Chapter 4

DISCUSSION

The Minimal Inhibitory Concentrations of preservatives were determined by the tube dilution method. This method was used mostly in determination of the strength of any preservative to be incorporated in pharmaceutical preparations. However, this method was not often used when the preservatives or bacteriostatic agents caused precipitation or cloudiness to nutrient medium. In the case of phenolic compounds and colored fluid types, the Minimal Inhibitory Concentrations were determined by the plate dilution technique of Blair, et al (12).

The Minimal Inhibitory Concentrations gave the approximate amount of preservative to be used in the formula. Such concentration was not corrected when some other ingredients were added in the formula of liquid preparation. The preparation of two-phase system presented the interesting problems. The results from these experiments indicated that concentration of preservative incorporated in the formula was reduced by many factors. In oil-water system the preservative distributed in both oil-phase and water-phase, and it was found that the preservative concentration in aqueous phase was reduced. Formulation and quality of oil could have a marked influence on the available amount of preservative used in cream or emulsion. The partition coefficients of arachis oil-water

system were high when compared with those of liquid paraffin-
water system. This showed that creams prepared from vegetable
oil gave more trouble on preservation than those prepared from
mineral oil.

The initial contamination of microorganisms in pharma-
ceutical preparations have some effect on the available amount
of preservative, the higher an initial level of contamination
the lower the amount of preservative remained in the system.
The uptake of preservatives by the bacterial cells was repre-
sented by absorption isotherms which was shown in Figure 1 to
13 (page 54 to 66). Those of organomercurial compounds (phenyl-
mercuric nitrate and thimerosal) were the exception, the
isotherms for the uptake of preservatives by Pseudomonas
aeruginosa were resembled to the tupe L isotherm of Giles and
others (31). The uptake isotherms of phenylmercuric nitrate
and thimerosal were resembled to the type S isotherm.

Bean and Das (1966) described an absorption isotherm
of phenols by Escherichia coli in which the initial portion
was linear, and suggested that for dilute solution the amount
taken up was proportion to the initial concentration of the
bulk aqueous phase, an uptake mechanism behaved as though the
phenol was partitioned between the cells and aqueous solution.
At higher concentrations the absorption isotherms indicated
an increase in uptake of phenol by the cells, and this continued
to rise with increasing bulk concentration (9).

The results of uptake pattern in these experiments showed that an increase in the concentration of preservatives led to an arising in uptake until the plateau was reached and the uptake pattern of all preservatives were varied. Many ingredients used in the formulation of pharmaceutical preparation were liable to be contaminated themselves, as well as forming ideal substrates for the growth of microorganism. This problem could be avoided by ensuring that the initial level of contamination was low and that an adequate concentration of the preservative was used, not merely the Minimal Inhibitory Concentration.

Equilibrium dialysis has proved satisfactory for evaluating interaction between a number of preservatives and surface active agent. The disadvantage was the time taken, about 4 to 5 days required for equilibrium to be reached. The results in Table 6 indicated that the ratio, R, of total preservative in solution to the concentration of free preservative was a function of the concentration of Tween 80. These data showed some evidence that considerable interaction had taken place between the preservative and surfactant. R value used to determine the quantity of preservative added to the preparation containing a known concentration of Tween 80 in order to make the required concentration of free preservative. Patel and Kostenbauder (1958) suggested that the multiplying of the required concentration of free preservative

by the appropriate R value gave the concentration of total
preservative which must be employed (47).   The plot of R ratio
against the concentration of nonionic (Tween 80) regression
was a straight line passing through R = 1 in the absence of
nonionic even though several concentrations of nonionic were
used, the slope must be independent of the preservative con-
centration.   In other words the degree of binding between
preservative and Tween 80 was dependent on the nonionic con-
centration and independent on the preservative concentration.
The slope of the regression was a parameter of a preservative-
nonionic combination.   The greater the slope the greater the
binding between preservative and nonionic.