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Origins of Life on Earth: Mineral Effects on Vesicle Formation and Permeability

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ABSTRACT: Understanding how life could have emerged on Earth is a very old problem that is still far from being resolved. Minerals have long been proposed to play a role in the formation of the first cell, but only few experimental data were reported concerning the mineral-lipid interactions. In the present work, calcein leakage assay, a fluorescence based experiment, and dynamic light scattering measurements were used to examine the effects of mineral properties and of $MgCl_2$ on the formation and the stability of vesicles formed from single-chain amphiphiles (SCA), which are believed to have been present on early Earth. The results showed that minerals do not have significant effect on SCA vesicles, but the latter appeared to be very sensitive to $MgCl_2$ (a fatal concentration of ~7 mM, under the tested conditions). These results demarcate the parameters of the viability of a protocell and suggest an early Earth's site for the emergence of life where high mineral loading could be present, but only low Mg^{2+} concentrations could be tolerated.

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i. Introduction

It is still unclear how life could have emerged on Earth ~4 billion years ago, despite extensive recent advances. Many hypotheses have been proposed over years, each with significant set of experimental evidences, however, the RNA world hypothesis is still the most accepted scenario to date [for reviews, please see (Orgel 2004, Bernhardt 2012, Robertson and Joyce 2012)]. Briefly, RNA, because of its versatility, has been proposed to have ensured simultaneously the role of DNA in carrying the genetic information, and the role of proteins in executing essential biochemical reactions. Another major component to the earliest form of life is a boundary formed of lipid amphiphiles. By encapsulating RNA, this entity would form a prebiotic cell or a protocell, which, by replicating and evolving, would give birth to the first cellular life. In the last decade, many studies have focused on creating a self-sustained protocell from simple molecules (Mansy et al. 2008, Adamala and Szostak 2013). However, the role of minerals and rocks, in the formation and stability of a protocell was underestimated - this is probably because of the difficulty of their experimental analysis. Indeed, while non-enzymatic RNA polymerization has been repeatedly proven on mineral surfaces (Ferris and Ertem 1992, Ferris 2005, Joshi and Aldersley 2013), little is known about the

effect of minerals on the lipid amphiphile self-assembly and stability. In our laboratory, it is believed that minerals could have played key roles in the emergence and evolution of a life. But before diving in a complex systems such as multi-component prebiotic soups from where protocells could have emerged (Kaddour and Sahai 2014), it is important to first understand the lipid/mineral interactions. In the present study, we focused on the role of a wide range of mineral properties on the encapsulating efficiency of simple amphiphilic molecules such as short single-chain amphiphiles. These amphiphiles were chosen in this study, because they have been found in meteorites (Yuen and Kvenvolden 1973) and are more likely to have formed on early Earth (Powner and Sutherland 2011). Longer chain amphiphiles, such as oleic acid (OA), and phospholipids (double chain amphiphiles) were also tested for their encapsulation efficiency, as comparison (figure 1). Calcein, a fluorescent dye, was used as proxy to nutrients such as single RNA nucleotides (Figure 2). At high concentration, calcein dimerize and self-quench. When released from the vesicle, it gets diluted, the dimers dissociate and the monomers fluoresce. In this experiment, the increase of fluorescence was monitored over time and the percentage of leakage was reported.

In another set of experiments, the effect of siderite, a natural iron carbonate mineral that is believed to be abundant on early Earth, was tested on the vesicle formation, using dynamic light scattering (DLS). Briefly, when vesicles start forming, the amount of scattered light increases significantly. Measuring light scattering at different lipid concentrations allows thus the determination of the critical vesicle concentration (*cvc*), above of which the vesicles start forming. Because siderite is known to leach ions, its effect as well as the effect of its supernatant were both tested. Moreover, the fatal concentration of $MgCl_2$, above which vesicles get disrupted, was determined by both DLS and calcein leakage assay.

So far, under the tested conditions, minerals were not found to have significant effect on the viability of lipid vesicles, but were very sensitive to $MgCl_2$ salts.

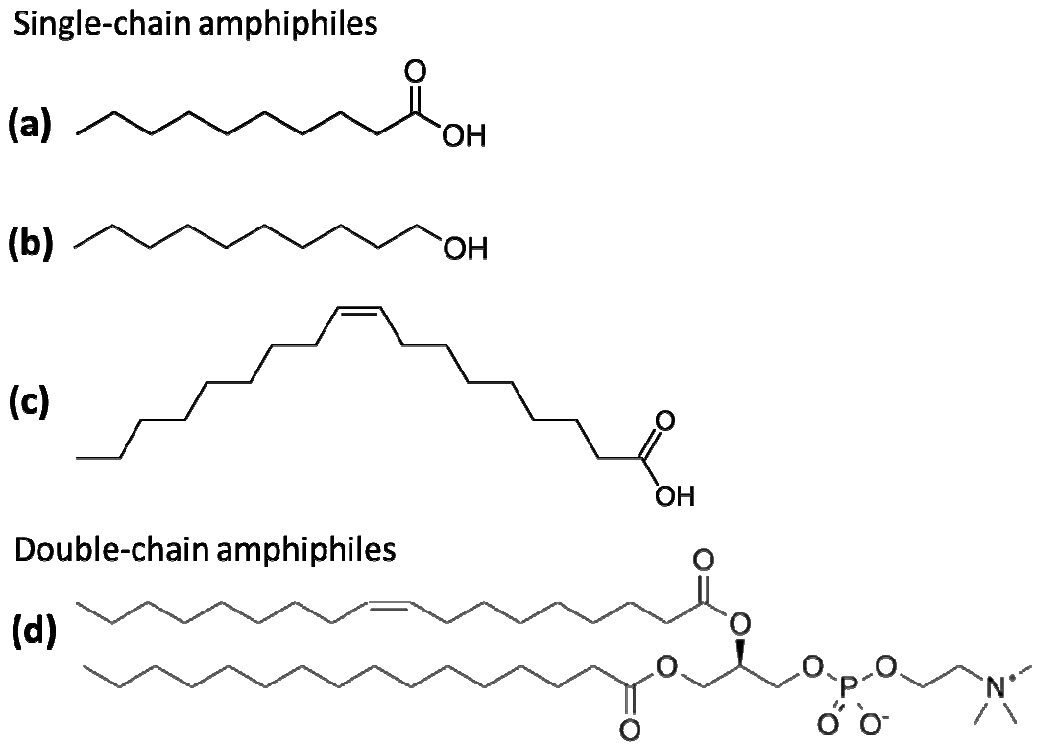


Figure 1. The structures of lipid amphiphiles used in this study. (a) decanoic acid (DA); (b) 1-decanol (DOH); (c) oleic acid (OA); and (d) 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

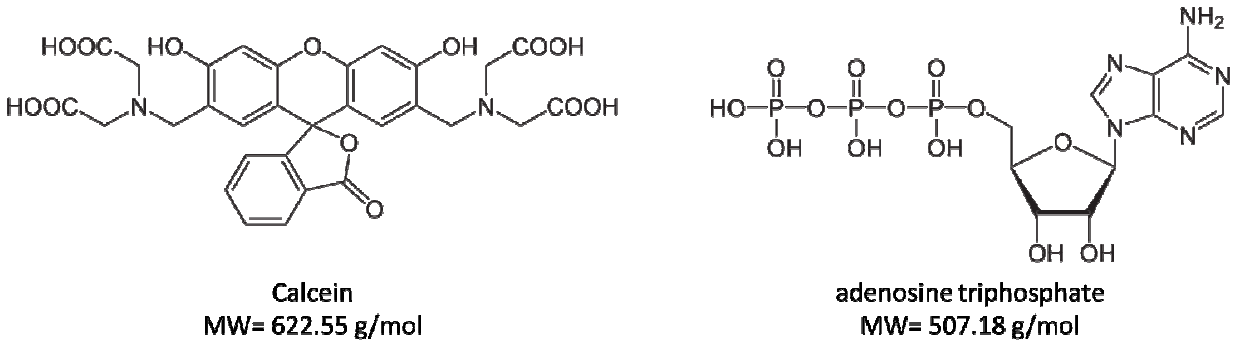


Figure 2. Chemical structure of calcein (a) used in this study as a proxy for adenosine triphosphate nucleotide (b). These molecules have similar molecular weight and are both highly negatively charged under the tested conditions in this study.

ii. Materials and Methods

All chemicals were purchased from Sigma Aldrich and were of the highest available purity. Minerals were either synthetic (□-alumina (Al_2O_3), Aerosil-300 (SiO_2), zincite (ZnO) and pyrite (FeS)), or natural (Volclay SPV 200, komatiite, siderite, and tonalite).

Calcein leakage assay

Decanoic acid (DA) and 1-decanol (DOH) vesicles were prepared in 2:1 ratio at a total lipid concentration of 300 mM. Lipids were dissolved in chloroform to ensure proper mixture. Chloroform was removed using a stream of compressed nitrogen and then the sample was placed under vacuum for approximately 3 hours to ensure complete solvent evaporation. The lipid film was rehydrated with a solution of 20 mM calcein in 200 mM bicine buffer pH 8. The sample was then vortexed and sonicated. The pH was checked; if necessary, NaOH was added to raise the pH to 8, where vesicle formation would best occur. The sample was then allowed to tumble overnight. Vesicles were extruded through a 200 nm polycarbonate membrane using a mini-extruder (Avanti Polar Lipids) to form homogenous, monodisperse and single-bilayer vesicles. Low pressure-size exclusion chromatography was used to separate the dye-encapsulating vesicles from the un-encapsulated free dye. The column (25 cm x 1 cm) was filled with sephadex G-50 medium beads (Sigma Aldrich). The mobile phase was a 200 mM bicine buffer at pH 8. The flow rate was maintained at 1.5 mL/min (econo gradient pump, Bio-Rad) and the injection volume was of 300 µL. Fractions were collected (FC204, Gilson) in a 96-micro-wellplate. Fluorescence was measured in a plate reader (Synergy H1, BioTek) and the fractions containing vesicles were pooled. Purified vesicles were mixed with different minerals at a final particle loading of 0.1 mg/mL, in the presence and absence of Triton^{TM}-100X (0.1%), each condition in triplicate. Kinetics of release of calcein was monitored for 18 hours (at ex./em of 495/530 nm). The percent of encapsulation was calculated according to the following equation: $Encapsulation\,(\%) = 100 \times \left(1 - \frac{F_t - F_0}{F_f - F_0}\right)$, where F_t is the fluorescence at time t, F_0 is the fluorescence at time zero and F_f is the fluorescence after addition of Triton.

DLS measurements

DA/DOH (2:1) mixtures were prepared in concentrations ranging from 0.2-27 mM, in the presence of 1 mg/mL siderite in 200 mM bicine buffer pH 8. Samples were rotated (40 rpm) at room temperature overnight to allow

mineral/vesicle interaction. DLS measurements were taken (Zetasizer Nano ZS, Malvern) and both the mean count rate (in kilo counts per second (kcps)) and z-average diameter (nm) were plotted as a function of lipid concentration. Similarly, the *cvc* of DA/DOH (2:1) was tested in the presence of siderite supernatant. The supernatant was obtained by centrifugation of a 10 mg/mL siderite in bicine (4000 rpm, benchtop Fisher Scientific centrifuge) and filtration through 0.22 μm filters.

Lastly, the fatal concentration of $MgCl_2$ was determined at 10 mM of DA/DOH. Both the mean count rate and z-average diameter were plotted as a function of $MgCl_2$ concentration.

iii. Results and Discussion

Characterization of the minerals

Some of the properties of the minerals that have been used in this study are summarized in Table 1. The values were either previously determined in the laboratory or found in literature.

Table 1. mineral characterization

mineral	chemical formula	density (g/cm^3)	IEP	ζ-Potential (mV) pH 7[a]	ζ-potential (mV) pH 8[b]	B.E.T. surface area (m^2/g)
amorphous silica	SiO_2	2.7	2.0	-32.93 ± 3.9	-24.3 ± 5.9	288
tonalite	Hadean continental crust	2.7	2.4	-43.13 ± 2.5	-40.4 ± 4.2	5.8
komatiite	Hadean oceanic crust	2.9	2.8	-42.3 ± 1.6	-34 ± 3.4	5.7
montmorillonite *SPV 200*	$Na_{0.2}Ca_{0.1}Al_2Si_4O_{10}(OH)_2(H_2O)_{10}$	2.1	3.0	-46.33 ± 4.8	-42.1 ± 4	32.8
pyrite	FeS_2	5.0	6.0	2.56 ± 2.0	-29.7 ± 5.1	0.5
siderite	$FeCO_3$	3.8	7.5	7.56 ± 0.8	-16.7 ± 4.1	8.7
zincite	ZnO	5.6	7.5	-2.95 ± 0.5	-6.3 ± 3.1	3.2
γ-alumina	γ-Al_2O_3	4.0	9.3	25.2 ± 2.1	9.6 ± 1.5	119

[a] In 200 mM HEPES pH 7, [b] In 200 mM bicine pH 8

Calcein leakage assay

The fluorescence intensity of the fractions indicates the separation of vesicles on the column. The vesicles encapsulating the dye ran off first (excluded from the beads) and the last eluent corresponds to the un-encapsulated free dye (Figure 3). One difference in the elution profiles of DA/DOH as comparing to OA and POPC systems was

that the deconvolution of the two peaks could never be completely resolved, no matter what was the length of the column or the injection volume (Figure 3). This result by itself demonstrates that DA/DOH system is less stable as comparing to OA and POPC, where calcein start leaking out during the separation, as soon as a gradient of calcein concentration between the inside and the outside of the vesicles occurs.

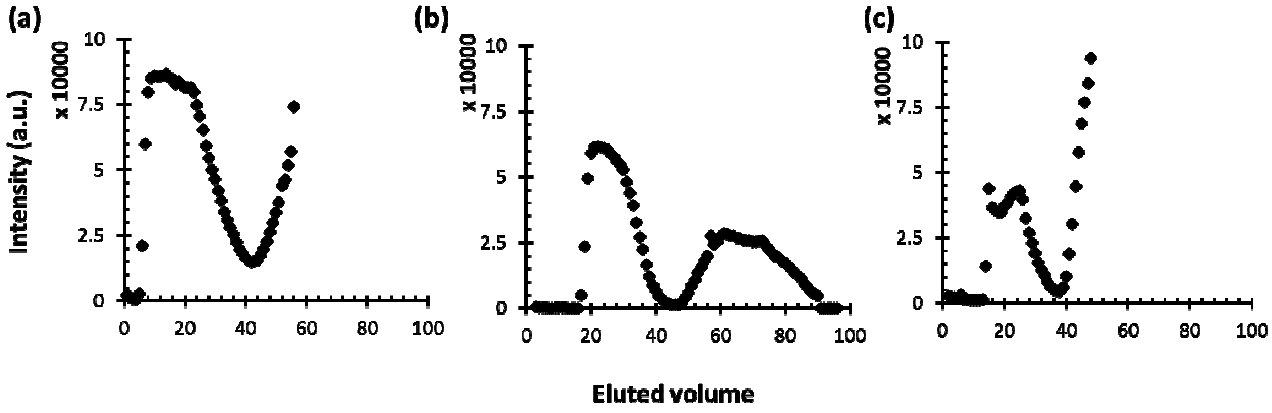


Figure 3. Elution profiles of calcein loaded vesicles. (a) DA/DOH; (b) OA; (c) POPC

The fractions of the first peak were combined and their volume was measured to calculate the final concentration of lipid. When following the calcein leakage over time (Figure 4), the result proves the previous interpretation, where DA/DOH is very leaky as comparing to OA and POPC systems. This is most likely due to the size of hydrophobic tail of the lipid (Figure 1). The longer the tail, the more stable the vesicles are.

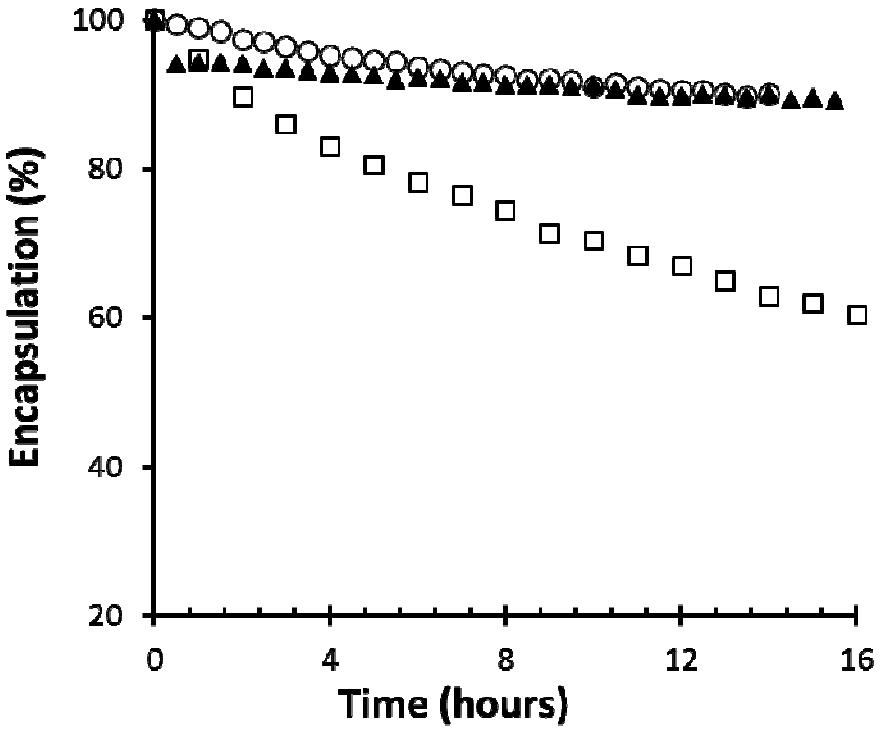
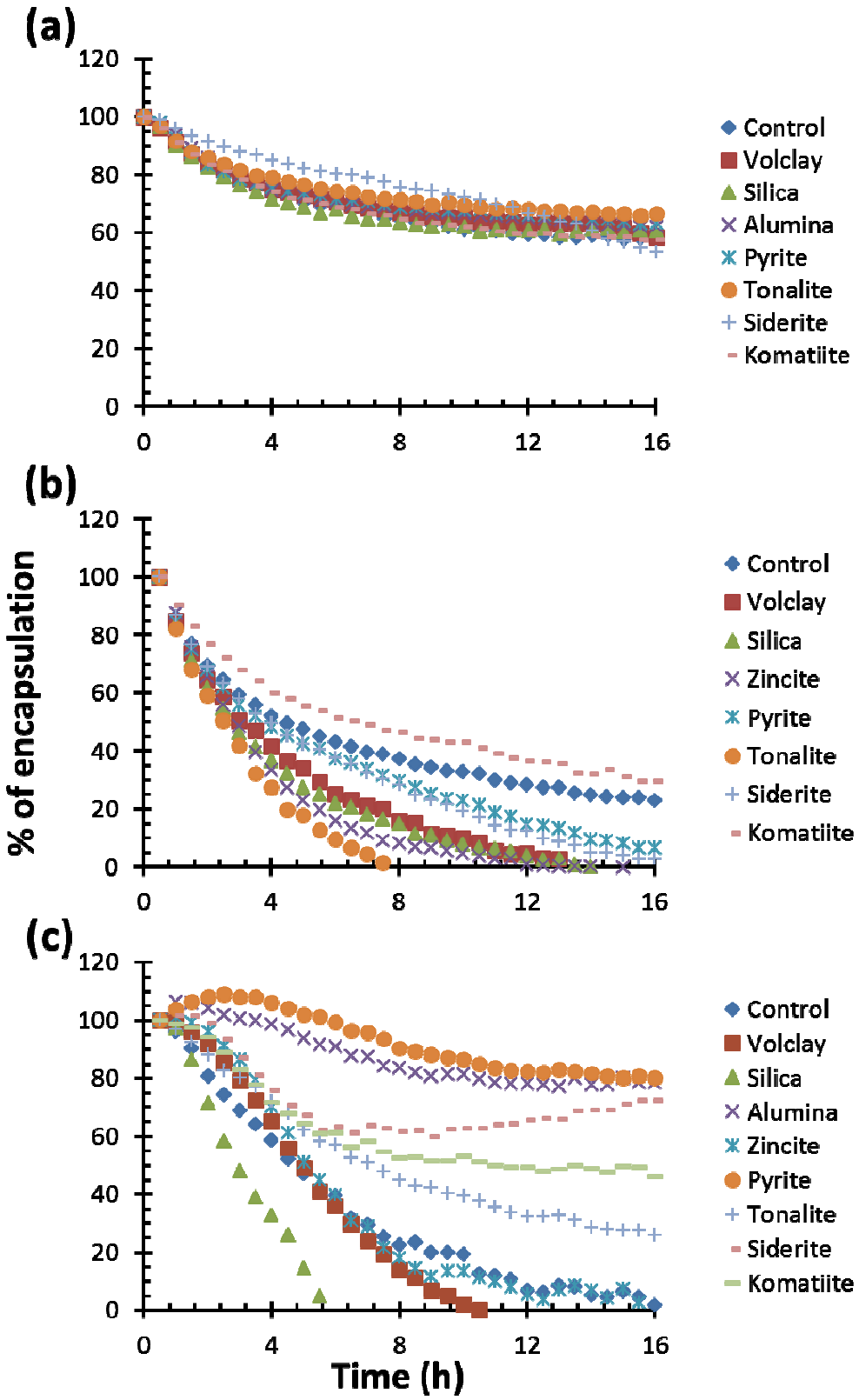


Figure 4. Calcein leakage assay for DA/DOH (open squares), OA (closed triangles) and POPC (open circles).

The effect of the minerals (0.1 mg/mL) on the leakage of calcein was tested on the DA/DOH system, at three different lipid concentrations of 25, 10 and 7 mM (Figure 5). When the lipid concentration decreases, the leakage of calcein becomes faster (Figure 5). At 25 mM DA/DOH (figure 5a), leakage was steady and no significant effect of any mineral was observed. By examining the 10 mM DA/DOH test closely, one can notice that within the first 2 hours of the measurements the minerals followed the same curve as the no mineral sample. As more time passed the vesicles in the presence of minerals began leaking at a slightly faster rate. Komatiite had almost no effect on the vesicles; it had the closest values to the no mineral sample. DA/DOH in the presence of pyrite was slightly leakier than the control. Vesicles in the presence of volclay, silica, and zincite had almost identical trends with complete leakage of dye around 13 hours. Tonalite and siderite caused the vesicles to be completely disrupted after about 7 hours. At 7 mM DA/DOH the vesicle leakage did not follow any pattern. The reason pyrite, alumina, and siderite samples showed extreme difference from the control is due to the samples that contained Triton decreasing in fluorescence overtime (see materials and methods). Although the samples without Triton leaked over time, the

Triton samples negated these results in the graph. All minerals without Triton behaved as expected and showed leakage. There may be too much leakage in the time it takes to run the column and begin the plate reader which made the determination of the encapsulation percent difficult. The effect of the same minerals was also tested on OA and POPC vesicle systems (data not shown) and, similarly, no obvious trend could be correlated to the mineral properties. The difficulty of the determination of such a trend may be due to the ratio of mineral loading to vesicles concentrations. However, lowering the lipid concentration did not help in this situation because it made the vesicles leakier, as shown in Figure 5. An experiment was thus done at higher particle loading (up to 50 mg/mL of mineral with 25 mM DA/DOH), and again, no significant effect of minerals could be detected. This result is not uninteresting, because it shows that vesicles are not disrupted by the presence of minerals, which were found to be essential for prebiotic RNA polymerization (Ferris et al. 1996, Ferris 2005).


(a)
120
100
80
60
40
20
0
0
4
8
12
16
Control
Volclay
Silica
Alumina
Pyrite
Tonalite
Siderite
Komatiite
(b)
% of encapsulation
120
100
80
60
40
20
0
0
4
8
12
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Control
Volclay
Silica
Zincite
Pyrite
Tonalite
Siderite
Komatiite
(c)
120
100
80
60
40
20
0
0
4
8
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16
Time (h)
Control
Volclay
Silica
Alumina
Zincite
Pyrite
Tonalite
Siderite
Komatiite

Figure 5. Effects of minerals (0.1 mg/mL) on the calcein leakage assay of DA/DOH system. (a) 25 mM; (b) 10 mM; and (c) 7 mM of total lipid concentration.

Effect of siderite on the cvc of DA/DOH

To further investigate the lipid-mineral interactions, the effect of siderite, a natural iron carbonate mineral, on the *cvc* of DA/DOH vesicle system, was studied using dynamic light scattering. The presence of siderite at 1 mg/mL particle loading shifted the *cvc* from 1.5 ± 0.5 mM to 4.5 ± 0.5 mM (Figure 6a,b). The values of *cvc* were graphically determined, where the scattered light starts increasing exponentially, or where the size of the particles starts stabilizing, in the range of ~400 nm (for the accuracy of the measurements, please see appendix 5). A natural siderite usually contains magnesium and calcium, and sometimes also zinc and cobalt, which substitute the ferric cations. When put in solution, siderite dissolve at some extent and all the ions leach into the solution until an equilibrium between the coordinated ions within the mineral crystal and the ions in the solution is reached. To know whether the shift of *cvc* is due to these leached ions or it is an effect of the mineral particle surface, the effect of the siderite supernatant on the *ta*minutes, 4000 rpm) and the supernatant was collected and passed through a 0.22 μm filter. An equivalent volume to a final particle loading of 1 mg/mL was used in this experiment. The result showed a *cvc* of 2 ± 0.75 mM (Figure 6c), not significantly different from the no mineral control (Figure 6a). This result proved unambiguously the role of the mineral surface chemistry in the formation of vesicles. Amphiphiles would have adsorbed on the mineral surface, decreasing thus the amount of lipid in the solution, available to self-assemble into vesicles.

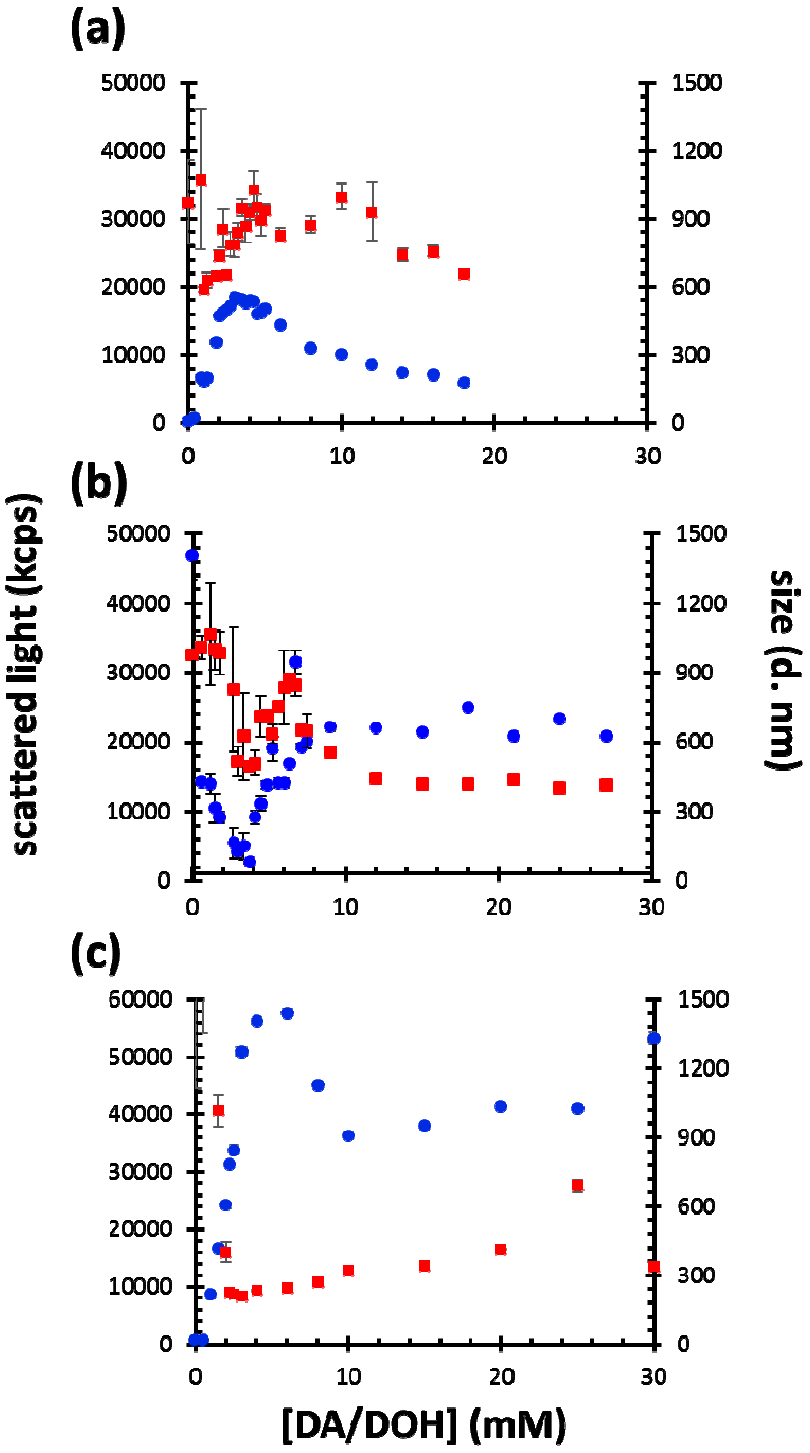

Figure 6. Effect of siderite on the *cvc* of DA/DOH, by light scattering measurements. (a) in the absence of mineral; (b) in the presence of 1 mg/mL siderite; and (c) in the presence of siderite supernatant. Blue circles and red squares correspond to scattered light and size, respectively.

Effect of $MgCl_2$ DA/DOH vesicles

$MgCl_2$ is known to disrupt the vesicles and precipitating the lipid. In this part of the study, we aimed to determine the fatal concentration of $MgCl_2$ on DA/DOH by both, light scattering and calcein leakage assay. In the first experiment, increasing amounts of $MgCl_2$ were used with 10 mM DA/DOH in 200 mM bicine buffer pH 8. The fatal Mg^{2+} concentration was determined to be 7 ± 1 mM (Figure 7a). This value was graphically determined, and confirmed by visual precipitations starting in the 6 mM $MgCl_2$ sample. The calcein leakage assay in Figure 7b also confirmed that the fatal concentration of Mg^{2+} is between 5 and 10 mM, where at 5 mM of $MgCl_2$ ~70% of calcein were instantaneously leaked out and total instantaneous leakage was observed at 10 mM of $MgCl_2$. This result indicates a major disruption of the vesicles within this range of $MgCl_2$ concentration.

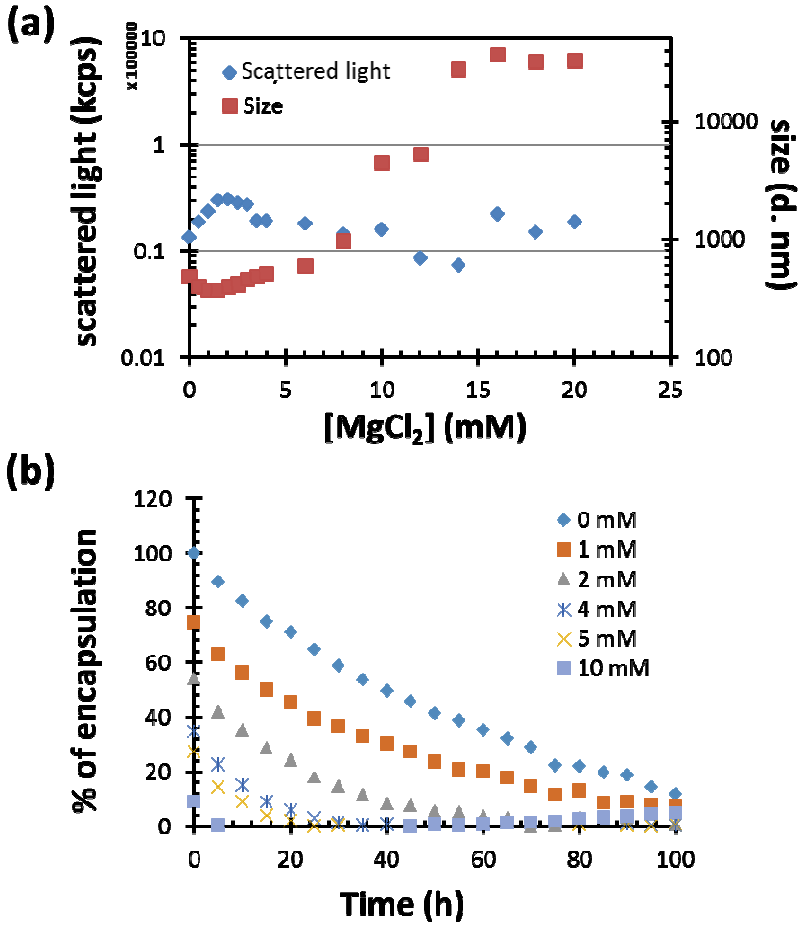

Figure 7. Effect of $MgCl_2$ on DA/DOH vesicles. (a) Effect of $MgCl_2$ on the cvc of DA/DOH, determined by DLS. (b) Effect of $MgCl_2$ on the stability of vesicles, determined by calcein leakage assay.

iv. Conclusion

In the present work, the possible interactions between prebiotically plausible amphiphiles and different mineral surfaces were investigated. We have showed that, under the tested conditions, the presence of minerals did not have drastic effects on the stability of DA/DOH vesicles, but these vesicles appeared to be very sensitive to Mg^{2+} (a fatal concentration of ~7 mM, under the tested conditions). It was also shown that the concentration of lipids is also an important variable. At 25 mM of lipid, vesicles were fairly stable, while at 7 mM these vesicles became exceptionally leaky. These results combined constraint the geochemical sites of emergence of life on early Earth, and come in agreement with a recent illustrated scenario of origins of life (Damer and Deamer 2015).

v. References

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Appendix 1: Safety Considerations

Safety precautions were taken at all times in lab. Protective gear was always worn, including: gloves, goggles, closed toed shoes, long pants, and lab coats, to ensure skin contact with chemicals did not occur. Always conduct experiments under properly ventilated hoods to ensure chemicals are not inhaled. Proper disposal of waste is essential to avoid contamination. Extreme care was taken with the handling of chemicals. These measures are shown below.

Decanoic Acid: If inhaled, take victim to well-ventilated area and allow them to rest. Upon skin contact, have victim wash the contaminated skin area with disinfectant soap and anti-bacterial cream and seek medical attention. If contact to eyes occurs, make sure the victim is not wearing contact lenses, if they are remove them and then use an eye wash station and seek immediate medical attention. If ingested, do not induce vomiting; make sure breathing has not stopped and alert medical attention.

Decanol: If inhaled, take victim to well-ventilated area and allow them to rest. Upon skin contact, have victim wash the contaminated skin area with disinfectant soap and anti-bacterial cream and seek medical attention. If contact to eyes occurs, make sure the victim is not wearing contact lenses, if they are remove them and then use an eye wash station and seek immediate medical attention. If ingested, do not induce vomiting; make sure breathing has not stopped and alert medical attention.

Calcein: If inhaled, take victim to well-ventilated area and allow them to rest. Upon skin contact, have victim wash the contaminated skin area with disinfectant soap and anti-bacterial cream and seek medical attention. If contact to eyes occurs, make sure the victim is not wearing contact lenses, if they are remove them and then use an eye wash station and seek immediate medical attention. If ingested, do not induce vomiting; make sure breathing has not stopped and alert medical attention.

Chloroform: If inhaled, take victim to well-ventilated area and allow them to rest. Upon skin contact, have victim wash the contaminated skin area with disinfectant soap and anti-bacterial cream and seek medical attention. If contact to eyes occurs, make sure the victim is not wearing contact lenses, if they are remove them and then use an eye wash station and seek immediate medical attention. If ingested, do not induce vomiting; make sure breathing has not stopped and alert medical attention.