A NEW ENZYME PREPARATION.

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The inadequacy of the form in which enzyme preparations are at present available on the market for general laboratory use, led us to the preparation of enzyme papers which promise to offer a very convenient mode of handling enzymes quantitatively with rapidity and ease. Most of the enzyme solutions, now ordinarily employed, do not keep long, and fresh batches have to be prepared each time to ensure a desired standard of activity. If enzymes could be uniformly distributed on filter-papers to secure a definite activity per unit area of the paper, such a preparation would constitute a valuable laboratory reagent.

A successful preparation of this type should fulfil the following conditions.—(i) The active surface of the absorbing medium should be large to ensure maximum concentration of the enzyme; (ii) the adsorbed enzyme must be capable of being eluted or freed from the preparation and act upon the corresponding substrate; (iii) the concentration of the enzyme per unit area should be uniform; and (iv) the preparation must preserve its activity for a reasonable period.

Filter paper, previously employed by Wood (*J. Soc. Chem. Ind.*, 1918, **37**, 313), for purification of enzymes, was found to respond to all these requirements and has accordingly been chosen for this work.

EXPERIMENTAL.

DIASTASE.

Materials.—Substrate 0.1% solution of Lintner's soluble starch (B.D.H. analytical reagent).

Enzyme: B.D.H. diastase: A 1% solution was prepared and the filtrate incubated at 38°C. till the solution gave no colour with iodine.

Filter-paper used for absorption.—Quantitative filter paper (W. R. Balston Ltd., Genuine Whatman, No. 41, 9 cm.).

Methods.—Qualitative: Equal strips of the above filter-paper of known dimensions (20 sq. cms. and 12 sq. cms.) were cut out and 1, 2, 3, 4 and 5 drops respectively of enzyme solution placed on 5 strips. The different lots were then dried in a desiccator over sulphuric acid. These dried strips, after fine shredding (subsequently shown to be unnecessary), were introduced into a series of test tubes,

each containing 10 c.c. of substrate and after half an hour the enzyme activity tested by the Wohlgemuth method (*Biochem. Z.*, 1908, **9**, 1 ; *Ibid.*, 1909, **21**, 432). A control experiment, with the corresponding number of drops of the enzyme extract and with the same amount of substrate, was conducted.

TABLE I.

Substrate : 0·1% solution of starch, 10 c.c.
Time of reaction : Half an hour.
Temperature : Room temperature (23°).

Enzyme	Number of drops of enzyme solution				
	1	2	3	4	5
	Colour with iodine				
On strips of 12 sq. cms.	Blue	Red-yellow	Yellow	Yellow	Yellow
,, 20 ,,	,,	,,	,,	,,	,,
Pure solution	,,	,,	,,	,,	,,

Quantitative:—The results (Table I) show qualitatively that the enzyme gets eluted from the filter-paper when put into substrate solution and is active to the same extent as the corresponding enzyme extract. These experiments were repeated quantitatively, the sugar formed by the enzymic hydrolysis of starch, being estimated by Hartmann and Schaffer's Micro method (*J. Biol. Chem.*, 1920, **45**, 365). The enzyme-preparations for these experiments were obtained as follows :—The 9 cm. filter-papers used in the previous case were cut into 4 strips and after addition of just a drop (0·1 c.c.) of enzyme solution, delivered by a capillary pipette, the strip was held by a copper wire and air-dried for 24 hours.

TABLE II.

Enzyme	Volume of substrate	Time of reaction	Thiosulphate required (0·0046N)	Mg. of copper
1 drop of pure enzyme solution ..	2 c.c.	30 mins.	5·1	1·49
1 drop of enzyme solution on ¼ strip F.P.	,,	,,	,,	,,

The results (Table II) confirm the fact that the filter-paper introduced as an absorbent, does not interfere with the activity of the enzyme preparation.

EMULSIN.

Materials :—Substrate: B.D.H. salicin, 0·2% solution.
Enzyme: B.D.H. emulsin, 1·0% ,,

Methods.—The sugar obtained as a result of enzyme hydrolysis of salicin was estimated quantitatively, as in the case of diastase, by Hartmann and Schaffer's method (*loc. cit.*).

As with diastase, comparison was made between the activity of the pure enzyme solution and that absorbed by the filter-paper. A little improvement was effected in the mode of preparing the enzyme filter-paper. The filter-papers were divided into small areas by wax lines and a known number of drops of enzyme solution was put into each of these wax-circumscribed strips. These strips, after drying, were cut out and their activity determined. The wax at the edges has the advantage of limiting the loss of enzyme while handling the preparations as also of preventing creeping and concentration of the enzyme at the edges.

TABLE III.

Enzyme	Substrate in c.c.	Time of reaction	Thiosulphate required (0·0047N)	Mg. of copper
5 drops of pure enzyme	2	15 mins.	12·8 c.c.	3·86
,, ..	,,	,,	,,	,,
5 drops on F. P. ..	,,	,,	12·6 c.c.	3·80
,, ..	,,	,,	12·8 c.c.	3·86

INVERTASE.

Experiments with invertase extract absorbed on filter-paper gave a low value compared with the free enzyme extract showing that the enzyme was not completely eluted from the filter-paper. Yeast autolysate, filtered through Kieselguhr, with a total solid content of about 2 per cent. was employed as the source of the enzyme and the yeast gum associated with such a preparation is probably responsible for the inefficiency of this filter-paper preparation.

Preparation of coarse filter-paper for enzyme absorption.—With a view to increase the absorption of the enzyme extract and thereby

secure a higher concentration of the enzyme, a coarse type of filter-paper, with loosely bound fibres, was prepared. Filter-paper shredded to a fine suspension in water was spread into thin sheets on wire gauze, most of the water drained off and then dried in a hot-air oven. The dried sheets were cut into circular strips and used for enzyme preparations under conditions identical with those described previously.

The efficiency of the coarse-filter preparation, offering a greater volume of capillary space for the retention of the enzyme extract as compared with the ordinary filter-paper, was found to be greater (Table IV).

TABLE IV.

Filter-paper	Weight of solid per gm. weight of filter-paper	Time taken to hydrolyse 5 c.c. of 1% starch solution (Wohlgemuth)
Ordinary	106 mg.	32 mins.
Coarse	326 mg.	12 ,,

Preservation Experiments.—9 cm. filter-paper used formerly with an average weight of 670 mg., were dipped for 10 minutes in enzyme solution and dried in a desiccator over sulphuric acid. The quantity of solid retained by the paper was found in each case.

TABLE V.

Weight of filter-paper ..	671 mg.	679 mg.	615 mg.
Weight of solid absorbed ..	72·4 mg.	76 mg.	65·2 mg.
Weight of solid absorbed per gram of the filter-paper ..	107·9 mg.	111·9 mg.	106 mg.

For preservation, the filter-paper with the maximum absorption, namely, 111·9 mg. of solid per gram weight of paper, was used. The preparation was preserved in a stoppered bottle and the activity determined after over a year.

TABLE VI.

Date	Substrate	Quantity of F. P.	Time for reaching end-point (Wohlgemuth)
11–5–1931 ..	3 c.c. of 1% starch	¼ F. P.	20 mins.
13–7–1932 ..	Same solution	,,	,,

DISCUSSION.

Experiments with diastase and emulsin have shown (Tables I, II and III) that these enzymes absorbed on filter-paper act on their corresponding substrates to the same extent as when they are free in extracts. In the case of invertase, however, the enzyme papers gave a lower value, which was found to be due to the associated yeast gum present in the yeast autolysate. This emphasises the need for purifying enzymes, if a more general application of the method is desired. A greater absorption of the enzyme extract resulting in a greater concentration of the enzyme (Table IV) is secured by employing coarse filter-paper. Diastase-enzyme preparations have been found to retain their activity unimpaired for over a year (Table VI). Obviously this technique cannot be extended to hygroscopic enzyme preparations.

This investigation has indicated the possibility of preparing enzyme-papers and they have been employed in this laboratory with advantage; it is believed that its usefulness will be appreciated by those who are called upon to handle enzymes frequently in their analytical work. Urease papers, for example, would be very useful to medical men, diastase papers to starch chemists, while invertase papers should prove useful in the analytical laboratory of sugar factories. It is hoped that ere long this process will be commercialised.

The work is being extended to the preparation not only of other enzyme papers but also of substrate papers, both of which should constitute useful laboratory reagents.

SUMMARY.

1. The preparation of enzyme filter-papers offers a convenient method of handling enzymes quantitatively with rapidity and ease.

2. Preparations of diastase and emulsin have been shown to be as active as the corresponding quantities of the enzyme extract in their free states, thus establishing that the filter-paper introduced as an absorbent, does not interfere with the activity of these preparations.

3. In the case of invertase, however, the filter-paper preparations gave a lower value, which has been attributed to the yeast gum associated with our invertase extract.

4. The activity of the preparations has been found to keep unimpaired for over a year.

5. The possibility of manufacturing these enzyme papers and also the corresponding substrate papers as useful reagents, is indicated.

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