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REFERENCE: Short Term Scientific Mission (STSM), COST P9
Beneficiary: Edyta SULAK, Institute of Applied Radiation Chemistry – Technical University of Lodz
Host: Chantal HOUEE – LEVIN, LCP Orsay
Period: from 15/11/2004 to 29/11/2004 Place: Orsay Cedex – 91405 (F)
Reference code: COST-STSM-P9-00202

TO WHOM IT MAY CONCERN

With reference to requirement for the reimbursement of my STSM executed by me at LCP Orsay under the supervision of Prof. Chantal HOUEE – LEVIN.

SCIENTIFIC REPORT

PURPOSE OF THE VISIT

The research programme of my STSM had two research objectives. The first one was to compare pulse radiolysis data already gathered in my home institution with those obtained in Orsay. Another one very important aspect of the visit to the Centre Universitaire was to gain experience with the use of analytical techniques (gel electrophoresis and liquid chromatography) allowing separation of the products of irradiation of samples containing drugs used in photodynamic anti-tumour therapy (PDT). These analytical techniques are not available in my home institution.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

The pulse radiolysis studies of aqueous solutions containing chlorin e6 (chle6) and its complex with polymer (photolon) were carried out. The purpose of the research was to study reaction with superoxide radicals using very low doses of ionising radiation (electron pulse duration 0.5μs, 1 μs, and the dose below 15 Gy). In order to study the product formation some γ-radiolysis measurements were carried out in the oxygen saturated solutions containing chle6.

Two analytical techniques, namely, Tricine – Sodium Dodecyl Sulfate – PolyAcrylamide Gel Electrophoresis (SDS – PAGE) and High Pressure Liquid Chromatography (HPLC) were applied to analyse samples containing chle6.

A high molecular weight molecule such as a protein was analysed by electrophoresis in denaturating conditions.

HPLC method was used to determine composition of samples containing chle6. The properly adjusted conditions of HPLC experiments (an elution gradient with the mobile phases

(A) methanol-water (4:1, v/v) containing 1% (v/v) acetic acid and (B) methanol, flow rate 0.5 ml/min.) allowed to collect and characterise chemical compounds presented in biomedical samples containing chle6. Separation was based on hydrophobicity (using a Beckman column, 25×0.45 cm, packed with C18) and mass (using a Zorbax column, 25×0.45 cm, packed with G25).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The pulse radiolysis and γ-radiolysis studies of reactivity of chle6 with superoxide radicals clearly revealed that chle6 did not react with superoxide radicals. So it suggested that the transient absorption band with maximum at 420 nm recorded in the pulse radiolysis studies of photolon and chle6 solutions in the presence of oxygen at the Institute of Applied Radiation Chemistry was not a result of reaction of dye with superoxide radicals. The results obtained in LCP indicate that another explanation for origin of this transient absorption band should be proposed.

The samples containing chle6 used in PDT has been isolated from plant materials and has contained mainly chle6 but also its derivatives.

Applying HPLC method using Zorbax G25 column two fractions were collected for further analysis. Absorption spectra were recorded for these fractions using Beckman DU-600 spectrophotometer.

After HPLC analysis using Beckman C18 column 17 and 8 fractions of analysed samples of chle6 and photolon were collected, respectively. These fractions will be subject to further analysis in Poland to improve the quality of the drug.

The experimental studies were discussed with scientists from host institution. I received also very valuable information about working with amino acids and peptides what is very profitable for further study relevant to my PhD thesis.

In my opinion it is also very important I was introduced to a new field of radiation chemistry concerning influence of ionising radiation on amino acids and peptides.

FUTURE COLLABORATION WITH HOST INSTITUTIONS

The future collaboration with host institution will involve exchanging our opinions and experience concerning the damage to biomolecules induced by ionising radiation.

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Lodz, 09.12.2004.