



**PAPER CHROMATOGRAPHIC SEPARATION OF COMPONENTS OF ROSE BENGAL LABELLED WITH IODINE - 131**

SEPARAÇÃO CROMATOGRÁFICA DOS COMPONENTES DE ROSA DE BENGALA MARCADA COM I - 131

*FAUSTO W. LIMA e RÔMULO R. PIERONI*

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**INSTITUTO DE ENERGIA ATÔMICA**  
Caixa Postal 11049 (Pinheiros)  
CIDADE UNIVERSITÁRIA "ARMANDO DE SALLES OLIVEIRA"  
SÃO PAULO — BRASIL

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OF ROSE BENGAL LABELLED WITH IODINE-131.<sup>+</sup>



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PAPER CHROMATOGRAPHIC SEPARATION OF COMPONENTS  
OF ROSE BENGAL LABELLED WITH IODINE-131.

Rose bengal (tetraiodotetrachlorofluorescein) labelled with iodine-131 is used in medicine to test hepatic function. The most used tests are those of Blahd and Nordyke<sup>1</sup> and Taplin, Meredith and Kade<sup>2</sup>. In both tests it is assumed that the dye, injected endovenously, is eliminated only by the liver. Experiments on the elimination of the dye were made with labelled rose bengal - supplied by a well-known laboratory specializing in radio-pharmaceuticals<sup>3</sup>. The study showed that the elimination curve could be resolved into two exponential curves. This suggests that the dye is not eliminated only by the liver, perhaps because the dye is not a single chemical substance. Stowe, Delprat and Weeks<sup>4</sup> have directed attention to the fact that the liver only eliminates rose bengal when it has eight halogens, that is, when it is the pure chemical compound tetraiodotetrachlorofluorescein.

We attempted to separate the components of the dye by paper chromatography. One-dimensional chromatograms were run using a mixture of butanol and acetic acid (20 per cent) as solvent. Two components were identified, one with  $R_F$  zero and one with  $R_F$  0.98 (Fig. 1). By the count-ratio the amount of

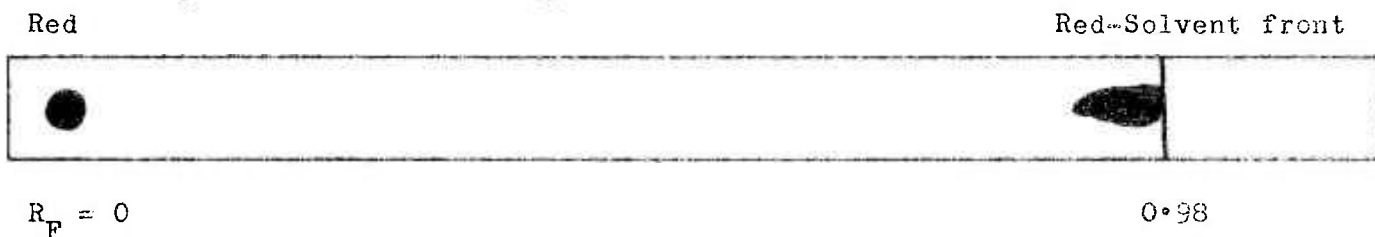


Fig.1. - Chromatogram of rose bengal using butanol-acetic acid as solvent.

substance with  $R_F$  zero was found to be 18 per cent of the amount of substance with  $R_F$  0.98. The original red colour of the dye usually fades on chromatograms developed with butanol-acetic acid. The colour is restored by exposing the strip to ammonia vapour. A parallel chromatogram was run with the same solvent, adding potassium iodide to the rose bengal to calculate the  $R_F$  for free iodide; the iodide spot was identified with lead acetate with  $R_F$  0.1. The spot was not radioactive, indicating that no exchange took place with the rose bengal.

Ishida et al.<sup>5</sup>, using ethanol-ammonia as solvent found an  $R_F$  value of 0.60 for rose bengal. We tried a mixture of 25 per cent ethanol, 5 per cent ammonia 1 : 1 made up with 70 per cent water as solvent and found that by running the chromatograms and allowing the solvent to drip from the end of the strips, the spot at  $R_F$  0.60 resolved into three red spots, all active ( Fig. 2). With this same mixture as solvent an active colourless spot with  $R_F$  0.35 was always found; it is not free iodide, which had  $R_F$  0.65 with the same solvent.

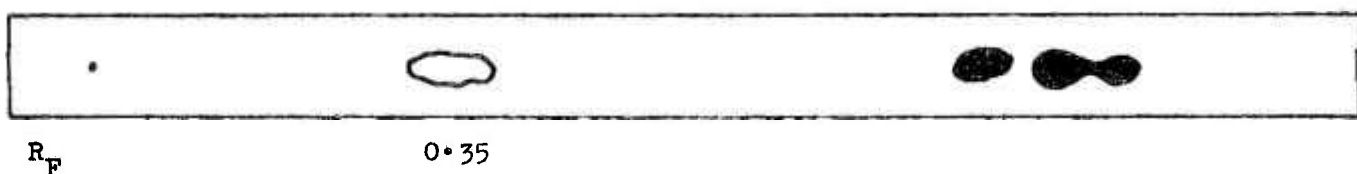


Fig. 2. - Chromatogram of rose bengal using ethanol-ammonia as solvent.

Two-dimensional chromatograms were run with butanol-acetic acid and ethanol-ammonia. Four active components were found: one colourless and three others with the original colour of rose bengal. Separations by paper column chromatography, with these solvents, are being carried out in order to separate the four components for injection and to follow the elimination of each component.

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