Trace amounts of renewable surfactants enable rapid and high-density methane storage in clathrate hydrates: Sustainable alternatives to synthetic promoters

Elaheh Sadeh,[a] Abdolreza Farhadian,[a*] Zherui Chen,[b,c] Abolfazl Mohammadi,[d] Fei Wang,[e,*] Nastaran Sayyari,[f] Rustam R. Davletshin[a]

[a] Department of Petroleum Engineering, Kazan Federal University, Kremlevskaya str. 18, 420008 Kazan, Russian Federation

[b] Future Technology School, Shenzhen Technology University, Shenzhen 518118, P. R. China

[c] College of Applied Sciences, Shenzhen University, Shenzhen 518060, P. R. China

[d] Department of Chemical Engineering, University of Bojnord, 9415615458 Bojnord, Iran

[e] College of Electromechanical Engineering, Qingdao University of Science & Technology, Qingdao, China

[f] School of Medicine, Qom University of Medical Sciences, 3716993456 Qom, Iran.

Corresponding Authors

*Email: AFarhadian@kpfu.ru, and elliot_wang@qust.edu.cn

Characterization methods

The CHNS/O analysis was carried out on an Eager 300 Flash EA1112 elemental analyzer (Waltham, MA, USA). The elemental analysis of the synthesized renewable surfactants provided essential quantitative data on their composition, which was critical for confirming their molecular structures and purity. These results were consistent with the expected stoichiometric ratios based on the synthetic routes employed, including acid-base reaction. The renewable surfactants with metallic counterions (LO, SO, and PO), exhibited trends in carbon percentage (LO: 74.95%, SO: 71.01%, PO: 67.40%) that inversely correlated with the atomic mass of their respective cations ($Li^+ < Na^+ < K^+$). The absence of nitrogen and sulfur in these compounds confirmed their simple oleate salt structures. For the ammonium and ethanolamine oleates (AO, EAO, DEAO), the nitrogen content (AO: 4.67%, EAO: 4.09%, DEAO: 3.63%) confirmed the incorporation of ammonium or ethanolamine groups. The higher oxygen content in EAO and DEAO (O: 13.99% and 16.53%, respectively) compared to AO (O: 10.71%) reflected the additional hydroxyl groups from ethanolamine. The data show the successful incorporation of the expected cationic variations (Li^+, Na^+, K^+, NH_4^+, ethanolamine derivatives) into the structure of the renewable surfactants.

Table S1. Elemental analysis of the renewable surfactants.

Sample	C	H	O	Na	S	N	K
LO	74.95	11.58	11.1	-	-	-	-
SO	71.01	10.75	10.40	7.84			-
PO	67.40	10.42	9.98	-	-	-	12.2

AO	72.13	12.49	10.71	-	-	4.67	-
EAO	69.90	12.05	13.99	-	-	4.09	-
DEAO	68.15	11.50	16.53	-	-	3.63	-

MTT assay

In the MTT assay, the cytotoxicity of the samples was evaluated using A549 and NIH/3T3 cell lines obtained from the Pasteur Institute in Tehran, Iran. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, and incubated at 37 °C. For the assay, 1×10^4 cells were seeded per well in 96-well plates and incubated overnight. Subsequently, varying concentrations (1-100 ppm) of each sample dissolved in phosphate-buffered saline (PBS) were added to the wells, with DMEM alone serving as the negative control. After a 24-hour exposure period, 25 µL of MTT solution (5 mg/mL in DMEM) was added to each well, followed by a 3.5-hour incubation at 37 °C. Then, 70 µL of dimethyl sulfoxide was added to each well to dissolve the formazan crystals, and the plates were incubated at room temperature for 30 min. Absorbance was measured at 570 nm with a reference wavelength of 630 nm. Each condition was tested in quadruplicate (n = 4). Cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = [(A_s - A_b) / (A_c - A_b)] \times 100$$

Where A_s, A_b, and A_c are absorbance of sample, blank, and control, respectively.

Biodegradability method

Stock solutions were prepared in MilliQ water at concentrations of 2 g/L and 4 g/L. For the closed bottle tests, the 2 g/L stock was added to the seawater test medium to achieve a final concentration of 2 mg/L. For the respirometer experiments, the 4 g/L stock solution was used to obtain a final concentration of 33 mg/L. Closed bottle tests were conducted in 290 mL glass bottles with ground-glass stoppers in accordance with OECD 306 guidelines. Each bottle was filled to overflowing with seawater test medium supplemented with mineral nutrients as specified in OECD 306, with or without the reference substance, and then sealed to ensure the absence of headspace. The bottles were incubated for 7 days at 20 °C in a climate-controlled room and shielded from light using black plastic. Biodegradation was quantified based on the decrease in dissolved oxygen, corrected for oxygen consumption in blank controls. To enable continuous monitoring of biodegradation, respirometer tests were performed using WTW OxiTop®-C systems following OECD 301 F, with mineral nutrient supplementation as defined in OECD 306. Respirometer bottles were filled with 250 mL of test medium, leaving a 250 mL headspace. These systems were incubated in the dark at 20 °C for 28 days with continuous stirring, and sodium hydroxide was added to absorb produced CO_2. No liquid samples were withdrawn from the respirometers; however, closed bottle blanks were included for microbial community analysis. Dissolved oxygen in closed bottle tests was measured after 7 days using an electrochemical oxygen probe (WTW Inolab Oxi 7310). Oxygen consumption in the respirometer tests was recorded continuously over 28 days using OxiTop®-C measuring heads with sodium hydroxide as the CO_2 absorbent. Microbial samples were analyzed on the day of collection without preservation using flow cytometry (FCM). Samples were diluted 1:10 with MilliQ water and stained with SYBR Green I (10,000× stock in DMSO; working solution diluted 1:100 in Tris buffer and further diluted 1:100 in the sample) for at least 20 minutes at room temperature. SYBR Green I binds to bacterial DNA and is routinely used for total bacterial

enumeration. A volume of 50 µL per stained sample was analyzed using an Accuri C6 (BD) flow cytometer equipped with a 488 nm blue laser, with green (533/30 nm) and red (>670 nm) fluorescence channels used for detection. Bacterial populations were distinguished from background noise through electronic gating.

Calculation of kinetic parameters

Pressure changes during methane hydrate formation were measured to determine the methane consumption, water conversion degree, and storage capacity. These are crucial parameters for assessing methane hydrate formation in pure water, SDS, and DCSs solutions. Using Eq. (1), the moles of methane consumed (Δn) at a specific time can be calculated [1]. In this equation, T_0, V_0, P_0, R symbolize the initial cell temperature, initial gas volume, initial pressure and standard gas constant (8.314 J. mol $^{-1}$. K^{-1}), respectively. T_t and P_t stand for the cell's temperature and pressure at a given moment in time. Using the Peng-Robinson equation, the values of Z_0 and Z_t represent the gas compressibility factors in the reactor at the initial moment and at time t, respectively [2].

$$\Delta n = \frac{P_0 V_0}{Z_0 R T_0} - \frac{P_t V_t}{Z_t R T_t} \quad (1)$$

The volume of gas in the reactor, denoted as V_t, continually changes as the hydrate grows because of the varying densities of water and hydrate. Eq. (2) was used to calculate the value of V_t [1]. Where V_{Ht} indicates the volume of the formed hydrate, V_{So} is the solution's volume as well as V_{cell} and V_{RWt} are the cell volume and the volume of converted water, respectively.

$$V_t = V_{cell} - V_{S0} + V_{RWt} - V_{Ht} \quad (2)$$

Assuming the molar volume of the hydrate is equal to the molar volume of the empty hydrate lattice, the volume of the produced hydrate can be determined using the following equation:

$$V_{H_t} = M \times \Delta n \times \upsilon_w^{MT} \tag{3}$$

Where υ_w^{MT} is the molar volume of water in the empty hydrate lattice.

The value of storage capacity was calculated using Eq. (4) [1]:

$$\text{SC} = \frac{V_{STP}}{V_H} = \frac{\Delta_n RT_{STP} / P_{STP}}{V_H} \tag{4}$$

where abbreviation STP displays standard temperature and pressure conditions. The water conversion degree was calculated using Eq. (5) [2], which indicates the quantity of water molecules transformed into hydrates per original mole of water. M denotes the hydration number in this equation.

$$\text{Water conversion degree} = \frac{M \times \Delta n_{gas}}{n_w} \tag{5}$$

Molecular dynamics

To analyze the influence of these surfactant molecules on hydrate growth at the microscopic level, we further performed molecular dynamics (MD) simulations to investigate hydrate formation in their presence. The initial computational model consisted of two components: a 4×4×2 sI hydrate crystal structure at the bottom and a supersaturated methane aqueous solution above it. This setup included 5,888 water molecules and 1,024 methane molecules. Ten surfactant molecules were placed 1.5 nm above the hydrate crystal, initially minimizing their interaction with the hydrate and

representing the early, separated state. The simulation box dimensions were 4.7487 nm × 4.7487 nm × 10 nm. MD simulations were conducted using Gromacs 2024.4 [3,4]. The TIP4P-ice model was employed for water molecules [5], GAFF for the surfactant molecules [6], and TRAPPE for methane. This combination of force fields has been shown to accurately describe hydrate properties and is widely used in hydrate-related MD studies. Simulations were carried out at a low temperature of 260 K and a high pressure of 50 MPa to accelerate hydrate formation. Such low-temperature, high-pressure protocols are commonly adopted in MD simulations of hydrate growth [7–9]. Furthermore, as the initial model contained both hydrate crystals and excess methane in solution, water molecules preferentially converted to hydrate rather than ice, even at this temperature.

[1] E. Sadeh, A. Farhadian, M. Maddah, M.E. Semenov, A. Mohammadi, F. Wang, Branched sulfonated promoter: Achieving high methane uptake and foam-free gas recovery for solidified gas storage, Chem. Eng. J. 487 (2024) 150674.

[2] E. Sadeh, A. Farhadian, A. Mohammadi, M. Maddah, M. Pourfath, M. Yang, Energy-efficient storage of methane and carbon dioxide capture in the form of clathrate hydrates using a novel non-foaming surfactant: An experimental and computational investigation, Energy Convers. Manag. 293 (2023) 117475.

[3] D. Van Der Spoel, E. Lindahl, B. Hess, G. Groenhof, A.E. Mark, H.J.C. Berendsen, GROMACS: fast, flexible, and free, J. Comput. Chem. 26 (2005) 1701–1718.

[4] H.J.C. Berendsen, D. van der Spoel, R. van Drunen, GROMACS: A message-passing

parallel molecular dynamics implementation, Comput. Phys. Commun. 91 (1995) 43–56.

[5] J.L.F. Abascal, E. Sanz, R. García Fernández, C. Vega, A potential model for the study of ices and amorphous water: TIP4P/Ice, J. Chem. Phys. 122 (2005) 234511.

[6] J. Wang, R.M. Wolf, J.W. Caldwell, P.A. Kollman, D.A. Case, Development and testing of a general amber force field, J. Comput. Chem. 25 (2004) 1157–1174.

[7] P. Nakate, B. Ghosh, S. Das, S. Roy, R. Kumar, Molecular dynamics study on growth of carbon dioxide and methane hydrate from a seed crystal, Chinese J. Chem. Eng. 27 (2019) 2074–2080.

[8] Y. Liu, C. Chen, W. Hu, W. Li, B. Dong, Y. Qin, Molecular dynamics simulation studies of gas hydrate growth with impingement, Chem. Eng. J. 426 (2021) 130705.

[9] Z. Chen, A. Farhadian, P. Naeiji, D.A. Martyushev, C. Chen, Molecular-Level insights into kinetic and agglomeration inhibition mechanisms of structure I and II gas hydrate formation, Chem. Eng. J. 511 (2025) 162194.