aim of the investigations was to study the validity of the "cubic rule" [1,2] close to the critical point. The determination of the co-existence curves themselves, and the constant of formula (1) for the two mixtures was regarded as being of secondary importance.

The investigation of the triethylamine-water system was complicated by the fact that, at temperatures above, but closer to the critical, the two phases formed did not show the homogeneous composition which is to be expected. Furthermore, at temperatures below the critical, fog was occasionally seen to form inside the prism. After about an hour the fog would disappear, and the section of the concentration curve previously veiled by fog was then seen to have attained an arched form different from the arched concentration curves obtained by diffusion.

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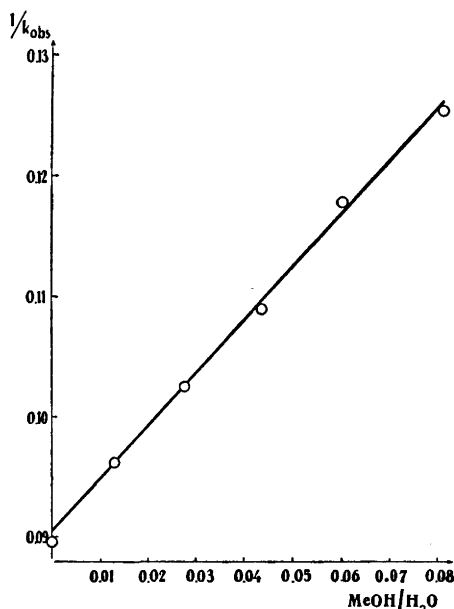3. Lorentzen, H. L. and Bergsnov Hansen, B. *Acta Chem. Scand.* **11** (1957) 893.

Fig. 1. Weight ratio of methanol and water in the resin phase *versus* $1/k_{obs}$ at the concentration range of 0 to 5 % methanol in the outside solution.

Reaction Kinetic Determination of the Hydroxyl-Alkoxide Equilibrium in Anion Exchangers

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As a sequel to a previous work on the reaction mechanism between estradiol benzoate and an anion exchange resin [1], a kinetic method was developed which is reported here. This method makes it possible to follow the rate of hydrolysis (or alcoholysis) at very low ester concentrations. The method has been used for the investigation of the hydrolysis of methyl benzoate in water and in water-methanol mixtures of low methanol content. The experiments were carried out at $25 \pm 0.02°C$, by a batch-wise technique with constant stirring. At suitable time

intervals samples were taken from the reaction mixture by means of a constant pressure apparatus. The amount of unreacted ester was estimated spectrophotometrically in the ultraviolet region.

According to Samelson and Hammet [2], the alkaline hydrolysis by anion exchangers in ethanol-water mixtures is a second order reaction. The present results show that when the hydroxyl ion concentration (the resin) is high compared with the ester, the rate constant can be calculated from the first order equation.

On adding methanol to the resin-water mixture the hydroxyl ions are in part converted into alkoxide ions owing to the reaction:

$$MeOH + HO^- \rightleftharpoons MeO^- + H_2O$$
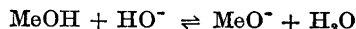
and the hydroxyl ion concentration in the resin phase is decreased. If this reaction is the dominant factor in reducing the hydrolysis rate, it should be possible in theory to calculate the state of this equilibrium from the observed kinetic data. In fact, it was found that at low methanol

concentrations, up to about 10 % (w/w), the equilibrium constant

$$K = \frac{[MeO^-][H_2O]}{[MeOH][HO^-]}$$

can be calculated from the following equation:

$$\frac{[MeOH]}{[H_2O]} \cdot K + 1 = \frac{k}{k_{obs}}$$

where k is the true rate constant, *i.e.*, the same as in aqueous solution, k_{obs} the observed rate constant, and [MeOH] and [H$_2$O] the molar concentrations of methanol and of water, respectively, in the resin phase. The linear relationship between the values of [MeOH]/[H$_2$O] and $1/k_{obs}$ was verified by the data obtained from Fig. 1, and the resulting value for K was found to be 11.1. This equilibrium in anion exchangers has thus been determined for the first time.

A detailed description of the procedure as well as of the mathematical treatment will be published in the near future. Investigations on the hydrolysis of ethyl benzoate in water-ethanol mixtures are still in progress. Furthermore, the study of the saponification of several phenolic esters by this method has been started.

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Action of Group Blocking Agents and Enzymes on Intrinsic Factor Preparations

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The binding of vitamin B$_{12}$ appears to be of importance for the physiological action of intrinsic factor [1,2]. It therefore

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seemed of interest to study the mode of binding of B$_{12}$ to the B$_{12}$-binding principle contained in intrinsic factor preparations. This was performed by subjecting hog intrinsic factor concentrates and pepsin-inactivated [1] human gastric juice to group blocking agents. During the course of attempts to break down the B$_{12}$-binding principle with proteolytic enzymes some data were obtained bearing upon the mode of binding of B$_{12}$, and these results are included in the following together with some data on the physiological activities of the chemically modified preparations.

Vitamin B$_{12}$-binding was assayed by adding radioactive ^{60}Co labeled B$_{12}$, whereupon the free and bound vitamin was separated with dialysis [1] or ultrafiltration [3].

The action of the group blocking agents is apparent from Table 1. A clearcut effect is observed with fluorodinitrobenzene, iodine, and monochromatic (280 mμ) ultraviolet light, whereas treatment with *p*-chloromercuribenzoate, acetic anhydride, and nitrous acid lacked effect. The absence of effect of the three last mentioned compounds makes the involvement of SH- and NH$_2$-groups less likely, whereas the involvement of phenol or imidazole groups seems more probable. An active tyrosinase preparation (Worthington) lacked effect, however. This does not eliminate the possibility of a phenolic group being involved, because tyrosinase has been found to lack effect on tyrosine groups of several proteins [4]. The mode of binding of B$_{12}$ to intrinsic factor preparations seems to be much the same as in the cobalamin protein of sow's milk [3].

When both gastric juice and hog stomach preparations were subjected to thorough digestion with crystalline pepsin, trypsin, and highly purified chymotrypsin and mucinase [5] preparations, addition of B$_{12}$ prior to digestion was found to have a marked protective action against chymotrypsin. A slight similar effect was observed in the case of pepsin, whereas B$_{12}$ did not influence the effect of trypsin and mucinase. The latter were found not to have any effect on B$_{12}$-binding.

The protective action of B$_{12}$ against chymotrypsin seemed to be limited to very few components of the intrinsic factor preparations. This was shown by digesting two identical samples, one in presence of B$_{12}$, and the other one in its absence. After digestion, vitamin B$_{12}$ was added to the second sample and both samples