

STUDY OBJECTIVE

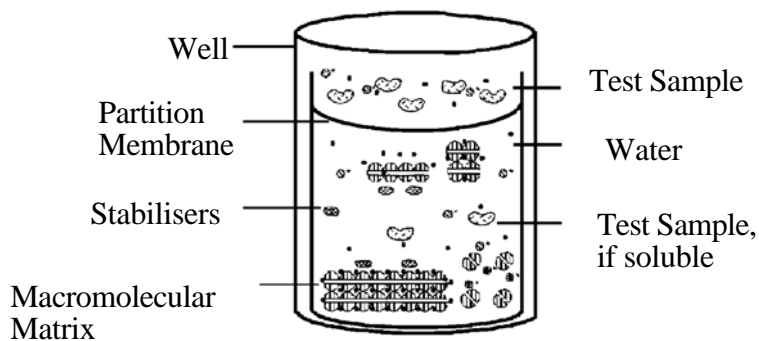
A sample provided by Example was evaluated with the Irritation Assay System in order to predict potential to cause irritation. The test was conducted on 20/8/2002.

To achieve this objective, a standard volume-dependent dose-response study was performed using the Ocular Irritation test method.

BACKGROUND

The proprietary Irritation assay is a standardized and quantitative *in vitro* test which utilizes changes of relevant macromolecules to predict the acute ocular irritancy of chemicals and chemical formulations. This assay depicted schematically in Figure 1 below, is based on the principle that chemical compounds will promote measurable changes in target biomolecules and macromolecular structures. Previous studies have clearly demonstrated that the processes of protein denaturation and disaggregation that are induced in this *in vitro* assay mimic the effects that are produced when these types of irritants are applied to the eye. Consequently, this *in vitro* test may be employed to predict the *in vivo* toxic effects of chemicals and formulations.

Figure 1. The Irritation Model



Additionally, the Irritation assay system provides significant benefits when compared to the *in vivo* Draize test method. The quantitative Irritation *in vitro* assay has been found to be highly reproducible. Of even greater relevance, the Irritation assay method can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, the test serves as an extremely useful screening tool that facilitates all stages of raw material selection, formulation development and final product selection. The optical density was detected spectrophotometrically at a wavelength of 405 nm

MATERIALS AND METHODS

The Irritation assay is a quantitative *in vitro* test method that mimics an acute ocular irritation test. To perform this standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution.

The irritancy potential of a test sample is expressed as an Irritation Draize Equivalent (IDE) score. This score is defined by comparing the changes in optical density (OD405) produced by the test material to a standard curve that is constructed by measuring the increase in OD405 produced by a set of Calibration substances.

Table 1. Relationship of Irritation Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritation Test Method

Irritation Draize Equivalent (IDE)	Predicted Ocular Irritancy Classification
0.0 - 12.5	Minimal Irritant
12.6 - 30.0	Mild Irritant
30.1 - 51.0	Moderate Irritant
51.1 - 80.0	Severe Irritant

A detailed description of the Irritation test procedure may be found in InVitro International's Irritation® Assay System Instruction Manual. All data are calculated and analyzed via a computer program which determines assay result acceptance based upon qualification parameters defined in the program. In general, the program has been designed to accept sample data as qualified if the following criteria are met: the OD values of Calibrators and internal Quality Control samples fall within previously specified ranges; sample blanks are less than 500 optical density (OD) units; the net sample OD is greater than -15; and an Inhibition Check is negative.

