error, 0.139, is for the acid-catalyzed hydrolysis of acetate esters in water at 25° (see footnote, Table II). Equation (1) covers variations in relative rate as large as approximately one million-fold. All comparisons of rates have been made at a temperature of 25° (or 30° in a few cases).

For ortho-substituted benzoates, Eq. (1) fails for the substituents H, F, NH₂ and OH. The former, H and F, follow the Hammett relationship,[27] that is, they show no ortho effect, while the latter, NH₂ and OH, probably show departure because of internal H bonding.[28]

Corresponding values of the substituent constant, A, for acid and base catalysis, listed in Table I,

(27) Reference 1, p. 206.

(28) G. E. K. Branch and D. L. Yabroff, This Journal, **56**, 2568 (1934).

in most cases differ widely and bear no immediately obvious relationship to one another or to the English school theory of polarity of substituents.

The small effect of solvent on f indicates that the freezing out of solvent molecules in the formation of the transition state by bulky o-benzoate substituents or highly branched aliphatic groups in the acyl or alkyl component of an ester cannot be an important factor in determining relative rates for these reactions.

The much greater dependence of f values on structure in the acyl than the alkyl component may be the result of greater rigidity in the latter required by resonance in the carbalkoxy function.[29]

(29) See discussion by G. W. Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 69, 70.

State College, Penna.

The Hydrogenation of Ethylene over Copper. I. Reproducing the Activity of the Catalyst

By Charles Law McCabe and G. D. Halsey, Jr.[1]

One of the factors which determines the activity of a reduced copper catalyst toward the hydrogenation of ethylene is the amount of chemisorbed hydrogen on the catalyst surface. The kinetic experiments show that this hydrogen does not serve as a reactant. It thus can be considered to function as part of the catalyst. Three distinct methods of getting varying amounts of chemisorbed hydrogen of the surface are employed and the activity of the catalyst as a function of the parameter governing the amount of chemisorbed hydrogen on the surface is reported. Control of this coverage of chemisorbed hydrogen allows the activity to be reproduced to within 5% over a series of 50 runs.

Introduction

In recent years the technique of making evaporated metal films[2] has been adapted to the study of catalytically active films; indeed, this technique has largely replaced the older hydrogen-reduced catalysts of the type investigated here. Although this new method allows the preparation of generally more active and "cleaner" catalysts, it suffers from one unfortunate defect. The films so prepared cannot be restored to their original activity or put through any cycle that tests the reversibility of a loss in activity. For example, Beeck, Smith and Wheeler's nickel films, after each run on ethylene hydrogenation following the initial run, suffered small but progressive losses in activity which can be variously ascribed to impurities in the reactant gases, progressive poisoning of the surface by reactants or by-products, or loss of surface area by gumming or sintering.

We have reinvestigated the copper catalyst of Pease[3,4] for the hydrogenation of ethylene to ethane. The catalyst we have investigated, while never free of the hydrogen used in preparing it, can be returned at will to a standard activity by treatment with hydrogen. This ability to return to reference activity shows that the actual copper surface is not irreversibly altered. In the work reported here,

(1) University of Washington, Seattle 5, Washington.

(2) O. Beeck, A. E. Smith and A. Wheeler, Proc. Roy. Soc. (London), **177**, 60 (1940).

(3) R. N. Pease, This Journal, **45**, 1196 (1923).

(4) R. N. Pease and L. Stewart, ibid., **47**, 1235 (1925).

the copper surface and the initial pressures of the reactant have been maintained unchanged.

Experimental

(a) **Materials and Apparatus.**—The catalyst was prepared by the reduction of 249.0 g. of Mallinckrodt CuO (wire form) with hydrogen at 130° for four days. The temperature was then raised gradually to 315° over a period of 3 hours; the temperature was lowered and held at 200° for 5 hours. The hydrogen outlet was then sealed off, and the catalyst was cooled slowly to room temperature with 1 atmosphere of hydrogen over it.

Circulation of reactant gases was unnecessary since the catalyst completely filled the bulb. The catalyst was protected from mercury vapor by Dry Ice traps inserted between the manometer and catalyst bulb. Poisoning by mercury is irreversible (Pease, ref. 3), and thus the ability to return to standard activity that emerges below is sufficient proof that the Dry Ice trap is adequate protection against mercury.

Electrolytic hydrogen was purified by passing it over platinized asbestos at 200° and freezing out the water formed with a Dry Ice trap followed by a liquid-nitrogen trap packed with glass wool. Mathieson C.P. grade ethylene was purified by condensation with liquid nitrogen, followed by alternate melting, freezing and pumping until air was apparently removed. As a final precaution the ethylene was passed through an activated-charcoal trap at −79°, condensed, and pumped off until it was all condensable. Mathieson helium was passed through an activated-charcoal trap in liquid nitrogen and stored with no other precaution. Absence of any poisoning effect showed this purification to be adequate.

The catalyst bulb was connected to the usual vacuum system. Pressure was measured on a mercury manometer equipped with a buzzer. A reading telescope was used to determine coincidence with the graduations of a mirrored scale to better than 0.1 mm.

The dead space of the reaction bulb was 100 cc. The

BET surface area was 0.28 sq. meter per gram, corresponding to V_m = 12.5 cc. for the entire catalyst.

(b) **Measurement of Activity.**—A 1:1 mixture of hydrogen and ethylene prepared in advance and mixed over a hot light bulb was introduced from a thermostated gas buret. The amount introduced was sufficient to produce an initial pressure of 35.0 cm. on the basis of helium dead space. On the basis of this quantity of mixture, the activity was arbitrarily defined as 100 times the reciprocal of the *time in minutes that it took the pressure to decrease* from 28.50 to 22.35 cm. This arbitrary activity figure was very closely proportional to the apparent initial slope of the pressure *versus* time curve, but could be reported with considerably greater accuracy because of the necessary uncertainty of taking the derivative of an extrapolated curve. The product of total reaction was completely condensable and showed no ethylene upon infrared analysis. No attempt was made to measure isotherms for the separate gases, since the mixed isotherm of the three gases present during a reaction is a non-equilibrium isotherm not directly related to the separate isotherms. We are investigating here the problem of catalyst reproducibility, as a preliminary to elucidating the kinetics. Our experiments were designed therefore to measure activity in the most accurate manner, rather than the most significant manner. For example, we used a simple manometer for accuracy and convenience, rather than a constant or zero volume manometric system. All activities were determined at 0°.

The Standard Activity and the Pressure of Reduction.—The initial activity of the catalyst after the first reduction is rapidly lost in a series of conventional experiments. A repetition of all, or of the final part of the initial reduction schedule, although it revives the activity of the catalyst, does not restore the original activity with any accuracy. Reoxidation and rereduction is no more satisfactory. If the customary flow process of reduction is used, the final activity of the catalyst is a sensitive function of the exact purity of the hydrogen and other inherently ill-controlled variables. If the catalyst is cooled before flow is stopped the activity declines as the volume of flow increases. Clearly, then, the original reduction procedure is not suitable for attaining a standard activity. If the catalyst is stored with hydrogen at room temperature and pressure for several weeks the activity rises and even surpasses the initial activity.

If the catalyst is stored in a vacuum, hydrogen is evolved, and the activity declines. It is evident from all these facts that a standard activity is difficult to reach and difficult to maintain.

We found that a reproducible standard activity could be achieved by the following procedure. After a run, to completion or otherwise, or after any other experiment, the catalyst is kept in a hydrogen atmosphere at 15 cm. and 0° for one-half hour. This procedure is designed to remove gross quantities of ethylene that might conceivably affect further procedure. The catalyst is then heated with 15 cm. of fresh hydrogen (measured at 0°) for one hour at 200°. It is then cooled at the natural cooling rate of the oven to room temperature. Finally it is cooled to 0°, and pumped out. This procedure reproduces the activity to within ±5% over a series of fifty runs.

We make use of the fact that the usual drift of 5–10% in activity between successive runs can be all but eliminated by only the first part of the procedure above, a treatment with hydrogen at 0°. This treatment does not suffice if the temperature has been raised or the catalyst stored for a protracted period.

The use of a static hydrogen treatment removes the problem of the accumulation of the traces of impurities in the hydrogen. Since no material is removed from the catalyst except at 0° any water or other poison remains with the catalyst throughout a series of experiments. The achievement of reproducibility demonstrates the adequate purity of the reagent gases under these conditions, in a direct manner not realizable in the absence of a reproducible standard activity.

A reproducible curve of activity *versus* the pressure of hydrogen during the static reduction at 200° can be determined (pressure measured at 0°) (Fig. 1). The two points at lowest pressure demonstrate the reproducibility achieved. The square indicates the activity some 30 runs previously. The data represented in Fig. 1 were taken after the data reported below.

The Role of Chemisorbed Hydrogen.—In a preliminary experiment directed toward removing hydrogen "contam-
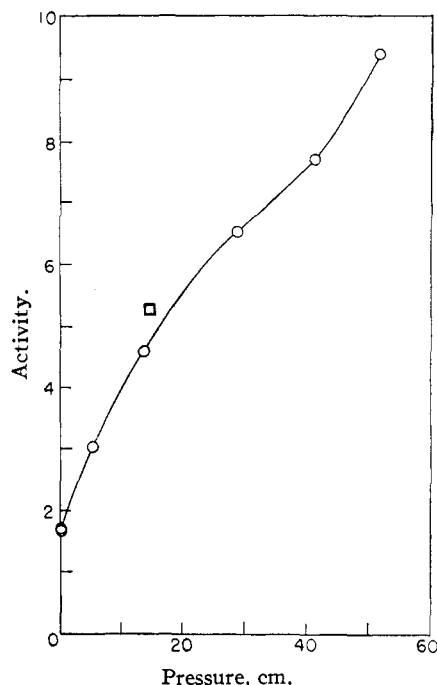

Fig. 1.—Activity as a function of reduction pressure.

inating" the surface we found that a catalyst pumped for three hours at 200° showed only one-seventh the activity of a similar catalyst pumped for one-half hour at 0°. The effect was not caused by sintering; the catalyst returned to approximately its original activity on being reduced again on the original schedule.

Treatment of the catalyst, pumped out at 200°, with hydrogen at 0° did not restore the activity, although as noted above, this treatment sufficed to eliminate drift between a series of runs with the catalyst maintained at 0° throughout. The restoration of activity is thus a rate process governed by an activation energy.

The activity was then measured as a function of hydrogen pumped off. It is difficult to pump widely varying amounts of hydrogen off at the same temperature, the first portions desorbed raise the pressure to several millimeters very quickly whereas the last portions are desorbed very slowly. Therefore the catalyst was pumped at temperatures between 0 and 200° for the same time to obtain various volumes. It was concluded that the gas pumped off was hydrogen since it was not condensable in liquid nitrogen.

The sequence of events was as follows: The catalyst was initially put through the process of section 3 to gain standard activity, but no run was made. It was then pumped out at the desired temperature using a diffusion pump discharging into a Toepler pump. Next, the volume of the hydrogen was measured. The catalyst was then cooled quickly and let sit in ice for one-half hour. Finally, the activity was measured, and the catalyst was returned to standard activity. Activity as a function of volume of hydrogen pumped off is shown in Fig. 2.

If, instead of proceeding in a systematic fashion, we remove different amounts of hydrogen by pumping at various temperatures for different lengths of time, a single-valued curve of activity *versus* hydrogen pumped off does not result. It is clear, then, that not only the amount of hydrogen on the surface, but its distribution affects the activity of the catalyst.

The same behavior can be shown in a different manner. If, after the catalyst has been heated with hydrogen at 200° in the standard manner, it is cooled to temperature T at the normal cooling rate of the furnace and then plunged into ice, the activity is lower the higher the quenching temperature. These data are given in Fig. 3. Here it is clear that hydrogen adsorbed between 100 and 140° is most important in fixing the activity. Adsorption experiments show that the excess of the volume of hydrogen adsorbed on slow cooling over that adsorbed on quenching is 0.2 cc. at S.T.P., approximately 2% of V_m.
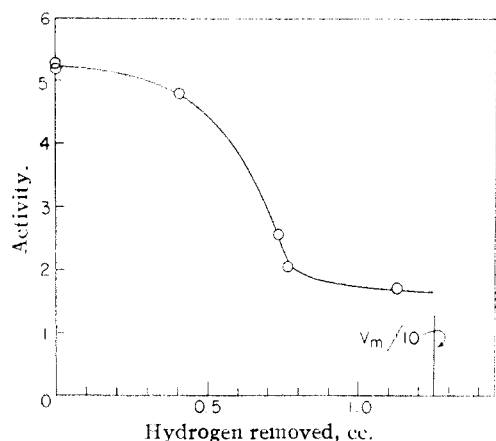
Fig. 2.—Activity as a function of the volume of hydrogen pumped off.

We at first supposed that the data of Fig. 2 could be explained by the diffusion of oxygen and water from the interior of the catalyst. However, the experiment of Fig. 3 shows that this is not so. In the pumping-off experiments the absence of hydrogen during the pumping and the varying temperatures of pumping indeed would allow differing concentrations of oxygen to accumulate on the surface. However in the quenching experiments the same high pressure of hydrogen is always present to remove oxygen. The short temperature range over which the loss of activity is encountered also indicates an activated process other than diffusion.

This second experiment also indicates that the hydrogen actively involved is not in ordinary bulk solution. The endothermic solution of hydrogen in copper would require more hydrogen to be dissolved at high temperature and thus quenching, if anything, would cause more hydrogen to be retained than would slow cooling. The experiments show the opposite to be true.

Conclusion

It appears from these data that hydrogen

chemisorbed in the neighborhood of 120° has a marked effect on the activity of a reduced copper catalyst.
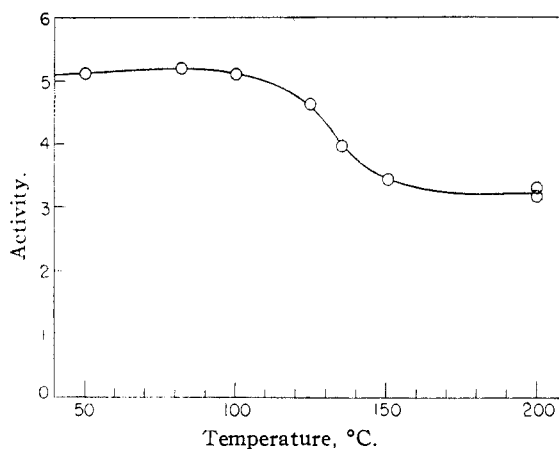

Fig. 3.—Activity as a function of quenching temperature following slow cooling from 200°.

The adsorption of this critical hydrogen does not take place at an appreciable rate at 0°; it is therefore activated adsorption.

Since, once this hydrogen is adsorbed, activity can be maintained by treating the catalyst with hydrogen at 0°, the chemisorbed hydrogen is not removed by reaction; it thus constitutes part of the catalyst, and is not a reactant.

Chromatography of Proteins.[1]   Frontal Analysis on a Cation Exchange Resin

By Herbert A. Sober, Gerson Kegeles and Frederick J. Gutter

Chromatographic frontal analysis on a column of a sulfonic acid cation exchange resin has been applied to a mixture of proteins from an egg white albumin fraction and to artificial mixtures of the purified proteins, bovine plasma albumin and human carbon monoxide hemoglobin. The effluent from the column was examined continuously by refractometric methods. Correct quantitative analyses have been obtained in the pH range of 5.6–8.6 when the protein mixtures were equilibrated with 0.01–0.10 ionic strength buffers. Cation exchange is not an essential prerequisite for adsorption and resolution of these protein mixtures although specific cation effects have been observed. Adsorption of protein by this cation exchange resin is small and concentration dependent. The effluent from frontal analysis of these protein mixtures through this cation exchange resin showed no change in charge or weight distribution. Quantitative elution analysis shows no irreversible binding of bovine plasma albumin. It is proposed that homogeneity in chromatographic analysis be considered one of the required criteria of protein purity.

Introduction

Chromatography, a technique of considerable versatility, has had little application to the study of proteins, probably because they are easily denatured and must be handled with very mild techniques. Enzyme chemists, following the lead of Willstätter, however, have been using adsorption and elution in batch processes[2] for some time. Al-

though occasional reports have appeared on the use of chromatography in the purification of crude solutions of proteins,[3] it is only in recent years, since the appearance of the optical methods of Tiselius[4,5] and Claesson,[6,7] the partition chromatography of

(1) Presented in part at the 117th National Meeting of the American Chemical Society, Philadelphia, Penna., April, 1950.

(2) E. Bamann and K. Myrbäck, "Die Methoden der Enzymforschung," G. Thieme, Leipzig, 1940, pp. 1452-1466.

(3) L. Zechmeister, "Progress in Chromatography, 1938-1947," Chapman and Hall, London, 1950.

(4) A. Tiselius, Trans. Faraday Soc., 33, 524 (1937).

(5) A. Tiselius, Arkiv. Kemi, Mineral., Geol., 14B, No. 22 (1940).

(6) S. Claesson, ibid., 23A, No. 1 (1946).

(7) S. Claesson, Ann. N. Y. Acad. Sci., 49, 183 (1948).