

ABSTRACT

Purpose. Nitric oxide (NO) is a diatomic free radical endogenously generated by nitric oxide synthase (NOS) that functions as a crucial effector and chemical messenger in diverse physiological and pathophysiological processes. Especially NO plays a key role in the innate defense against foreign pathogens. However, clinical application of NO is hampered owing to its gaseous property and short half-life (2-3 sec). The synthesis of exogenous NO (NO donor) as a means for storing and releasing NO in a sustained manner is indispensable. Biodegradable and biocompatible biopolymer such as alginate can be a potent and promising NO donor scaffold. The purpose of this study was to synthesis NO-releasing functionalized alginate with diethylenetriamine (DETA) as alkyl amine precursors (Alg-DETA/NO), confirming the successful synthesis and examine the *in vitro* NO release profile.

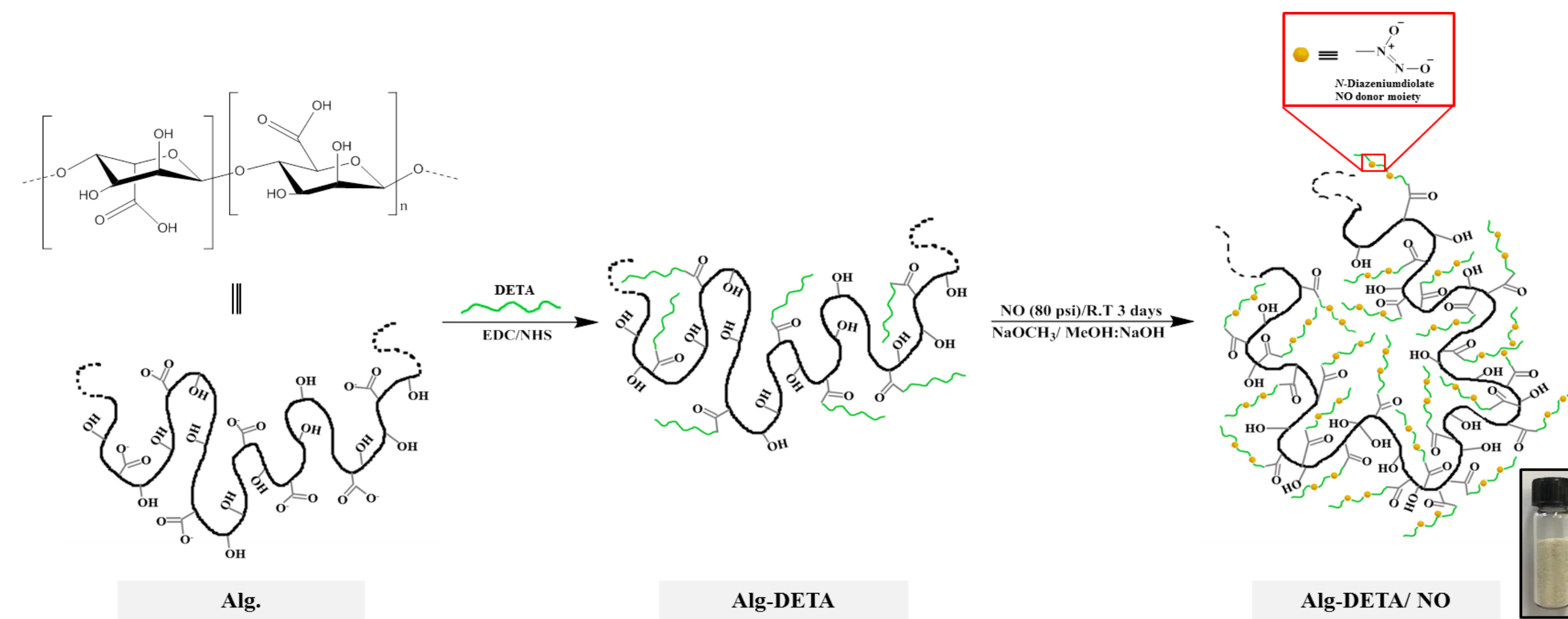
Methods. The covalent attachment of DETA to alginate (Alg-DETA) was done prior Alg-DETA/NO synthesis. Carboxylic acid group of alginate was conjugated to the primary amino group of DETA to form amide bonds. Alg-DETA/NO was synthesized via the reaction between the secondary amine groups of Alg-DETA with NO (gas) under high pressure (80 psi) at room temperature for 3 days. Successful preparation of Alg-DETA/NO was confirmed by proton nuclear magnetic resonance (¹H NMR), fourier transform infrared spectroscopy (FTIR), and ultraviolet/visible (UV/Vis) spectrophotometry. The real-time NO release of Alg-DETA/NO was evaluated in phosphate buffered saline (PBS) pH 7.4 at 37 °C using a Sievers 280i chemiluminescence NO analyzer.

Results. ¹H NMR, FTIR, and UV/Vis spectrophotometer verified the conjugation between Alg-DETA and NO. The total NO released (*t*[NO]) was 0.23 ± 0.006 μmoles/mg polymer. The real time NO release showed the maximum flux of NO ([NO]_{max}) was 27 ppb/mg polymer. Alg-DETA/NO displayed a prolonged release of NO over a period of 4 days with half-lives (*t*_{1/2}) of ≈13 h.

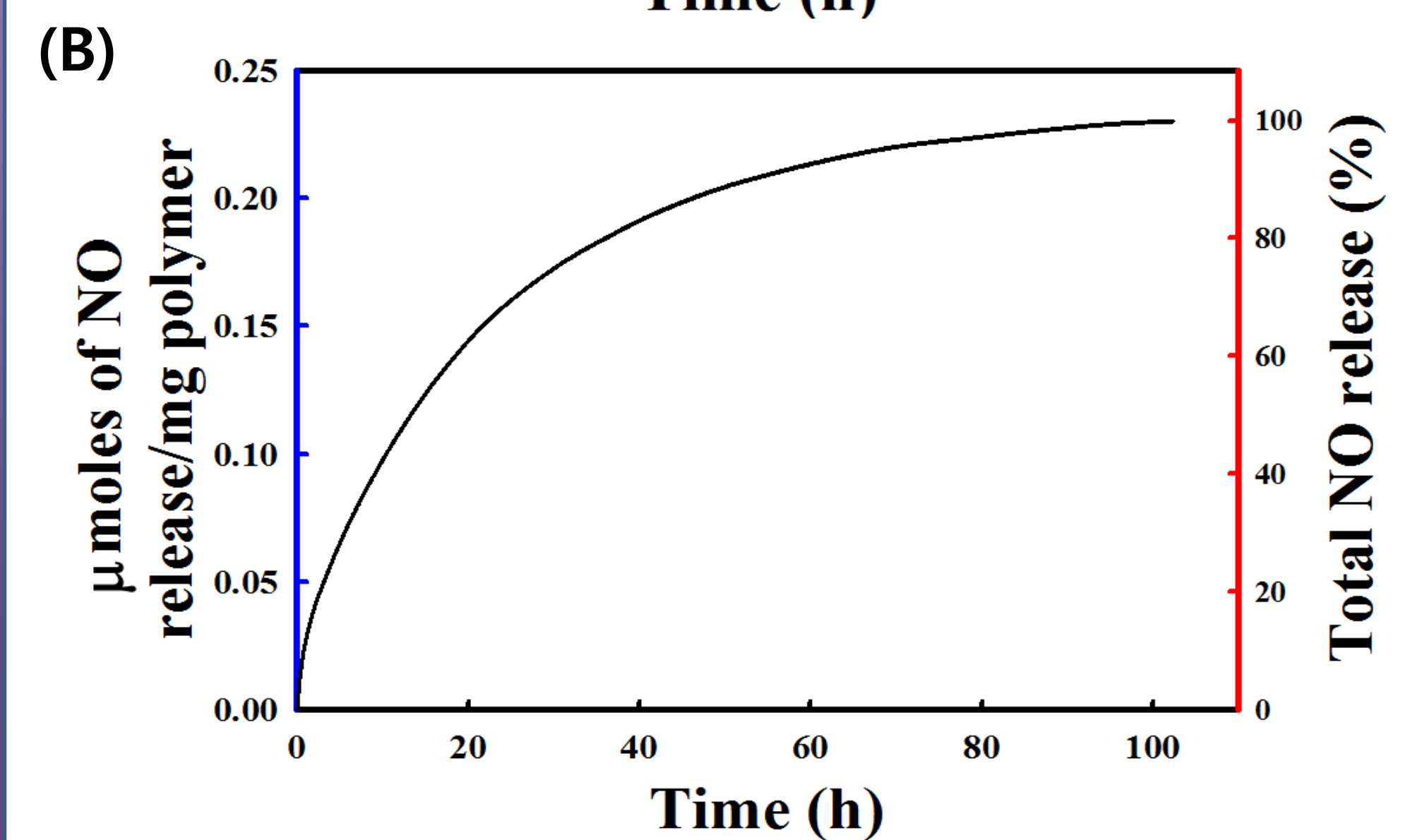
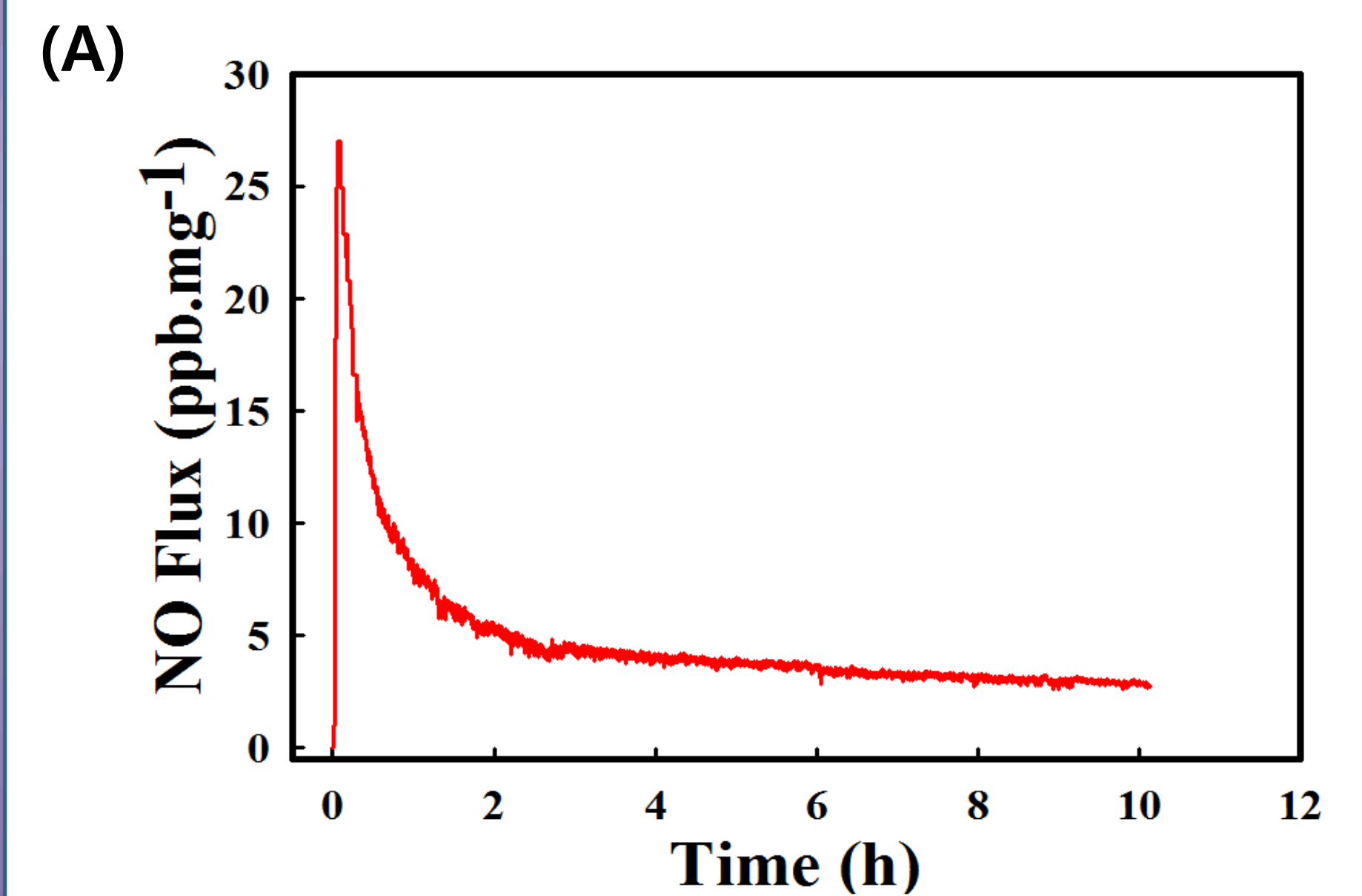
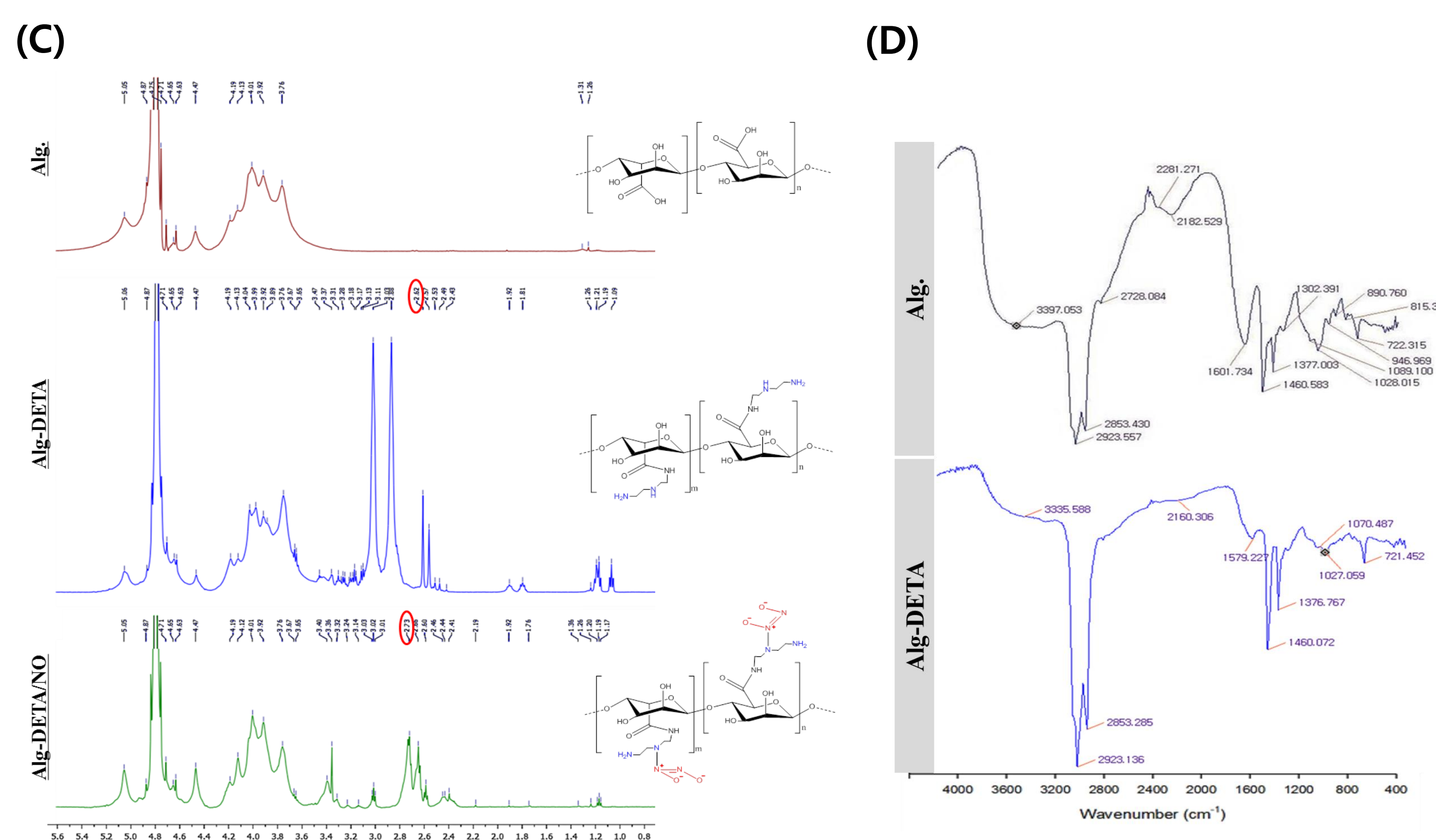
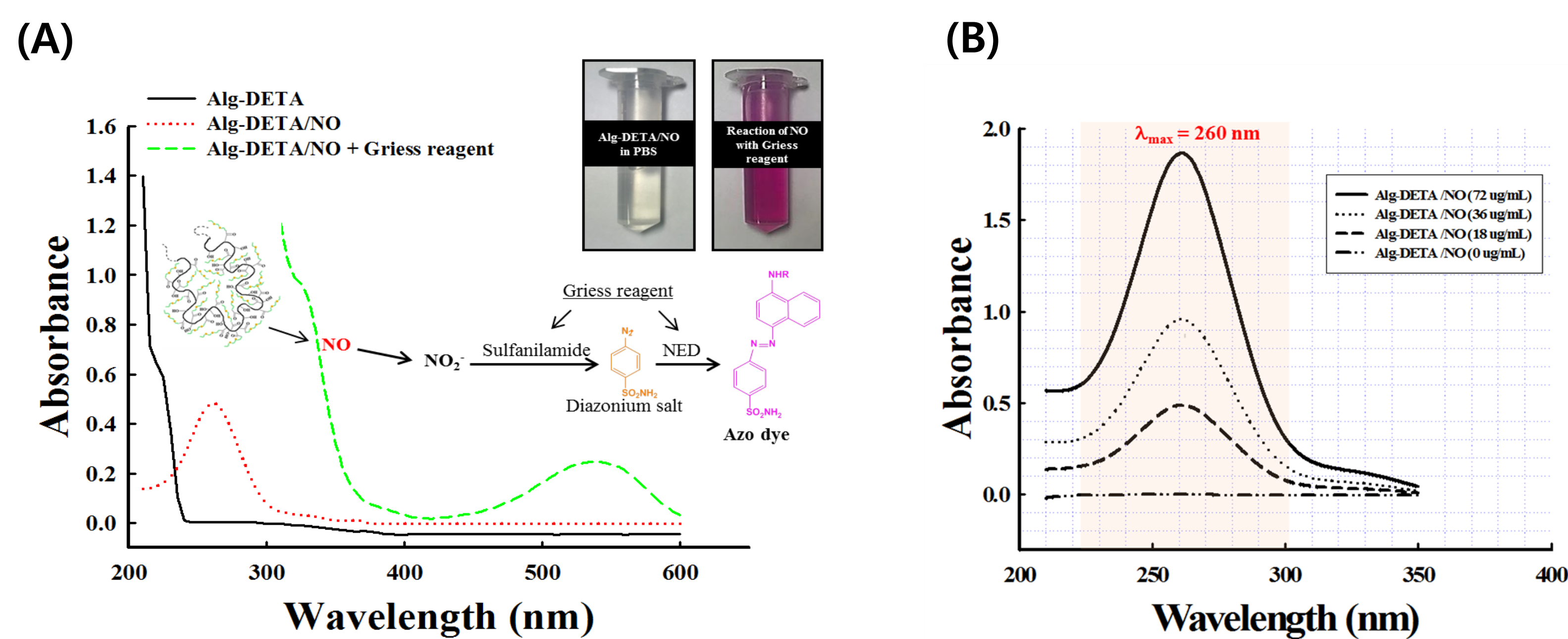
Conclusion. In this study, NO-releasing functionalized alginate has been successfully synthesized. The Alg-DETA/NO also exhibited a remarkably prolonged NO release profile over 4 days.

RESULTS

Synthesis of Alg-DETA/NO



Characterization of Alg-DETA/NO



(A) Real time NO-release for the first 10 h.
(B) Plot of percentage total NO release and amount of NO release vs time measured via NOA in PBS (pH 7.4, 37 °C).

CONCLUSION

- NO-releasing functionalized alginate has been successfully synthesized.
- UV-Vis spectra, characteristic peak in FTIR spectra and proton signal from ¹H NMR measurement verified the successful synthesis of Alg-DETA/NO.
- The Alg-DETA/NO also exhibited a remarkably prolonged NO release profile over a period of 4 days.

ACKNOWLEDGMENTS

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In vitro NO release

Nitric oxide release properties of Alg-DETA NONOates in PBS (pH7.4, 37°C).

NO donor	<i>t</i> [NO] ^a (μmoles/mg)	[NO] _{max} ^b (ppb/mg)	<i>t</i> _{1/2} ^c (h)	<i>t</i> _d ^d (h)
Alg-DETA/NO	0.23 ± 0.006	27.02 ± 3.107	13.35 ± 0.066	102.40 ± 0.003

Values are expressed as mean averages ± SD of three different measurements. ^aTotal NO released. ^bMaximum flux of NO release. ^cNO-release half-life. ^dDuration of NO release.