Multiplexed biomarker detection using phase change nanoparticles

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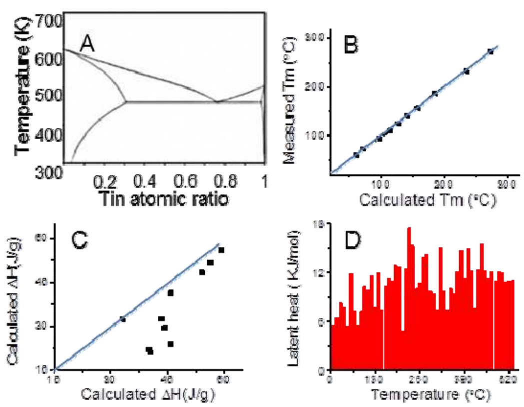
Statement of Purpose: Many biomarkers are specific for early stage cancer detection, but no individual biomarker is ideal to distinguish lethal cancer from indolent ones due to lack of tumor specificity. Parallel to efforts of finding specific biomarkers, a feasible way of providing better predictive value is to detect multiple biomarkers from a sample and collectively assess response pattern of more biomarkers. But, existing techniques such as microarrays and nanoparticles are not suitable to detect multiple low concentration biomarkers in a tiny amount of sample due to low sensitivity, low multiplicity and extensive sample preparation. We have detected multiple DNA and protein biomarkers with a panel of phase change nanoparticles of metals and alloys. Nanoparticles attached on a substrate through ligand-biomarker interactions are readout using differential scanning calorimetry, where the peak position and area reflect presence and concentration of biomarkers. This research is innovative for several reasons. (1) It uses an unexplored phenomenon of solid materials, i.e., the temperature of a solid will not rise above its melting point until the entire solid is molten. (2) Sharp melting peak, large thermal scan range and wide choices of materials enhance multiplicity this detection without adding system complexity. (3) The surface grafting density of ligands on nanoparticles is controlled to detect multiple biomarkers with concentrations differing several orders of magnitude.

Methods: Ten different metals that form binary eutectic alloys among any two of them are identified from phase diagram database. These metals (i.e., aluminum, bismuth, cadmium, copper, gadillium, indium, lead, magnesium, palladium, silver) can form 45 binary eutectic alloys, 120 ternary eutectic alloys, 210 quaternary eutectic alloys, and so on. The total number of metals and eutectic alloys are 1,023. Pandat 8 software (CompuTherm) has been used to derive compositions of ternary and higher order eutectic alloys formed by these ten metals. Two thermophysical properties (melting temperature and latent heat of fusion at eutectic composition) had been extracted from data. After obtaining eutectic compositions, nanoparticles had been made by thermally decomposing of organometallic precursor in a high boiling point solvent (ethylene glycol) by using polyvinyl alcohol (PVA) as surfactant.

Results: Fig. 1A shows a calculated binary phase diagram of lead and tin, where the melting temperature at eutectic composition (Sn63Pb37) corresponds with the reported value (183°C). Fig. 1B shows the comparison between the measured and calculated melting points of ten metals and alloys. The calculation provides atomic ratios of eutectic alloys, from which the latent heats of fusion of alloy had been derived. Fig. 1C shows the comparison between the calculated and measured latent heats of fusion of ten types of metals and eutectic alloys, where alloys with higher melting points are close to the line with 45 degree slope, but those with lower melting points are largely below the line. Fig. 1D shows calculated melting temperatures and latent heats of fusion of 50 eutectic alloys selected from a group of 600 eutectic alloys, where temperature variation between adjacent alloys is ~5±0.2°C.



Thermal detection has been used to detect proteins and single strand DNA (ssDNA). Fig. 2A shows DSC curves of indium nanoparticles attached on aluminum substrates after forming DNA duplex. The lowest concentration of ssDNA is 80 pM. Two target ssDNAs has been detected simultaneously. Fig. 2B shows two melting peaks from indium and bismuth nanoparticles, where the existence of two peaks at according melting temperatures excludes the possible cross-talk among different types of nanoparticles during melting process. The thermal ramp rates had been maintained to detect indium nanoparticles, and changed from 1 to 10°C/min to readout bismuth nanoparticles. The variable ramp rate in the same scan allows adjustment of sensitivity for 10 times. Fig. 2C are DSC curves of lead-tin eutectic alloy nanoparticles on aluminum substrates after detecting avidin of different concentrations (0, 5, 10, 20, and 50 ng/ml from bottom to up), where heat flow increases as avidin concentration increases. Four different proteins, prostate specific antigen, human and rabbit immunoglobulin antigens, and avidin had been detected simultaneously using four types of nanoparticles (tin, tin-lead alloy, indium, and bismuth). Fig. 2D are DSC curve of four sharp peaks of nanoparticles, where melting peaks are the same as according bulk materials.