This Week's Citation Classic


A bifunctional reagent, ferric chloride in acetic acid, was designed for the determination of serum cholesterol. One function was to precipitate the serum proteins while leaching cholesterols from their lipoproteins and solubilizing them in the acetic acid. The other function was to become the color reagent when an aliquot was mixed with sulfuric acid. [The SC!~ indicates that this paper has been cited in over 445 publications since 1961.]

B. Zak
Department of Pathology
Wayne State University School of Medicine
and
Detroit Receiving Hospital
University Health Center
Detroit, MI 48201

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"It is devastating when one is criticized in the literature for what appears to be a serious error. It is even worse that such a happening could warrant any credence. As a novice clinical chemist at the time, the potential procedural interference that surfaced in my case was the possibility of a Hopkins-Cole reaction with the tryptophane of the serum proteins. This generated an overlapping spectrum to the one formed by cholesterol, the analyte of interest, when using a ferric chloride reaction in a sulfuric acid-acetic acid medium. The tryptophane reaction requires that glyoxal, a natural impurity of acetic acid, be present. Without it, no such side reaction would take place. In addition, and ideally for the interference, the Hopkins-Cole reaction works well when ferric iron is present. We had inherited 24 one-lb. bottles of glyoxal-free glacial acetic acid from a recently departed professor who had obtained it for some project of his own, a project about which we knew nothing. Using this purified solvent, we optimized a reaction we had developed for the determination of cholesterol in rabbit serum and then applied it to a direct reaction using whole serum rather than an organic extract in the reaction medium. Because of the absence of glyoxal, we obtained results that were similar to those that we arrived at on comparing this direct procedure to an acceptable extraction.

"We described the process as one using 100 percent glacial acetic acid. Had others known this and used a purification process such as a Kuhn-Roth treatment, then the tryptophane interference could have been avoided as it was in our own studies using the manufacturer purified glacial acetic acid. This publication was popular and it became a Citation Classic."

"Another naturally occurring interference was bilirubin. It, like tryptophane, has been a critical substance because it generates a side reaction on its easy oxidation to a stable bilirubin. This resulted in an additive error of 0.7 mg of cholesterol per mg of bilirubin. However, the popular Liebermann-Burchard reaction, which is still commonly used in automation, results in an error of at least 5 mg of cholesterol per mg of bilirubin. Because of some criticism on the tryptophane problem, we began work on the development of procedures that would minimize it as an interference and thus evolved two extraction procedures that removed the proteins and their tryptophane as well as much of the bilirubin. The first of these, also a Citation Classic, became popular with researchers looking for what they must have considered to be more suitable cholesterol methodology than those available to them at the time.

"I believe that the method that was developed became a Citation Classic because it offered a simple way to remove all proteins, at the same time easily leaching the cholesterols from their binding sites on the lipoproteins. In a somewhat novel approach, the precipitating agent was also the color reagent when it was mixed with sulfuric acid, the final reagent. Another advantage afforded to investigators along with procedural simplicity was the formation of a stable, intensely colored equilibrium form that provided a molar absorbivity about eight times that of the unstable Liebermann-Burchard reaction. This allowed one to apply the procedure and its stable reaction to a large number of small samples, making it eminently suitable for small animal research.

"On surveying the literature for a comparison to other procedures for the determination of cholesterol in biological fluids early on, a review of the available methodologies resulted. Since that time, requests have been received to write four subsequent reviews where the latest contributions include a description of the more modern use of enzymes as reagents in spectrophotometric procedures allowing a more selective approach to the assay of serum and cerebrospinal fluid cholesterol."


