Abstract: Spray-dried poly(lactic-co-glycolic acid) (PLGA) peptide-loaded microspheres have demonstrated similar long-term in vitro release kinetics compared to those produced by conventional methods and commercial products. However, the difficult-to-control initial burst release (0-24 hr) presents an obstacle to product development and establishing bioequivalence. Currently, detailed information about underlying mechanisms of the initial burst release from spray-dried microspheres is very limited. We investigated the mechanism and extent of initial burst release using 16 previously developed spray-dried microsphere formulations of the hormone drug, leuprolide acetate, with similar composition to the commercial 1-month Lupron Depot® (LD). The burst release kinetics was measured with a previously validated continuous monitoring system as well as traditional sample-and-separate methods. The potential mechanism of aqueous pore diffusion through opening and closing pores was investigated through SEM imaging and the uptake of bodipy-dextran pore probe. In vitro results were compared to pharmacokinetics in rats over the same interval. Spray-dried microspheres had distinctive pore opening/closing behaviors and pore diffusion relative to commercial LD, which is prepared by the solvent evaporation method. High-burst, spray-dried microspheres were differentiated in the well-mixed continuous monitoring system but reached an upper limit when measured by sample-and-separate. The exact length of bursts (tburst) relative to the formulation’s characteristic pore diffusion time (τ) were highly correlated to burst extent for spray dried particles. Of the four spray-dried formulations administered in vivo, three spray-dried microspheres with similar polymer density showed nearly ideal linear correlation between in vivo absorption and well-mixed in vitro release over the first 24 hours. By contrast, the more structurally dense LD and a more-dense in-house formulation showed a slight lag phase during in vivo absorption kinetics relative to that in vitro. Furthermore, in vitro tburst/τ were highly correlated with PK parameters for spray-dried microspheres but not for LD. While the correlation of increases in effective diffusion and burst release suggests aqueous pore diffusion as a major release mechanism both in vitro and in vivo, a fixed lower limit for burst release implies an alternative release mechanism such as polymer phase diffusion of ion pairs of peptide and very low molecular weight acids in the polymer.