Received: 13 September 2020 | Revised: 13 December 2020 | Accepted: 14 December 2020

DOI: 10.1002/poc.4182

RESEARCH ARTICLE

Journal of Physical Organic Chemistry WILEY

Aromatic nucleophilic substitution (s_nar) reactions of halo-substituted dinitrobenzene in liposome reaction media: Effect of reaction medium and role of halogen leaving group

Jyoti Dutta | **Shraeddha Tiwari**

Department of Chemistry, Institute of Chemical Technology, Mumbai, Maharashtra, India

Correspodence
Shraeddha Tiwari, Department of Chemistry, Institute of Chemical Technology, Mumbai, Maharashtra 400019, India.
Email: ss.tiwari@ictmumbai.edu.in

Funding information
Council of Scientific and Industrial Research, India, Grant/Award Number: 09/991(0053)2K19; Department of Science and Technology, India, Grant/Award Number: IFA13-CH95; Science and Engineering Research Board, Grant/Award Number: SB/FT/CS-183/2013

Abstract

S_NAr reactions constitute an important pathway for the synthesis of many crucial organic derivatives from polyhaloaromatic compounds. The sluggish nature of the reaction in many cases makes it a challenging pathway and limits its potential applications. In the present report, liposomes have been used as model membrane systems to study nucleophilic substitution reactions of halo-substituted dinitrobenzene with morpholine. The results show an interesting dependence of the reactivity on the size and composition of liposomes. The extent of rate acceleration in liposomes is strongly dependent on the identity of the halogen which undergoes the substitution—for example, chloro-substituted aromatic compounds show the most sensitivity to the presence of lipsomal reaction media. The observed behavior correlates with the reported reactivity of halobenzenes while revealing interesting details which may be critical in harnessing the reactivity of less reactive substrates. The results explore the viability of employing liposomes as promising alternatives for synthetic protocols.

KEYWORDS

kinetics, liposome, nucleophilic substitution, reaction mechanism

1 | INTRODUCTION

Aromatic nucleophilic substitution (S_NAr) reactions of 1-halo-2, 4-dinitrobenzenes are synthetically important class of reactions.[1–8] This class of reactions is very sensitive to the composition of the reaction medium.[9, 10] However, the general reactivity of substrates in organic solvents and aqueous media has observed to be low, thus hampering extensive application of this class of reactions. The sluggish nature of nucleophilic substitution reactions (coupled with concern over the environmental hazards of conventional organic solvents) has prompted the use of numerous unconventional reaction media such as ionic liquids,[11–13] deep eutectic solvents,[14] and nanomicelles.[15] The high yields of S_NAr reaction in nanomicelles indicated the possible role of aqueous-organic interface towards enhancing the reactivity. In continuation with our previous efforts towards developing an environmentally sustainable synthetic strategy for S_NAr reactions,[14,16] further exploration of S_NAR reactions in microheterogeneous media such as liposomes (or vescicles) was relevant and logical extension of the previous attempts.

The complex chemistry of liposomal assemblies has been harnessed for improved synthetic protocols and was recently demonstrated by Iwasaki and coworkers,[17] who

reported high enantioselectivity for alkylation of *N*-(diphenylmethylene)glycine-*tert*-butyl ester in liposomes. Recent studies on use of liposomes as reaction media for 1,3-dipolar cycloaddtion reactions[18] and Michael addition reaction[19] have also highlighted the critical role of liposomal interface in accelerating the rates of these reactions. Kinetic and mechanistic investigations of chemical processes in liposomal assemblies have been very few in number. For example, several modes of liposomal reactivity under identical conditions were investigated by Menger and Azob[20] as a cytomimetic model. The observed rates were justified on the basis of hydrophilic nature of the functional groups present. Further investigation revealed the critical role of interliposomal transfer of reactant molecules during collisions between liposomes.[21]

An additional incentive for studying chemical processes in liposomes is that it provides an opportunity to model phospholipid membranes in vitro while overcoming many of the experimental limitations associated with *in vivo* studies. These membranes are fundamental building blocks for cellular structures and are capable of controlling the outcome of numerous biological processes.[22–25] In recent years, this approach has been explored extensively to design protocells that can mimic cellular complexity in the form of cascading enzymatic reactions,[26] synthesis of proteins[27] and the effect of compartmentalization over peptide synthesis.[28,29]

The present work explores the model S_NAr reaction of 1-halo-2,4-dinitrobenzene (DNXB) with morpholine in liposomal assemblies composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). Various factors that could influence the reaction outcome such as location of the substrate vis-à-vis the liposomal assembly, composition of the liposomes, and concentration of lipid constituting the liposomes were examined. Varying the halide substituent affected the sensitivity of the substrate to the liposomal reaction medium and thus influenced the rate of the reaction. The results are discussed in terms of the hydrophobicity and leaving group ability of the substrate.

2 | RESULTS AND DISCUSSION

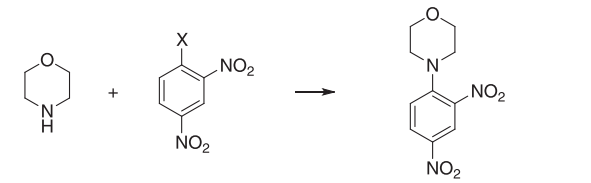
Unlike conventional kinetic studies in homogeneous reaction media, the kinetic studies for micro-heterogeneous media are complicated by the fact that the reaction dynamics may depend on the exact location of the reaction—in the bulk phase or at the interface. For example, reactions taking place in liposomal media may occur at any one of the three "locations"—in bulk water, inside the liposome or at the liposome-water interface. It may be possible that the reaction occurs at all three "locations" simultaneously with either one or two of them dominating the reaction outcome—depending on the conditions employed. This situation also complicates the quantitative discussion of kinetic data—especially for comparison of rates in bulk media with those occurring at the interface. In order to overcome this limitation, the rate constants in the present report have been designated as "apparent" rate constants, although all experimental kinetic data could be best modeled as first order (or pseudo first order) rate processes.

Another important consideration would be the pH of the solution, which can be influenced by the presence of a weak base (morpholine, pKa = 8.4). For the concentrations of morpholine used in this study, the pH of the aqueous medium was found to be greater than 9 and varied slightly for different concentrations of morpholine used. No buffer solution was used to control the pH of the reaction medium. The possibility of competitive reactions with hydroxide ions at such high pH cannot be completely ruled out. However, the stoichiometric conversion of the substrates to the product *N*-(2,4-dinitrophenyl)morpholine indicates that any competitive reactions may be negligible in extent.

3 | KINETICS IN LIPOSOME VERSUS AQUEOUS MEDIA

The primary mechanistic concern about any reaction carried out in liposomes would be to determine which location/s is/are most conducive for the reaction to occur. In order to determine whether the model S_NAr reaction primarily occurred in the aqueous phase, inside the liposome or at the interface, the rate of product formation was determined under three different reaction conditions. Initially, the rates of nucleophilic substitution reaction between 1-fluoro-2, 4-dinitrobenzene (DNFB) and morpholine were determined in the presence of liposomes.

Two different approaches were adopted to measure the kinetics of the reaction in liposomal media. In the first approach, the DNFB substrate was added to liposomal dry film before formation of liposomes and thus, got "encapsulated" inside the liposome due to its low solubility in water. In the second case, the substrate was allowed to get adsorbed on the liposome interface after the formation of liposome from the dry film; that is, it was "incubated" to yield the liposomal solution of DNFB. Given the low solubility of substrate and low membrane permeability of pure DPPC liposomes and our kinetic data, it can be safely assumed that majority of the



SCHEME 1 Model SNAr reaction between 1-halo-2,4-dinitrobenzene and morpholine

substrate was adsorbed on the surface of the liposome and the percolation of the encapsulated substrate was negligible during the progress of the reaction. An analogous kinetic run was carried out in the aqueous media, keeping all other reaction conditions identical, except the presence of liposomes. The third setup would be critical in determining the relative rate of such reactions in aqueous versus liposomal media. The results of the three kinetic runs are summarized in Figure 1. Maximum rate of product formation was observed for the "incubated" DNFB (i.e. reactant molecules adsorbed at liposomal surface) followed by reaction of "encapsulated" DNFB (i.e. reactant primarily located/encapsulated inside liposomes) whereas the reaction in water showed least rate of conversion. Rate acceleration was, thus, observed for reactions progressing at liposome–water interface as compared to those taking place in water, while the reaction in the interior of liposome showed a marginal acceleration in comparison to the reaction taking place in water. Similar trends were reported by Iwasaki et al[18] for 1,3-dipolar cycloaddition reactions, wherein the

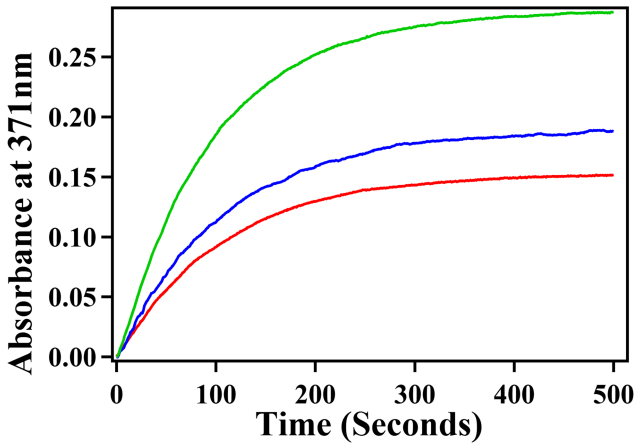


FIGURE 1 Time-dependence of product formation for the reaction of 0.01 M DNFB with 10 mM morpholine in aqueous medium (blue), interior of DPPC liposome (red), and liposome-water interface (green). The concentration of liposomes (wherever used) is 19 mM

substrates adsorbed on the surface of liposome were found to show a 3-fold increase in reactivity as compared to the substrates in bulk aqueous medium.

The possible explanation for an enhanced rate at liposomal surface can be attributed to the presence of optimum hydrophobic environment at liposomal surface comparable to organic solvents, promoting greater interaction between the hydrophobic substrate and water soluble secondary amine. In contrast, lower k_{obs} values for encapsulated substrate indicate that the interior of liposome was less accessible for the more water soluble substrate morpholine. The order of reactivity is, thus, consistent with the previously observed reaction rates in liposomes.[20,21] Considering the rate acceleration at liposome surface, all further kinetic experiments for liposomal assembly were carried out in conditions promoting interfacial interactions, that is, with substrate incubated on the liposome.

4 | EFFECT OF LIPID CONCENTRATION

The reactions taking place at the interface of the liposome are bound to be controlled by the total interfacial area available—which can be regulated by controlling the size of the liposomes or changing the concentration of liposomes in a monodisperse suspension. The rate of product formation for the DNFB-morpholine reaction was measured in monodisperse DPPC liposomes with lipid concentration ranging from 5 to 50 μM (refer Table S1 in Supporting Information). The variation in the observed pseudo first order rate constants in liposomes (k_{obs}) with change in lipid concentration was monitored (Figure 2). The increase in k_{obs} values with increasing lipid concentration can be easily related to availability of increase in interfacial area, adsorption, and conversion of DNFB molecules (since the size of the liposomes was maintained constant). However, after an initial increase, the k_{obs} values were found to level off at a maximum value and then decrease with further increase lipid concentration. It was critical to understand the nonlinear dependence of k_{obs} values on interfacial area, achieved by increasing concentration of liposomes in a monodisperse solution.

The liposomes used for the kinetic experiment were extruded through a polycarbonate membrane of fixed mean pore size resulting in a monodispersed liposomal system. Hence, the size of liposomes had no significance in explaining the decrease in k_{obs} values with increasing lipid concentration. It must be noted that previous literature studies have limited number of reports focused on the interfacial reactions in liposomes and their correlation with concentration of lipid or interfacial

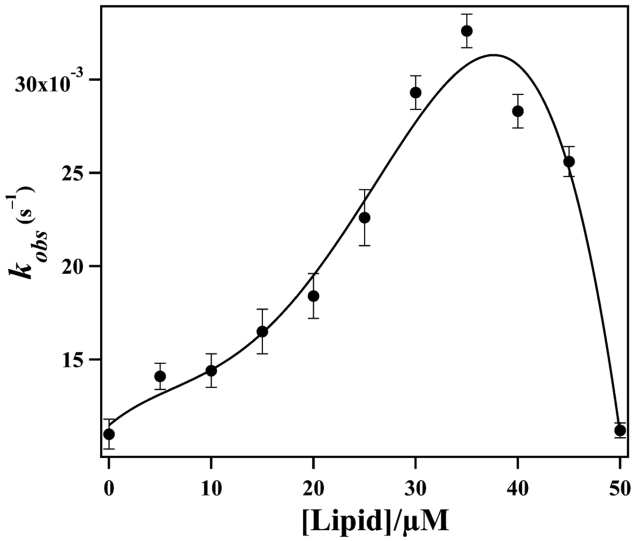


FIGURE 2 Observed pseudo first order rate constants (k_{obs}) for the reaction between 1-fluoro-2,4-dinitrobenzene (DNFB) and morpholine against varying lipid concentration of DPPC liposomes at 298 K. Final concentrations of DNFB and morpholine in a 2.5 ml reaction mixture were 0.012 mM and 10 mM, respectively

area—none of those report a nonlinear dependence of rates on concentration as observed in Figure 2. The possible explanation for the observed decrease in k_{obs} values with increasing lipid concentration can be the increase of liposomal surface area with increasing lipid concentration, which should promote the reactivity of any interfacial process. Above an optimum lipid concentration, the number of liposomes available in reaction system may increase significantly beyond the requirement for the reaction. In such circumstances, DNFB concentration available in the reaction system for adsorption at liposome surface would be lower than the saturation limit of the interface, leading to a decrease in the effective interfacial concentration (number of molecules adsorbed per unit area). The increase in surface area, under such conditions, may be counterproductive.[30,31]

5 | EFFECT OF COMPOSITION

The effect of composition of the liposome on the kinetics of the S_NAr reaction was also investigated. Liposomes composed of DPPC, DMPC, and their mixtures with cholesterol were prepared. The substrate (DNFB) was incubated over the liposomes under identical conditions and kinetics measurements were done. Rate acceleration was observed for DMPC based liposomes, similar to the observations in DPPC liposomes (Refer Tables S2 and S3 in Supporting Information for absolute rate constants). The k_{obs} value was marginally higher for DPPC liposome in comparison to DMPC liposome, suggesting the contribution to interfacial reactivity by hydrophobic chain length. For investigating mixtures of lipids, lipid-cholesterol mixtures with X_{chol} ranging from 0.1 to 0.4 were prepared (X_{chol} is mole fraction of cholesterol). The total concentration of lipid in the kinetic runs was kept constant at 19 mM, and only the relative compositions were altered. Cholesterol is known to modulate the physical properties and organization of lipid bilayer, affecting the membrane fluidity and permeability. This modulation of lipid membranes can be critical for the reactions proceeding at the surface of liposome. The k_{obs} value increased from liposome composed of only DPPC to liposome with

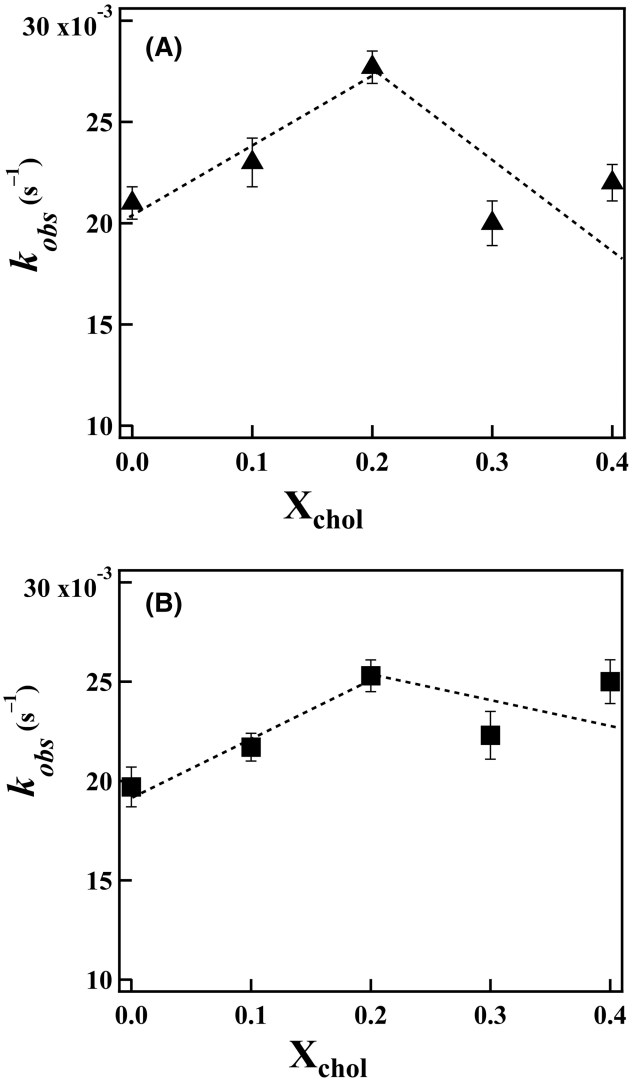


FIGURE 3 Observed pseudo first-order rate constants (k_{obs}) for the reaction of 1-fluoro-2,4-dinitrobenzene (DNFB) and morpholine at 298 K against mole fraction of cholesterol (X_{chol}) inreaction medium composed of (A) DPPC-cholestrol liposomes and (B) DMPC-cholestrol liposomes. The final concentrations of lipid, DNFB, and morpholine in a 2.5 ml reaction mixture were 19, 0.012 and, 10 mM respectively

(DPPC + X_{chol} = 0.2) but further increases in amount of cholesterol X_{chol} to 0.4 resulted in a lower k_{obs} value (Figure 3A). Similar trends were observed for DMPC + cholesterol mixture. However the change in k_{obs} value on increasing X_{chol} from 0.2 to 0.4 in DMPC liposomes was less significant (Figure 3B).

The kinetic outcome of the SNAr reaction was thus correlated with the structural alterations of the liposome interface induced by change in lipid composition. Liposomes constituting of pure lipids exist either in gel phase (S) or in liquid-disordered phase (Ld), whereas liposomes composed of binary mixtures of (lipid + cholesterol) possess an additional cholesterol-rich phase, known as liquid-ordered (Lo) phase.[32] The significance of cholesterol-rich phase can be observed in biological membranes possessing heterogeneous structures exhibiting different lipid domains—for example, lipid rafts present in biological membranes have close resemblance in properties with liquid ordered phase (Lo).

According to the literature on liposomal phase behavior, the (DMPC + Xchol = 0.2) composition lies in Ld + Lo phase above 24°C.[32] For DPPC liposomes, the phase transition temperature was reported to be around 42°C.[33,34] The SNAr reactions were performed at 298 K which corresponds closely to the T_m of DMPC. DPPC could possibly undergo a pretransition between S phase and ripple gel phase below the phase transition temperature, as observed for DPPC previously.[33] Considering the observed relative rates for (DMPC + X_{chol} = 0.2), it can be said that the Ld + Lo phase behavior of liposome composition was favoring the S_NAr reaction. (DMPC + X_{chol} = 0.4) composition exhibits mainly Lo phase for all the temperature ranges. The highly ordered arrangement of liposome in the Lo phase could not be favorable to S_NAr reactions progressing at liposomal interface, hence a deceleration is observed in relative rate constants. In DPPC liposome the temperature at which S_NAr reactions were carried out 298 K corresponds to pretransition temperature, and the existing phase lies between S phase and ripple phase, which could be a less ordered state in comparison to Ld + Lo, and hence, k_{obs} value is relatively much higher in comparison to that observed for highly ordered Lo phase of (DPPC + X_{chol} = 0.4).

5.1 | Substrate study

Kinetics of different 1-halo-2,4-dinitrobenzene substrates (fluoro-, chloro-, bromo- and iodo-substituted) with morpholine at 298 K in DPPC liposomes under identical conditions revealed interesting mechanistic details. For DNFB and 1-chloro-2,4-dinitrobenzene (DNCB), the substitution reactions were faster when carried out in presence of liposome compared to pure water (k_{rel} values in Table 1).

In contrast to the observation for the fluoro- and chloro-substituted subtrate, S_NAr reactions of 1-bromo-2,4-dinitrobenzene (DNBB) and 1-iodo-2,4-dinitrobenzene (DNIB) with morpholine showed a deceleration in rates when liposomal assemblies were used as reaction media instead of water. Interestingly, the trends in the absolute rates and relative rates for all four substrates had notable peculiarities. While DNFB showed a highest absolute rate in liposomal media among all the four substrates, the relative rates in liposomes (with respect to aqueous medium) were observed to be highest for DNCB. The results indicate that DNCB was the most sensitive to the presence of liposome-water interface.

Several factors can influence the interfacial reactivity for these substrates. It must be noted that, conventionally, the order of reactivity for the halogens for undergoing nucleophilic substitution reactions is F> > Cl > Br > I. Previous reports on mechanistic investigation of S_NAr reactions of DNFB with different amines have demonstrated an unusual reactivity of DNFB depending on the nature of reaction medium.[9] The discrepancy between the observed reactivity of fluorine substituted substrate as compared to other halo-substituted aromatic compounds is due to the

TABLE 1 Observed relative pseudo first order rate constants for the reaction of DNFB, DNCB, DNBB, and DNIB with different concentrations of morpholine

	DNFB			DNCB			DNBB			DNIB		
[Amine] (mM)	10^2k_w (s^{-1})	10^2k_{lip} (s^{-1})	k_{rel}	10^5k_w (s^{-1})	10^5k_{lip} (s^{-1})	k_{rel}	10^5k_w (s^{-1})	10^5k_{lip} (s^{-1})	k_{rel}	10^6k_w (s^{-1})	10^6k_{lip} (s^{-1})	k_{rel}
10	1.02	2.10	2.10	2.60	12.9	4.96	2.24	1.10	0.49	9.00	7.74	0.86
16	1.40	3.00	2.14	4.00	22.7	5.67	3.53	1.49	0.42	19.5	18.3	0.94
22	1.80	3.40	1.89	6.10	24.5	4.02	4.33	1.90	0.44	32.5	24.2	0.74

The final concentrations of DPPC and DNXB in a 2.5 ml reaction mixture were 19 μM and 0.012 mM, respectively. [b]$k_{lip} = k_{obs}$ in liposomal reaction media; $k_w = k_{obs}$ in water; $k_{rel} = k_{lip}/k_w$.

mechanistic differences for the halo-substituted dinitrobenzene substrates. In most of S_NAr reactions, formation of Meisenheimer intermediate is known to be rate determining step, and the reactivity of substrates towards nucleophile is governed by ability of leaving group to stabilize the negative charge in benzene ring in transition state and not the ability of heterolytic dissociation of C–X bond.[35] The enhanced reactivity of DNFB was attributed to the steric advantage available to DNFB during the nucleophilic attack,[36] which renders the formation of the intermediate as a facile and not a rate determining step. However, the departure of leaving group is the rate determining step, which can compete with rate limiting proton transfer in protic solvents.[37] Even in aprotic or nonpolar media, the formation of the Meisenheimer complex may still not be the rate determining step, if the halo-substituent is fluorine. Thus, the reaction of DNFB is mechanistically different from the rest of the halogen-substituted substrates, and hence, the difference in sensitivity to the reaction medium is not wholly unexpected.

In order to facilitate comparison of the reactivity and mechanism in different reaction media for the present work, few additional assumptions need to be incorporated in the model scheme. It should be assumed that the mechanism of the reaction is similar in water, in the interior of the liposomes, or at the liposome-water interface. Additionally, it should be assumed that an observed rate constant represents only the interfacial process in the presence of liposome and does not contain any contribution from the aqueous process.

It is observed that the k_{obs} values for DNCB are almost 2 to 3 orders of magnitude lower than those for DNFB in water as well as liposomes. This is agreement to the hypothesis that in DNFB, the formation of the complex is much more facile as compared to the other halo-substituted substrates. On changing the reaction medium from water to liposomal solution, the k_1 values for DNCB increase by almost five times as compared to the two times increase in the rate for DNFB. This observation supports the results from Table 1, showing that nucleophilic substitution of DNCB is sensitive to the presence of liposomes as compared to the substitution reactions of DNFB.

Another important factor to be considered is the hydrophobicity of the substrate which is critical in determining the location inside the liposome, and hence, its accessibility for the reaction. The aggregation of substrates at liposomal surface, within the bilayer or in the interior of the liposome is expected to be governed by the hydrophobicity of the substrate. Increasing the substrate hydrophobicity could increase substrate incorporation on or in liposomal assembly, favoring organic transformations of hydrophobic substrates. In case of highly hydrophobic substrate, the substrate is rendered inaccessible for the reaction to proceed. For example, retardation of rates has been reported for substrates being located at the micellar core.[31] The hydrophobicity parameters of the four substrates have been previously reported as DNIB > DNBB > DNCB > DNFB.[15] Higher hydrophobicity of DNBB and DNIB should induce the relocation of these substrates in the interior of liposome that has limited accessibility to promote reaction, leading to a decrease in rates relative to water (Table 1).[38] The high sensitivity of DNCB to liposomal reaction medium can be attributed to its optimum hydrophobicity.

The results can be correlated to the nature of DNCB as a standard contact allergen. The results also indicate that liposomes, and related assemblies can be potentially used as reaction media for the nucleophilic aromatic substitution reactions. Interesting mechanistic insights on the factors governing interfacial reactions can also be determined by further studies. In the present study, we have examined a model SNAr reaction in liposomal assembly, with an aim to explore the applicability of such systems as biomimetic substitutes. Future work in the area should aim to compare the results from such experimental models with those reported earlier from biological testing. The liposomal systems could easily be modified to incorporate more complex structural features, enhancing their similarity to living cells.

6 | CONCLUSION

The results show the promising potential of liposomal assemblies as synthetic and mechanistic tools for organic transformations. The reaction rates at the surface of liposome were found to be greater as compared to the interior of liposomes. The results indicate the important role of liposome-water interface in determining the reactivity as compared to the interior of the liposomes. The lower reactivity of the more hydrophobic substrates which tend to permeate towards the interior of the liposomes confirms that the conditions at the interface are the most optimum. Interesting trends in reactivity were observed as a function of different lipid concentration and composition. Interfacial reactivity showed a nonlinear dependence on the interfacial area, with an initial increase followed by decrease at higher values. Presence of cholesterol resulted in altered phase behavior of liposomes, modulating the feasibility of S_NAr reactions at liposome surface. The S_NAr reactions were found to be fastest in composition of liposome corresponding to moderately ordered phase Ld + Lo, whereas highly ordered cholesterol rich Lo phase and pure lipid phase had a relatively low rate acceleration. The reactivity trend for different halogen substituted substrates could be justified by

differences in mechanism and hydrophobicity. In summary, S_NAr reactions in liposomes are sensitive to several factors like interfacial area, phase behavior of the liposomal membrane, and substrate hydrophobicity. The present approach can potentially facilitate experimental verification of S_NAr modeling studies for skin sensitization potency, eliminating the need for animal testing. A robust liposome-based experimental setup and accurate computational models should be the ideal substitute for animal testing studies.

7 | EXPERIMENTAL SECTION

All chemicals used, including lipids and cholesterol, were of the highest purity available commercially and used without further purification. HPLC grade water was used for liposome preparation and dilution.

7.1 | Preparation of liposomes

Liposomes were prepared by conventional thin lipid film hydration method. A chloroform solution of requisite amount of lipid mixture was dried in a round bottom flask by rotary evaporator. The dried lipid film was kept in vacuum for 15 min, to ensure complete removal of chloroform. Thin lipid film was hydrated with HPLC water (volume of water in accordance with lipid concentration) and then, gently sonicated to ensure complete dislodging of lipid film. The resulting liposomal solution was extruded 20 times through a polycarbonate membrane with a mean pore size of 0.08 μm installed in Avanti polar mini extruder assembly. The extruded liposomes were analyzed by dynamic light scattering, and the average sizes of 99% liposomes were observed to be 0.08 μm with a poly dispersity index of 0.47.

7.2 | General procedure for aromatic nucleophilic substitution reactions

Typically, the reaction was initiated by addition of a stock solution of morpholine to the halo-substituted dinitrobenzene substrate (in aqueous/liposomal media). The product formation was followed by TLC. The product *N*-(2,4-dinitrophenyl)morpholine was isolated using flash column chromatography on 60-120 mesh size silica gel with 1:1 hexane:ethylacetate as eluent. The product was characterized using ^{1}H NMR spectroscopy (400 MHz, $CDCl_3$): δ 8.72 (d, J = 2.7 Hz, 1H), 8.30 (dd, J = 9.3, 2.7 Hz, 1H), 7.11 (d, J = 9.3 Hz, 1H), 3.91–3.84 (m, 4H), and 3.31–3.24 (m, 4H).

7.3 | Encapsulation of substrate

In a round bottom flask, 11.7 mg of DPPC was dissolved in 1 ml of chloroform, and the lipid solution was dried over rotary evaporator resulting in thin lipid film on walls of round bottom flask. The thin lipid film was vacuum dried for 15 min to ensure complete removal of solvent. A total of 1.2 μl of DNFB was added to the round bottom flask containing thin lipid film and further hydrated by addition of 998.8 μl of HPLC water; total volume of the solution was 1 mL. The mixture was sonicated gently until complete dislodge of lipid film from walls of round bottom flask. The resulting stock solution of DNFB encapsulated liposome was extruded 20 times through a polycarbonate membrane with mean pore size 0.08 μm installed in Avanti polar mini extruder assembly. The extruded liposomal DNFB stock solution was used for encapsulation studies. Final concentration of lipid and DNFB in stock solution was 19 mM and 0.01 M, respectively.

7.4 | Incubation of substrate

Liposome stock solution was prepared as described above by dissolving 11.7 mg of DPPC in 1 ml of chloroform followed by drying to give a thin film. The film was hydrated by adding 1 ml of water and sonicated gently to give a liposomal suspension. 998.8 μl of this liposomal solution was added in fractions (20 μl each) to a volumetric flask containing 1.2 μl of DNFB with gentle vortex. The resultant volume of liposomal DNFB stock solution was 1 mL. The final lipid and DNFB concentration in liposomal DNFB stock solution for a typical reaction was 19 mM and 0.01 M respectively. The liposomal DNFB stock solution was left for 1 h at 298 K to ensure adhesion of DNFB to liposomal surface. The liposomal DNFB stock solution prepared was used for incubation based kinetic measurements.

7.5 | Kinetic study

Kinetic study of the nucleophilic substitution reactions was performed on Shimadzu UV2450 spectrophotometer equipped with a Peltier temperature controller (accuracy of ±0.1 K). The progress of the reaction was monitored by appearance of product *N*-(2,4-dinitrophenyl)morpholine at fixed wavelength of 371 nm (the λ_{max} of the product, consistent in all reaction media, refer Figure S1 and S2 in the Supporting Information). All other reactant components including solvent did not show any significant absorbance at this wavelength. For a typical kinetic run 3 μl of 0.01 M stock solution of DNXB in respective

medium (water or liposomal solution) was added to 2.5 ml of 10 mM aqueous solution of morpholine at 298 K. All reactions were carried out in triplicate with an average standard deviation of ≤5%.

Due to the microheterogeneous nature of the reaction media, the order of the reaction was determined by comparing fitting parameters from various rate laws. The time-dependent change in absorbance data showed best fit to the first order rate equation:

$$\ln (A_\infty - A_t) = -k_{obs}t$$

where A_t is absorbance at time t and A_∞ is absorbance at t = infinity.

ACKNOWLEDGEMENTS

JD and SST gratefully acknowledge Department of Science and Technology, India (IFA13-CH95) and Science and Engineering Research Board (SB/FT/CS-183/2013) for funding. JD acknowledges Council for Scientific and Industrial Research for SRF fellowship (09/991(0053)2 K19).

ORCID

Shraeddha Tiwari https://orcid.org/0000-0001-7600-5738

REFERENCES

[1] R. Beugelmans, G. P. Singh, M. B. Choussy, J. Chastanet, J. Zhu, *J. Org. Chem.* **1994**, *59*, 5535.
[2] F. Fant, A. D. Sloovere, K. Matthijsen, C. Marlé, S. E. Fantroussi, W. Verstraete, *Environ. Pollut.* **2001**, *111*, 503.
[3] A. K. Nezhad, A. Zare, A. Parhami, M. N. S. Rad, G. R. Nejabat, *Can. J. Chem.* **2006**, *84*, 979.
[4] F. D'Anna, V. Frenna, R. Noto, V. Pace, D. Spinelli, *J. Org. Chem.* **2006**, *71*, 5144.
[5] E. E. L. Tanner, R. R. Hawker, H. M. Yau, A. K. Croft, J. B. Harper, *Org. Biomol. Chem.* **2013**, *11*, 7516.
[6] M. Gazitua, R. A. Tapia, R. Contreras, P. R. Campodonico, *New J. Chem.* **2014**, *38*, 2611.
[7] P. Pavez, D. Millán, M. Rojas, J. I. Morales, J. G. Santos, *Int. J. Chem. Kinetics* **2016**, *48*, 337.
[8] J. A. Espósito, R. Contreras, R. A. Tapia, P. R. Campodónico, *Chem. Eur. J.* **2016**, *22*, 13347.
[9] I. H. Um, S. W. Min, J. M. Dust, *J. Org. Chem.* **2007**, *72*, 8797.
[10] J. A. Espósito, R. A. Tapia, R. Contreras, P. R. Campodónico, *RSC Adv.* **2015**, *5*, 99322.
[11] J. S. Yadav, B. V. S. Reddy, A. K. Basak, A. V. Narsaiah, *Tetrahedron Lett* **2003**, *44*, 2217.
[12] I. Newington, J. M. Perez-Arlandis, T. Welton, *Org. Lett.* **2007**, *9*, 5247.
[13] X. Zhang, G.-P. Lu, C. Caia, *Green Chem.* **2016**, *18*, 5580.
[14] A. Valvi, S. Tiwari, *Eur. J. Org. Chem.* **2018**, 4933.
[15] N. A. Isley, R. T. H. Linstadt, S. M. Kelly, F. Gallou, B. H. Lipshutz, *Org. Lett.* **2015**, *17*, 4734.
[16] A. Valvi, S. Tiwari, *J. Phys. Org. Chem.* **2017**, *30*, 3615.
[17] F. Iwasaki, K. Suga, Y. Okamoto, H. Umakoshi, *ACS Omega* **2017**, *2*, 91.
[18] F. Iwasaki, K. Suga, H. Umakoshi, *J. Phys. Chem. B* **2015**, *119*, 9772.
[19] M. Hirose, T. Ishigami, K. Suga, H. Umakoshi, *Langmuir* **2015**, *31*, 12968.
[20] F. M. Menger, V. A. Azov, *J. Am. Chem. Soc.* **2000**, *122*, 6492.
[21] F. M. Menger, K. L. Caran, V. A. Seredyuk, *Angew. Chem. Int. Ed.* **2001**, *40*, 3905.
[22] F. Sanger, *Biochem. J.* **1945**, *39*, 507.
[23] S. Mann, *Angew. Chem. Int. Ed.* **2008**, *47*, 5306.
[24] W. Wang, W. X. Xu, Y. Levy, E. Trizac, P. G. Wolynes, *Proc. Nat. Acad. Sci. USA* **2009**, *106*, 5517.
[25] H. X. Zhou, G. Rivas, A. P. Minton, *Annu. Rev. Biophys.* **2008**, *37*, 375.
[26] P. L. Luisi, P. Stano, T. de Souza, *Orig. Life. Evol. Biosph.* **2014**, *44*, 313.
[27] R. J. R. W. Peters, M. Marguet, S. Marais, M. W. Fraaije, J. C. M. van Hest, S. Lecommandoux, *Angew. Chem. Int. Ed.* **2014**, *53*, 146.
[28] K. Nishimura, T. Matsuura, K. Nishimura, T. Sunami, H. Suzuki, T. Yomo, *Langmuir* **2012**, *28*, 8426.
[29] A. Grochmal, L. Prout, R. M. Taylor, R. Prohens, S. Tomas, *J. Am. Chem. Soc.* **2015**, *137*, 12269.
[30] G. T. Barnes, I. R. Gentle, The Gas-Liquid Interface: Adsorption; Films and Foams; Aerosols, in *Interfacial Science: an Introduction*, 2nd ed., Oxford University Press Inc., New York **2011**, *58*.
[31] L. G. Qiu, A. J. Xie, Y. H. Shen, *Colloids Surf. A* **2005**, *260*, 251.
[32] Z. Arsov, L. Quaroni, *Chem. Phys. Lipids* **2007**, *150*, 35.
[33] J. M. Holopainen, J. Lemmich, F. Richter, O. G. Mouritsen, G. Rapp, P. K. J. Kinnunen, *Biophys. J.* **2000**, *78*, 2459.
[34] T. Heimburg, *Biochim. Biophys. Acta* **1998**, *1415*, 147.
[35] M. B. Smith, J. March, *March's Advanced Organic Chemistry*, 5th ed., Wiley, New York **2001** 850.
[36] G. Bartoli, P. E. Todesco, *Acc. Chem. Res.* **1977**, *10*, 125.
[37] N. A. Senger, B. Bo, Q. Cheng, J. R. Keeffe, S. Gronert, W. Wu, *J. Org. Chem.* **2012**, *77*, 9535.
[38] C. Bobica, D. F. Anghel, A. Voicu, *Colloids Surf. A* **1995**, *105*, 305.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Dutta J, Tiwari S. Aromatic nucleophilic substitution (s_nar) reactions of halo-substituted dinitrobenzene in liposome reaction media: Effect of reaction medium and role of halogen leaving group. *J Phys Org Chem*. 2021; 34:e4182. https://doi.org/10.1002/poc.4182