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Evaluation of impact of temperature and pH alterations on the size and antigenicity of meningococcal serogroup A and X polysaccharides and conjugates

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ABSTRACT

The changes in the recommended storage conditions of the glycoconjugate vaccines against *Neisseria meningitidis* (Men) serogroup A and serogroup X can affect its activity or potency. Elevated temperature and the change in pH may result in the physical instability leading to the size degradation of the polysaccharide (PS) and subsequent loss of PS epitopes. Moreover, high temperature may also result in protein aggregation and altered tertiary structure of the protein in the conjugate. Consequently, the construction of a potent glycoconjugate is dependent on optimal temperature and pH. The changes in both these conditions can also affect the production of a capsular polysaccharide (PS) and its conjugation to a protein carrier and may also affect the integrity of the vaccine molecule including the maintenance of the protective epitopes. In our study we have used inhibition ELISA as a tool to assess the impact of temperature and pH alterations on the antigenicity of *N. meningitidis* serogroup A and X, PS and conjugates and their correlation with the size distribution analysis using high pressure size exclusion chromatography. The studies on pH alterations from 5 to 9 led to minimal impact on size and antigenicity of all antigens, however, an elevated temperature adversely impacted the antigen size as well as antigenicity to varying extent. Results indicate the higher stability of MenX PS and conjugate as compared to that for MenA counterparts at elevated temperatures. Furthermore, both the MenA and MenX conjugates appears to be more stable as compared to the corresponding PSs.

1. Introduction

The making of glycoconjugate vaccines involves T-cell dependent protein carrier conjugated to the polysaccharide (PS) and the developed glycoconjugate induces T-dependent immune responses which is long lasting. However, for the construction of meningococcal conjugate vaccines there are series of complex steps such as the production and purification of PS and carrier protein, conjugation of the purified PS to a suitable protein carrier. Consequently, a potent and stable vaccine formulation is prepared [1,2].

Glycoconjugate vaccines against meningitis are considered to be complex molecular assemblies and are susceptible to multiple factors which may affect their activity or potency. Among others, two of the most important parameters which can compromise its stability and hence potency are pH and temperature. The storage of the PS at −20 °C or lower and the conjugate at 2–8 °C are generally required to maintain its biological activity. Furthermore, the formulated meningococcal glycoconjugate vaccine is generally stable at pH 7 ± 0.5. Any change in the above conditions may affect the size and integrity of the PS and the conjugate and may compromise its activity or potency. Therefore, it is necessary to conduct stability studies under different conditions as a part of vaccine development [3].

The generation of data under different adverse conditions also helps in the identification of a suitable formulation and is essential for regulatory documentation as well. The guidelines of Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) also state the need to study various stability parameters which can affect drug product or bulk material with time under different environmental conditions [3].

Stability studies recognising the changes in the entity leading to degradation of the meningococcal PS and their conjugates have been reported however the evaluation is done using Nuclear Magnetic Resonance (NMR) and High-performance liquid chromatog-

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raphy (HPLC) [4]. World Health Organization (WHO) advises and recommends the use of Enzyme-Linked Immunosorbent Assay (ELISA) as an alternative test for the identity of PSs [5]. Moreover, immunological assays such as inhibition ELISA and sandwich ELISA have been used for the serotyping of group B streptococcal isolates and the quantification of meningococcal PSs respectively [6,7]. In this study, we have used high pressure size exclusion chromatography (HPSEC) to assess the effect of high temperature as well as the change in pH on the size and integrity of *Neisseria meningitidis* serogroup A (MenA) and *N. meningitidis* serogroup X (MenX) PS as well as their respective tetanus toxoid (TT) conjugates. Also, the impact of both the parameters (high temperature and change in pH) on the PS and the conjugates of both MenA and MenX, was evaluated using inhibition ELISA and correlated with HPSEC results.

2. Materials and methods

2.1. Polysaccharides, conjugates and primary antibodies

The MenA PS, MenX PS, MenA-TT and MenX-TT conjugates were prepared in-house at MSD Wellcome Trust Hilleman Laboratories Pvt. Ltd. using standardized processes. All the 4 antigens met the desired specifications as per World Health Organization Technical Report Series (WHO-TRS) 962 Annex 2 or in-house specifications. In-house primary antibodies were prepared by pooling of rabbit sera obtained after 2, 3 and 4 biweekly intramuscular immunizations with monovalent MenA-TT or MenX-TT conjugates containing 5 μg of respective polysaccharide per dose at a reputed Contract Research Organization with protocols approved by the Institutional Animal Ethics Committee. The hyper-immunization study was conducted following guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Indian Ministry of Environment and Forest.

2.2. Incubation of PS and TT conjugates of MenA and MenX at 45 °C and 90 °C

The MenA PS, MenX PS, MenA-TT and MenX-TT conjugates at a concentration of 1 mg/ml (or 1 mg saccharide/ml) were incubated individually at 45 ± 2 °C for different time points as 1, 3, 5, and 7 days and at 90 ± 2 °C for the time points of 1, 2, 4 and 6 hr. The samples were stored at 2–8 °C for conjugates and −20 ± 2 °C for PSs after experiment completion at different timepoints until analysis started within 2 days of the completion of the final timepoint. Consequently, samples were analysed using inhibition ELISA with the corresponding untreated PS and conjugate controls stored at −20 ± 2 °C and 2–8 °C for the PS and the conjugates, respectively and pH 7.0 ± 0.5 for both the PS and the conjugates.

2.3. Incubation of PS and TT conjugates of MenA and MenX at pH 5, 6, 8 and 9

The MenA PS, MenX PS (with storage at −20 ± 2 °C) and MenA-TT, MenX-TT conjugates (with storage at 2–8 °C) at a concentration of 1 mg/ml (or 1 mg saccharide/ml) were incubated individually at pH 5 ± 0.2, 6 ± 0.2, 8 ± 0.2 and 9 ± 0.2 for different time points as 1, 3, 5, and 7 days. The pH of each sample was adjusted to 7.0 ± 0.5 before storing them at 2–8 °C for conjugates and −20 ± 2 °C for PSs after experiment completion at different timepoints until analysis started within 2 days of the completion of the final timepoint. Consequently, samples were analysed using inhibition ELISA with the corresponding untreated PS and the conjugate controls as above.

2.4. HPSEC analyses

All the samples processed in this experiment were run on HPSEC (Waters, E2695) system equipped with size exclusion columns TSKgel 5000 PWXL and TSKgel 4000 PWXL (7.8 mm X 300 mm and 7 μm particle size; TOSOH) connected in series along with TSK gel guard column. The mobile phase was 0.1 M sodium nitrate, pH 7.2 ± 0.2, at the flow rate of 1.0 ml/min (isocratic mode for 30 min). The samples eluted from the columns were observed for their molecular weight in case of MenA and MenX PS and for the retention time and size distribution in case of MenA-TT and MenX-TT conjugates, respectively. The response on RI detector for each sample was monitored for evaluation. The calibration curve was prepared using Pullulan standards (Shodex Standard P-82) of known molecular weight range from 5 kDa to 800 kDa and the resulting chromatographic data was processed using Empower® software version 2 to estimate the molecular weight of the test samples. For size distribution evaluation, void (V_0 = 11.4 ml) and total column volumes (V_t = 23.32 ml) were determined with dextran, MW 5000–40000 kDa (Hi Media) and sodium azide (Sigma), respectively.

2.5. Inhibition ELISA protocol

The untreated controls and samples treated at altered temperature and pH for different time points were analyzed by qualified inhibition ELISAs. In this assay, the in-house rabbit primary antibodies raised against the respective serogroups of *N. meningitidis* (MenA and MenX) monovalent conjugates were incubated for 1 h (hr) at 37 °C with threefold dilutions of the respective control and the treated samples. Each well, finally contained 100 μl of 1:250 diluted primary antibody and 100 μl of the polysaccharide or conjugate samples or controls diluted to different concentrations as below. All the experiments had their respective control (of either PS or the conjugate) and the samples with the corresponding PS or the conjugates at various concentrations in duplicate as 0.03, 0.11, 0.33, 1, 3, 9 and 27 μg/ml diluted in assay buffer (phosphate-buffered saline (PBS) (pH 7.3 ± 0.1) containing 0.1% v/v Brij35 (Sigma) and 5% fetal bovine serum (FBS, Gibco)) in 96 well microtiter plate (Plate A). A separate plate (plate B) was coated with a 100 μl mixture of *methylated*-Human Serum Albumin (m-HSA) (NIBSC) with the respective in-house purified PS (MenA or MenX) each at 5 μg/ml (i.e. 500 ng/well) and subsequently blocked with assay buffer after overnight incubation at 2–8 °C. To this plate B, 100 μl antibody-antigen mix from plate A was added and incubated at 37 °C for 1.5 hr and 30 min at room temperature (RT) (25 ± 2 °C). The plate was washed and incubated for 1 hr at RT with 100 μl/well (1: 1000 dilution) of peroxidase labelled anti-rabbit IgG antibodies (Sigma). Plate was washed again and incubated for 10 min at RT with the 100 μl/well of 3,3′,5,5′-tetramethylbenzidine (TMB, Sigma). The reaction was stopped by adding 50 μl/well (2 M H_2SO_4). The plate absorbance was recorded at 450 nm ($A_{450\ nm}$) on an ELISA reader (Tecan micro plate reader) with a reference to $A_{630\ nm}$. ($A_{450-630}$ or OD). The "No Antigen Control" (NAC) consisted of coated wells incubated without the inhibitors (serum diluent buffer only) and had the maximum optical density. Percentage inhibition of OD in the duplicate wells containing a particular test sample/untreated controls dilution was calculated against the OD of the NAC with the following formula: *Percent inhibition = (NAC OD − Test sample OD)/NAC OD * 100* and the standard deviation of the replicates was calculated.

3. Results and discussion

PS vaccines have proven to be effective however the development of glycoconjugate vaccines in the latter half of the 20th and

early 21st century have been more effective in controlling the disease and are considered as the most significant health interventions [8]. For a glycoconjugate to be effective, it must be potent enough to induce protection through the production of bactericidal antibodies and the potency relies on the effective conjugation of the purified PS to the carrier protein and the integrity of the vaccine molecule [2]. There are other factors also which can affect the potency of the glycoconjugate, and among these, temperature and pH can be considered as the critical ones. Alteration in temperature may also have impact on the pH of the solutions. High temperature may result in the physical instability such as the degradation in the size of the PS, protein aggregation and altered tertiary structure of the protein in the conjugate and therefore potency of the vaccine may be compromised. Data generated on the effect of high temperature and change in pH on the vaccine potency is beneficial as cold chain conditions may not be maintained (particularly) in the developing countries [3]. Also, the experimental exposure of the product to undesired conditions (such as altered temperature and pH) for a shorter period of time may reflect the compromise in quality of the product if any accidental exposure occurs in real conditions [3].

In the current study, the effect of high temperature and the changes in pH on the size and stability of the MenA PS, MenX PS, MenA-TT and MenX-TT conjugate were evaluated by HPSEC. The HPSEC analysis for MenA PS showed a very fast reduction in the molecular weight (MW) when incubated at 45 °C (for 1, 3, 5, and 7 days) (Table 1) and 90 °C (for 1, 2, 4, and 6 hrs) (Table 1). Compared to the initial MW of 380 kDa, it is reduced to 26 kDa and 3 kDa at the 7th day of 45 °C and at the 6th hr of 90 °C treatment, respectively.

HPSEC evaluation of MenX PS when treated at 45 °C and 90 °C, shows a decrease in the MW but not as fast as that of MenA PS. The initial MW of 326 kDa of the MenX PS is degraded to 250 kDa at the 7th day of 45 °C treatment (Table 1) whereas it degrades more prominently when treated at 90 °C (Table 1) with MW reducing to 59 kDa at the 6th hr. Consequently, the HPSEC profile of both MenA PS and MenX PS indicate degradation of the PS for both the serogroups however the MW reduction is more noticeable for MenA PS. Furthermore, any of the altered pH conditions (pH 5, 6, 8 and 9) did not reduce the MW substantially for both MenA and MenX PS (Table 1).

Similarly, MenA-TT and MenX-TT conjugate samples were analysed by HPSEC and due to lack of suitable MW standards, the retention time (Rt) of the main peak was considered for evaluation of change in conjugate size distribution. The HPSEC analysis of the MenA-TT when incubated at 45 °C (for 1, 3, 5 and 7 days) (Table 2), showed an increase in the Rt and was maximum at the 7th day at 15.2 min compared to the untreated MenA-TT conjugate Rt of 14.5 min. However, the treatment at 90 °C (for 1, 2, 4, and 6 hrs) (Table 2) decreased the Rt and reached to a minimum at the 6th hr at 12.9 min. The increase in the Rt for the MenA-TT conjugate when treated at 45 °C possibly indicates the hydrolysis of the PS whereas the decrease in the Rt when the MenA-TT conjugate is treated to 90 °C may be a consequence of both the hydrolysis of the PS indicated by increase in area of the second smaller peak as well as the aggregation of the protein, indicated by decrease in the Rt of the main conjugate peak. The appearance of second peaks for MenA-TT conjugate at increasing time intervals at 45 °C indicate degradation of PS (Fig. 1A) however the PS degradation is more pronounced for the conjugate with the increasing time intervals at 90 °C (Fig. 1B).

For MenX-TT conjugate, there is not much change in the Rt when treated at both 45 °C and 90 °C (Table 2) and the Rt at all the time points for the conjugates is comparable to the Rt of untreated conjugate as 12.9 min suggesting no or minor degradation. The HPSEC chromatograms for MenX TT conjugates when treated at 45 °C at increasing time intervals also indicate no degradation of the conjugate (Fig. 2A) however at 90 °C, smaller second peaks are observed indicating minor release of degraded PS with the increasing time intervals (Fig. 2B). Furthermore, for the conju-

Table 1
HPSEC analysis of the effect of temperature and pH on the molecular weight of MenA, MenX polysaccharides.

Experimental condition	Time interval	MenA PS Molecular weight (kDa)	MenX PS Molecular weight (kDa)
Untreated control	Not applicable	380	326
45 °C	1 day	150	324
	3 day	64	275
	5 day	38	273
	7 day	26	250
90 °C	1 hr	20	160
	2 hr	9	113
	4 hr	4	76
	6 hr	3	59
pH 5	1 day	376	325
	3 day	371	314
	5 day	363	312
	7 day	367	308
pH 6	1 day	374	334
	3 day	374	331
	5 day	374	327
	7 day	386	312
pH 8	1 day	395	340
	3 day	379	335
	5 day	383	337
	7 day	387	341
pH 9	1 day	352	331
	3 day	349	326
	5 day	349	321
	7 day	352	330

Table 2
HPSEC analysis of the effect of temperature and pH on the retention time of MenA-TT and MenX-TT conjugates.

Experimental condition	Time interval	MenA-TT conjugate Retention time (min)	MenX-TT conjugate Retention time (min)
Untreated control	Not applicable	14.5	12.9
45 °C	1 day	14.7	12.9
	3 day	15.0	12.9
	5 day	15.1	12.9
	7 day	15.2	12.9
90 °C	1 hr	14.1	12.8
	2 hr	14.1	12.8
	4 hr	13.5	12.9
	6 hr	12.9	12.8
pH 5	1 day	14.6	12.7
	3 day	14.5	12.7
	5 day	14.6	12.7
	7 day	14.9	12.7
pH 6	1 day	14.6	12.8
	3 day	14.6	12.8
	5 day	14.6	12.8
	7 day	14.6	12.8
pH 8	1 day	14.6	12.8
	3 day	14.6	12.8
	5 day	14.6	12.8
	7 day	14.6	12.8
pH 9	1 day	14.6	12.8
	3 day	14.6	12.8
	5 day	14.6	12.8
	7 day	14.6	12.8

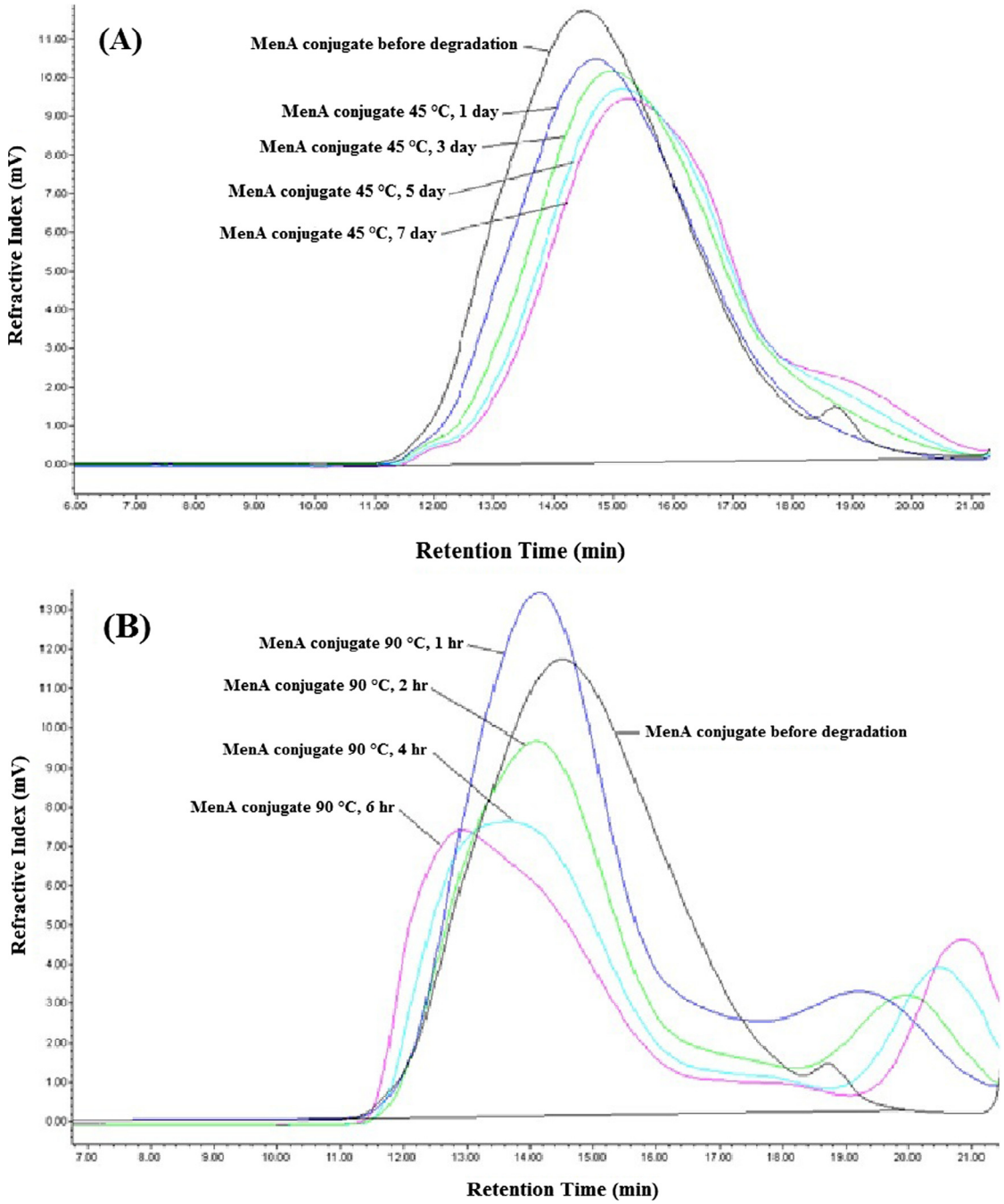

Fig. 1. HPSEC profile of MenA-TT conjugate at 45 °C (A), 90 °C (B) eluted with 0.1 M sodium nitrate buffer on TSK 5000–4000 PWXL columns. Data recorded using RI detector.

gates of both MenA and MenX (Table 2), the altered pH conditions (pH 5, 6, 8 and 9) did not change the Rt indicating negligible PS degradation.

Other studies have also shown depolymerisation or hydrolysis of the PS and the changes in the conformation of the protein at elevated temperature for *N. meningitidis* serogroup C-CRM_{197} and *Haemophilus influenzae* type b-CRM_{197} conjugates [9,10]. Furthermore, as TT is considered to be a stable carrier protein, therefore it may not have undergone denaturation and aggregation at 45 °C for the MenA-TT conjugate, however at 90 °C, denaturation leading to aggregation may have decreased the Rt in our results [11,12].

Like other PSs, the integrity of MenA and MenX capsular PSs may be sensitive to changes in the storage temperature. The potential loss of epitopes may reduce their antigenicity. This loss of immunogenicity is usually evaluated by in vivo mice immunization. However, in vivo studies in mice are variable, expensive and time consuming. Alternatively, the change in the PS integrity and subsequent loss of protective epitopes could also be evaluated by a simple and rapid competitive Inhibition ELISA. In our study, we have also used an inhibition ELISA based method to determine the effect of high temperature (45 °C and 90 °C) and the change in pH (pH 5, 6, 8 and 9) on the antigenicity of MenA PS, MenX PS as well as the TT conjugates of both the serogroups.

The range of percent inhibition (at 27 μg/ml antigen concentration) was between 50 ± 10% and 70 ± 13% for the untreated controls of MenA PS and MenX PS, respectively both kept at a storage temperature of −20 °C and pH 7 ± 0.5. Whereas it was

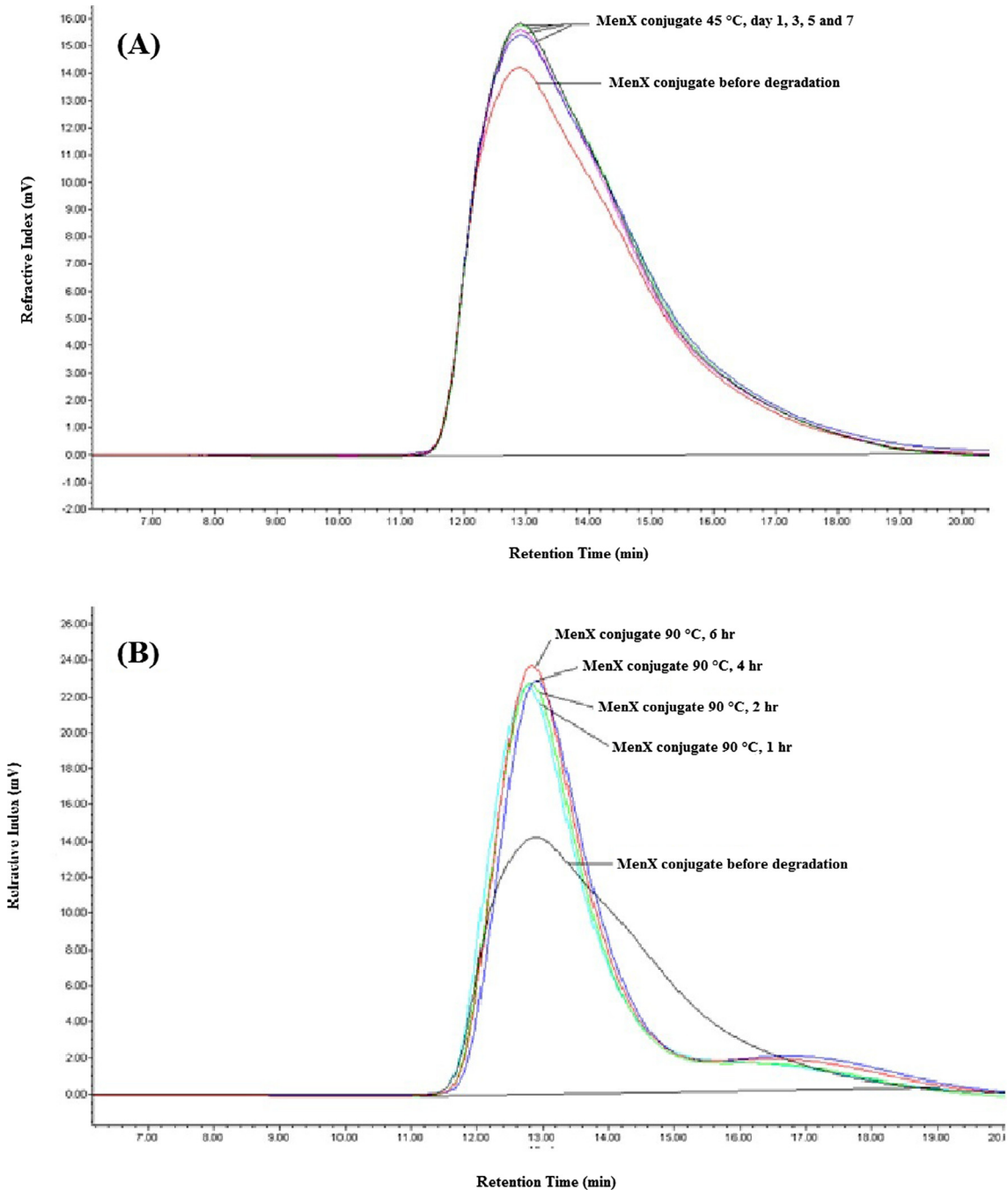

Fig. 2. HPSEC profile of MenX-TT conjugate at 45 °C (A), 90 °C (B) eluted with 0.1 M sodium nitrate buffer on TSK 5000–4000 PWXL columns. Data recorded using RI detector.

between 60 ± 5% and 65 ± 8% for MenA-TT and MenX-TT conjugate untreated controls, respectively kept at a storage temperature of 2–8 °C and pH 7 ± 0.5. However, when the MenA PS is treated to 45 °C (Fig. 3A) the percent inhibition starts to decrease with the increase in the time interval and is lowest at the 7th day at 27% at a concentration of 27 μg/ml antigen. Furthermore, the treatment of MenA PS at 90 °C (Fig. 3B) drastically decreases the percent inhibition after just 1 hr of treatment to 17% at 27 μg/ml and the percent inhibition becomes almost negligible with the increase in time as the PS is significantly degraded. Alternatively, MenX PS when treated to 45 °C (Fig. 3C), the percent inhibition at all the time points is comparable to the percent inhibition of MenX PS control (82% at 27 μg/ml) however, the percent inhibition of MenX PS starts to decrease slightly with the increase in the time intervals when treated at 90 °C (Fig. 3D) and is lowest at the 6th hr at 43% at 27 μg/ml compared to the 56% inhibition of the MenX PS control at 27 μg/ml. The above results indicate the degradation as well as loss of antigenicity of MenA PS at both 45 °C and 90 °C but the degradation as well as reduction in antigenicity is observed for MenX PS at 90 °C only.

The treatment of MenA-TT conjugate at 45 °C (Fig. 4A) caused a decrease in the percent inhibition at increasing time points and across various concentrations compared to the control. A similar trend in the decline in the percent inhibition was observed when the MenA-TT conjugate was treated at 90 °C (Fig. 4B) however the decline was more prominent at all the time points and concentrations compared to control. Of particular relevance is the percent inhibition of 2% (at 27 μg/ml) at the 6th hr which signifies major

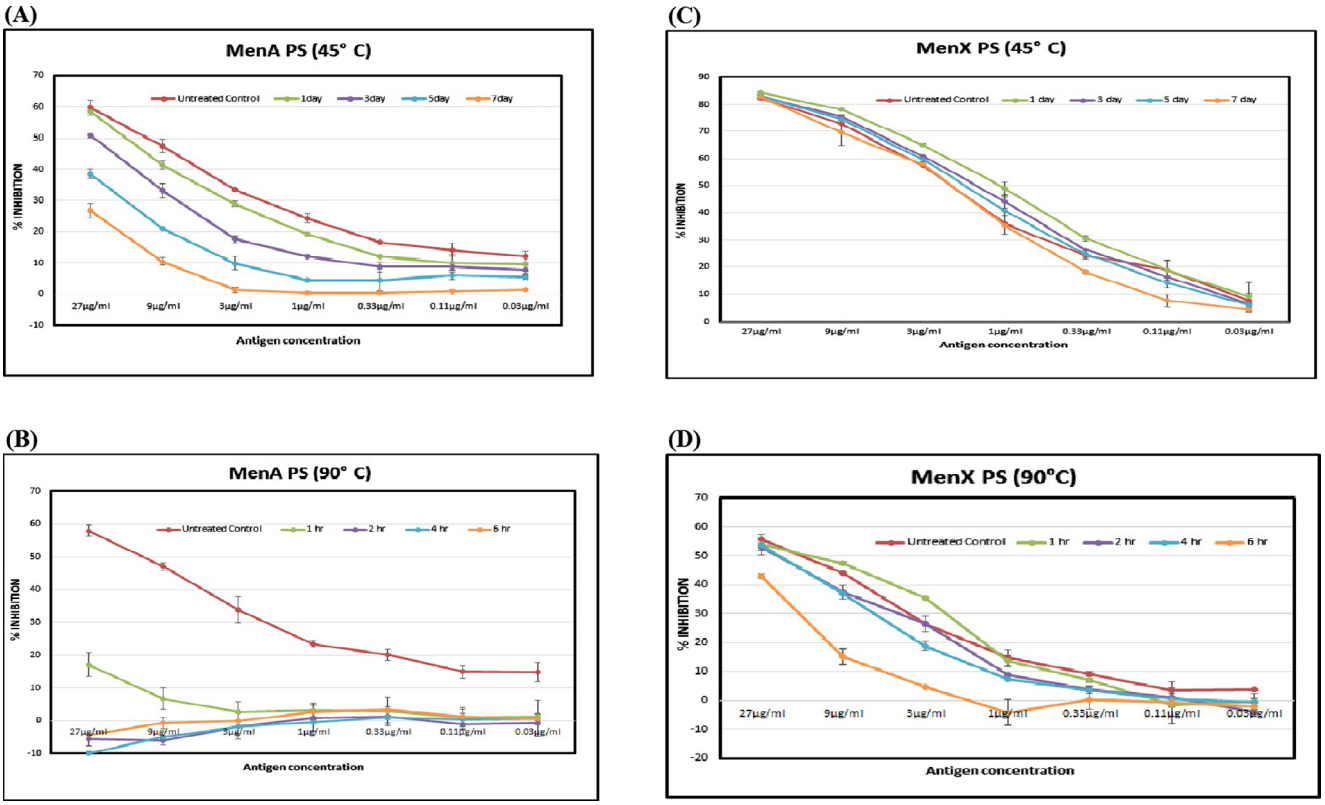

Fig. 3. Inhibition ELISA curves showing percent inhibition for MenA PS treated at 45 °C (A), 90 °C (B) and MenX PS treated at 45 °C (C), 90 °C (D) compared to their respective untreated controls. Error bars represent standard deviations of the per cent inhibition of the replicates.

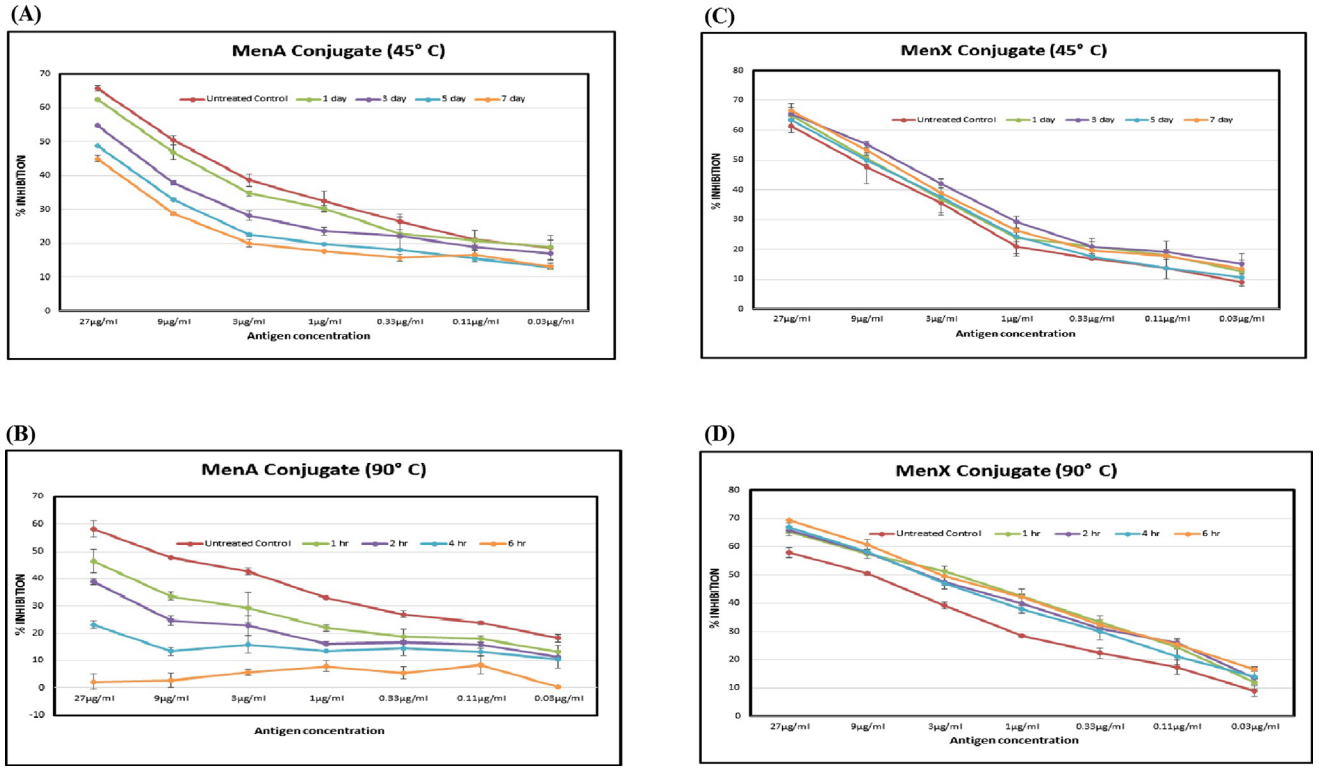

Fig. 4. Inhibition ELISA curves depicting percent inhibition for MenA-TT conjugate treated at 45 °C (A), 90 °C (B) and MenX-TT conjugate treated at 45 °C (C), 90 °C (D) compared to their respective untreated controls. Error bars represent standard deviations of the per cent inhibition of the replicates.

antigenicity loss in the conjugate. The treatment of MenX-TT conjugate at 45 °C (Fig. 4C) and 90 °C (Fig. 4D) showed comparable percent inhibition at all the time points and at all the concentrations relative to its control. Compared to the percent inhibition of the untreated MenX-TT conjugate control of 61% (at 27 μg/ml), the percent inhibition of the treated conjugate was highest at the 7th day at 66% at 45 °C. Similarly, the percent inhibition for MenX-TT conjugate was highest at the 6th hr at 69% when treated at 90 °C compared to the 58% percent inhibition of the MenX-TT control at 27 μg/ml. The results further indicate the much higher impact on antigenicity of MenA-TT conjugate compared to MenX-TT conjugate at both 45 °C and 90 °C. The decreased percent inhibition after exposure to higher temperatures for both MenA and MenX PS and MenA conjugates is attributed to the depolymerization and possibly due to loss of epitopes also.

MenA and MenX PS treatment at pH 5 and pH 9 (Fig. 5A–D) possibly did not alter the antigenicity at all the time intervals. Moreover, there was no change in the antigenicity of both the serogroups when treated at pH 6 and 8 also (results not shown). Similar results were obtained for MenA-TT and MenX-TT conjugates when treated at pH 5, 6, 8 and 9 as the trend in the percent inhibition for both the conjugates was comparable to their respective conjugate untreated controls (results not shown). However, the pH experiments involving the PS and the conjugates of both MenA and MenX had minimal but within acceptable assay variations. It is reported that the change in pH can lead to depolymerization and degradation [2,4,13] but pH induced degradation or loss of antigenicity has not been observed in our studies in both MenA and MenX PSs as well as in their respective conjugates however this observation may be due to short time frame of the experiment.

Earlier studies [4,14] have compared the stability of MenA PS with MenX PS and have concluded that MenX PS is more stable than MenA PS. The thermostability of MenX PS has been described well by Xie et al. [14]. The authors observe that the HPSEC profile of stability samples of MenA PS following two weeks at 37 °C showed a substantial shift in the elution of the main peak whereas for MenX PS, shift in the elution of the main peak was observed but not as significant as that of MenA PS. The stability of MenX PS is also explained by Berti et al., [4]. MenX PS has alpha 1 → 4 phosphodiester bond and equatorial orientation of glucosamine N-acetyl at C2 position whereas for MenA PS it is 1 → 6 phosphodiester bond and axial orientation of mannosamine N-acetyl at C2 position. The authors state that for, MenA PS, the axial orientation of the mannosamine N-acetyl group may facilitate the cleavage of the phosphate group but for MenX PS, the equatorial orientation of the glucosamine N-acetyl group deters the same mechanism to occur [4]. In agreement with other authors [4,14], our results also suggest that MenX PS is more stable than MenA PS. We also conclude that MenX-TT conjugate is also more stable when compared with the MenA-TT conjugate. Furthermore, both the TT conjugates of MenA and MenX appears to be more stable as compared to their plain PS.

The degradation of MenA PS has also been acknowledged as the unconjugated MenA PS vaccine in a WHO campaign was unable to produce a protective immune response [15]. Consequently, the degradation of the PS may affect the efficacy of the PS vaccine. Although the same PS size is not required for conjugate as it is needed for the PS vaccines [16] however it is crucial to control the size of the PS because its degradation may affect the potency of the conjugate consequently.

The identification of 45 °C as one of the temperature points in our studies was chosen deliberately as the temperature may rapidly increase to 45 °C owing to common and frequent power failures in the developing country settings. The selection of 90 °C for the experimental studies as another temperature point is based on the assumption that the observed degree of degradation at 90 °C for a shorter duration may correlate to the degradation of the PS and the changes in the conjugate, if kept at lower temperatures such as 45 °C for longer duration [17]. Moreover, the studies

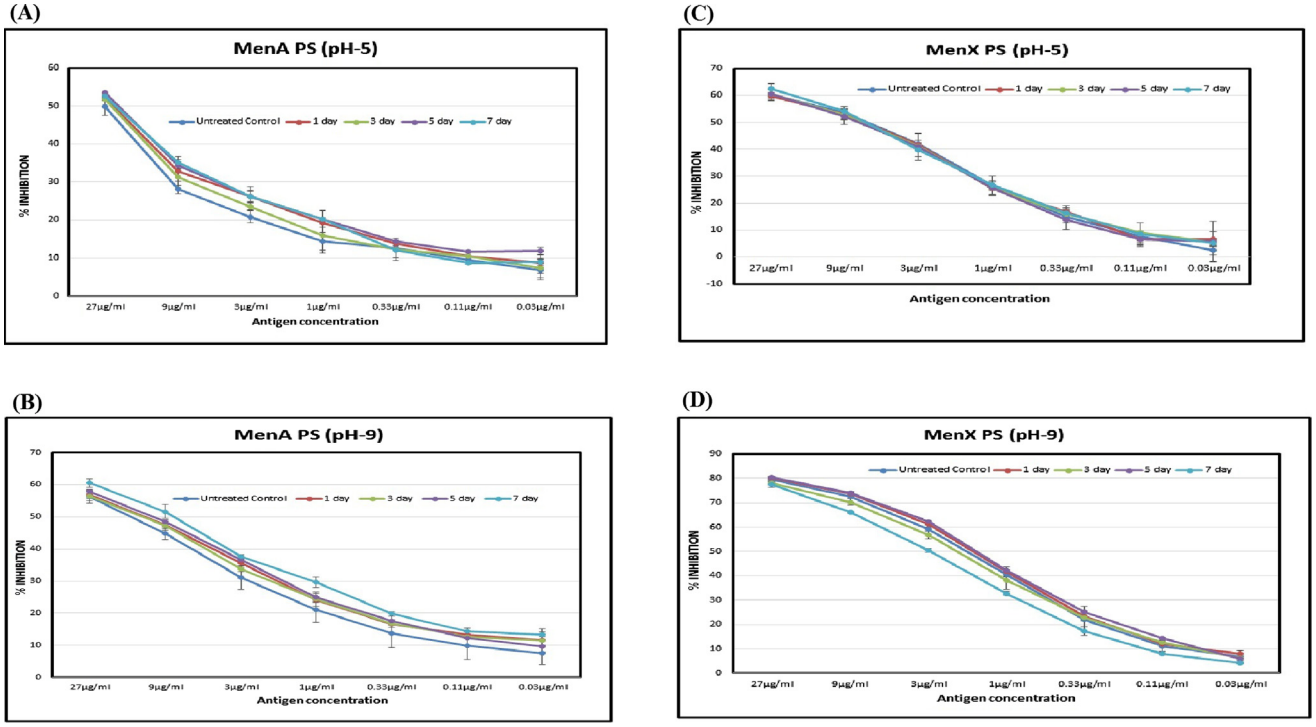

Fig. 5. Inhibition ELISA curves showing percent inhibition for MenA PS treated at pH 5 (A), pH 9 (B), and MenX PS treated at pH 5 (C), pH 9 (D) compared to their respective untreated controls. Error bars represent standard deviations of the per cent inhibition of the replicates.

involving the impact of various pH conditions compared to the routine storage pH of the PS and conjugate, were selected because it very likely that these pH conditions may be reached accidently in real storage settings of the PS and the conjugate. The studies on such experimental conditions may help in identifying the changes in the size and integrity of the PS and the conjugate with exposure to such altered pH conditions.

Compared to the MenX PS and MenX-TT conjugates, the extent of decrease of percent inhibition is more prominent at all the time points for MenA PS as well as for MenA-TT conjugates at both 45 °C and 90 °C. Consequently, our inhibition ELISA results suggest and indicate that the degradation of the PS as well as the conjugate of MenA may lead to the damage or alteration of epitopes and therefore may affect the antigenicity. The reduction in the antigenicity as assessed by an inhibition ELISA may indicate towards a possibility of decrease in vaccine potency after exposure to above adverse conditions. However, there is no clear evidence if the antigenicity of an antigen is directly proportional to the in vivo protective efficacy of the same. This is due to the variety of epitopes which may be important for two types of analyses.

There are studies already, which identify that MenA PS is less stable or degrades faster than the MenX PS however the impact of temperature and pH stress on the antigenicity has not been evaluated yet using inhibition ELISA. The hallmark of this study is the utilization of an inhibition ELISA for studying the impact of these parameters on the antigenicity of meningococcal PSs confirming MenX PS as more stable than MenA PS and also identifying that both MenA and MenX conjugates as more stable than respective PSs. In conclusion, the studies presented in this paper are the first to show the use of Inhibition ELISA for the evaluation of antigenicity of the PS and the conjugate and its correlation with the size distribution analysis using HPSEC.

Contributors

MKC conceptualized and designed the experiments. NS and RR conducted the experiments. NS and SH drafted the manuscript. MKC and DU reviewed the manuscript.

Conflict of interest statement

None of the authors has conflicts of interest.

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