Electronic Supplementary Information

Development and characterization of a novel L-asparaginase/MWCNT nanobioconjugate

Raquel O. Cristóvão[1], Mafalda R. Almeida[2], Maria A. Barros[1], João C.F. Nunes[2], Rui A.R. Boaventura[1], José M. Loureiro[1], Joaquim L. Faria[1], Márcia C. Neves[2], Mara G. Freire[2], Valéria C. Ebinuma-Santos[3], Ana P.M. Tavares[2,*], Cláudia G. Silva[1,*]

[1]*Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua do Dr. Roberto Frias, 4200-465, Porto, Portugal*

[2]*CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

[3]*Department of Engineering Bioprocess and Biotechnology, School of Pharmaceutical Sciences, UNESP-University Estadual Paulista, Araraquara, Brazil*

*Corresponding authors:

Ana P. M. Tavares:
Tel: +351 234 401 520; e-mail: aptavares@ua.pt
Cláudia G. Silva:
Tel: +351 220 414 874; e-*mail:* cgsilva@fe.up.pt

Table S1. Effect of pH on immobilization yield and relative recovered activity obtained with the immobilization of 8.6×10^{-5} g.mL^{-1} of ASNase on 2 mg of MWCNTs for 60 min of contact time.

pH	Relative recovered activity (%)	Immobilization yield (%)
5	26.7 ± 0.5	99 ± 2
6	19.7 ± 0.4	100 ± 2
7	32.6 ± 0.7	100 ± 2
8	35.5 ± 0.7	94 ± 2

Table S2. Effect of contact time on immobilization yield and relative recovered activity obtained with the immobilization of 8.6×10^{-5} g.mL^{-1} of ASNase on 2 mg of MWCNTs at pH 8.

Contact time (min)	Relative recovered activity (%)	Immobilization yield (%)
15	69 ± 1	74 ± 2
45	51± 1	90 ± 2
60	36 ± 1	94 ± 2
90	15.4 ± 0.3	100 ± 2
120	20.8 ± 0.4	100 ± 1

Table S3. Effect of enzyme concentration on immobilization yield and relative recovered activity obtained during the immobilization of ASNase on 2 mg of MWCNTs at pH 8 for 45 min.

ASNase concentration ($\times 10^{-3}$ g.mL^{-1})	Relative recovered activity (%)	Immobilization yield (%)
0.040	11 ± 2	98.6 ± 0.3
0.086	35 ± 1	97 ± 1
0.160	72.4 ± 0.3	97 ± 4
0.320	80 ± 1	99.0 ± 0.1
1.500	90.9 ± 0.4	90 ± 10
3.000	90 ± 5	90 ± 5

Table S4. Amount of adsorbed active ASNase (U) per gram of MWCNT (U.g^{-1}) for the immobilization of different concentrations of ASNase on 2 mg of MWCNTs at pH 8 during 45 min, used for the prediction of Langmuir and Freundlich isotherm models.

ASNase concentration ($\times 10^{-3}$ g.mL^{-1})	q (U.g^{-1})
0.040	5.89
0.086	42.81
0.160	96.19
0.320	123.74
1.500	133.40
3.000	131.15