LABELLING OF VITAMIN A (RETINOL) WITH ^{131}I

Maria Teresa COLTURATO, Marycel Figols de BARBOZA,
Nécia M. J. MIRANDA, Rosana HERRERIAS,
Maria A. T. de ALMEIDA, Constância P.G. da SILVA
and Carlos FARALDO

Comissão Nacional de Energia Nuclear
Instituto de Pesquisas Energéticas e Nucleares
Caixa Postal 11049 – Pinheiros
05499 – São Paulo – Brasil

ABSTRACT

Vitamin A a derivative of certain carotenoids occurs in fish oils as a mixtures of the free alcohol and esters of fatty acids. It's found in the blood stream mainly in the ester form, esterification occurring in the intestinal epithelium and liver. The aldehyde is presumably an intermediate in the formation of the vitamin from its carotenoid precursors. Moreover, "retinene" an intermediate in the rodopsin (visual purple) cycle, has been identified as Vitamin A aldehyde.

Preliminary communication on the preparation of retinol were reported by Perry and Liebman (1963–1965). Retinyl acetate and all trans–retinoid acid labelled with tritium with specific activities ranging from 10 to 40 Curies /mmole and the preparative details of these isotopically labelled compounds were presented by Liebman (1990) in recent publication. The aim of this study was the labelling of retinol (Vit–A) with 131I and the establishment of the chemical and radiochemical control.

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INTRODUCTION

Vitamin A derives its alphabetical designation from the fact that, although not isolated until some year later, it was the first substance to be placed in this category due nutritionally essential factors(1913). It was originally recognized as a substance present in egg–yolk and butter fat, in the absence of which form the diet rats could not maintain growth. This growth factor was designated"unidentified dietary factor fat–soluble A".

Vitamin A is a derivative of certain carotenoids. The carotenoid pigments,as can be deduced from the hidrocarbon type of structure, are lipid soluble ,hence are found associated with lipids in nature. In fact, the yellowish color ascribed to many fats is not due the fats themselves but to dissolved carotenoid pigments.

Carotenoids and A Vitamins are classified as lipids in some systems, and combination with proteins are consequently regarded as lipoproteins (1).

The most important carotenoid precursors designated pro–vitamin A, are: α, β and γ carotene, which are $C_{40}H_{56}$–hydrocarbon. Such compounds are subject to cis–trans isomerization and when considered from a three dimensional standpoint, there are a large number (148) of possible steroisomers of the pro–vitamins. In general, the trans–isomers are active and the cis inactive. One important exception is encountered in the synthesis of rhodopsin (visual purple), an important function of Vit–A in which apparently only cis–isomers are active.

Vitamin A and the pro–Vitamins are practically insoluble in water and very soluble in most fat solvents. One of the most important of the physical properties of this vitamin and its precursors is their spectral absorption, which is used for their identification. β carotene (in chloroform) shows two peaks at 466 and 497 nm. Vit–A ,on the other hand, shows absorption bands in the ultraviolet portion of the spectrum and, the absorption maximum for Vit–A (in chloroform) being 328 nm

By virtue of its alcoholic structure "in vivo", Vit–A could form esters and can be oxidized to an aldehyde. The Vit–A is found in liver oils as mixture of the free alcohol and esters of fatty acids. Vit–A in the blood stream is chiefly in ester form, esterification occurring in the intestinal epithelium and liver. The aldehyde is presumably an intermediate in the formation of the Vit–A from its carotenoid precursors. Moreover, "retinene" an intermediate in the rodopsin cycle (visual purple) has been identified as Vit–A aldehyde. (4)

Preliminary communication on the preparation of retinol were reported by Perry and Liebman (1963 – 1965) (3). Retinyl acetate and all trans–retinoid acids labelled with tritium with specific activities ranging from 10 to 40 Curies/m moles and the preparative details of these isotopically labelled compounds were presented by Liebman(1990) in recent publication (2).

The aim of this study was the labelling of retinol (Vit–A) with ^{131}I and the establishment of a methodology on chemical and radiochemical quality control.

MATERIAL AND METHOD

Vitamin A (retinol) was obtained from Roche Laboratories. The chemical purity was determined by espectrophotometry and HPLC technique. The espetrophotometry study was carry out with Beckman–Mod. 25

espectrophotometer at 430–340 nm. High pressure liquid chromatography assay was performed in HPLC Waters–Mod. 510 on C_{18} Partisil 5–ODS–3 Whatman column using as mobile phase acetonitrile:methanol(10:90) at a flow rate 0.6 ml/min, UV detector at 325 nm.

Iodine monochloride (ICL) iodination was applied; 1.0 ml of 0.005N ICl was mixed with 50 µl of Na–^{131}I (IPEN–CNEN/SP). The iodine was extracted with 5.0 ml of ether. The etherea phase was added to 2.0mg Vit–A/9.0ml ether and Na_2SO_4. This solution was kept in darkness for 1 hour, them dried under N_2 and the residue was recovered with 1.0–2.0ml of ethanol:Tween 80:0.9% saline solution (3.16,90.4).

The labelled compound was purified through an anionic resin column(Lewatit M–600, Vetec) for iodine retention.

The radiochemical quality control was evaluated by different chromatographic systems: Whatman 1 paper (30 X 2cm) absorbed in paraffin, developed in methanol:butanol:H_2O (80:50:5) as a solvent; TLC–G and TLC–CF in ciclohexano:petroleum ether (80:20) as a solvent.

RESULTS AND CONCLUSION

The espectrophotometric assay (Fig. 1) showed a single peak in 364nm

The high efficiency HPLC analysis resented a retention time Rt=4min 57sec. for Vit–A from Roche Laboratories.(Fig. 2) similar to the Vit–A from Pharmacy University(USP).

The paper chromatography study developed during 6 hours showed two peaks with Rf=0.85 (Vit–A–^{131}I) and 0.64 (I^-) (Fig. 3). The yield of labelling varied from 40–60 % but with high radiochemical purity 80–90 % (Table 1) (Fig.4 A good separation in TLC–G or TLC–GF system was not obtained.

The procedure is simple to perform, labelling yields are acceptably high (40 – 60%), the technical problems are that ICl must be synthesized by the investigator because commercial ICl contain I_2, which decrease the labelling yield, and carrier iodine is introduced into the labelled product, thus lowering its specific activity.

Further studies on the biological distribution in rats are in progress in order to evaluate the selective localization of Vit–A–^{131}I in retine.

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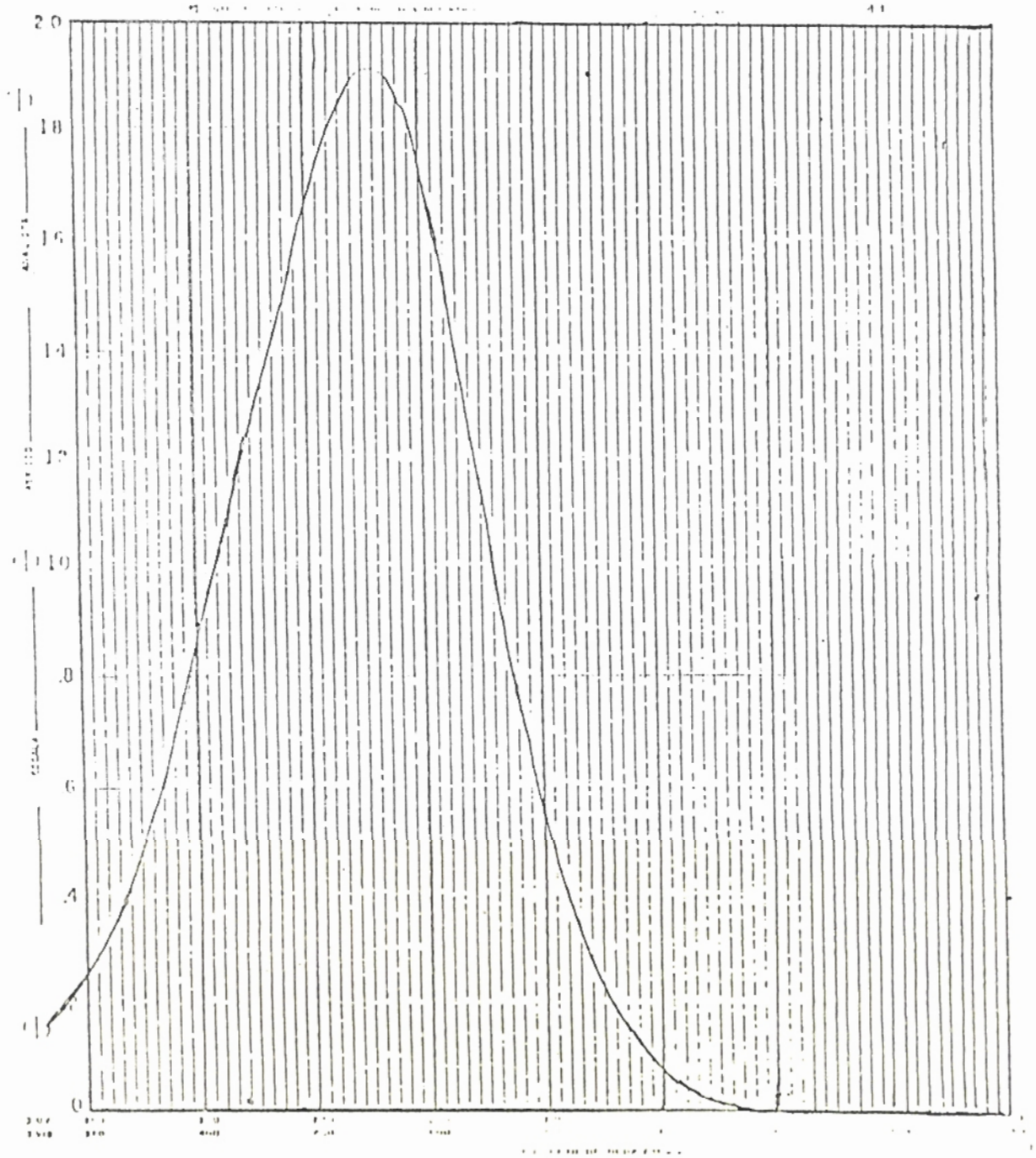

FIGURE 1 - ESPECTROPHOTOMETRIC STUDY OF VITAMIN -A (RETINOL) FROM ROCHE LABORATORY (= 364 NM)

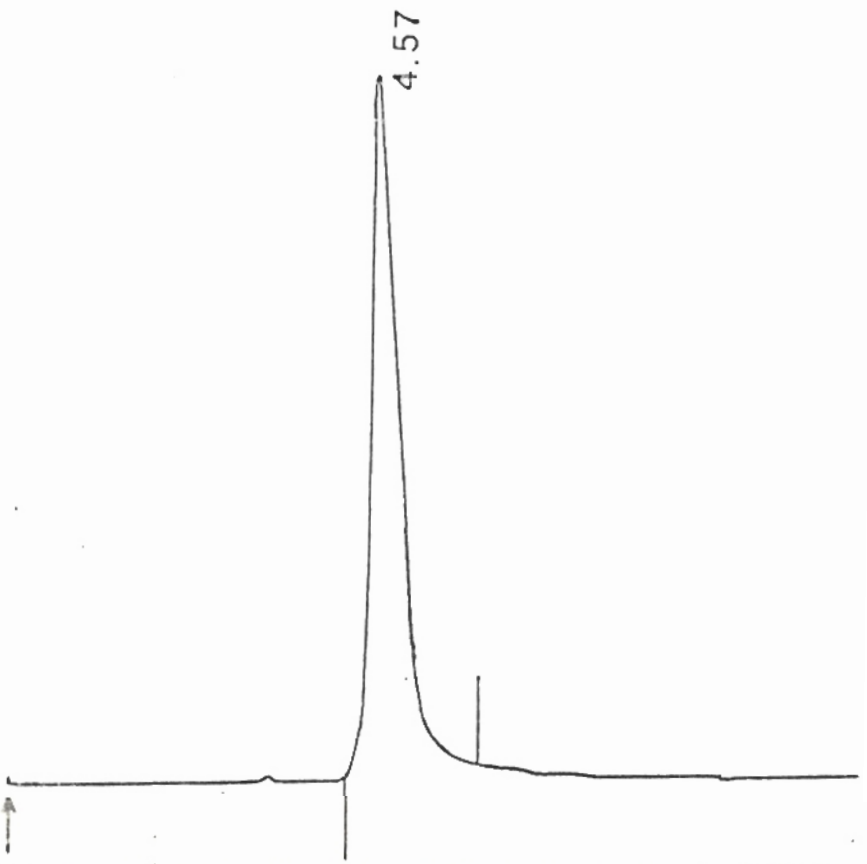

FIGURE 2- GRAPHIC REPRESENTATION OF HPLC ASSAY, IN WATERS MOD. 510 ON C_{18} PARTISIL S-ODS-3 WHATMAN COLUMN, FLOW RATE 0.6mL/MIN, UV DETECTOR 325NM, USING AS MOBILE PHASE ACETONITRILE:METHANOL:(10:90).

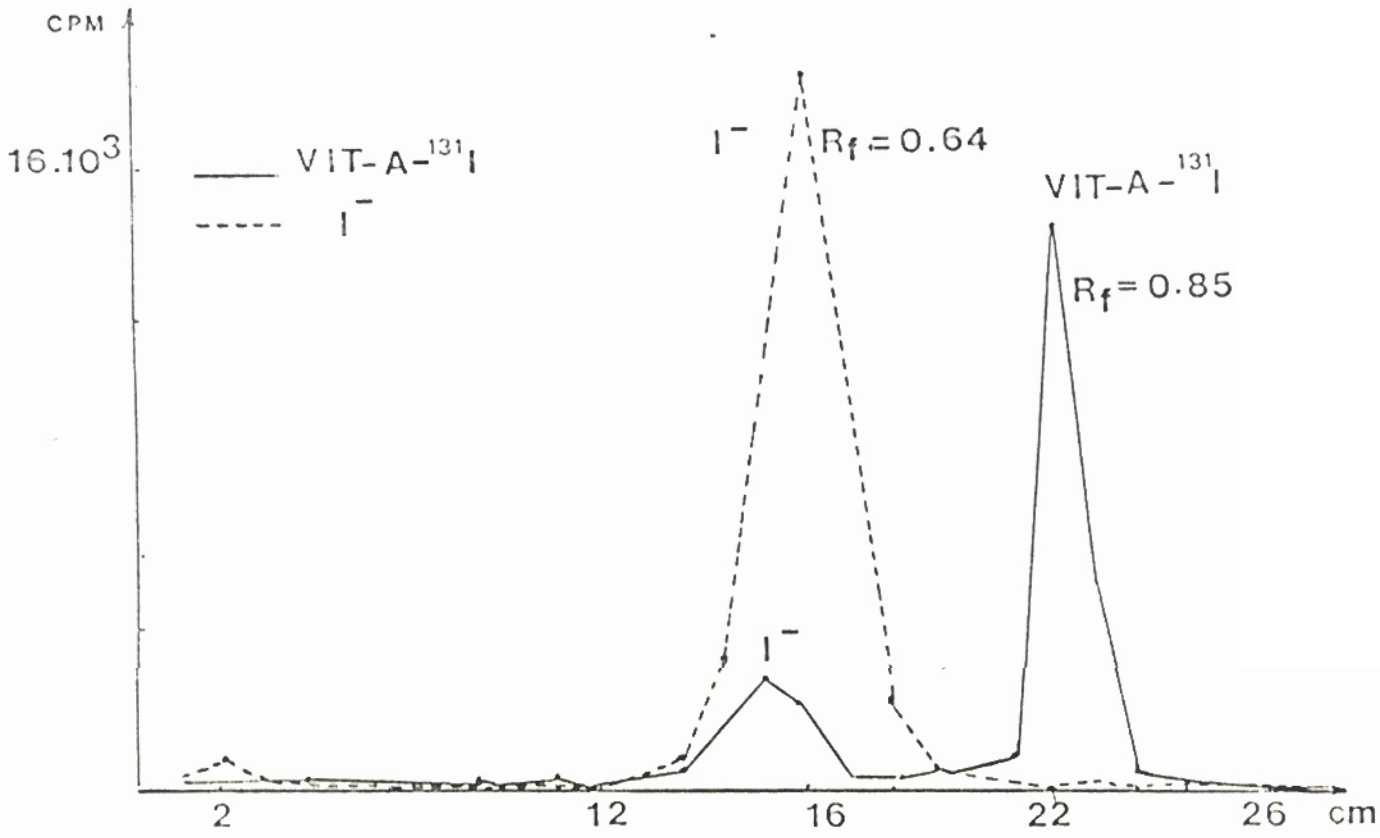

FIGURE 3 - CHROMATOGRAPHIC STRIP ACTIVITIES DISTRIBUTION OF VIT-A ^{131}I AND ^{131}I. (RF VALUES)

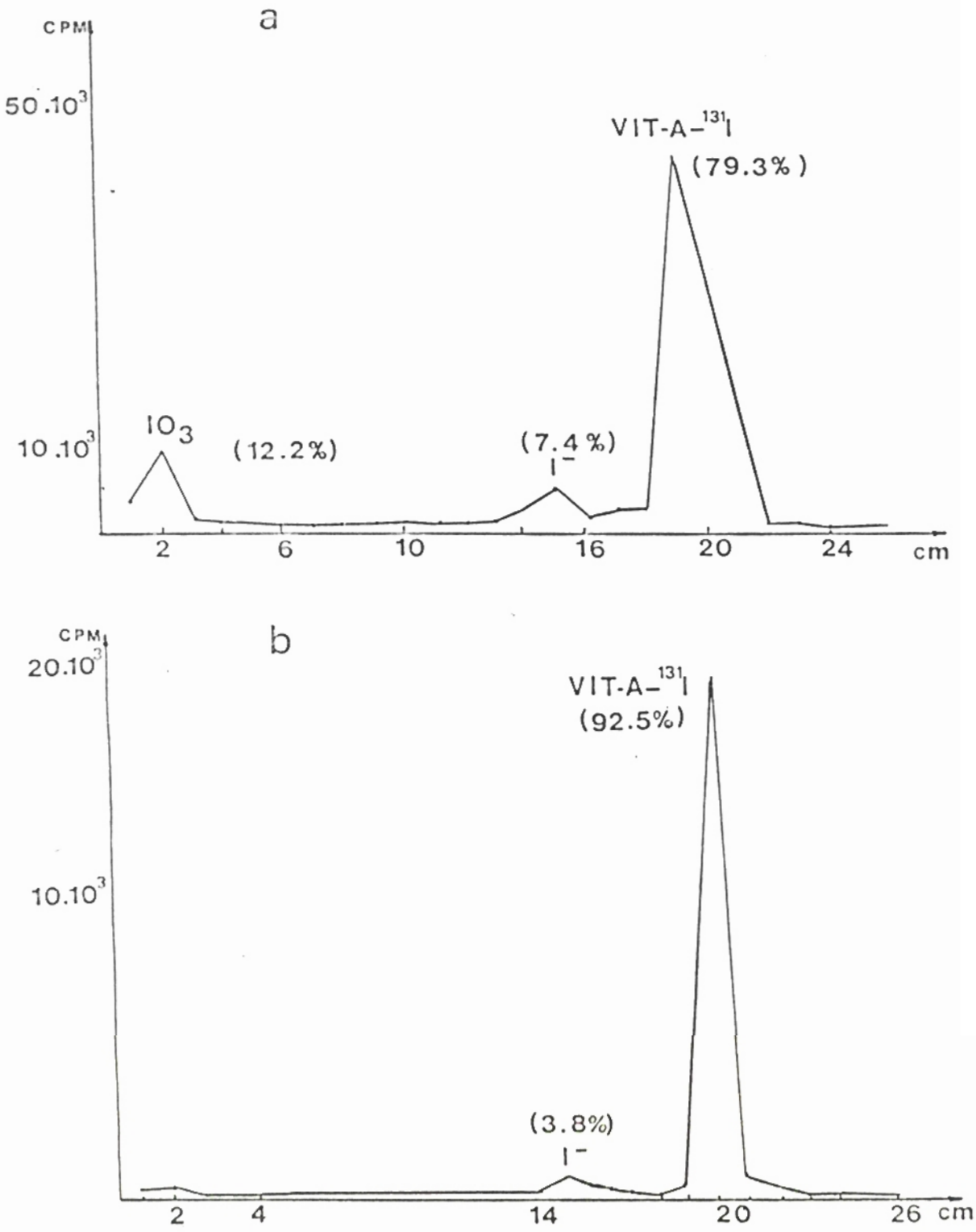

FIGURE 4 - CHROMATOGRAPHIC STRIP ACTIVITIES DISTRIBUTION OF VIT-A^{131}I A)BEFORE AND B) AFTER PURIFIED IN LEWATIT RESIN COLUMN FOR IODINE RETENTION.