Development of an In-Vitro Protein Binding Method for the Evaluation of Bioequivalence of Sucralfate Suspension
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Purpose
Sucralfate is an aluminum salt of sucrose octasulfate. It is a locally acting drug indicated for the short term treatment of active duodenal ulcer. Sucralfate is not absorbed systematically, traditional pharmacokinetic evaluation approaches will not offer meaningful information to evaluate the bioequivalence between generic and reference listed products. The development of standardized, discriminatory in vitro evaluation methods based on the mechanism of action of sucralfate is expected to be useful for the bioequivalence evaluation of generic drugs. The binding of sucralfate to the positively charged proteins overexpressed in the ulcer area is one of the mechanisms of action previously confirmed by in vitro and in vivo studies. The objective of the present study was to develop an in vitro method for sucralfate protein binding from its suspension as a possible approach for the evaluation of bioequivalence.

Methods
Bovine serum albumin (BSA) was used as the model protein. The protein binding of sucralfate was determined by adding sucralfate suspension into BSA solutions of known concentrations and analyzing free BSA concentration as a function of time. Two experimental set-ups were developed: centrifuge tubes and dissolution apparatus 2 with mini paddle. For the centrifuge tube method, the protein binding was conducted in conical centrifuge tubes incubated in 37°C water bath with continuous shaking. For the dissolution apparatus 2 method, the protein binding experiment was conducted using a semi-automated dissolution apparatus equipped with 200 ml mini vessels, mini paddles and an automated sample preparation module. The effects of sucralfate dose, BSA concentration, pH of medium, incubation time, paddle rotation speed, paddle position on the sucralfate-BSA protein binding were investigated. The sample was filtered through 0.45 μm PVDF filter and the concentration of free BSA in each aliquot was determined by a validated HPLC method. The percent binding was calculated using the following equation: Percent Binding = (C_{blank} - C_{freeBSA})*100/C_{blank}. For each test condition, at least 3 repeat samples were conducted. Three different lots of sucralfate suspensions were tested with the optimized test conditions.

Results
In all of the studies, only the placebo samples exhibited negligible change in albumin concentration over time indicating BSA was stable under the experiment conditions. The method employing dissolution apparatus 2 with mini paddles demonstrated higher reproducibility with RSD less than 10% for most sampling points as compared to the centrifuge tube method and the auto-sampler enabled more sampling points for this method which revealed the whole binding profile rather than only equilibrium binding. The 150 rpm paddle speed provided enough agitation to keep sucralfate particles suspended. Sucralfate exhibited high protein binding% only in acidic medium (pH 1-2), while very limited binding% was observed in medium with pH higher than 2.5. The binding rate, affinity constant and capacity constant were calculated for all samples tested. The method utilizing dissolution apparatus 2 with mini paddles was effective for comparing the protein binding of various sucralfate formulations manufactured in house.

Conclusion
Since the activity of sucralfate involves binding to the proteinaceous exudate at the ulcer site, studying the protein binding of sucralfate is crucial to evaluate its performance. Experimental factors that were found to impact the BSA protein binding of sucralfate were sucralfate dose, BSA concentration, time of incubation and pH of the medium. With the implementation of standardized instrumentation, the dissolution apparatus procedure was found to be a robust and discriminating in vitro method for the in vitro bioequivalence evaluation of sucralfate suspension.