Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Amine-functionalized magnetic nanocomposite particles for efficient immobilization of lipase: effects of functional molecule size on properties of the immobilized lipase

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Removing of the unattached lipase molecules after immobilization procedure

According to the zeta potential values results (**Fig. S1**), the free lipase exhibits an isoelectric point of 5; however its surface charge does not significantly change at various pH values as the enzyme molecules involve different functional molecules and can tune its surface charge at various buffer solutions. It is also indicated that the surface charge of the PEI1-MS and PEI-MS at all the pH values are positive; henece, some lipase molecules may adsorb on these particles via electristatic interactions as the immobilization procedure was carried out at pH 7.4. Therefore, after three times washing with a sodium phosphate buffer solution of pH 7.4, the prepared samples were washed with acetate buffer solutions of two pH values (0.1 mol L^{-1}, pH 3 and 5, twice with each solution) and then with sodium phosphate buffer solutions of three pH values (0.1 mol L^{-1}, pH 6, 8 and 9, twice with each solution) to ensure the removal of all the noncovalently-attached or loosely-bounded lipases. **Fig. S2** shows the amount of the loosly-bonded lipase molecules removed after each washing step with various buffer solutions for the immobilization procedure carried out with 2 mg mL^{-1} lipase initial concentration equaling to a lipase content of 5 mg in the immobilization media. The last right columns in this figure show the total amount of un-immobilized lipase molecules which is the sum of all the removed lipase molecules at each washing step. The L-Amine-MS, L-PEI1-MS and L-PEI2-MS were first washed with a sodium phosphate buffer solution (pH 7.4) until we did not measure any lipase molecules in the washig solution, as it was analysized by the Bradford method. The samples were then washed twice with a sodium acetate buffer solution of pH 3 at which the surface charge of the samples and the lipase are possitve and the repolsive forces are maximum. **Fig. S2** shows that the most of the loosely-bonded lipase molecules was removed over this washig step (pH 3). Then the samples were washed with other buffer solutions and no significant amount of the unatached lipase were observed in the washing solutions. In the case of the L-Amine-MS sample, since both the sample and lipase have low negative surface charge states, plenty of the unattached lipase molecules werre removed at the first stage of the washing with a buffer solution of pH 7.4. The most of the remaind un-immobilized lipase were washed at the second washing stage with a sodium acetate buffer solution of pH 3.

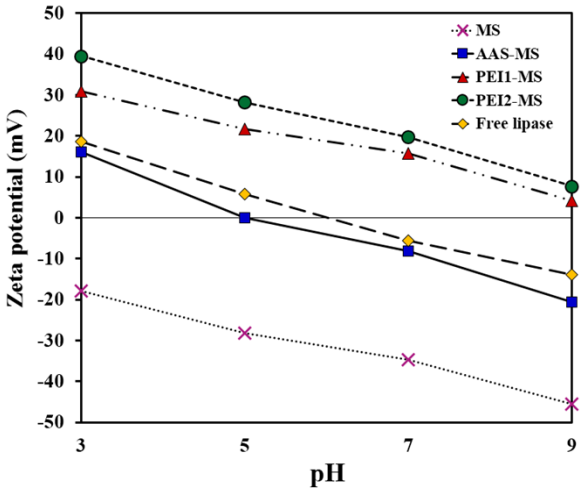

Fig. S1 Zeta potential values of the lipase molecules and the nanocomposite particles as a function of pH.

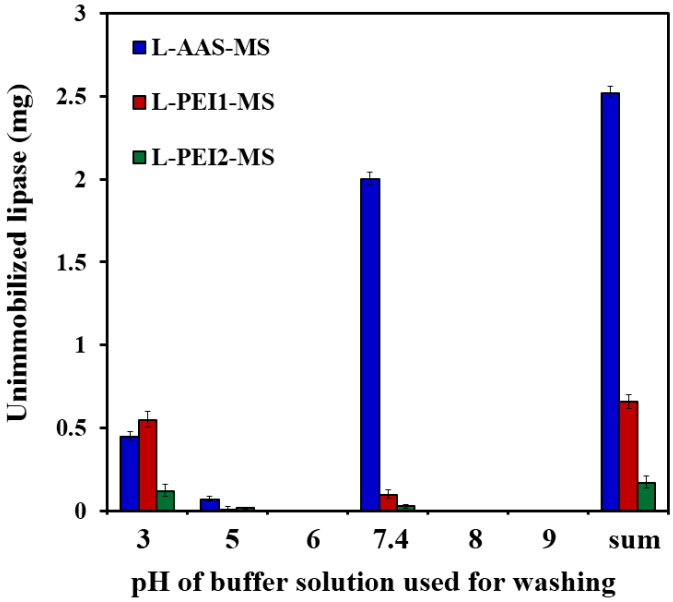

Fig. S2 The amount of un-immobilized lipase removed through each washing step with various buffer solutions and the total amount of the un-immobilized lipase represented in last columns. Lipase initial concentration of 2 mg mL^{-1} (= 5 mg in the system) was used in the immobilization procedure.

Kinetics of the enzymatic hydrolysis reactions

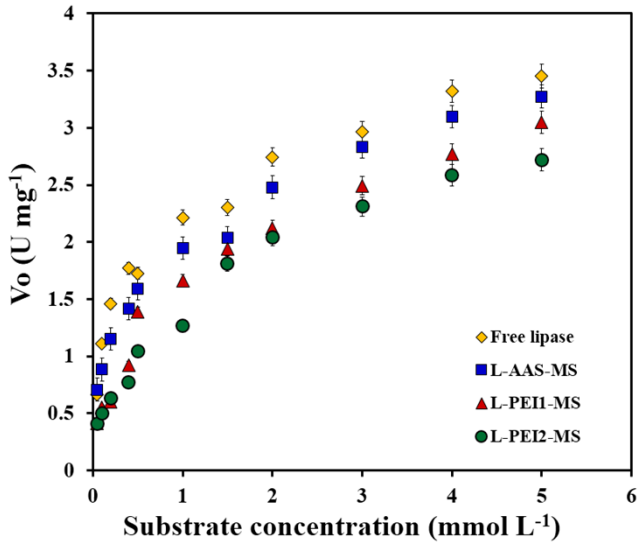

Fig. S3 Effect of initial substrate concentration on initial reaction rate at pH 7.4 and 40 °C.

Thermal stability of the free and immobilized lipases at 40 °C

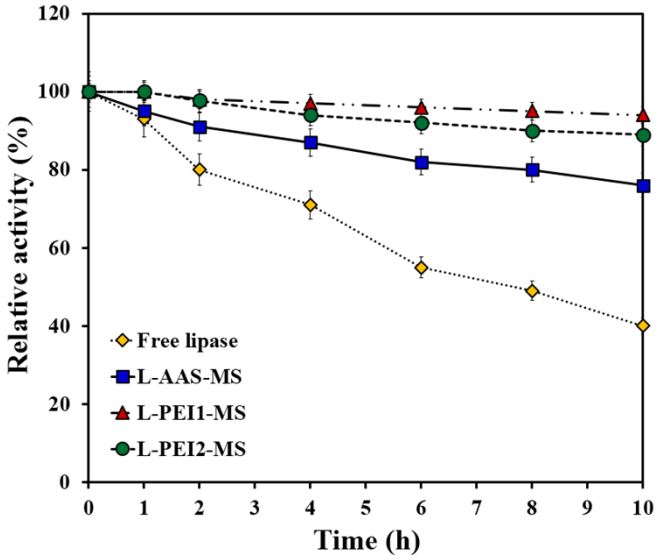

Fig. S4 Thermal stability of the free and immobilized lipases at 40 °C; the enzymatic activities were measured at 40 °C and pH 7.4.

Circular dichroism (CD) analysis results performed on the free and immobilized lipases in thermal and storage stability assays

Table S1 Secondary structural content (%) of lipase in free and different immobilized states.

Sample	α-Helix (%)				β-Sheet (%)				β-Turn (%)				Random coil (%)			
	[a]4 °C	[b]S-4 °C	[c]50 °C	[d]T-50 °C	4 °C	S-4 °C	50 °C	T-50 °C	4 °C	S-4 °C	50 °C	T-50 °C	4 °C	S-4 °C	50 °C	T-50 °C
Free lipase	31.6	22.3	22.7	7.7	20.4	13.6	16	6.5	18.1	25.4	25	37.6	29.9	38.7	33.3	48.2
L-AAS-MS	30.2	24.1	23	17.7	19.9	14.9	15.5	7.4	18.9	27.7	24.5	30.5	31	33.3	35	44.4
L-PEI1-MS	28.1	26.7	21.5	20.7	18.7	17.1	25	23	20.5	22.9	20.5	22	32.7	33.3	32.6	34.3
L-PEI2-MS	26.3	23.2	17	12	17.1	14.3	23.1	18.9	23.4	25.1	25.9	29.5	33.2	37.4	34	39.6

CD analysis of lipases pretreated at different condition: (a) fresh lipases dispersed in a buffer solution at 4°C and CD recorded at 4 °C (reported in the paper), (b) lipases pretreated in a buffer solution at 4 °C for 20 days and CD recorded at 4 °C, (c) lipases pretreated in a buffer solution at 50 °C for 15 min and CD recorded at 50 °C (reported in the paper), and (d) lipases pretreated at 50 °C for 10 h and CD recorded at 50 °C.