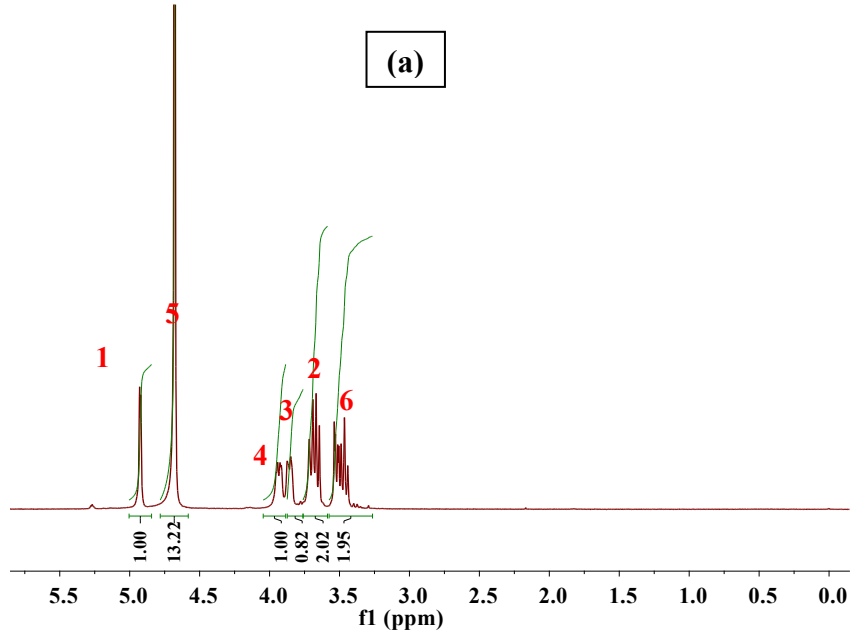
Supporting information

pH Sensitive Acetalated-Dextran/PLGA Based Double-layered Microparticles and Their Application in Food Preservation

Agni Kumar Biswal, Aiswarya Thattaru Thodikayil, Sampa Saha*

Department of Materials Science and Engineering, Indian Institute of Technology, Delhi, India-110016

Corresponding Author: *ssaha@mse.iitd.ac.in



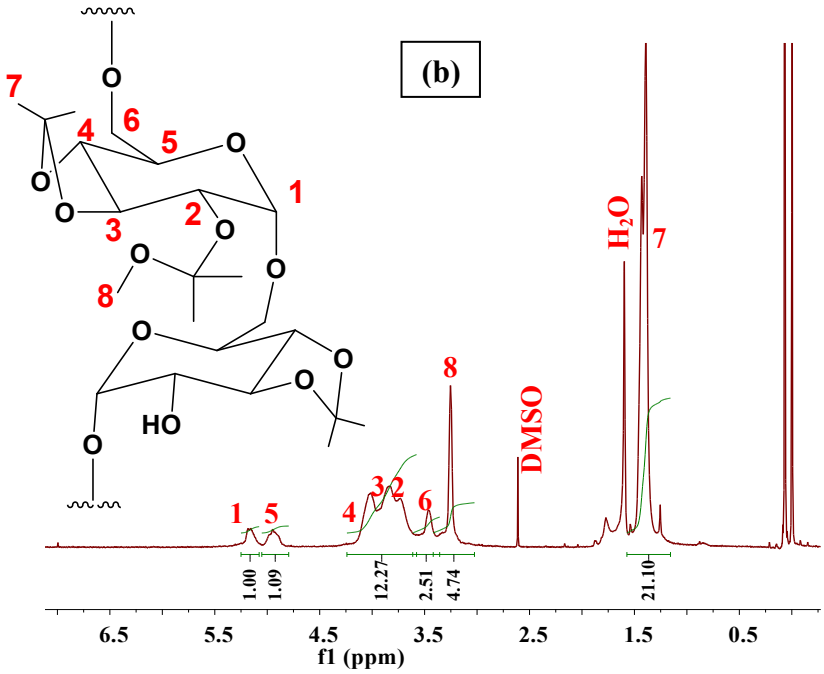


Figure S1. [1]HNMR for (a) dextran and (b) acetalated-dextran.

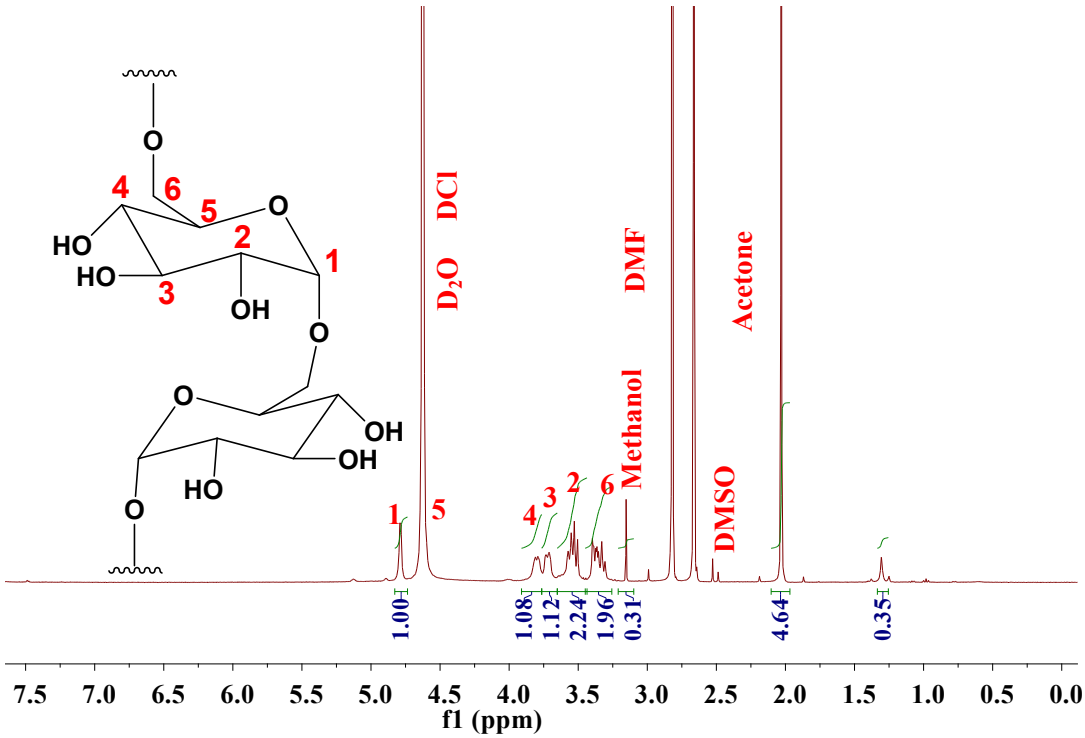


Figure S2.[1]HNMR for Ac-dextran in D_2O after 25 days of degradation study carried out at pH ~5.

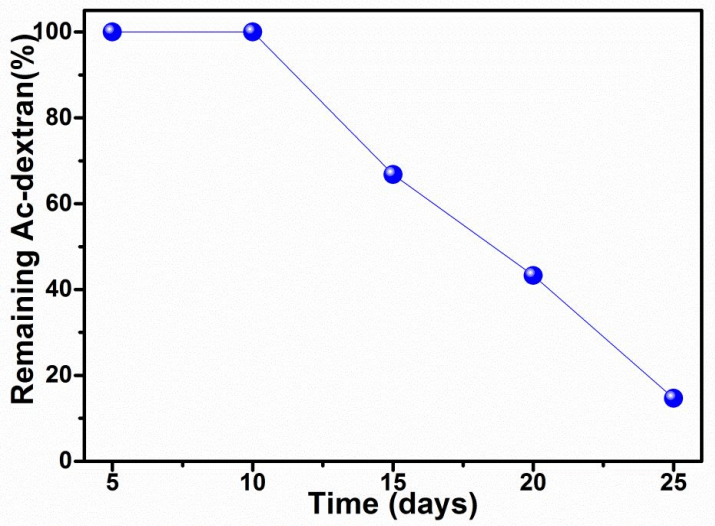


Figure S3. Degradation of Ac-dextran at pH 5 (calculated from proton NMR).

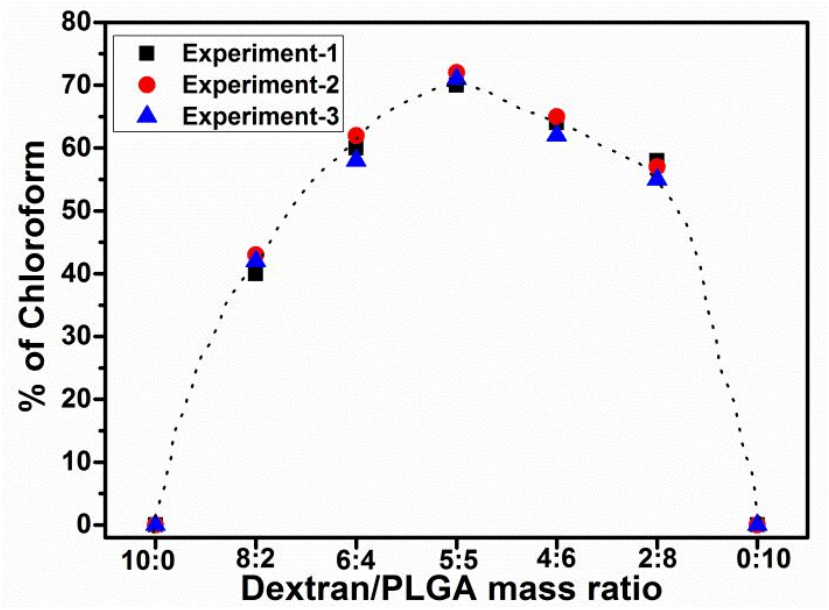


Figure S4. Evolution of cloud points for Ac-dextran/PLGA mixtures taken at different ratios.

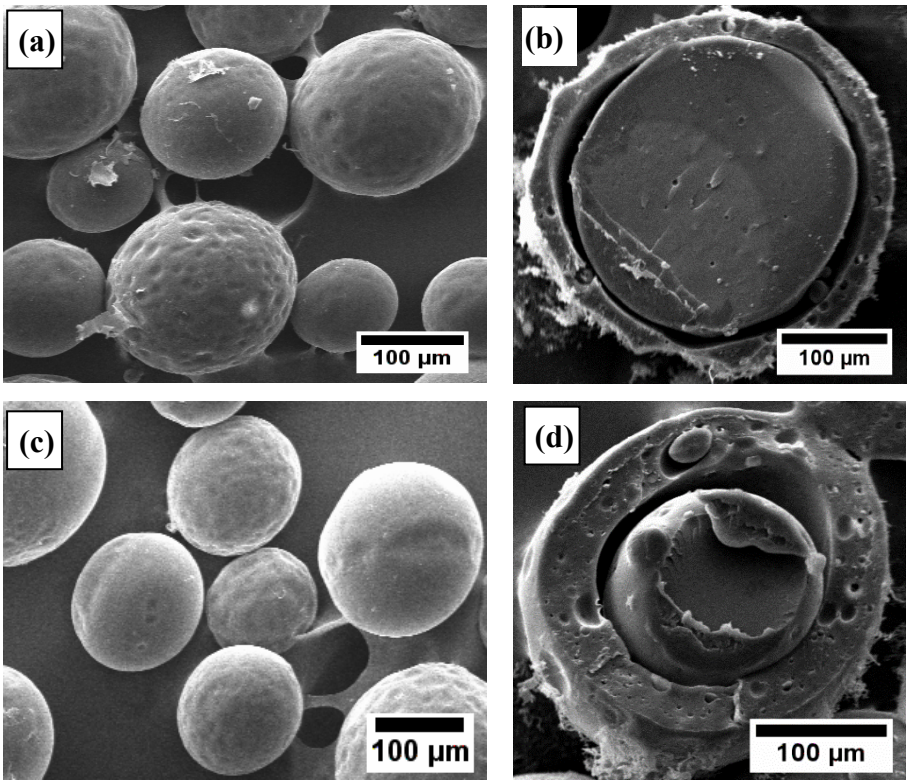


Figure S5. Representativewhole and cross-sectional SEM images of dual actives loaded (a,b) S1 and (c,d) S2 microparticles.

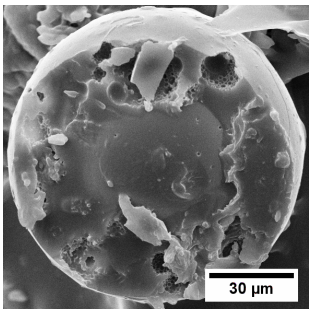


Figure S6. Representative cross-sectional SEM images for dual actives loaded system S4 (Ac-dextran/PLGA(I.V.: 0.61dL/g) fabricated at pH 8.

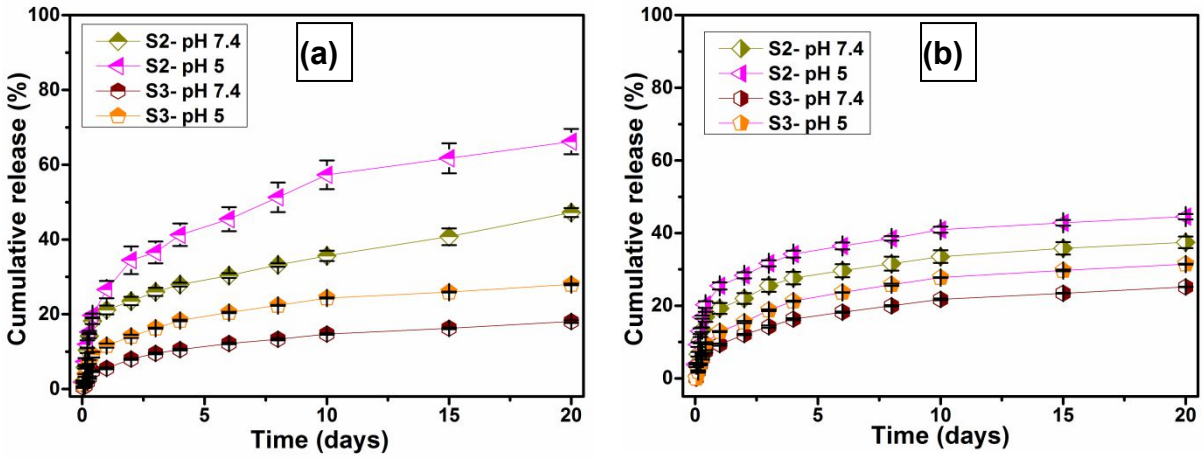


Figure S7. *In vitro* release study of (a) benzoic acid and (b) tocopherol from S2 and S3 in PBS pH 7.4 and 5.

Determination of active release mechanism from dual active loaded microparticles using power law model

Before fitting to the Ritger-Peppas model, different mathematical models such as zero order ($M_t/M_\infty=K_0t+C$), first order ($\ln(1-M_t/M_\infty)= K_1t+C$), Higuchi model ($M_t/M_\infty=K_Ht^{0.5}+C$ [1-2]) were applied and fitting parameters were reported in **Table** S1. None of the models can be well fitted into the release data obtained for different systems at different pHs.

Table S1 Fitting parameters calculated from the *in vitro* release of actives

Benzoic acid		
Samples	**pH**	**Mathematical Models**

		Zero Order		First Order		Higuchi	
		K_0	R^2	K_1	R_1^2	K_H	R_H^2
S1	7.4	0.0173	0.8121	-0.024	0.867	0.085	0.945
	5	0.419	0.749	-0.146	0.9765	0.209	0.922
S2	7.4	0.0180	0.798	-0.249	0.863	0.087	0.930
	5	0.0263	0.729	-0.041	0.877	0.129	0.915
S3	7.4	0.0089	0.816	-0.008	0.836	0.043	0.965
	5	0.012	0.776	-0.153	0.809	0.062	0.943
Tocopherol							
S1	7.4	9.47	0.694	-	-	48.11	0.886
	5	10.54	0.662	-	-	54.29	0.868
S2	7.4	8.73	0.711	-	-	44.15	0.899
	5	10.14	0.670	-	-	51.99	0.871
S3	7.4	6.93	0.777	-	-	34.36	0.945
	5	8.736	0.746	-	-	43.78	0.927

Finally, we used Ritger-Peppas model (power law model) to evaluate the release mechanism of actives from the polymeric microparticles.[3-7] The model can be expressed as:

$$\frac{M_t}{M_\infty} = kt^n$$

$$log(M_t/M_\infty) = nlogt + logk$$

Where, M_t and M_∞ are the cumulative release of encapsulated actives at time t and at infinite, k is the rate constant and n is a release exponent which can be used to predict the release mechanisms in following way.

n=0.43, Fickian diffusion
0.43<n<0.85, non-fickian diffusion
n<0.43, combination of diffusion and erosion
n≥0.85, swelling controlled release

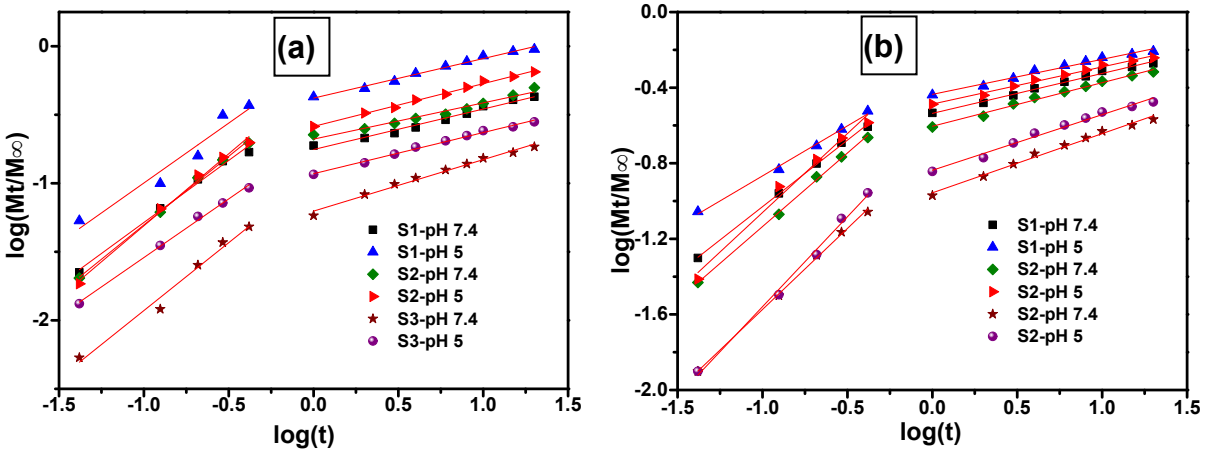


Figure S8. Plots for $log(M_t/M_\infty)$ Vs log(t) obtained from *in vitro* release study (**Figure**

7 and S7) for (a) benzoic acid (b) tocopherol using above model and their linear fittings at stage 1 (0-10h) and stage 2 (1-20 days).

Table S2 Release kinetic parameters [(R^2 correlations) and n (release exponent)] calculated from **Figure** S8

For benzoic acid				
Sample			**n**	**R^2**
pH 7.4	**S1**	stage1	0.90	0.98
		stage2	0.29	0.97
	S2	stage1	1.00	0.99
		stage2	0.26	0.96
	S3	stage1	0.99	0.97
		stage2	0.37	0.98
pH 5	**S1**	stage1	0.87	0.92
		stage2	0.28	0.98
	S2	stage1	1.05	0.99
		stage2	0.31	0.99
	S3	stage1	0.85	0.99
		stage2	0.30	0.99
For tocopherol				
pH 7.4	**S1**	stage1	0.70	0.99
		stage2	0.21	0.99
	S2	stage1	0.77	0.99
		stage2	0.23	0.98
	S3	stage1	0.85	0.99
		stage2	0.31	0.98
pH 5	**S1**	stage1	0.52	0.99
		stage2	0.18	0.98
	S2	stage1	0.83	0.98
		stage2	0.19	0.98
	S3	stage1	0.95	0.99
		stage2	0.29	0.98

Determination of cytocompatibility of microparticles

Briefly, RAW264.7 cells were seeded at a density of $5x10^4$ cells per well in 96-well plate in incomplete media (without FBS). Cells were allowed to adhere for 3h, after which freshly prepared suspension of system S1 and S2 particles were added to cells in four different concentrations i.e., 50 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml (n=4 for each concentration). Cell viability was measured after 24h of incubation with cells. At the end of exposure time, media was removed completely and the cells were washed once with 1X PBS followed by addition of 200 µl MTT reagent (0.25mg/ml) to all the wells. After 3h, MTT reagent was removed and 100 µl of DMSO was added to dissolve formazan crystals formed due to interaction of cells with MTT reagent. Cells incubated with cell culture medium alone were used as positive control. Absorbance readings were measured at 570 nm and % cell viability was calculated as given

$$Cell\ Viability\ (\%) = \frac{Absorbance\ of sample}{Absorbance of control} X100$$

Where,

Absorbance of sample = cells incubated with various concentrations of System S1 and S2.

Absorbance of control = cells incubated only with media

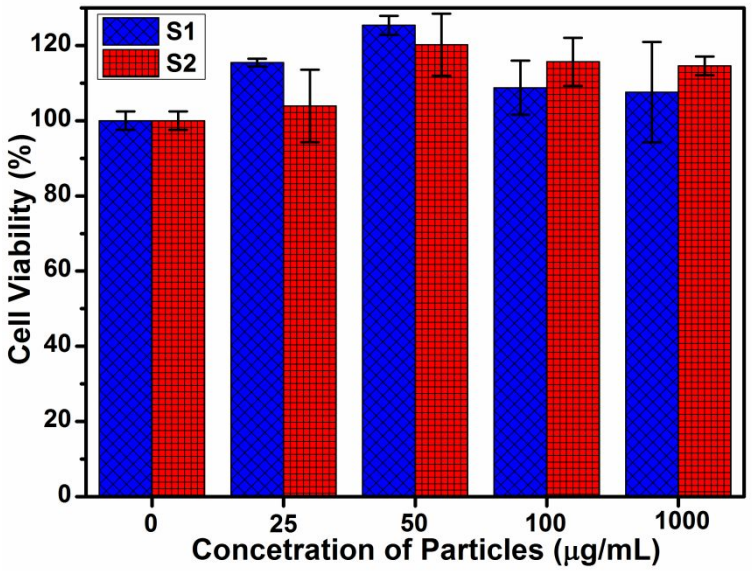


Figure S9. % of cell viability of system S1 and S2

Both the samples were found to be completely cytocompatible at all tested concentrations.

References

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