Supplementary Electronic Information for:

Triggered Cell Release from Shellac-Cells Composite Microcapsules

Shwan A. Hamada\textsuperscript{a}, Simeon D. Stoyanov\textsuperscript{b} and Vesselin N. Paunov\textsuperscript{**}

\textsuperscript{a} Surfactant \& Colloids group, Department of Chemistry, University of Hull, Hull, HU67RX, UK.

\textsuperscript{b} Unilever R\&D Vlaardingen, Olivier van Noortlaan 120, 3133 AT Vlaardingen, the Netherlands; Laboratory of Physical Chemistry and Colloid Science, Wageningen University, 6703 HB Wageningen, The Netherlands; Department of Mechanical Engineering, UCL, Torrington Place, London, WC1E7JE, UK.
Movie 1 and Movie 2: Video footage of the process of instantaneous disintegration of a sample of composite shellac-yeast cells microcapsules doped with polyacrylic acid in aqueous solution upon addition of a drop of 0.1 M NaHCO₃ solution. The microcapsules have been produced by spray-precipitation of aqueous solution of 7% wt ammonium shellac, 4%wt polyacrylic acid and 10% wt yeast in a solution of 3% wt acetic acid followed by washing on the filter and drying at room temperature.

In Figure S1, the composite microcapsules start to disintegrate after stirring them in the aqueous solution of 0.1 M sodium bicarbonate for 20 minutes and as the stirring times increase, more microcapsules disintegrate until after approximately 80 minutes of stirring all the microcapsules successfully disintegrated and released the yeast cells. The disintegration process was shortened by doping the ammonium shellac solution with 0.52 % wt. carboxymethyl cellulose. The idea is, when carboxymethyl cellulose starts to swell, it would produce voids in the structure of the microcapsules by co-swelling them and this increase the speed of disintegration, because the microcapsules will be more accessible for the aqueous solution. Using images similar to those in Figure S1, the number of the remaining intact micropcapsules counted and the data was used to plot a graph in Figure S2 to illustrate the trend of the triggered release of the yeast cells inside the fabricated microcapsules in different stirring time. Figure S3 shows sequential screen shots of a disintegrating composite shellac-yeast microcapsule doped with 4% wt sodium polyacrylate.

If a dispersion of yeast in ammonium shellac solution was sprayed through a column with hot air, the water content of the droplets start to evaporate and the droplets dry up which result in the increase of the concentration of the shellac solution which starts to precipitate and produce microcapsules loaded with high percentage of yeast cells and dried solid shellac. We collected the falling dry microcapsules in a Petri dish which contained milli-Q water at the base of the spray-drying column. Microcapsules fabricated by using this strategy are shown in Figure S4. Figure S5 shows the morphology of the microcapsules after stirring them for two hour in the alkaline solution at pH 8. The microcapsules started to open up which provided more access of their surface to the aqueous solution which continued until their complete disintegration after stirring in the solution for 2 hours. Figure S6 shows the process of the disintegration of shellac/PAA/yeast composite microcapsules at different values of the pH of the medium. Figure S7 shows the comparison of the growth of microencapsulated yeast in shellac microcapsules dispersed in a starch gel and culture medium which is compared with the blank sample containing only starch and culture media. The formation of carbon dioxide bubbles in the gel with shellac-cell microcapsules confirms the viability of the encapsulated cells.
Figure S1: Disintegration of composite shellac-yeast microcapsules fabricated by spray co-precipitating dispersion of 10 % wt. yeast cells in 7 % wt. ammonium shellac solution doped with 0.52 % wt carboxymethyl cellulose sprayed over 3% vol. acetic acid aqueous solution. The microcapsules were transferred in a solution of sodium bicarbonate at pH 8 with stirring. Images were taken after: (a) 0 min, (b) 20 min, (c) 40 min, (d) 60 min, (e) 70 min, and (f) 80 min, while (g) and (h) show the viable yeast cells released from the disintegrated microcapsules, using fluorescence microscopy after treating them with FDA solution.
**Figure S2:** The relationship between remaining composite shellac-yeast cell microcapsules and time of transferring and stirring them in aqueous solution of sodium bicarbonate at pH 8.
Figure S3: The composite shellac/yeast cells microcapsules fabricated by spray co-precipitating yeast cells in ammonium shellac solution doped with 4 % wt. sodium polyacrylate in 3 % vol. aqueous solution of acetic acid. The microcapsules swelled at pH 8 when exposed to a drop of 1M sodium bicarbonate and they started to disintegrate and release the yeast cells.
Figure S4: Composite shellac-yeast microcapsules fabricated by spray co-drying a dispersion of 10 \% wt yeast cells in 7 \% wt ammonium shellac solution through a column with hot air at 80 °C. The yeast cells inside the microcapsules treated with FDA solution and observed by FTIC fluorescence microscopy in micrographs (c) and (d).
Figure S5: The magnetised composite shellac-yeast microcapsules transferred into a aqueous solution of pH 8 and stirred for one hour in (a)-(b) and two hours in (c)-(f) then small samples were collected to be imaged by optical microscopy.
Figure S6: Composite microcapsules of shellac-yeast cells transferred into 0.1 M of phosphate buffer solutions at different pH and stirred on a magnetic stirrer while aliquots were withdrawn at regular intervals and checked by optical microscopy. The above micrographs illustrate the time it takes for the complete disintegration of the microcapsules at different pH. (a is after 24 hours at pH = 5, the microcapsules remained intact), (b is after 7 hours at pH = 6.5) (c is 3.2 hours at pH = 7), (d is 2 hours at pH = 7.5), (e is 1 hour at pH = 7.9), (f is 0.3 hours at pH = 9).
Figure S7: The culture medium was sterilised by boiling at 100 °C for 1 hour and then cooled down to 37 °C. Micrograph (a) represents the gel systems before incubation, (a1) Composite microcapsules were dispersed in the sterilised culture medium after they were washed with milli-Q water several times. A gel was prepared from the suspension of the microcapsules in culture medium by dissolving starch powder. (a2) Starch powder was dissolved in the sterilised culture medium without the composite microcapsules. Micrograph (b) shows the gel systems after incubation at 37 °C for 12 hours; only (b1) which contained encapsulated yeast cells is grown, while (c) is a zoomed in image of (b1).