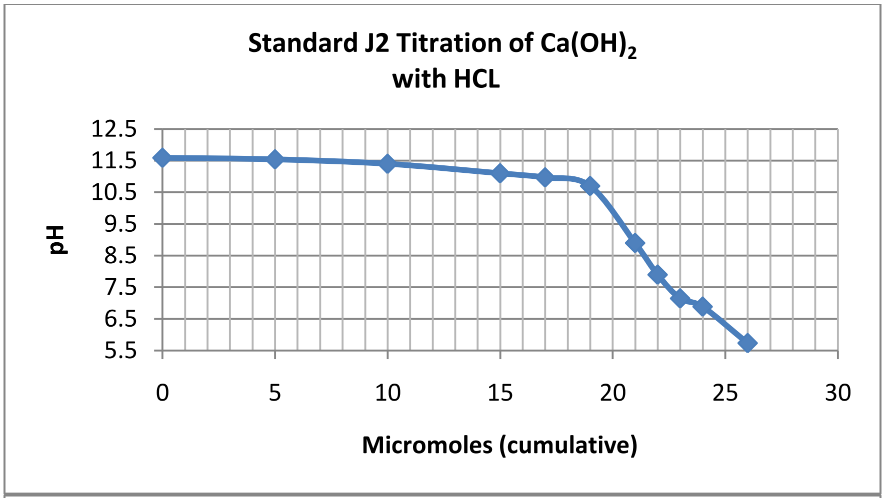


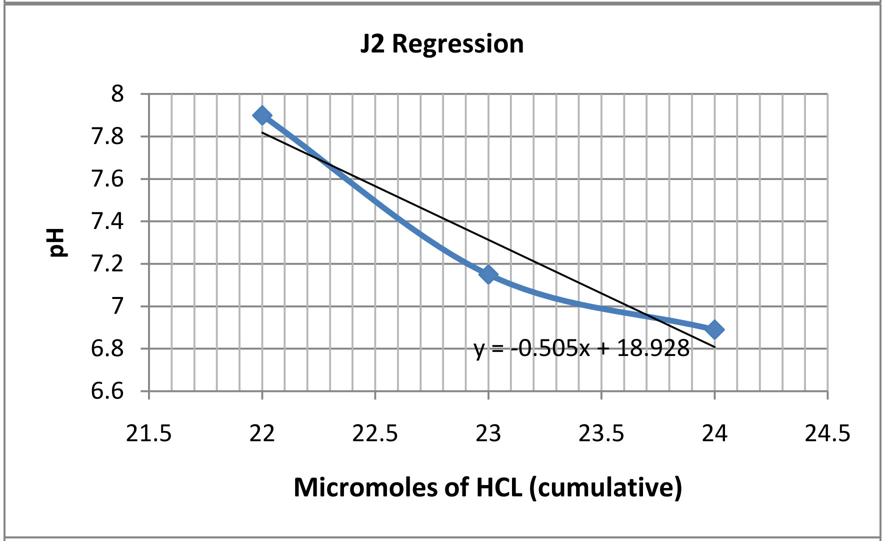
Standard J1 Titration of $Ca(OH)_2$ with HCL

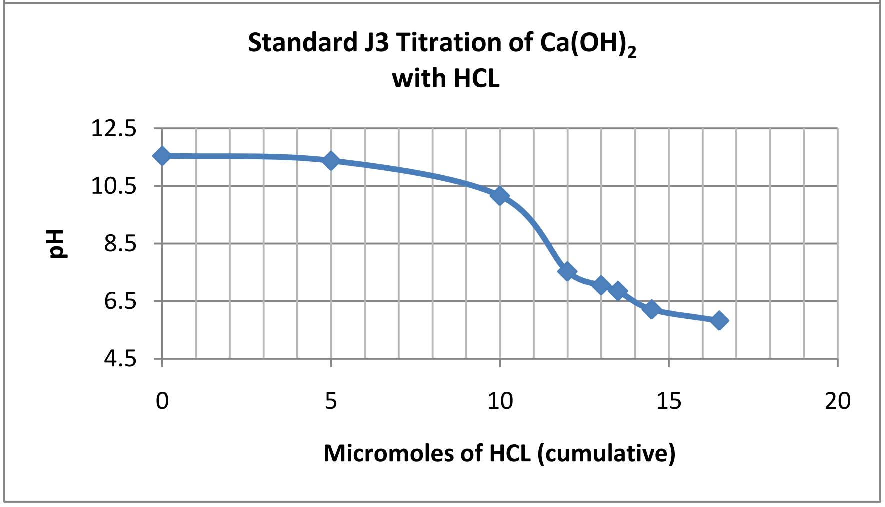


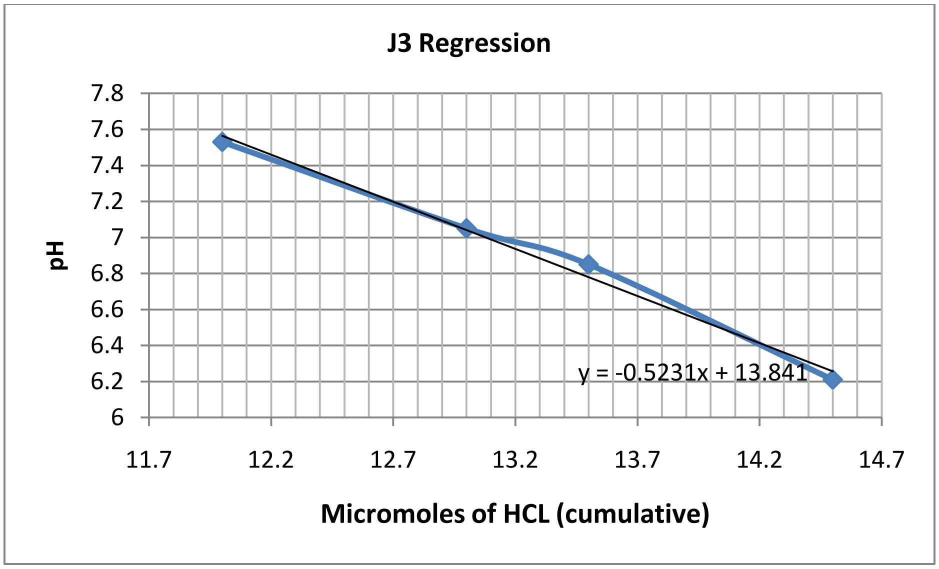
J1 Regression




Standard J2 Titration of Ca(OH)2 with HCL
pH
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
0
5
10
15
20
25
30
Micromoles (cumulative)


J2 Regression
pH
8
7.8
7.6
7.4
7.2
7
6.8
6.6
y = -0.505x + 18.928
21.5
22
22.5
23
23.5
24
24.5
Micromoles of HCL (cumulative)


Standard J3 Titration of Ca(OH)2 with HCL
pH
12.5
10.5
8.5
6.5
4.5
0
5
10
15
20
Micromoles of HCL (cumulative)


J3 Regression
pH
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
11.7
12.2
12.7
13.2
13.7
14.2
14.7
Micromoles of HCL (cumulative)
y = -0.5231x + 13.841

Appendix VII.

Titration data for the second standards. Columns from left to right: pH readings, concentration of HCL added, volume of HCL added, moles of HCL added, cumulative moles of HCL added, cumulative micromoles of HCL added, and pH (listed again).

Second Standard 2A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumula	Micromol	pH
10.81				0	0	10.81
10.58	0.1	0.00001	0.000001	0.000001	1	10.58
10.2	0.1	0.00001	0.000001	0.000002	2	10.2
9.5	0.05	0.00001	0.0000005	0.0000025	2.5	9.5
6.14	0.05	0.00001	0.0000005	0.000003	3	6.14
5.44	0.025	0.00001	2.5E-07	3.25E-06	3.25	5.44
4.33	0.05	0.00001	0.0000005	3.75E-06	3.75	4.33
2.73	0.1	0.00001	0.000001	4.75E-06	4.75	2.73

Second Standard 2B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumula	Micromol	pH
10.87				0.00000000	0	10.87
10.62	0.1	0.00001	0.000001	0.00000100	1	10.62
9.99	0.1	0.00001	0.000001	0.00000200	2	9.99
7.46	0.05	0.00001	0.0000005	0.00000250	2.5	7.46
6.18	0.025	0.00001	2.5E-07	0.00000275	2.75	6.18
5.36	0.025	0.00001	2.5E-07	0.00000300	3	5.36
4.16	0.05	0.00001	0.0000005	0.00000350	3.5	4.16
2.74	0.1	0.00001	0.000001	0.00000450	4.5	2.74

Second Standard 2C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumula	Micromol	pH
10.43				0	0	10.43
10.23	0.05	0.00001	0.0000005	0.0000005	0.5	10.23
9.5	0.05	0.00001	0.0000005	0.000001	1	9.5
7.6	0.025	0.00001	2.5E-07	1.25E-06	1.25	7.6
7.75	0.025	0.00001	2.5E-07	0.0000015	1.5	7.75
6.45	0.025	0.00001	2.5E-07	1.75E-06	1.75	6.45
5.8	0.025	0.00001	2.5E-07	0.000002	2	5.8
4.9	0.05	0.00001	0.0000005	0.0000025	2.5	4.9
2.9	0.1	0.00001	0.000001	0.0000035	3.5	2.9

Second Standard 3A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumula	Micromol	pH
11.33				0	0	11.33
11.21	0.1	0.00001	0.000001	0.000001	1	11.21
10.96	0.2	0.00001	0.000002	0.000003	3	10.96
10.76	0.1	0.00001	0.000001	0.000004	4	10.76
10.36	0.1	0.00001	0.000001	0.000005	5	10.36
5.47	0.2	0.00001	0.000002	0.000007	7	5.47
3.29	0.1	0.00001	0.000001	0.000008	8	3.29
2.27	0.2	0.00001	0.000002	0.00001	10	2.27

Second Standard 3B						
ph	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		ph
10.94				0	0	10.94
10.24	0.2	0.00001	0.000002	0.000002	2	10.24
5.52	0.1	0.00001	0.000001	0.000003	3	5.52
4.91	0.025	0.00001	2.5E-07	3.25E-06	3.25	4.91
4.25	0.025	0.00001	2.5E-07	0.0000035	3.5	4.25
2.66	0.1	0.00001	0.000001	0.0000045	4.5	2.66
2.16	0.2	0.00001	0.000002	0.0000065	6.5	2.16

Second Standard 3C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.3				0	0	11.3
11.04	0.2	0.00001	0.000002	0.000002	2	11.04
10.83	0.1	0.00001	0.000001	0.000003	3	10.83
10.7	0.1	0.00001	0.000001	0.000004	4	10.7
10.57	0.1	0.00001	0.000001	0.000005	5	10.57
9.71	0.1	0.00001	0.000001	0.000006	6	9.71
7.02	0.05	0.00001	0.0000005	0.0000065	6.5	7.02
6	0.025	0.00001	2.5E-07	6.75E-06	6.75	6
4.59	0.05	0.00001	0.0000005	7.25E-06	7.25	4.59
2.43	0.2	0.00001	0.000002	9.25E-06	9.25	2.43

Second Standard 4A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.34				0	0	11.34
11.17	0.2	0.00001	0.000002	0.000002	2	11.17
10.92	0.2	0.00001	0.000002	0.000004	4	10.92
10.22	0.2	0.00001	0.000002	0.000006	6	10.22
7.25	0.1	0.00001	0.000001	0.000007	7	7.25
5.33	0.05	0.00001	0.0000005	0.0000075	7.5	5.33
3.16	0.1	0.00001	0.000001	0.0000085	8.5	3.16
2.24	0.2	0.00001	0.000002	0.0000105	10.5	2.24

Second Standard 4B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		
11.23				0	0	11.23
11.11	0.2	0.00001	0.000002	0.000002	2	11.11
10.67	0.2	0.00001	0.000002	0.000004	4	10.67
6.46	0.2	0.00001	0.000002	0.000006	6	6.46
5.43	0.025	0.00001	2.5E-07	6.25E-06	6.25	5.43
3.11	0.1	0.00001	0.000001	7.25E-06	7.25	3.11
2.19	0.2	0.00001	0.000002	9.25E-06	9.25	2.19

Second Standard 4C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		
11.35				0	0	11.35
11.1	0.2	0.00001	0.000002	0.000002	2	11.1
11.03	0.2	0.00001	0.000002	0.000004	4	11.03
10.66	0.2	0.00001	0.000002	0.000006	6	10.66
10.09	0.1	0.00001	0.000001	0.000007	7	10.09
6.51	0.1	0.00001	0.000001	0.000008	8	6.51
3.86	0.1	0.00001	0.000001	0.000009	9	3.86
2.28	0.2	0.00001	0.000002	0.000011	11	2.28

Second Standard 5A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.58				0	0	11.58
11.24	0.5	0.00001	0.000005	0.000005	5	11.24
11.13	0.2	0.00001	0.000002	0.000007	7	11.13
10.84	0.2	0.00001	0.000002	0.000009	9	10.84
10.24	0.2	0.00001	0.000002	0.000011	11	10.24
7.53	0.1	0.00001	0.000001	0.000012	12	7.53
6.41	0.025	0.00001	2.5E-07	1.225E-05	12.25	6.41
4.73	0.05	0.00001	0.0000005	1.275E-05	12.75	4.73
2.43	0.2	0.00001	0.000002	1.475E-05	14.75	2.43

Second Standard 5B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.39				0	0	11.39
10.75	0.5	0.00001	0.000005	0.000005	5	10.75
7.56	0.2	0.00001	0.000002	0.000007	7	7.56
6.03	0.025	0.00001	2.5E-07	7.25E-06	7.25	6.03
5.35	0.025	0.00001	2.5E-07	0.0000075	7.5	5.35
4.18	0.05	0.00001	0.0000005	0.000008	8	4.18
2.3	0.2	0.00001	0.000002	0.00001	10	2.3
1.77	0.5	0.00001	0.000005	0.000015	15	1.77

Second Standard 5C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.51				0	0	11.51
11.06	0.5	0.00001	0.000005	0.000005	5	11.06
10.7	0.2	0.00001	0.000002	0.000007	7	10.7
9.66	0.2	0.00001	0.000002	0.000009	9	9.66
7.97	0.05	0.00001	0.0000005	0.0000095	9.5	7.97
7.42	0.025	0.00001	2.5E-07	9.75E-06	9.75	7.42
7.14	0.025	0.00001	2.5E-07	0.00001	10	7.14
6.28	0.025	0.00001	2.5E-07	1.025E-05	10.25	6.28
4.98	0.05	0.00001	0.0000005	1.075E-05	10.75	4.98
1.96	0.5	0.00001	0.000005	1.575E-05	15.75	1.96

Second Standard 6A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.59				0	0	11.59
11.3	0.5	0.00001	0.000005	0.000005	5	11.3
10.85	0.5	0.00001	0.000005	0.00001	10	10.85
9.82	0.2	0.00001	0.000002	0.000012	12	9.82
5.89	0.1	0.00001	0.000001	0.000013	13	5.89
3.61	0.1	0.00001	0.000001	0.000014	14	3.61
1.81	0.5	0.00001	0.000005	0.000019	19	1.81

Second Standard 6B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.34				0	0	11.34
10.94	0.5	0.00001	0.000005	0.000005	5	10.94
10.39	0.2	0.00001	0.000002	0.000007	7	10.39
9.88	0.1	0.00001	0.000001	0.000008	8	9.88
8.08	0.05	0.00001	0.0000005	0.0000085	8.5	8.08
6.81	0.025	0.00001	2.5E-07	8.75E-06	8.75	6.81
4.91	0.05	0.00001	0.0000005	9.25E-06	9.25	4.91
2.45	0.2	0.00001	0.000002	1.125E-05	11.25	2.45
1.8	0.5	0.00001	0.000005	1.625E-05	16.25	1.8

Second Standard 6C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.48				0	0	11.48
11.27	0.5	0.00001	0.000005	0.000005	5	11.27
10.82	0.5	0.00001	0.000005	0.00001	10	10.82
10.11	0.2	0.00001	0.000002	0.000012	12	10.11
9.29	0.05	0.00001	0.0000005	0.0000125	12.5	9.29
7.16	0.05	0.00001	0.0000005	0.000013	13	7.16
6.27	0.025	0.00001	2.5E-07	1.325E-05	13.25	6.27
5.42	0.025	0.00001	2.5E-07	0.0000135	13.5	5.42
4.29	0.05	0.00001	0.0000005	0.000014	14	4.29
1.97	0.5	0.00001	0.000005	0.000019	19	1.97

Second Standard 7A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.02				0	0	12.02
11.7	0.5	0.00001	0.000005	0.000005	5	11.7
11.51	0.2	0.00001	0.000002	0.000007	7	11.51
11.4	0.2	0.00001	0.000002	0.000009	9	11.4
11.3	0.2	0.00001	0.000002	0.000011	11	11.3
11.1	0.2	0.00001	0.000002	0.000013	13	11.1
10.43	0.2	0.00001	0.000002	0.000015	15	10.43
8.55	0.1	0.00001	0.000001	0.000016	16	8.55
3.27	0.05	0.00001	0.0000005	0.0000165	16.5	3.27
1.74	0.5	0.00001	0.000005	0.0000215	21.5	1.74

Second Standard 7B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.55				0	0	11.55
11.35	0.5	0.00001	0.000005	0.000005	5	11.35
10.82	0.5	0.00001	0.000005	0.00001	10	10.82
10.1	0.2	0.00001	0.000002	0.000012	12	10.1
7.1	0.1	0.00001	0.000001	0.000013	13	7.1
6.8	0.025	0.00001	2.5E-07	1.325E-05	13.25	6.8
5.34	0.025	0.00001	2.5E-07	0.0000135	13.5	5.34
3.62	0.1	0.00001	0.000001	0.0000145	14.5	3.62
1.81	0.5	0.00001	0.000005	0.0000195	19.5	1.81

Second Standard 7C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.54				0	0	11.54
11.27	0.5	0.00001	0.000005	0.000005	5	11.27
10.4	0.5	0.00001	0.000005	0.00001	10	10.4
6.84	0.2	0.00001	0.000002	0.000012	12	6.84
6.78	0.025	0.00001	2.5E-07	1.225E-05	12.25	6.78
4.77	0.1	0.00001	0.000001	1.325E-05	13.25	4.77
3.1	0.2	0.00001	0.000002	1.525E-05	15.25	3.1
1.76	0.5	0.00001	0.000005	2.025E-05	20.25	1.76

Second Standard 8A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.65				0	0	11.65
11.42	0.5	0.00001	0.000005	0.000005	5	11.42
11	0.5	0.00001	0.000005	0.00001	10	11
10.43	0.2	0.00001	0.000002	0.000012	12	10.43
7.84	0.1	0.00001	0.000001	0.000013	13	7.84
6.59	0.025	0.00001	2.5E-07	1.325E-05	13.25	6.59
4.92	0.05	0.00001	0.0000005	1.375E-05	13.75	4.92
3.55	0.1	0.00001	0.000001	1.475E-05	14.75	3.55
1.77	0.5	0.00001	0.000005	1.975E-05	19.75	1.77

Second Standard 8B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.58				0	0	11.58
11.39	0.5	0.00001	0.000005	0.000005	5	11.39
11.03	0.5	0.00001	0.000005	0.00001	10	11.03
10.65	0.2	0.00001	0.000002	0.000012	12	10.65
10.05	0.1	0.00001	0.000001	0.000013	13	10.05
6.91	0.1	0.00001	0.000001	0.000014	14	6.91
5.46	0.025	0.00001	2.5E-07	1.425E-05	14.25	5.46
4.43	0.05	0.00001	0.0000005	1.475E-05	14.75	4.43
2.59	0.2	0.00001	0.000002	1.675E-05	16.75	2.59

Second Standard 8C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.03				0	0	12.03
11.89	0.5	0.00001	0.000005	0.000005	5	11.89
11.7	0.5	0.00001	0.000005	0.00001	10	11.7
11.58	0.5	0.00001	0.000005	0.000015	15	11.58
7.66	0.5	0.00001	0.000005	0.00002	20	7.66
6.02	0.025	0.00001	2.5E-07	2.025E-05	20.25	6.02
4.27	0.05	0.00001	0.0000005	2.075E-05	20.75	4.27
1.78	0.5	0.00001	0.000005	2.575E-05	25.75	1.78

Second Standard 9A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		
11.92				0	0	11.92
11.9	0.5	0.00001	0.000005	0.000005	5	11.9
11.61	1	0.00001	0.00001	0.000015	15	11.61
11.35	0.5	0.00001	0.000005	0.00002	20	11.35
10.69	0.5	0.00001	0.000005	0.000025	25	10.69
7.68	0.2	0.00001	0.000002	0.000027	27	7.68
6.1	0.05	0.00001	0.0000005	0.0000275	27.5	6.1
2.36	0.2	0.00001	0.000002	0.0000295	29.5	2.36
1.59	0.5	0.00001	0.000005	0.0000345	34.5	1.59

Second Standard 9B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.95				0	0	11.95
11.75	0.5	0.00001	0.000005	0.000005	5	11.75
11.47	0.5	0.00001	0.000005	0.00001	10	11.47
11.34	0.2	0.00001	0.000002	0.000012	12	11.34
10.93	0.2	0.00001	0.000002	0.000014	14	10.93
5.81	0.2	0.00001	0.000002	0.000016	16	5.81
4.27	0.05	0.00001	0.0000005	0.0000165	16.5	4.27
1.75	0.5	0.00001	0.000005	0.0000215	21.5	1.75

Second Standard 9C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
11.5				0	0	11.5
11.46	0.5	0.00001	0.000005	0.000005	5	11.46
11.25	1	0.00001	0.00001	0.000015	15	11.25
10.55	0.5	0.00001	0.000005	0.00002	20	10.55
7.34	0.2	0.00001	0.000002	0.000022	22	7.34
6.35	0.025	0.00001	2.5E-07	2.225E-05	22.25	6.35
4.62	0.1	0.00001	0.000001	2.325E-05	23.25	4.62
1.82	0.5	0.00001	0.000005	2.825E-05	28.25	1.82

Second Standard 10A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.26				0	0	12.26
12.08	1	0.00001	0.00001	0.00001	10	12.08
11.74	1	0.00001	0.00001	0.00002	20	11.74
7.53	0.5	0.00001	0.000005	0.000025	25	7.53
6.88	0.025	0.00001	2.5E-07	2.525E-05	25.25	6.88
5.43	0.025	0.00001	2.5E-07	0.0000255	25.5	5.43
1.73	0.5	0.00001	0.000005	0.0000305	30.5	1.73

Second Standard 10B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.31				0	0	12.31
12.16	1	0.00001	0.00001	0.00001	10	12.16
11.85	1	0.00001	0.00001	0.00002	20	11.85
11.62	0.5	0.00001	0.000005	0.000025	25	11.62
8.62	0.5	0.00001	0.000005	0.00003	30	8.62
7.93	0.025	0.00001	2.5E-07	3.025E-05	30.25	7.93
7.3	0.025	0.00001	2.5E-07	0.0000305	30.5	7.3
6.46	0.025	0.00001	2.5E-07	3.075E-05	30.75	6.46
1.78	0.5	0.00001	0.000005	3.575E-05	35.75	1.78

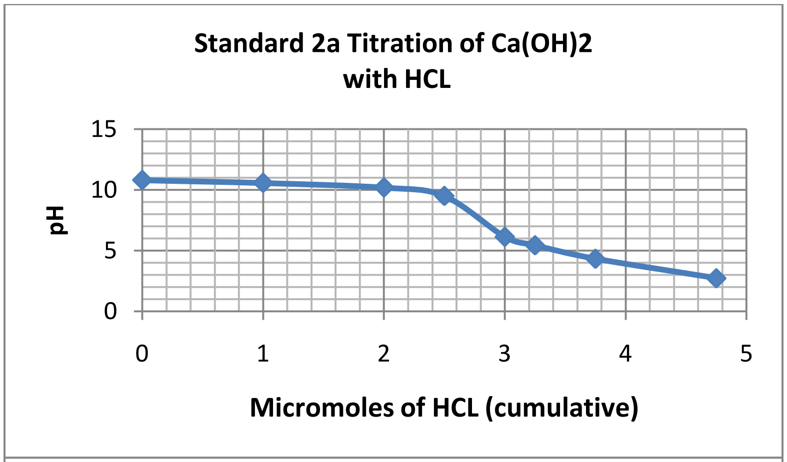
Second Standard 10C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.23				0	0	12.23
11.99	1	0.00001	0.00001	0.00001	10	11.99
11.5	1	0.00001	0.00001	0.00002	20	11.5
7.85	0.5	0.00001	0.000005	0.000025	25	7.85
6.9	0.025	0.00001	2.5E-07	2.525E-05	25.25	6.9
5.87	0.025	0.00001	2.5E-07	0.0000255	25.5	5.87
4.31	0.1	0.00001	0.000001	0.0000265	26.5	4.31
1.88	0.5	0.00001	0.000005	0.0000315	31.5	1.88

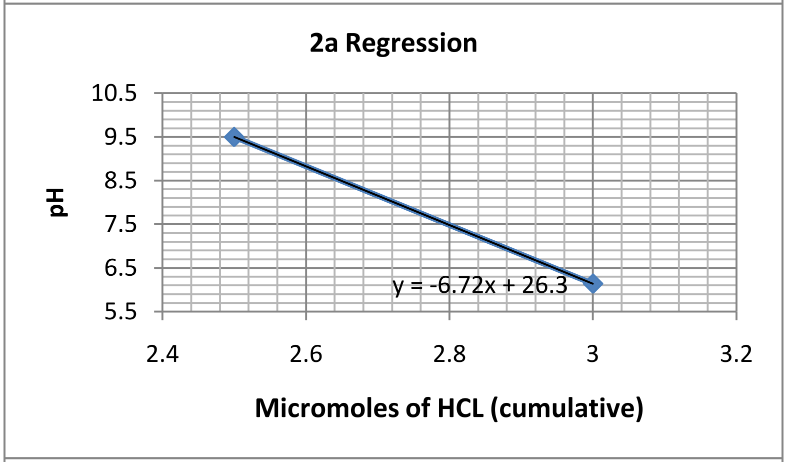
Second Standard 11A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.24				0	0	12.24
12.05	1	0.00001	0.00001	0.00001	10	12.05
11.85	1	0.00001	0.00001	0.00002	20	11.85
11.5	0.05	0.00001	0.0000005	0.0000205	20.5	11.5
11.42	0.2	0.00001	0.000002	0.0000225	22.5	11.42
11.13	0.2	0.00001	0.000002	0.0000245	24.5	11.13
10.22	0.2	0.00001	0.000002	0.0000265	26.5	10.22
7.48	0.1	0.00001	0.000001	0.0000275	27.5	7.48
6.5	0.025	0.00001	2.5E-07	2.775E-05	27.75	6.5
3.28	0.1	0.00001	0.000001	2.875E-05	28.75	3.28

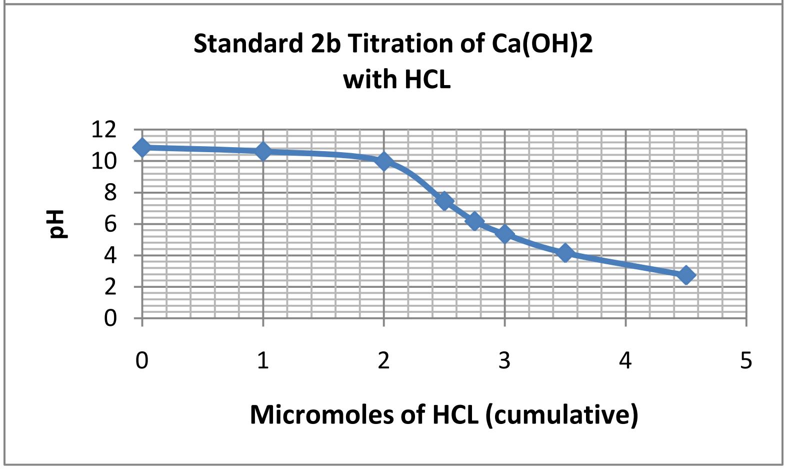
Second Standard 11B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.11				0.00000000	0	12.11
12.01	1	0.00001	0.00001	0.00001000	10	12.01
11.5	1	0.00001	0.00001	0.00002000	20	11.5
10.82	0.5	0.00001	0.000005	0.00002500	25	10.82
10.45	0.1	0.00001	0.000001	0.00002600	26	10.45
8.3	0.1	0.00001	0.000001	0.00002700	27	8.3
7.72	0.025	0.00001	2.5E-07	0.00002725	27.25	7.72
7.23	0.025	0.00001	2.5E-07	0.00002750	27.5	7.23
6.2	0.025	0.00001	2.5E-07	0.00002775	27.75	6.2
2.1	0.5	0.00001	0.000005	0.00003275	32.75	2.1

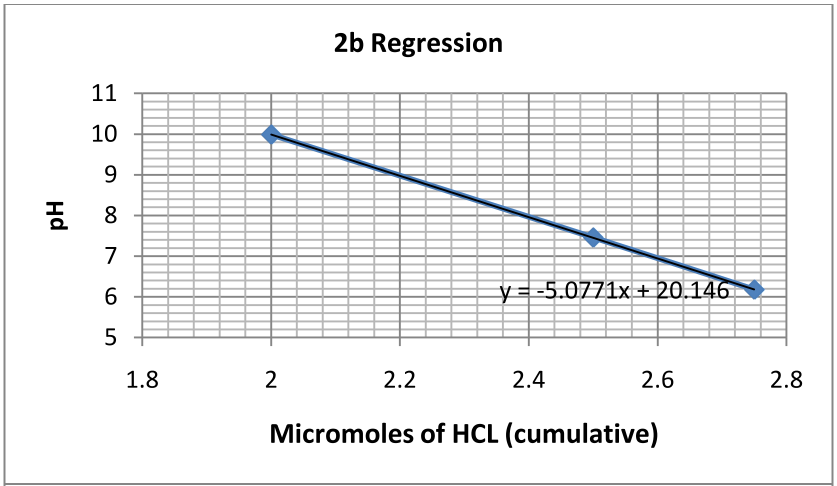
Appendix VIII.

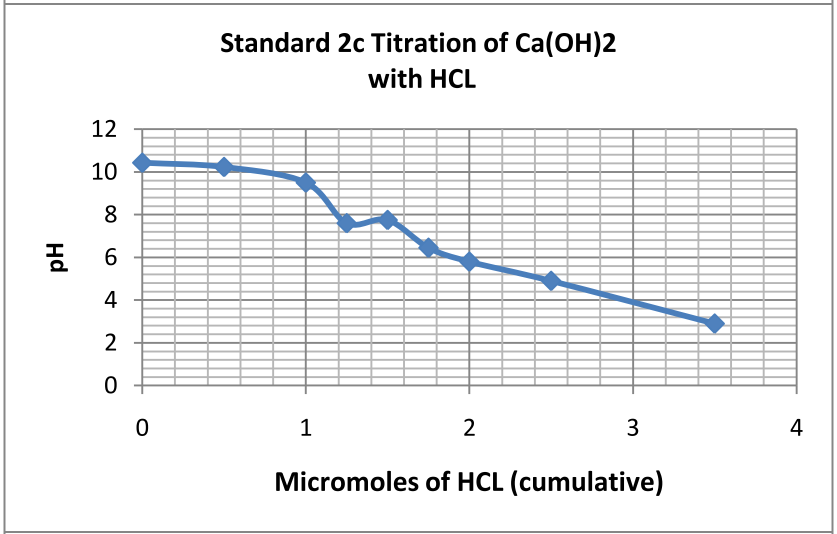
Titration graphs and linear regression graphs for second standard curve.

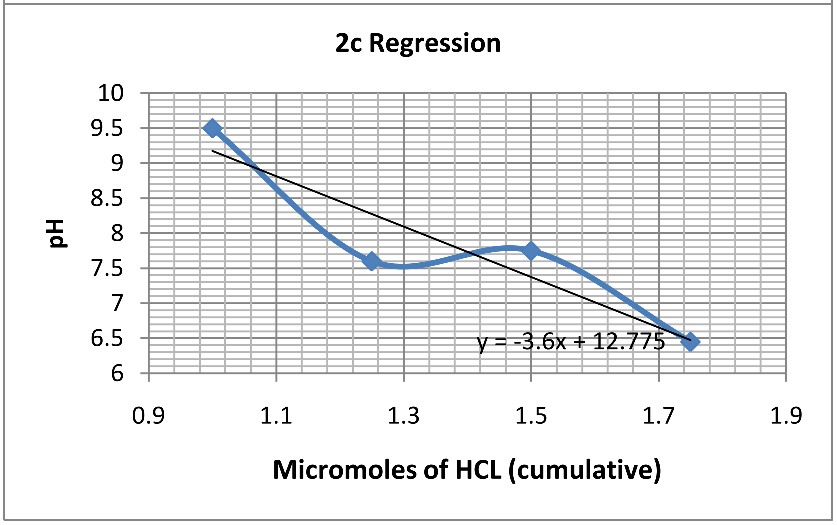


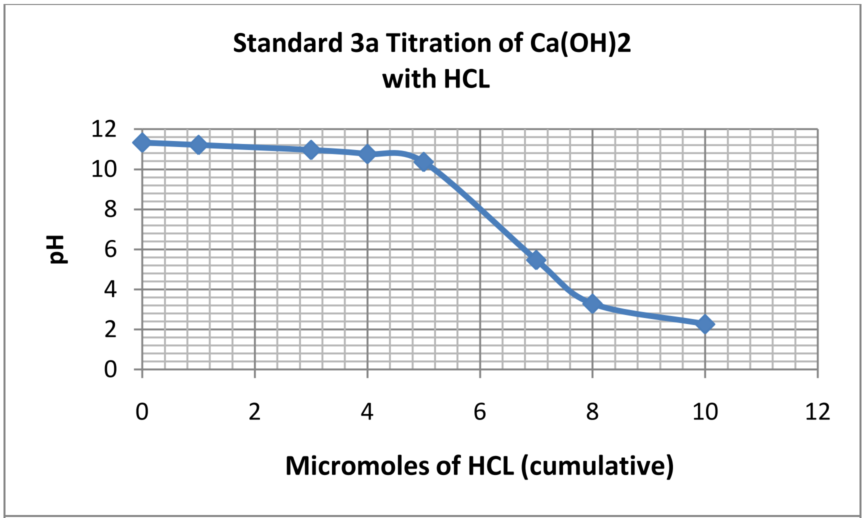


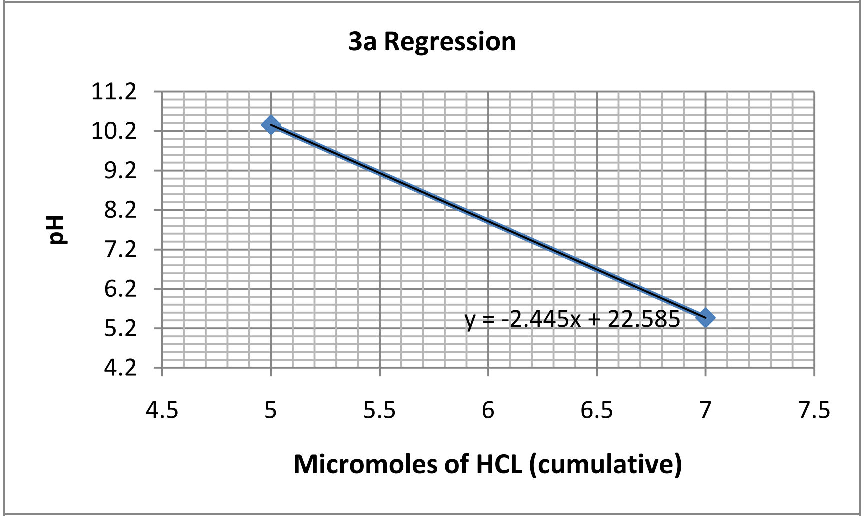


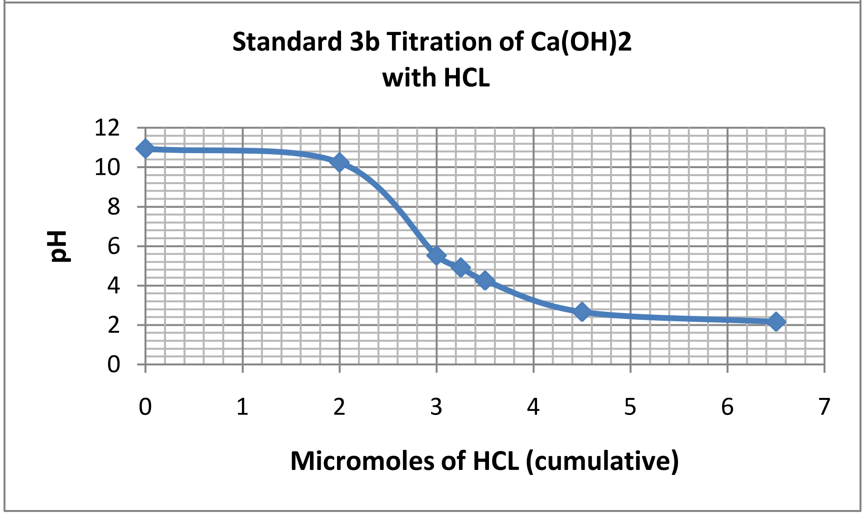

2b Regression

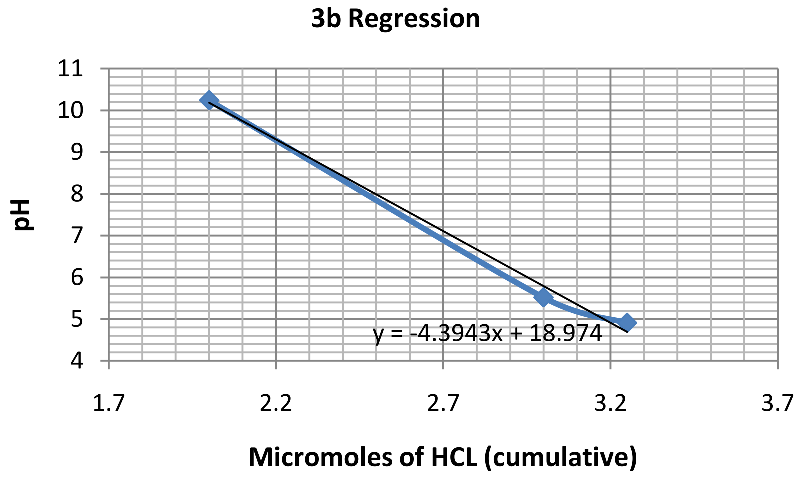

Standard 2c Titration of Ca(OH)2 with HCL

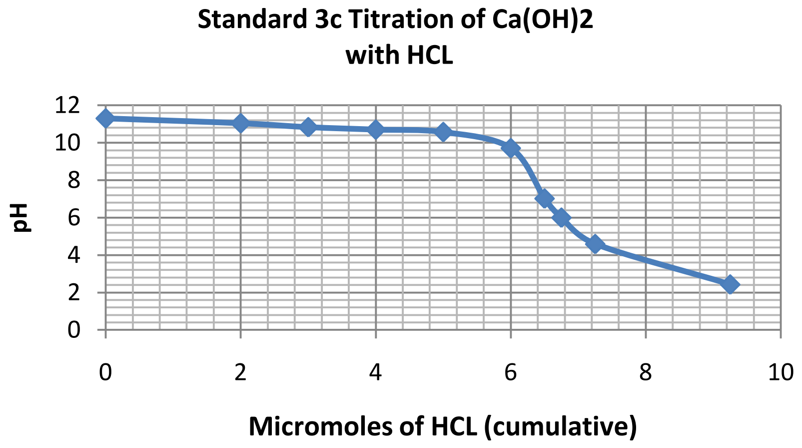

2c Regression

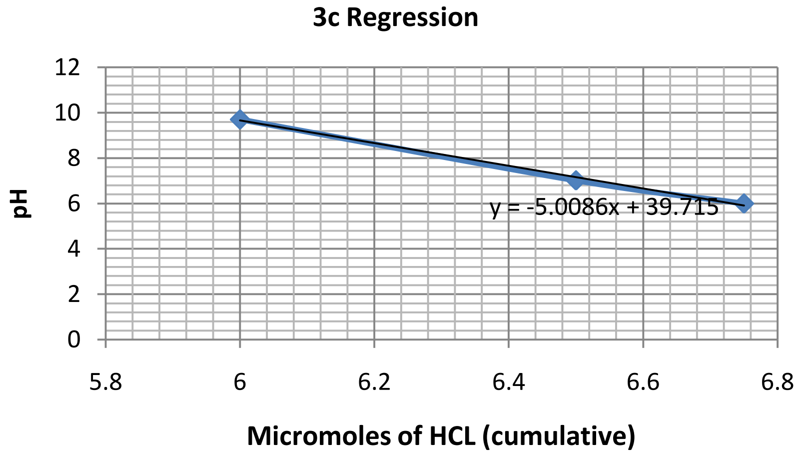

Standard 3a Titration of Ca(OH)2 with HCL

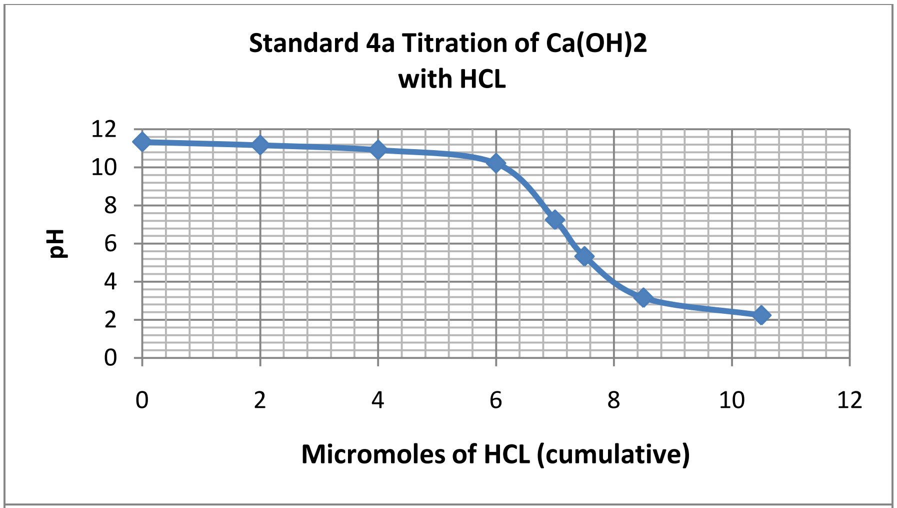

3a Regression

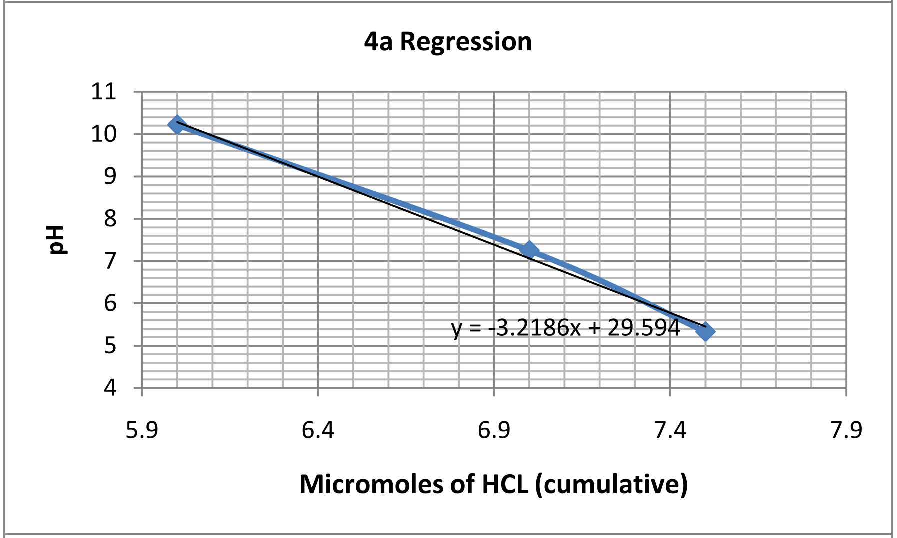

Standard 3b Titration of Ca(OH)2 with HCL

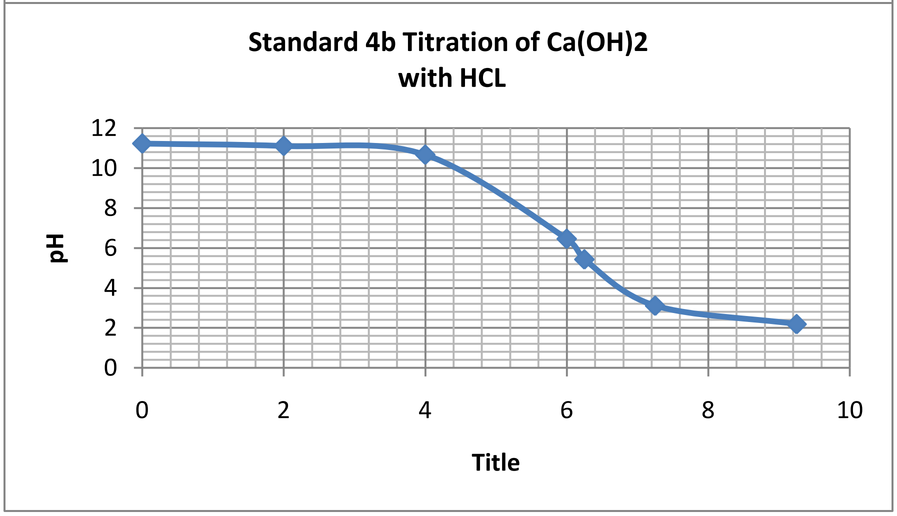

3b Regression
pH
11
10
9
8
7
6
5
4
1.7
2.2
2.7
3.2
3.7
Micromoles of HCL (cumulative)
y = -4.3943x + 18.974

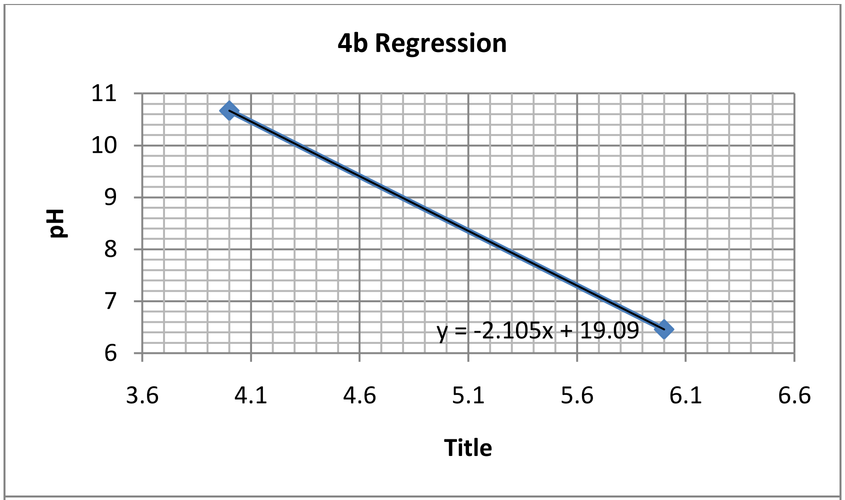

Standard 3c Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
Micromoles of HCL (cumulative)

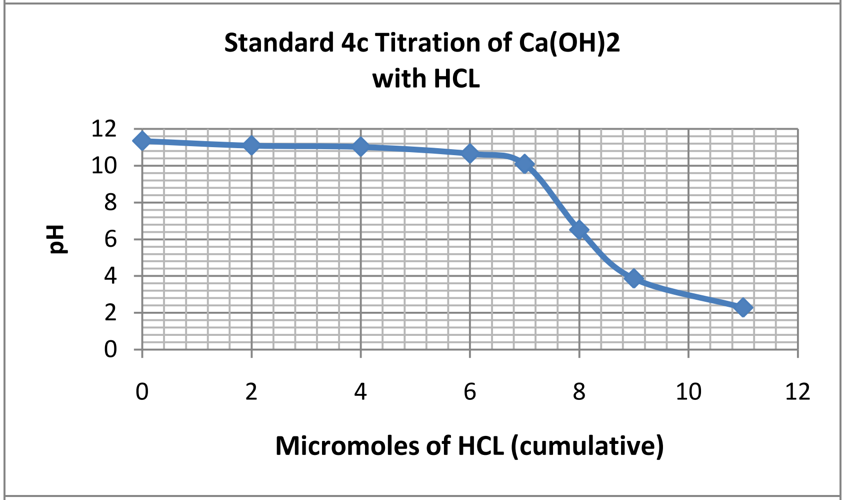

3c Regression
pH
12
10
8
6
4
2
0
5.8
6
6.2
6.4
6.6
6.8
Micromoles of HCL (cumulative)
y = -5.0086x + 39.715

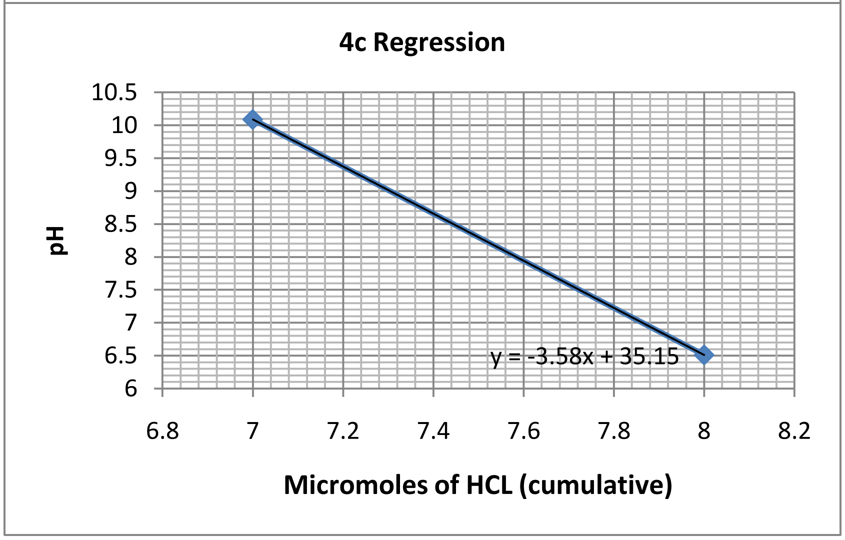

Standard 4a Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
12
Micromoles of HCL (cumulative)

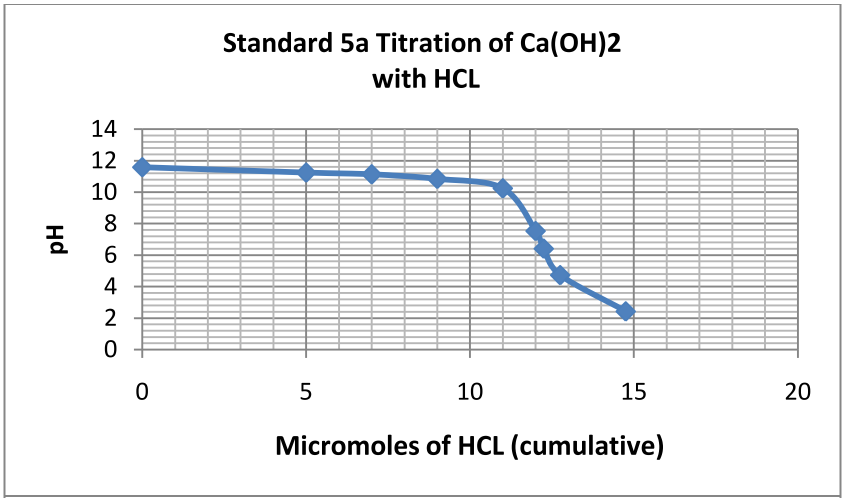

4a Regression
pH
11
10
9
8
7
6
5
4
y = -3.2186x + 29.594
5.9
6.4
6.9
7.4
7.9
Micromoles of HCL (cumulative)

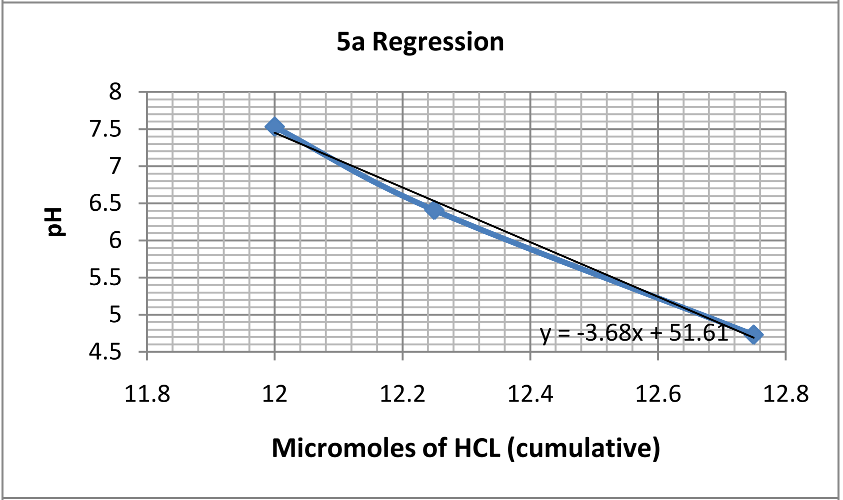

Standard 4b Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
Title

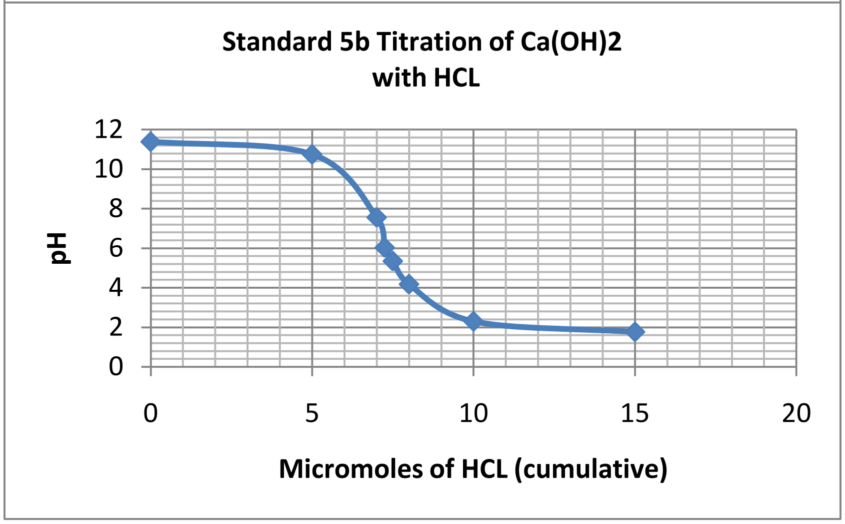

4b Regression
pH
11
10
9
8
7
6
3.6
4.1
4.6
5.1
5.6
6.1
6.6
Title
y = -2.105x + 19.09

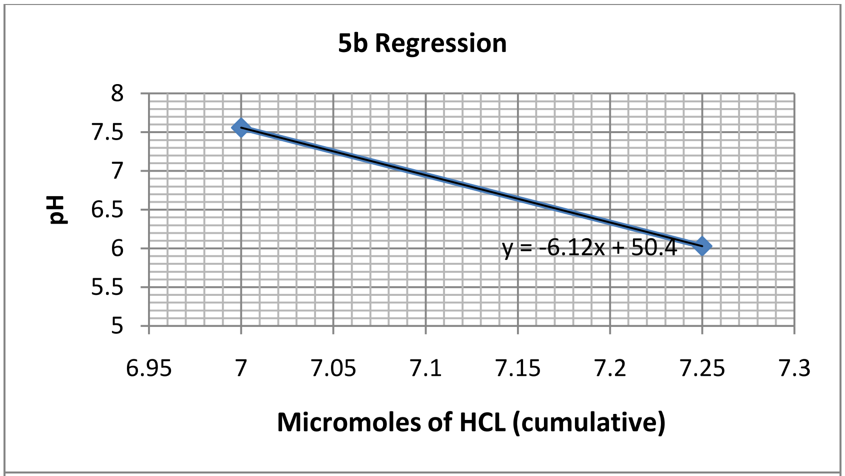

Standard 4c Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
2
4
6
8
10
12
Micromoles of HCL (cumulative)

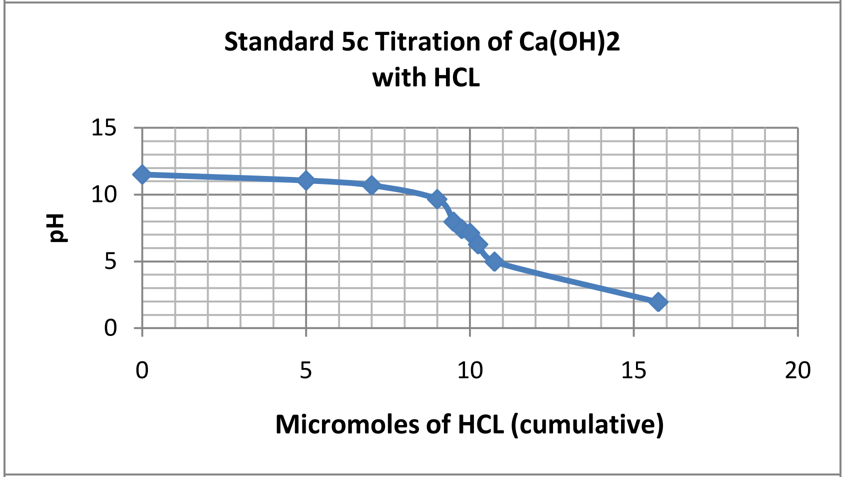

4c Regression
pH
10.5
10
9.5
9
8.5
8
7.5
7
6.5
6
6.8
7
7.2
7.4
7.6
7.8
8
8.2
Micromoles of HCL (cumulative)
y = -3.58x + 35.15

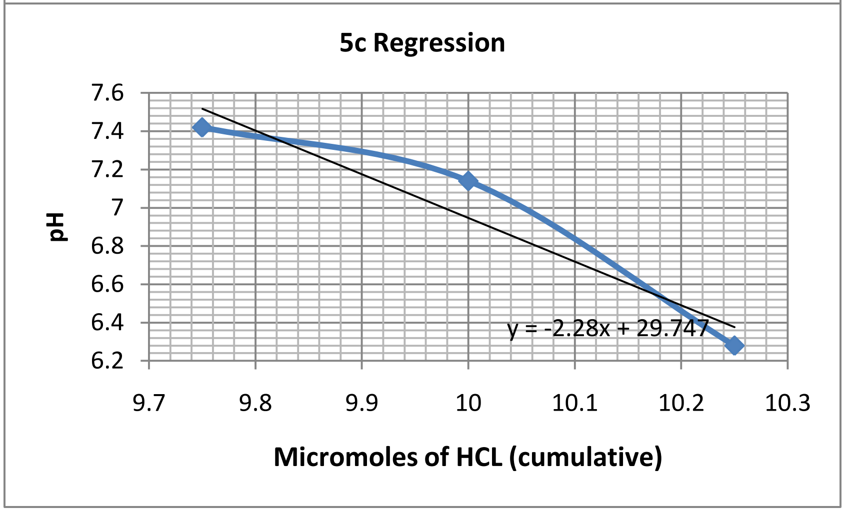

Standard 5a Titration of Ca(OH)2 with HCL

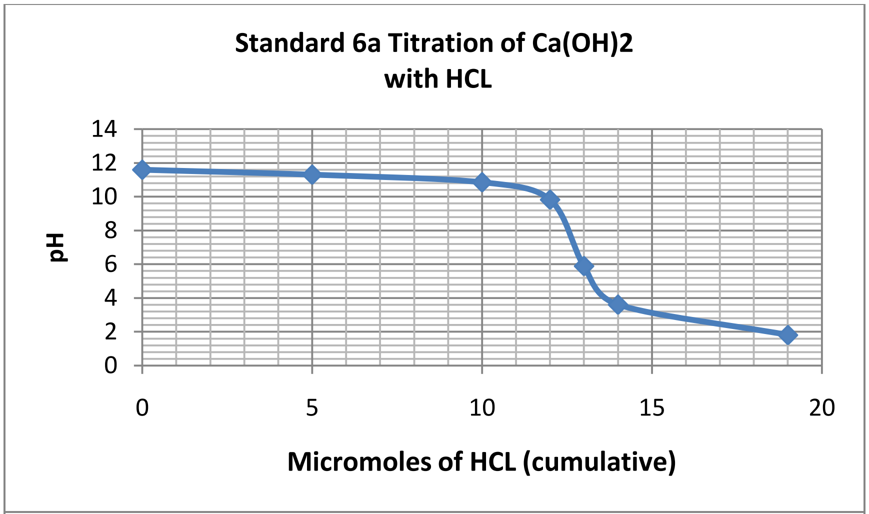

5a Regression

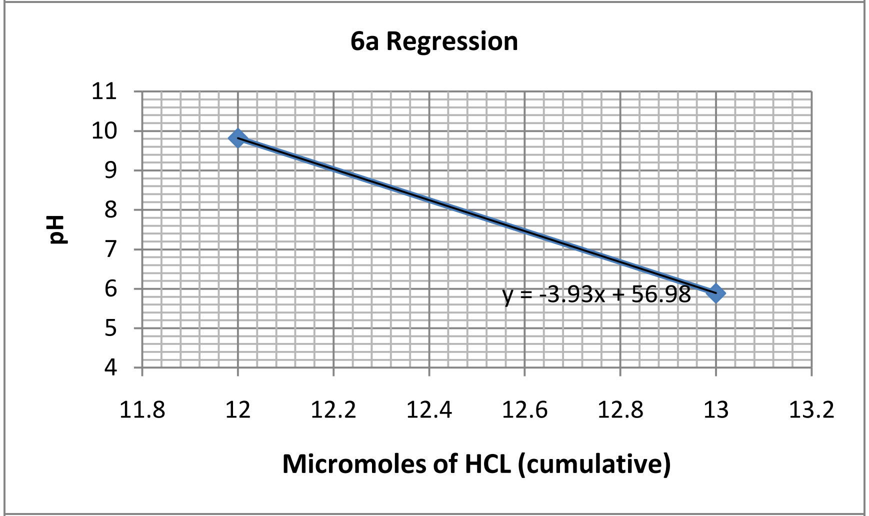

Standard 5b Titration of Ca(OH)2 with HCL

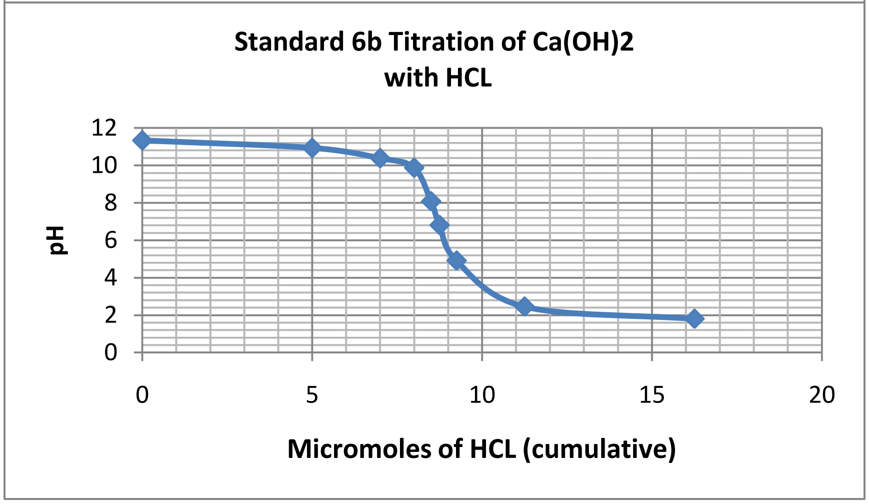

5b Regression

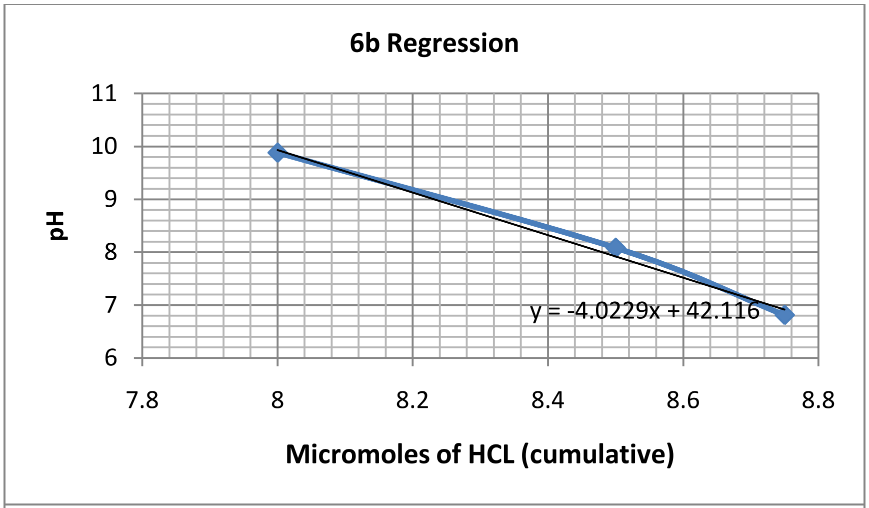

Standard 5c Titration of Ca(OH)2 with HCL

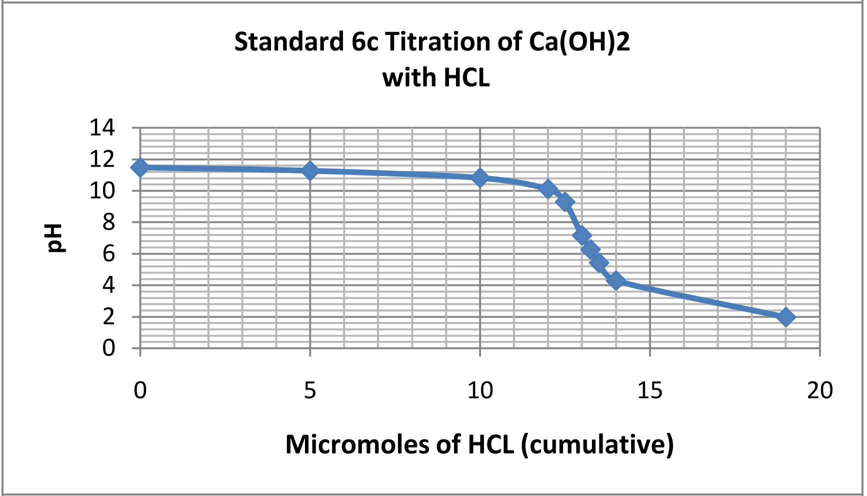

5c Regression

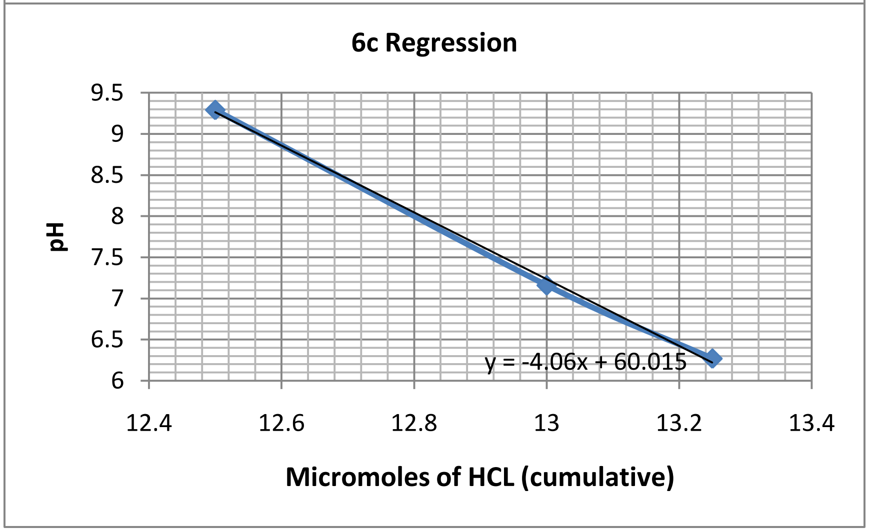

Standard 6a Titration of Ca(OH)2 with HCL
pH
14
12
10
8
6
4
2
0
0
5
10
15
20
Micromoles of HCL (cumulative)

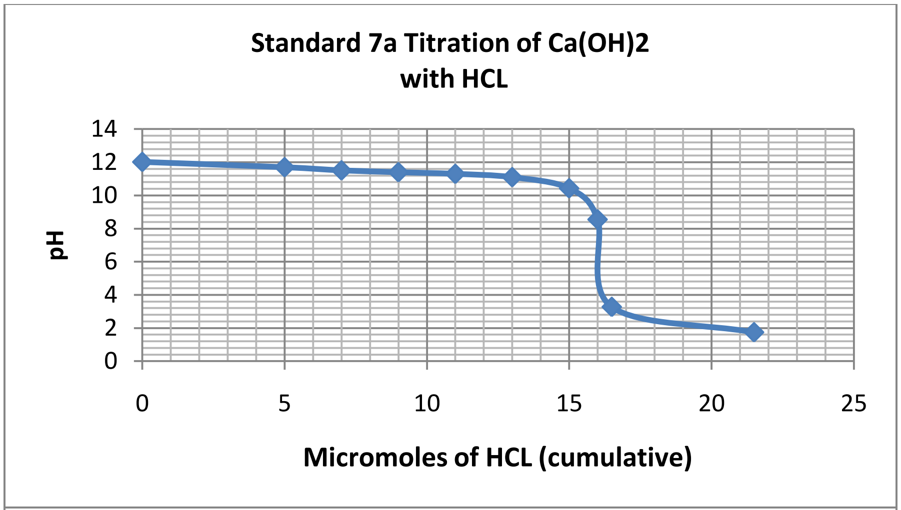

6a Regression
pH
11
10
9
8
7
6
5
4
y = -3.93x + 56.98
11.8
12
12.2
12.4
12.6
12.8
13
13.2
Micromoles of HCL (cumulative)

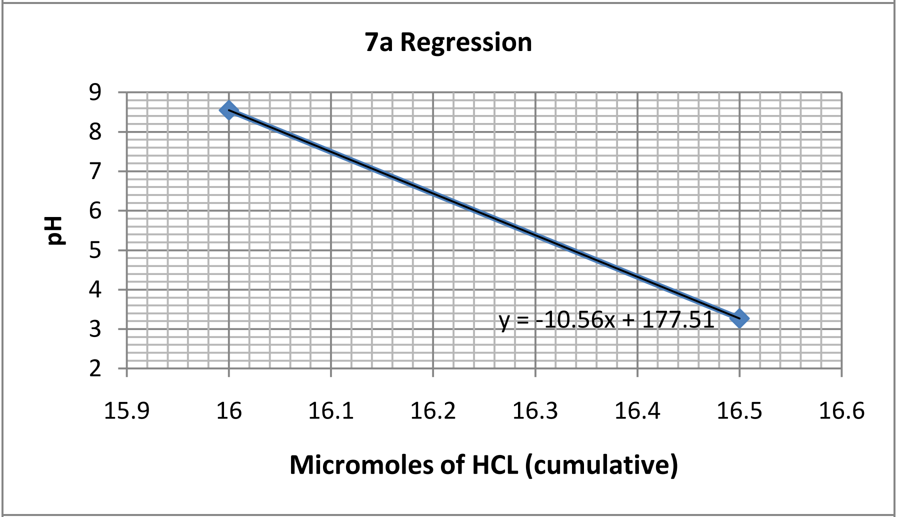

Standard 6b Titration of Ca(OH)2 with HCL
pH
12
10
8
6
4
2
0
0
5
10
15
20
Micromoles of HCL (cumulative)

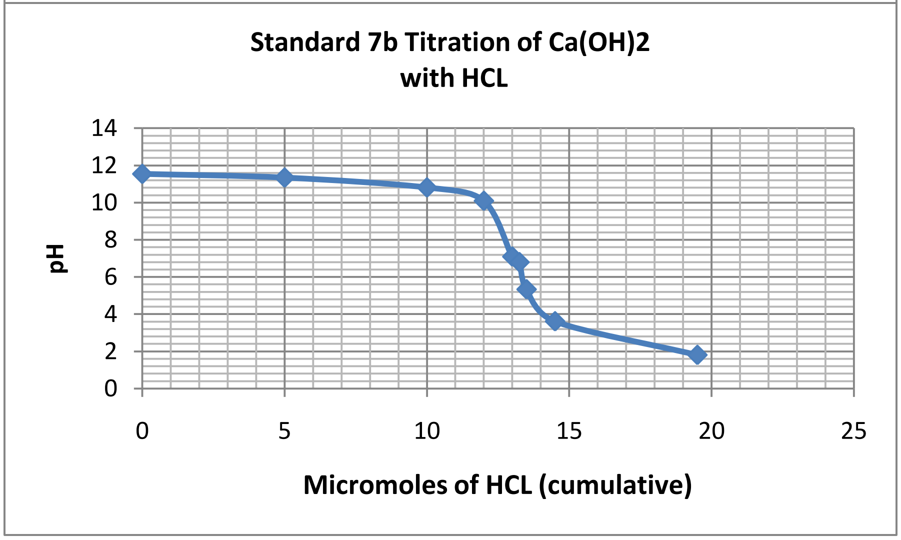

6b Regression
11
10
9
8
7
6
pH
y = -4.0229x + 42.116
7.8
8
8.2
8.4
8.6
8.8
Micromoles of HCL (cumulative)

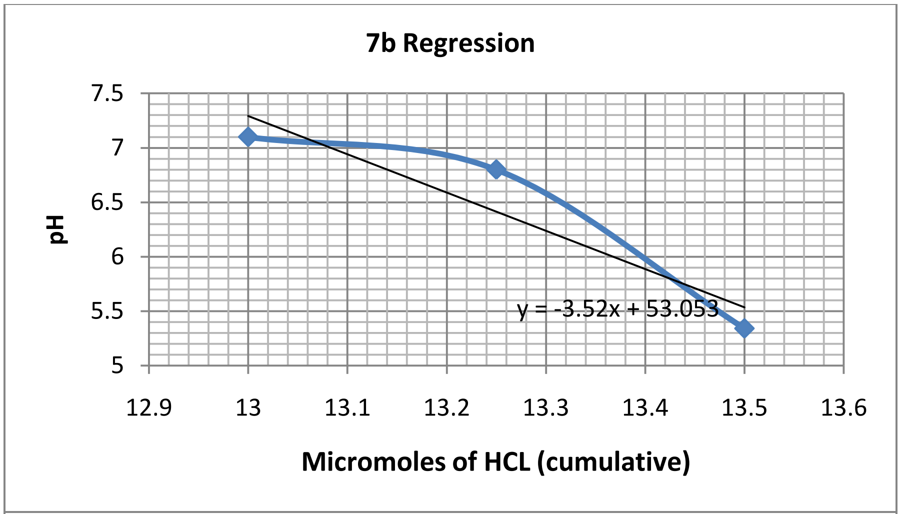

Standard 6c Titration of Ca(OH)2 with HCL
14
12
10
8
6
4
2
0
pH
0
5
10
15
20
Micromoles of HCL (cumulative)

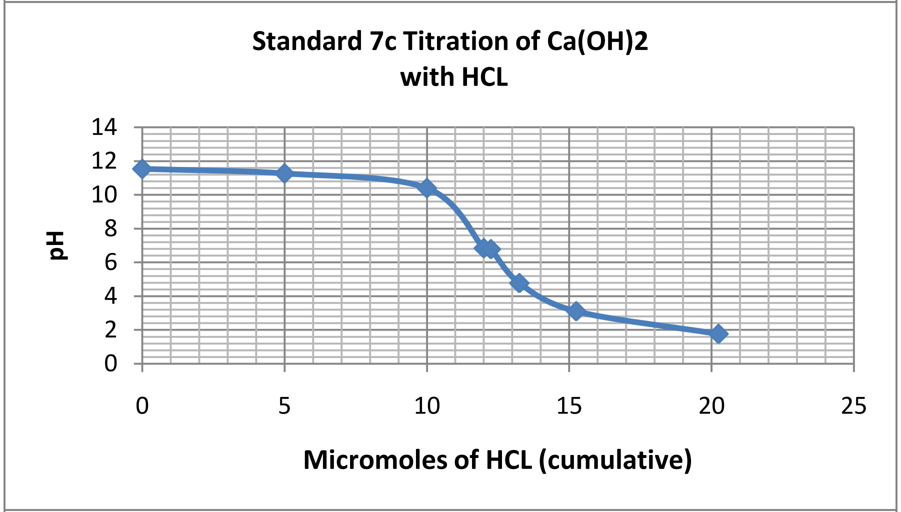

6c Regression
9.5
9
8.5
8
7.5
7
6.5
6
pH
y = -4.06x + 60.015
12.4
12.6
12.8
13
13.2
13.4
Micromoles of HCL (cumulative)

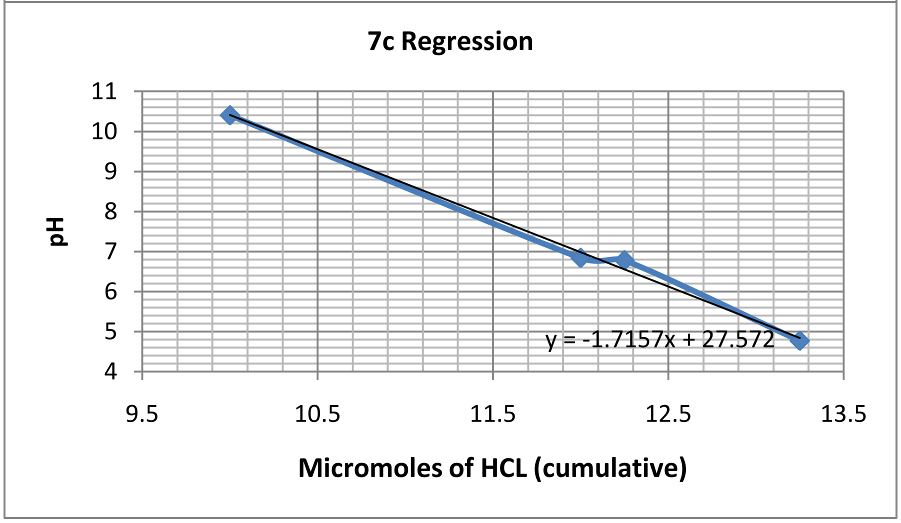

Standard 7a Titration of Ca(OH)2 with HCL

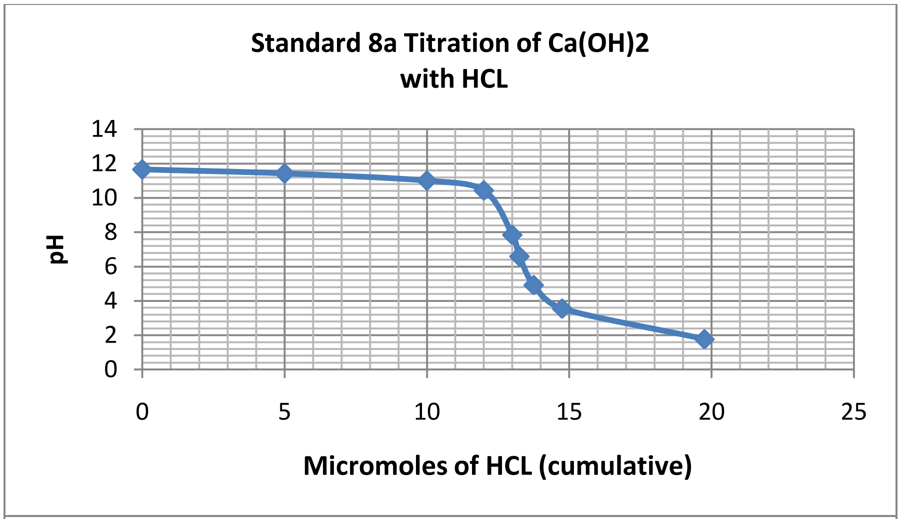

7a Regression


Standard 7b Titration of Ca(OH)2 with HCL

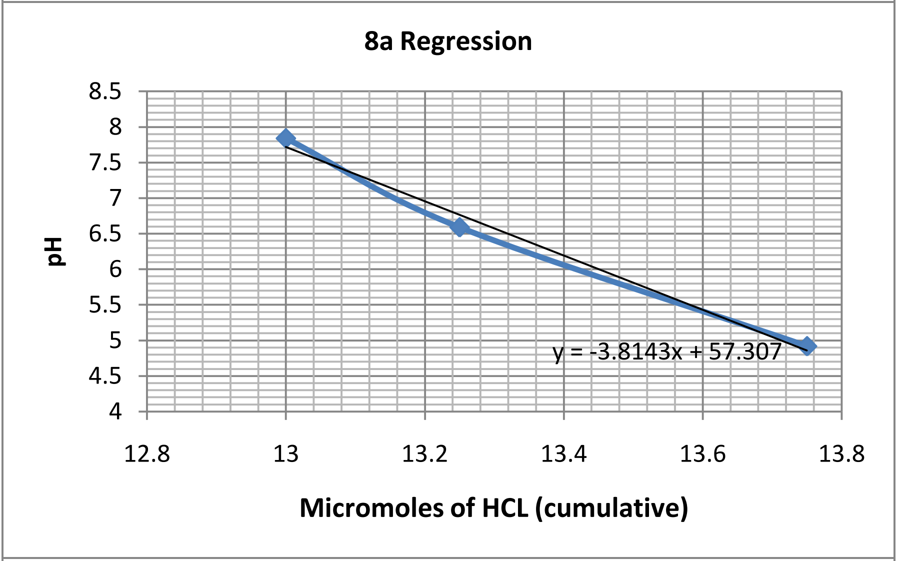

7b Regression
7.5
7
6.5
6
5.5
5
pH
12.9
13
13.1
13.2
13.3
13.4
13.5
13.6
y = -3.52x + 53.053
Micromoles of HCL (cumulative)


Standard 7c Titration of Ca(OH)2 with HCL
14
12
10
8
6
4
2
0
pH
0
5
10
15
20
25
Micromoles of HCL (cumulative)

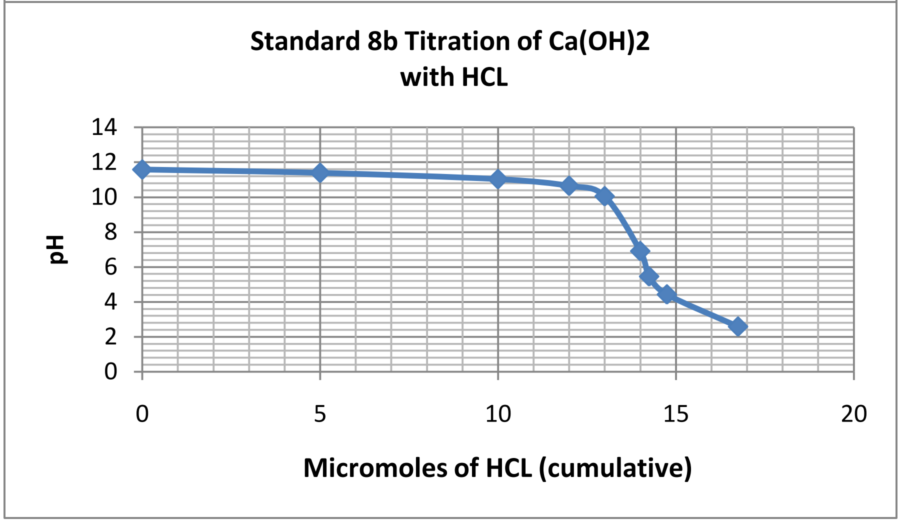

7c Regression
11
10
9
8
7
6
5
4
pH
9.5
10.5
11.5
12.5
13.5
y = -1.7157x + 27.572
Micromoles of HCL (cumulative)



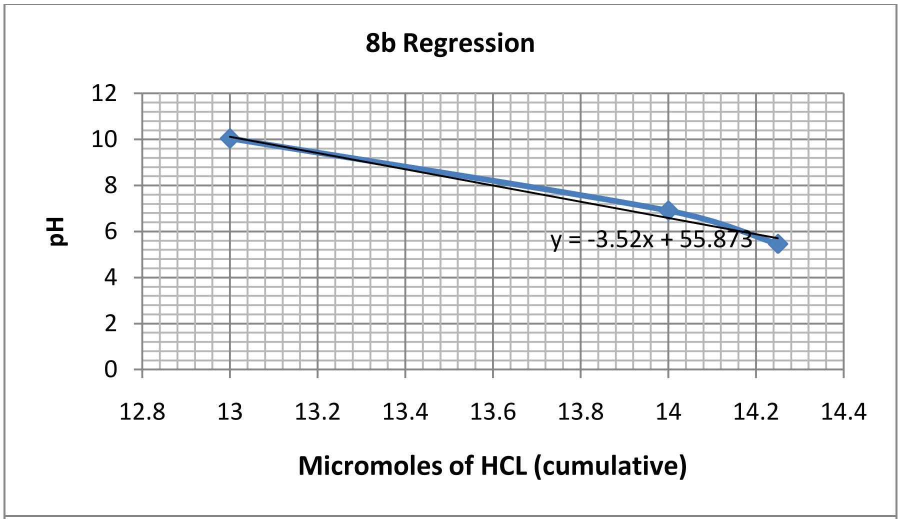
Standard 8a Titration of Ca(OH)2 with HCL

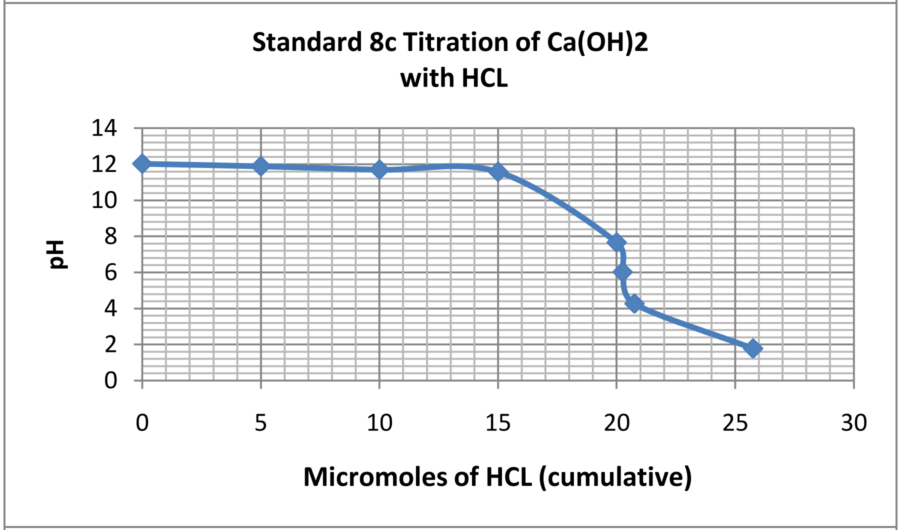


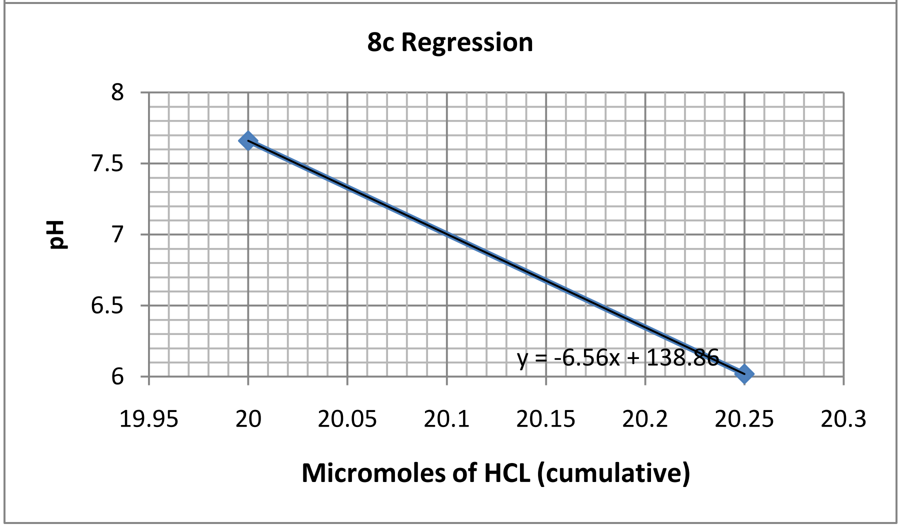
8a Regression

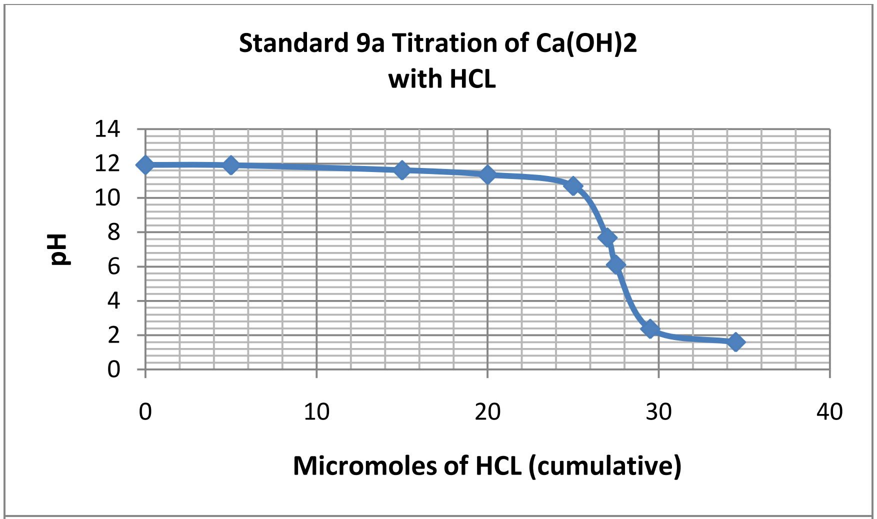


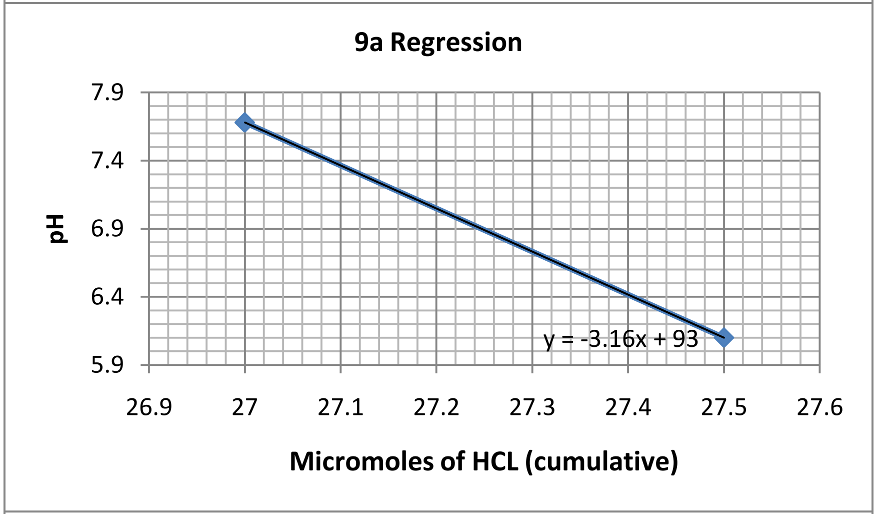
Standard 8b Titration of Ca(OH)2 with HCL

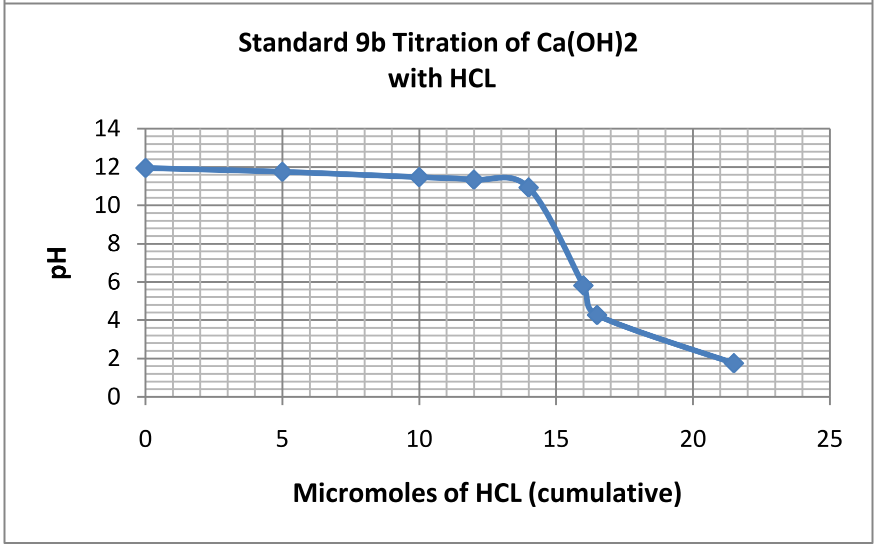

8b Regression

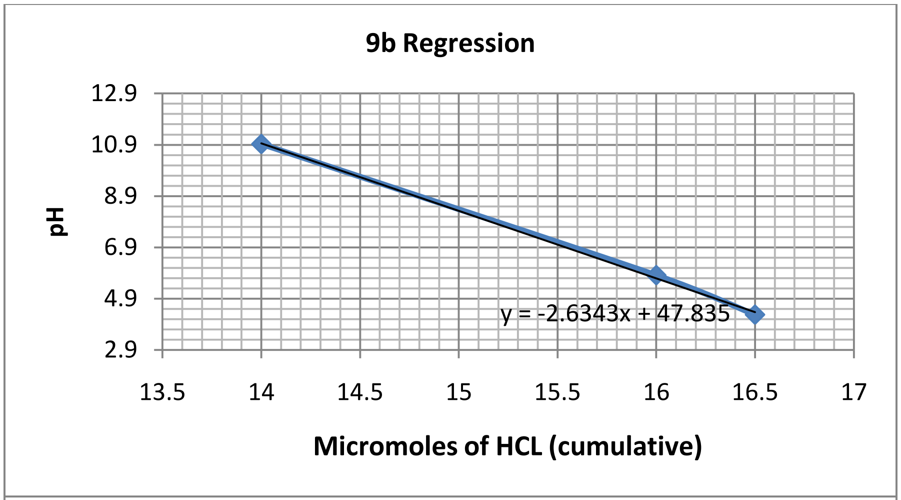

Standard 8c Titration of Ca(OH)2 with HCL

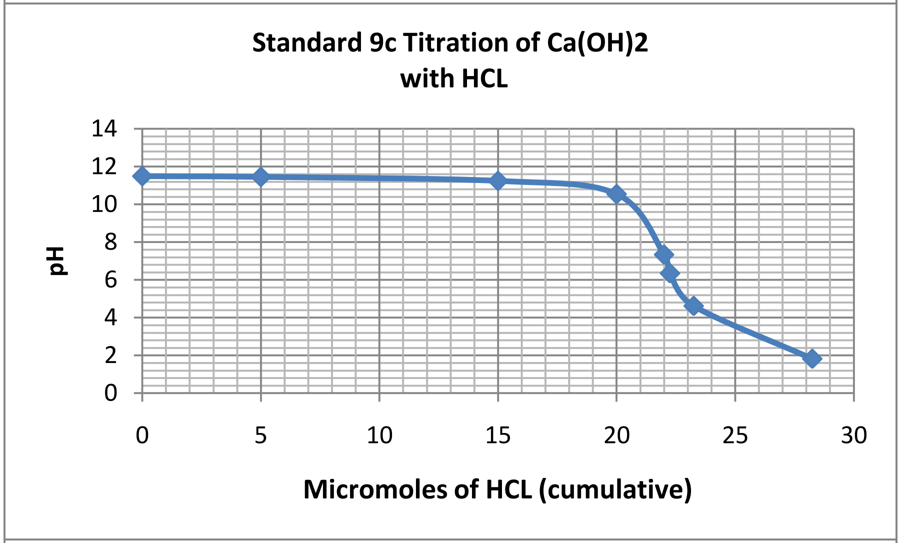

8c Regression

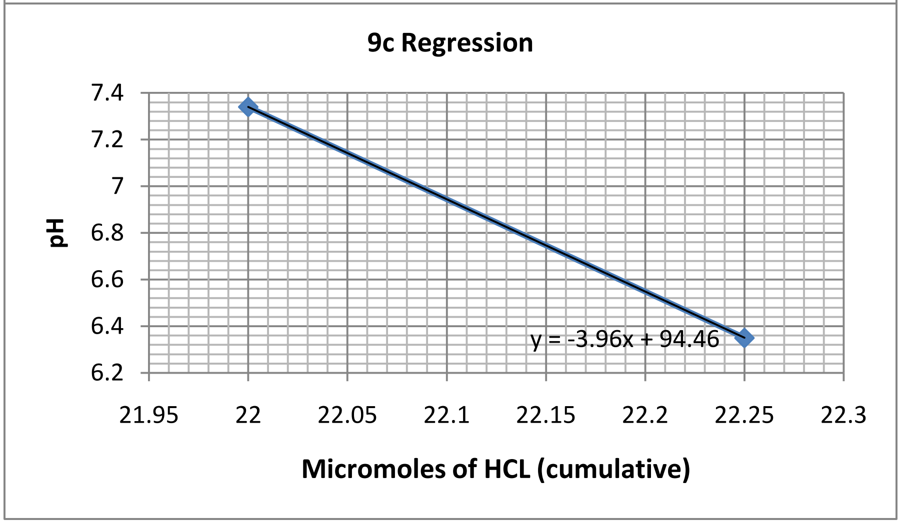

Standard 9a Titration of Ca(OH)2 with HCL

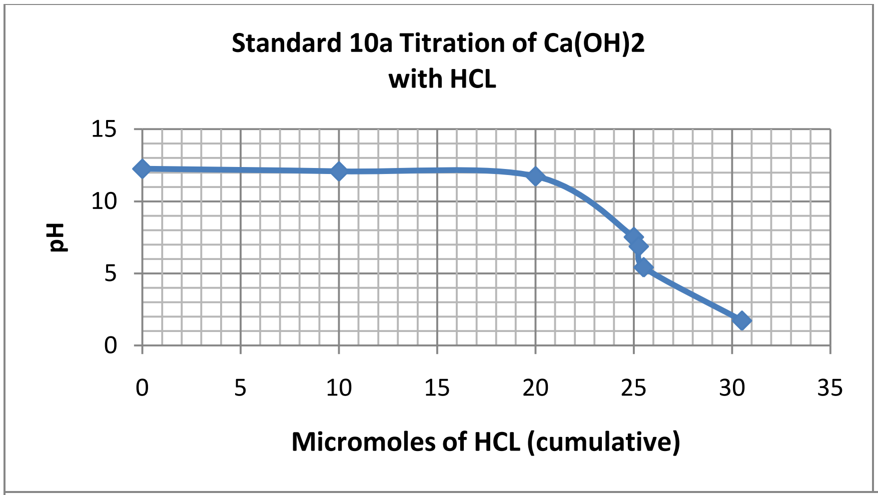

9a Regression

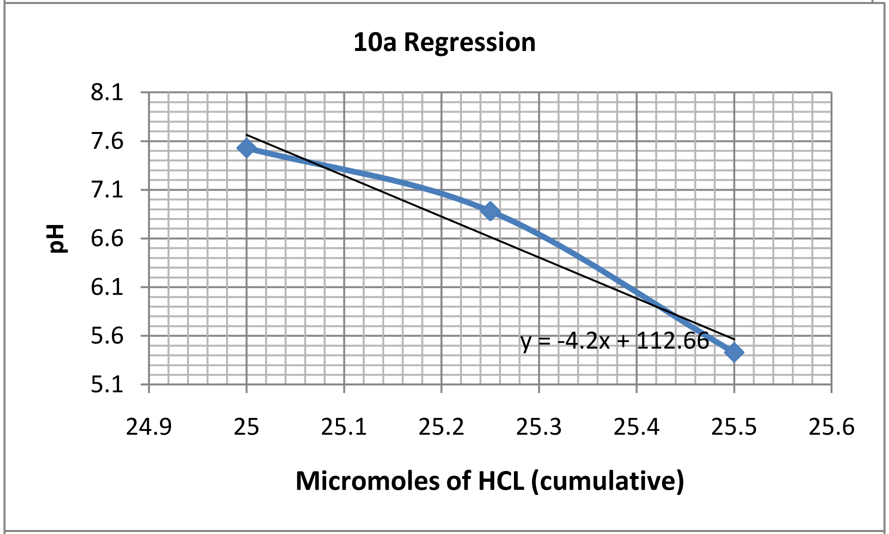

Standard 9b Titration of Ca(OH)2 with HCL

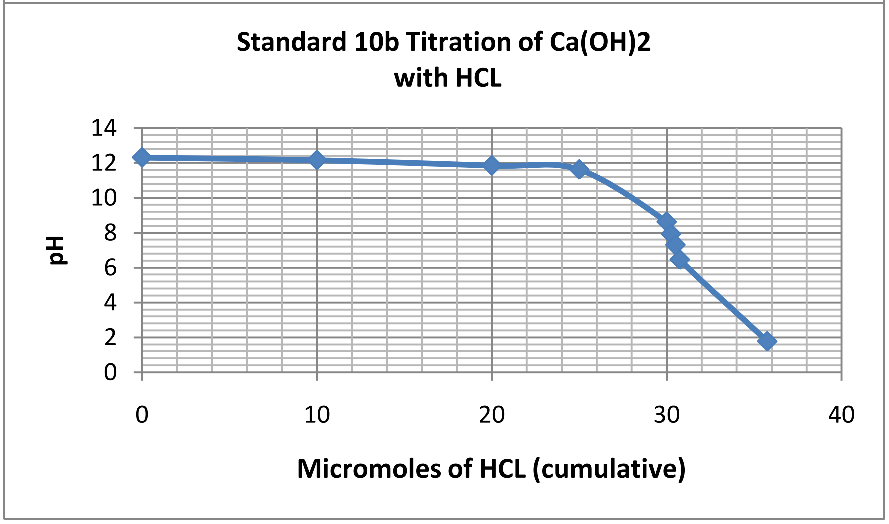

9b Regression
12.9
10.9
8.9
6.9
4.9
2.9
pH
y = -2.6343x + 47.835
13.5
14
14.5
15
15.5
16
16.5
17
Micromoles of HCL (cumulative)

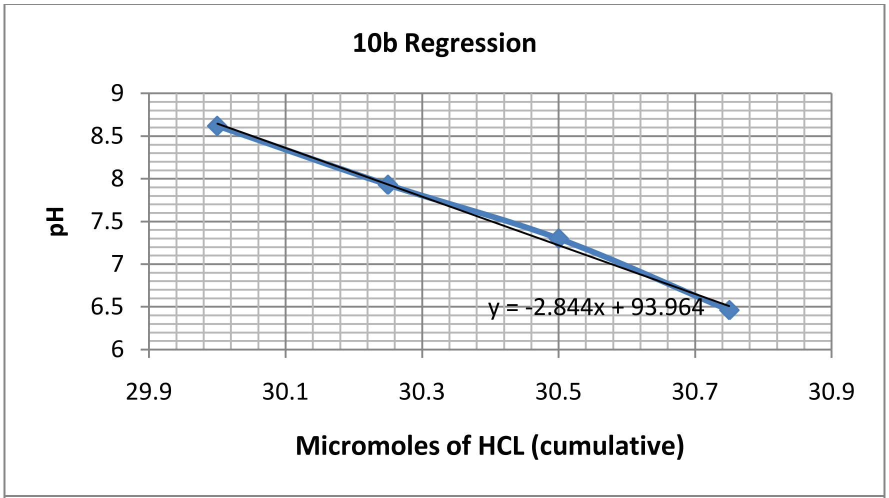

Standard 9c Titration of Ca(OH)2 with HCL
14
12
10
8
6
4
2
0
pH
0
5
10
15
20
25
30
Micromoles of HCL (cumulative)

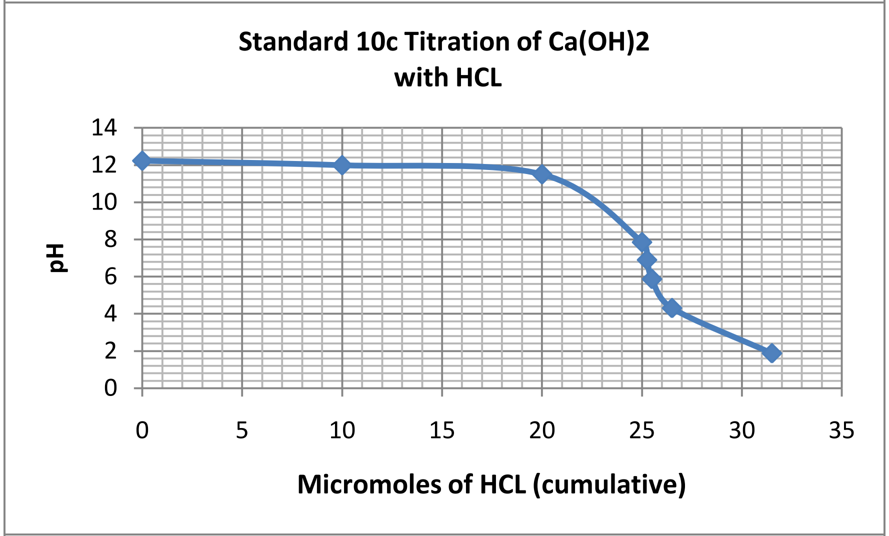

9c Regression
7.4
7.2
7
6.8
6.6
6.4
6.2
pH
y = -3.96x + 94.46
21.95
22
22.05
22.1
22.15
22.2
22.25
22.3
Micromoles of HCL (cumulative)

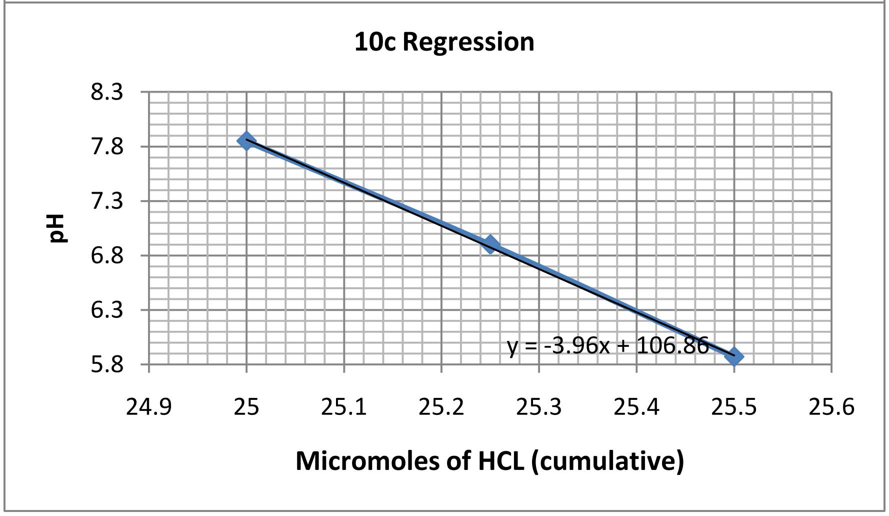

Standard 10a Titration of Ca(OH)2 with HCL
15
10
5
0
pH
0
5
10
15
20
25
30
35
Micromoles of HCL (cumulative)

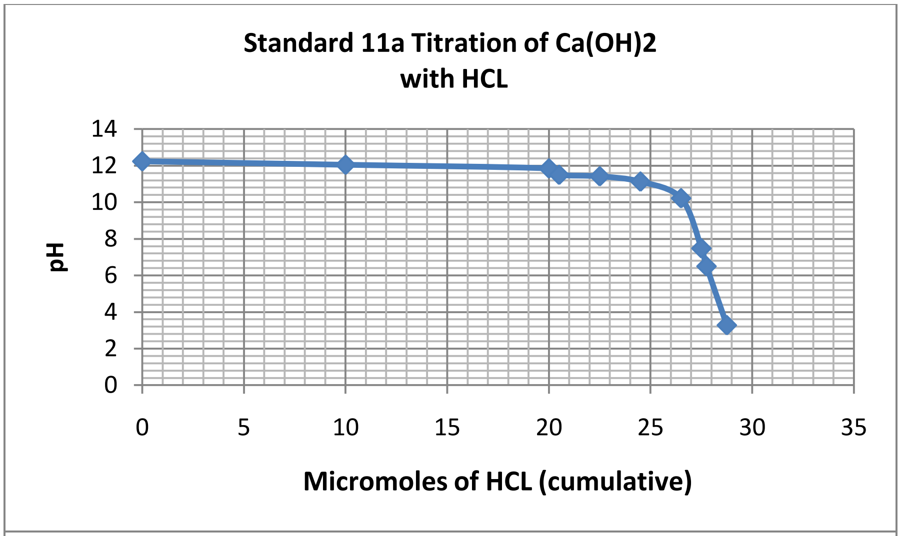

10a Regression
8.1
7.6
7.1
6.6
6.1
5.6
5.1
pH
y = -4.2x + 112.66
24.9
25
25.1
25.2
25.3
25.4
25.5
25.6
Micromoles of HCL (cumulative)

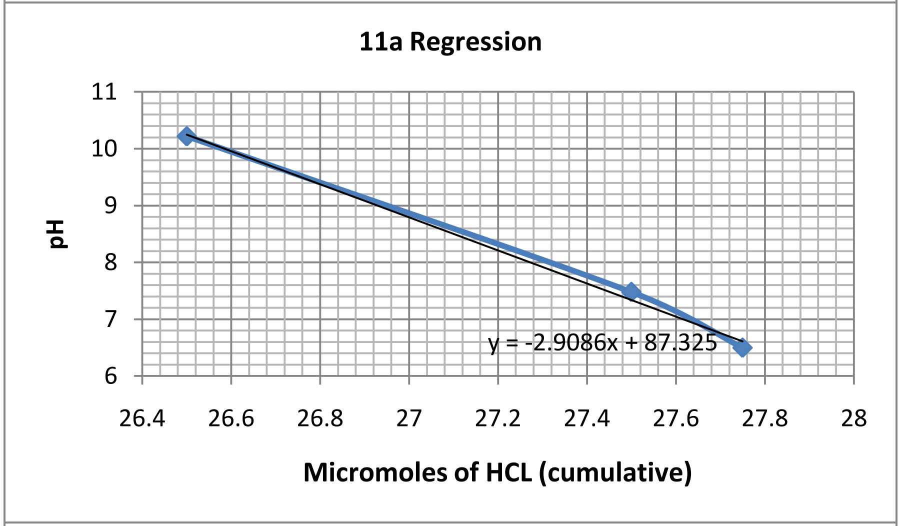

Standard 10b Titration of Ca(OH)2 with HCL
14
12
10
8
6
4
2
0
pH
0
10
20
30
40
Micromoles of HCL (cumulative)

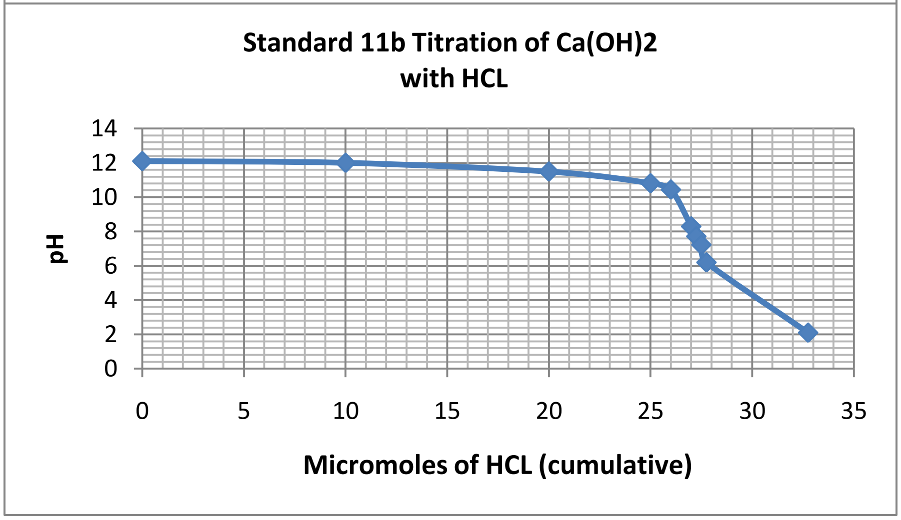

10b Regression
pH
9
8.5
8
7.5
7
6.5
6
29.9
30.1
30.3
30.5
30.7
30.9
y = -2.844x + 93.964
Micromoles of HCL (cumulative)

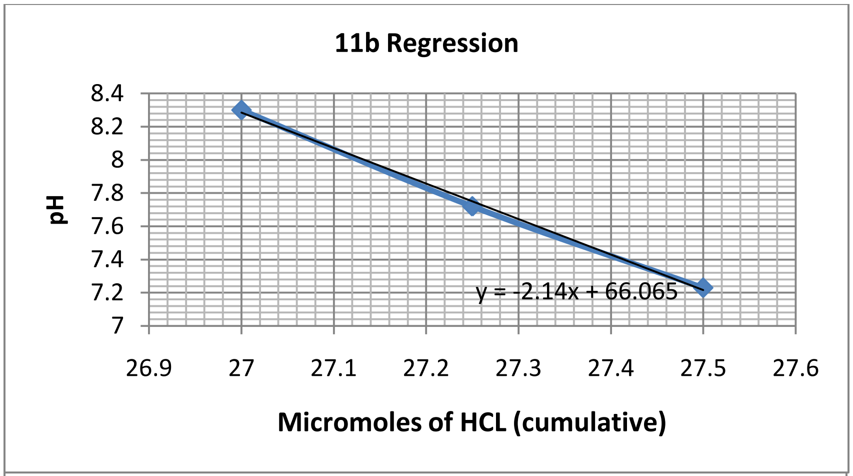

Standard 10c Titration of Ca(OH)2 with HCL
pH
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
Micromoles of HCL (cumulative)

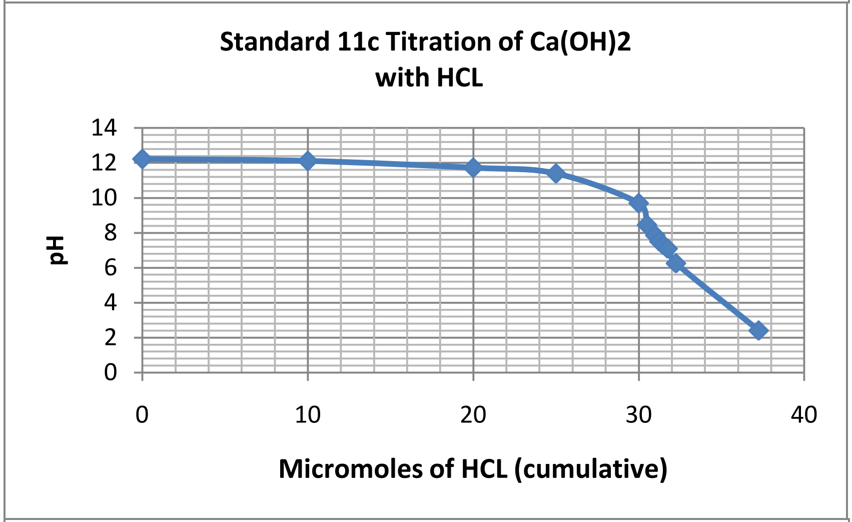

10c Regression
pH
8.3
7.8
7.3
6.8
6.3
5.8
24.9
25
25.1
25.2
25.3
25.4
25.5
25.6
y = -3.96x + 106.86
Micromoles of HCL (cumulative)

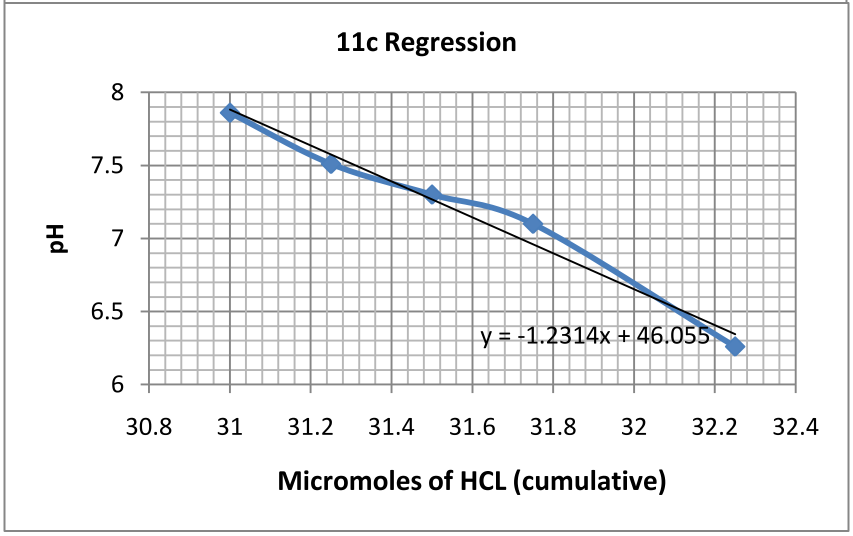

Standard 11a Titration of Ca(OH)2 with HCL
pH
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
Micromoles of HCL (cumulative)


11a Regression
pH
11
10
9
8
7
6
26.4
26.6
26.8
27
27.2
27.4
27.6
27.8
28
y = -2.9086x + 87.325
Micromoles of HCL (cumulative)


Standard 11b Titration of Ca(OH)2 with HCL
pH
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
Micromoles of HCL (cumulative)


11b Regression
pH
8.4
8.2
8
7.8
7.6
7.4
7.2
7
y = -2.14x + 66.065
26.9
27
27.1
27.2
27.3
27.4
27.5
27.6
Micromoles of HCL (cumulative)


Standard 11c Titration of Ca(OH)2 with HCL
pH
14
12
10
8
6
4
2
0
0
10
20
30
40
Micromoles of HCL (cumulative)


11c Regression
pH
8
7.5
7
6.5
6
y = -1.2314x + 46.055
30.8
31
31.2
31.4
31.6
31.8
32
32.2
32.4
Micromoles of HCL (cumulative)

Appendix IX.

Titration data for positive controls. Columns from left to right: pH readings, concentration of HCL added, volume of HCL added, moles of HCL added, cumulative moles of HCL added, cumulative micromoles of HCL added, and pH (listed again).

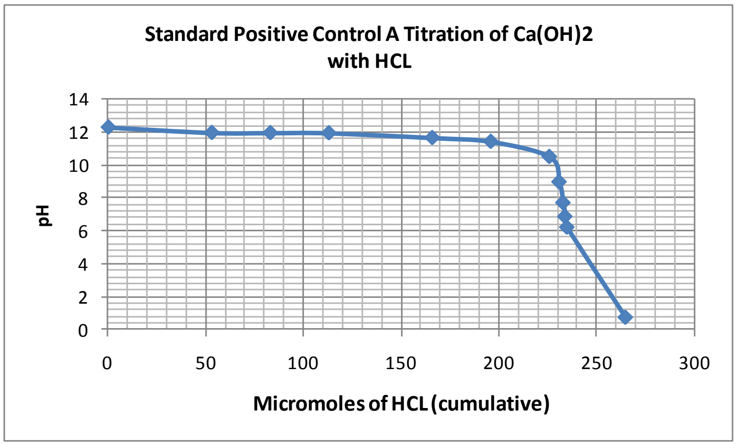
Pos Control A						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.29				0	0	12.29
11.96	5.3	0.00001	0.000053	0.000053	53	11.96
11.95	3	0.00001	0.00003	0.000083	83	11.95
11.94	3	0.00001	0.00003	0.000113	113	11.94
11.65	5.3	0.00001	0.000053	0.000166	166	11.65
11.42	3	0.00001	0.00003	0.000196	196	11.42
10.5	3	0.00001	0.00003	0.000226	226	10.5
8.96	0.5	0.00001	0.000005	0.000231	231	8.96
7.69	0.2	0.00001	0.000002	0.000233	233	7.69
6.85	0.1	0.00001	0.000001	0.000234	234	6.85
6.2	0.1	0.00001	0.000001	0.000235	235	6.2
0.71	3	0.00001	0.00003	0.000265	265	0.71

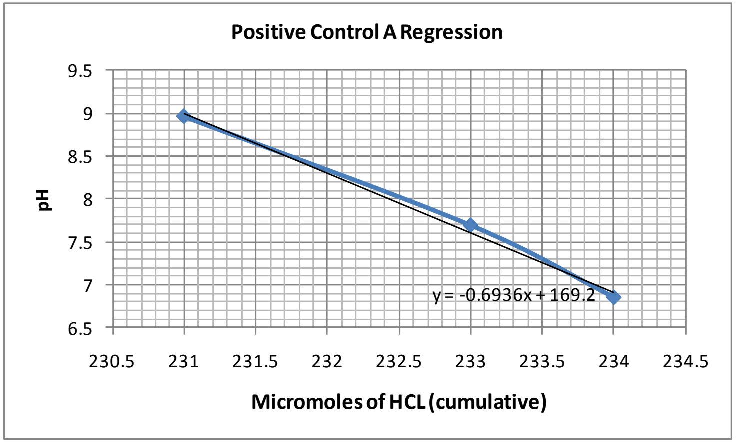
Positive Control B						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.27				0	0	12.27
11.96	5.6	0.00001	0.000056	0.000056	56	11.96
11.7	5.6	0.00001	0.000056	0.000112	112	11.7
11.67	3	0.00001	0.00003	0.000142	142	11.67
11.55	3	0.00001	0.00003	0.000172	172	11.55
11.55	3	0.00001	0.00003	0.000202	202	11.55
11.13	3	0.00001	0.00003	0.000232	232	11.13
1.36	3	0.00001	0.00003	0.000262	262	1.36

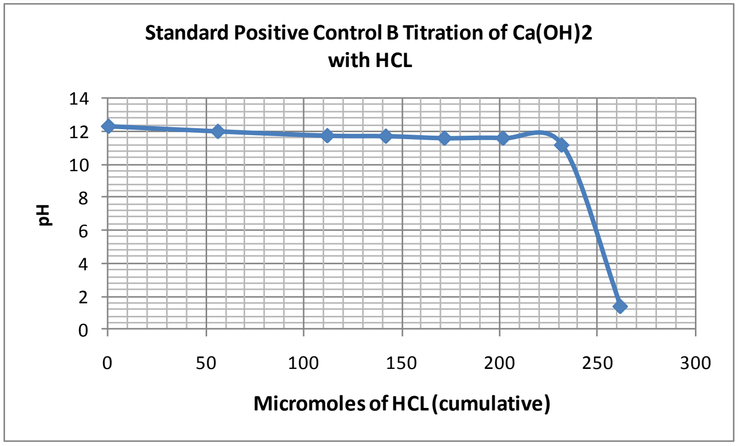
Positive Control C						
pH	[HCl]	HCl (L)	HCl Added	HCl Cumulative (mol)		pH
12.28				0	0	12.28
11.95	5.6	0.00001	0.000056	0.000056	56	11.95
11.66	5.6	0.00001	0.000056	0.000112	112	11.66
11.64	3	0.00001	0.00003	0.000142	142	11.64
11.6	3	0.00001	0.00003	0.000172	172	11.6
11.45	3	0.00001	0.00003	0.000202	202	11.45
8.8	3	0.00001	0.00003	0.000232	232	8.8
3.78	0.5	0.00001	0.000005	0.000237	237	3.78
0.67	3	0.00001	0.00003	0.000267	267	0.67

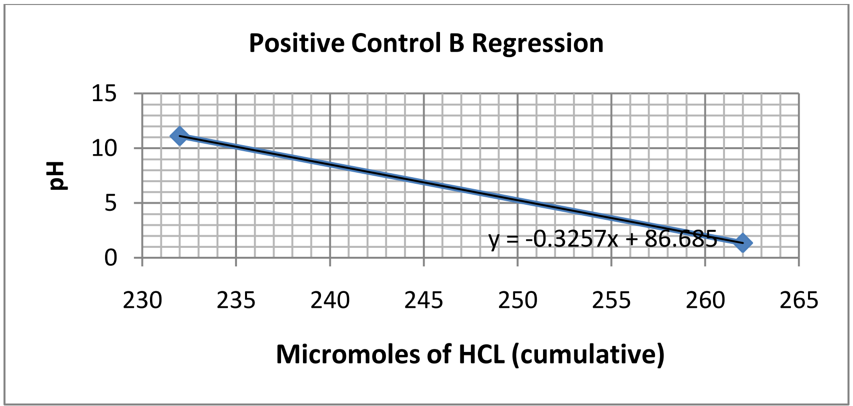
Appendix X.

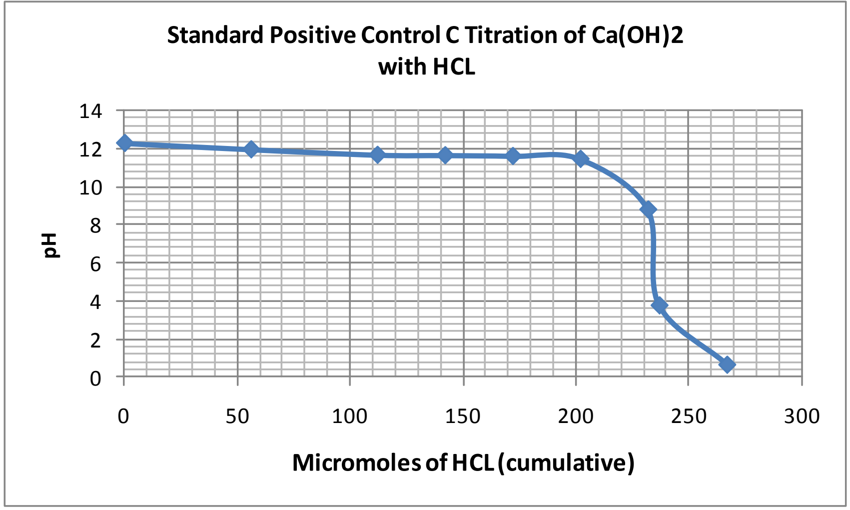
Titration graphs and linear regression graphs positive controls.

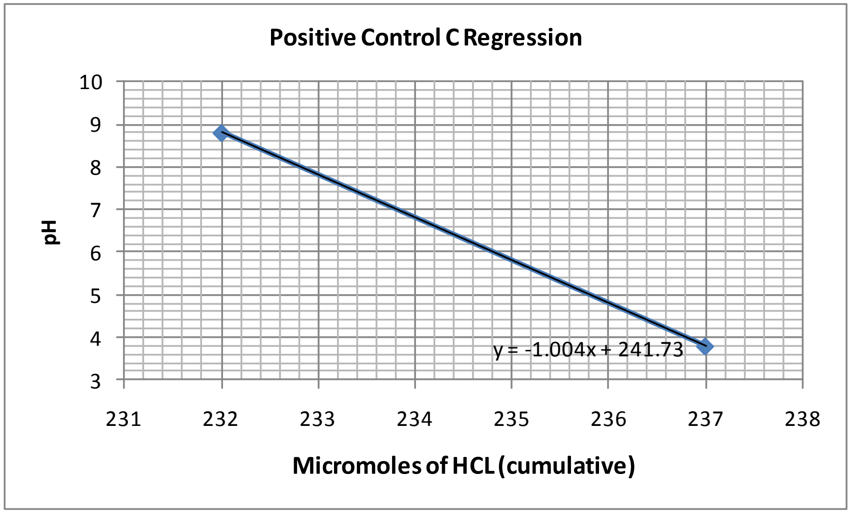







Positive Control B Regression


Standard Positive Control C Titration of Ca(OH)2 with HCL


Positive Control C Regression