would lead to useful results. Attempts have been made by Boscott[2], using cellulose acetate, and by Boldingh[3], using rubber powder, to hold the less polar phase on the inert support, and recently Howard and Martin[4] have described the use of kieselguhr, impregnated with silane, for a reversed-phase partition column.

However, all these methods involve difficulty in the preparation of a homogeneous support. We have now developed a reversed-phase chromatogram using a commercial chlorinated rubber ('Alloprene', I.C.I., Ltd.; extra high viscosity grade *E*) for the separation of the N-2 : 4-dinitrophenyl derivatives[5] of amino-acids by partition between butanol and aqueous buffer. Chlorinated rubber is a light cream-coloured, free-flowing powder, soluble in non-polar organic solvents (for example, benzene) but capable of retaining alcohols such as butanol in a partition chromatogram. The chlorinated rubber (150-200 mesh/in.) was prepared by shaking it with a suspension of butanol (4 ml. per 10 gm. of chlorinated rubber) in 0·2 *M* citrate – phosphate buffer previously saturated with butanol. The slurry thus obtained was used for packing the columns by filtering under slightly reduced pressure in the usual way. The *R* values of a number of dinitrophenyl amino-acids using butanol-saturated buffer of varying *p*H as the flowing solvent are given in the accompanying table. It is seen that the order of *R* values obtained is with a few exceptions the reverse of those obtained by Sanger[5] using silica gel columns and by Partridge and Davis[6] using buffered filter paper.

R-VALUES OF DINITROPHENYL AMINO-ACIDS ON CHLORINATED RUBBER COMPARED WITH R_F-VALUES ON BUFFERED FILTER PAPER[6]

Supporting substance	Allo-prene	Allo-prene	Allo-prene	Filter paper
Solvent	*n*-butanol	*n*-butanol	*n*-butanol	*Tert.*-Amyl alcohol
*p*H of buffer	*p*H 3	*p*H 4	*p*H 5	*p*H 6·75
ε-Dinitrophenyl-lysine	1·15	—	—	0·40
Dinitrophenyl-asparagine	0·83	—	—	0·23
,, -serine	0·61	1·14	—	0·26
,, -aspartic acid	0·40	0·96	—	0·03
,, -glycine	0·33	0·79	—	0·27
,, -alanine	0·18	0·58	—	0·43
,, -proline	0·17	0·54	1·14	0·51
,, -valine	0·10	0·11	0·52	0·74
,, -leucine	0·08	0·09	0·35	0·86

We have used the columns to separate various mixtures of dinitrophenyl amino-acids, including a mixture containing dinitrophenyl-glycine, -serine, -alanine, -valine, -leucine and ε-dinitrophenyl-lysine. The order in which the compounds were eluted from the column was confirmed by identifying the eluted bands by use of paper chromatograms. We have also shown that recovery of dinitrophenyl derivatives (estimated spectrophotometrically : λ, 358 mμ) from these columns is substantially quantitative. Dinitrophenyl-serine, for example, was eluted in 96–99 per cent recovery in four replicate experiments. However, dinitrophenyl derivatives of the aromatic amino-acids tyrosine and phenylalanine are not fully eluted from the column, and dinitrophenyl-glycine appears to suffer partial decomposition.

The shape of the concentration peaks in the effluent solution was investigated by taking fractions at frequent intervals and estimating the amino-acid present in each fraction. The peaks were sharp and usually nearly symmetrical; but in certain cases (for example, dinitrophenyl-valine and -leucine) the slope at the front of the peak was rather sharper than that of the rear. Full details of this work will be published elsewhere.

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Treatment of Paper for Chromatography of some Colloidal Electrolytes

THE surface of filter paper, in contact with water, has a slight negative charge[1], and this may account for the non-adsorption of certain colloidal electrolytes such as dyes of the 'acid' type, when developed with water, as these also carry a net negative charge in aqueous solution. By the application of principles similar to those described by Weiss[2], a 'primary adsorbate' may be applied to paper, in this case being so arranged that the paper now has a net positive charge in contact with water. A suitable compound for this purpose is cetyl trimethyl ammonium bromide, the paper being soaked in 0·03 per cent aqueous solution and dried before use.

By development with water, mixtures of tartrazine, geranine (I.C.I., Ltd.), eosin and fluorescein have been separated on such paper using the central-feed technique[3], a noteworthy feature being the sharpness of separation and the uniformity of zones, although there is little or no space between them. Similarly, detergents of the anionic type such as sodium stearate and 'Teepol' (Shell Chemicals, Ltd.) may be removed from aqueous solution.

The location of such colourless adsorbates is conveniently demonstrated by brushing a streak of dilute solution of a weakly adsorbed dye such as tartrazine from the centre of the paper outwards during development. The 'secondary adsorbate' such as 'Teepol' has a net negative charge and does not adsorb the tartrazine, whereas the unaffected area holds it, resulting in a break in the streak. Adsorbates may be eluted by solutions of more strongly adsorbed substances, such as sodium hydroxide.

The above principles have been utilized in the detection of traces of fluorescein used in locating faults in drainage systems; identification of amounts as small as one part in ten million parts of water is somewhat uncertain by viewing directly under ultra-violet light, whereas by allowing approximately 0·1 ml. of such a solution to feed up the 'tail' of a treated No. 3 Whatman paper[3], the fluorescein thus concentrated on the 'tail' and centre of the paper, when exposed to ultra-violet light, gives an unmistakable fluorescence.

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