Chapter I

Introduction

The Basis of Nuclear Magnetic Resonance

Certain atomic nuclei, such as the hydrogen nucleus ^{1}H or the phosphorus nucleus ^{31}P, posses a property known as spin. This can be visualized as a spinning motion of the nucleus about its own axis. Associated with the spin is a magnetic property, so that the nucleus can be regarded as a tiny bar magnet with its axis along the axis of rotation. If a magnetic field is applied to a sample containing such nuclei (e.g. to water in the case of ^{1}H NMR), we might expect the nuclear magnets to align along the field just as a compass needle aligns along a magnetic field. However, the nuclei have spin and obey the laws of quantum mechanics. We find that nuclei such as ^{1}H that have a spin quantum number I = 1/2 can have one of two orientations with respect to the applied field. These two orientations have slightly different energies and the energy difference between the two states is proportional to the magnitude of the applied field transitions between these states can be induced by applying an oscillating magnetic field of frequency (ν_o)

$$\nu_o = \gamma B_o / 2\pi$$

where Bo is the magnitude of the applied magnetic field, is known as the magnetogyric ratio of the nucleus and ν_o is the resonance frequency. The Magnetogyric ratio (γ) varies from one nuclear isotope to another, and this is why ^{1}H, ^{13}C and ^{31}P NMR for example, are all performed at different frequencies in a given field.

The resonance frequency of a nucleus is directly proportional to the local magnetic field experienced by the nucleus. The separation of resonance frequencies from an arbitrarily chosen reference frequency is termed the chemical shift and is expressed in terms of the dimensionless units of parts per million (p.p.m.). The intensities of N.M.R. signals, as measured from their areas, are proportional to the number of nuclei that contribute towards them. However, it should be noted that other factors, such as the spin-lattice relaxation time T, and the nuclear overhauser effect, can also affect signal intensities.

Because of its ability to draw molecular structure and interactions , NMR has some interesting possibilities for studying tissues in vivo. Especially, ^{31}P NMR is suitable for observing small but important molecules such as inorganic phosphates, sugar phosphates, phospholipids and nucleotides; more complex organic systems such as phospholylated proteins, poly-phosphates,

polynucleotides, cell walls and circulating lipoproteins; and also a variety of intact tissues and cellular organelles such as erythrocytes, reticulocytes, platelets, muscles, nerves, livers, kidneys, hearts, embryos, sperm, eggs, brains, tumor cells, mitochondria and yeast.

The applications of ^{31}P NMR in biological analysis fall into three categories:

1) determination of metabolites concentrations,

2) determination of the intracellular pH.,

3) detection of relaxation effect from line broadening.

For example, the ^{31}P NMR spectrum of an ATP solution has three signals corresponding to the α- , β- , γ- phosphates, so that we can detect the concentration of ATP in the living tissues without destroying them. In the case of inorganic phosphate, the NMR spectrum has only one signal, even though both $H_2PO_4^-$ and HPO_4^{2-} ions are present in a significant proportion in the given pH. With this principle, we can estimate the intracellular pH. of living tissues.

Recently, a powerful technique of intracellular pH measurement using ^{31}P NMR spectroscopy was developed for the study of various cellular systems such as red blood

cells, bakers' yeast, muscles, heart muscles, E. Coli, liver mitochondria, and sea urchin eggs. Since the pK's of several small molecules such as inorganic phosphates and sugar phosphate are around 7, their chemical shifts are very sensitive to pH alteration and hence can be used to measure pHi. This application of an NMR spectrometer as a pH meter is advantageous because of its nondestructive character and its potential for kinetic experiment.

The initial NMR study of living cells was carried out by Moon and Richards (1) using living red blood cells. They reported high - resolution ^{31}P NMR studies of intact red blood cells. They detected signals from 2,3-diphosphoglycerate, inorganic phosphate and ATP and showed how the spectra could be used to determine intracellular pH.

In 1974 it was reported that high resolution, ^{31}P NMR spectra could be obtained from intact muscle freshly excised from the hind leg of the rat (2). The signals could be assigned to the three phosphate groups of ATP, to phosphocreatine and to inorganic phosphate.

From the experiments of Hoult et.al (2),it was shown that high resolution ^{31}P NMR spectra could be obtained from frog sartorius muscles that were maintained in good physiological condition within the spectrometer.

More recently, developments in magnet technology and in radio frequency coil design have permitted high - resolution ^{31}P NMR spectra to be obtained from selected regions of live animals such as the rat. In these experiments (4) an anesthetized rat is positioned within the magnet and a radio frequency coil is placed in the required position against a leg of the rat. Remarkably clear ^{31}P NMR signals can be detected from a disc - shaped region of the leg immediately in front of the coil, in the studies described here this 'sensitive' region has a volume of about 1 ml. It should be stressed that the animal undergoes no surgery whatever; the experiment can be linkened to examining the condition of the heart with a stethoscope.

The high resolution nuclear magnetic resonance spectra of phosphorus metabolites in intact cells have been reported for erythrocytes (1, 3), heart (4), and muscle (2, 7, 9, 10, 11). This was made possible by the development of instrumentation capable of resolving phosphorus resonances of small molecule such as ATP, creatine phosphate, 2,3- diphosphoglycerate and inorganic phosphate (Pi). Furthermore, their phosphorus chemical shifts are very sensitive to pH and can be used to measure pH. The use of nuclear magnetic resonance signal for the determination of pH is advantageous because it is a nondestructive procedure.

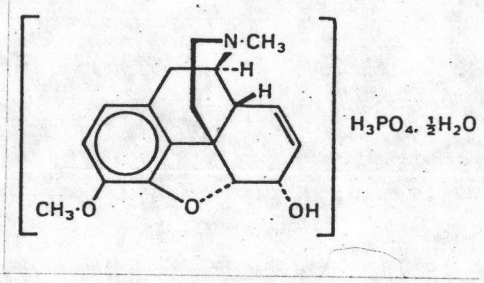
Casey et. al (6), used the chemical shifts of the γ - phosphate of ATP to detect pH changes within the vesicle of chromaffin granules from adrenal medulla.

Codeine Phosphate

Sources: Codeine is an alkaloid in opium which obtained from the milky exudate of the incised unripe seed capsules of the poppy plant, Papaver somniferum Linn. The milky juice is dried in the air and forms a brownish, gummy mass. This is further dried and powdered to make the official powdered opium. Codeine is obtained from opium directly or made by partial synthesis from morphine.

Codeine phosphate is prepared by dissolving codeine in an equimolecular quantity of aqueous phosphoric acid , adding alcohol, and allowing the salt to crystallize from solution.

Structure: 3-O- Methylmorphine phosphate hemihydrate.



Pharmacological Action

Codeine phosphate produces major effects on the central nervous system (CNS) and the bowel. The effects are remarkably diverse and include analgesia drowsiness changes in mood, respiratory depression, decreased gastrointestinal motility, nausea, vomiting, and alterations of the endocrine and autonomic nervous systems. Codeine also depress the cough reflex, at least in part by a direct effect on a cough center in the medulla.

Use

Codeine phosphate is employed as an analgetic, sedative, hypnotic, anticholinergic and antitussive agent.

Preparations: Codeine linctus, B.P. 1980

Codeine linctus, Diabetic, B.P.C. 1973

Codeine linctus, Paediatric, B.P.C. 1973

Aspirin, Phenacetin, and Codeine Tablets, B.P. 1973

Codeine phosphate Tablets, U.S.P. XXI

Codeine phosphate injection, U.S.P. XXI

Analytical Method of Codeine Phosphate

Many methods are used to analyze the quantity of codeine in several preparations. These can be clarified into three methods.

1) Aqueous Titration (Codeine phosphate injection (24), Codeine phosphate Tablet (24)

Render the codeine phosphate solution alkaline with 6N. ammonium hydroxide in order to form alkaloid. Extract the alkaloid with chloroform, Titrate the final solution with 0.02N sulfuric acid. Each ml. of 0.02 N. sulfuric acid is equivalent to 8.128 mg. of $C_{18}H_{21}NO_3 . H_3PO_4 . {}^1/_2 H_2O$

Codeine linctus (21), Codeine phosphate syrup (21), Aspirin and Codeine Tablets (21):

Add acetate buffer solution pH 2.8 and dimethyl yellow-oracet blue B solution, then extract with chloroform. Titrate the solution with 0.01 M dioctyl sodium sulphosuccinate solution. The end point is indicated when the colour of the chloroform layer changes from green to pinkish-grey. Repeat the operation without the preparation being examined. The difference between the titrations represents the amount of 0.01 M. dioctyl sodium sulphosuccinate VS. required by the sample. Each ml of

0.01 M. dioctyl sodium sulphosuccinate VS. is equivalent to 0.004064 g. of $C_{18}H_{21}NO_3H_3PO_4.{}^1/_2H_2O$. Determine the weight per ml and calculate the concentration of $C_{18}H_{21}NO_3H_3PO_4.{}^1/_2H_2O$, weight in volume.

2) High Performance Liquid Chromatography (Codeine phosphate syrup (12))

Diluting the syrup until the concentration of codeine phosphate in syrup is about 0.2 mg/ml with the chromatographic solvent and then analyzing the solution with H.P.L.C. Chlorpheniramine maleate was used as an internal standard for purposes of comparison. The chromatographic solvent was 0.05 M. KH_2PO_4 in water containing 13% (V/V) methanol. A high pressure liquid chromatograph equipped with a fixed wavelength (234 nm) detector and recorder was used.

3) Gas Chromatography (Codeine phosphate, Aspirin, phenacetin and Caffeine Tablets (18))

Transfer an accurately weighed portion of the finely powder of codeine phosphate, aspirin, phenacetin and caffeine tablets to a seperator, add sodium hydroxide, swirl to mix and then extract the solution with methylene chloride, filtering each extract through anhydrous sodium sulfate into a beaker. Evaporate the combined extracts to near dryness on a steam bath and complete the evaporation

to dryness with the aid of a current of the air, without heating. Dissolve the residue in Internal standard solution (a solution in chloroform containing 400 mcg of U.S.P. Propoxyphene HCl Reference Standard in each ml). To do a standard preparation as directed above but use powder of U.S.P. codeine phosphate. Then inject a portion of standard preparation into a suitable gas chromatograph equipped with a flame ionization detector and record the chromatogram. Measured the peak height of each component. Similarly, inject a portion of the sample preparation into the chromatograph and record the chromatogram under the same conditions as described for the standard preparation. Measure the peak height of each component calculate the quantity of codeine phosphate.

Statement of Problem

The above mentioned biological application of Phosphorus -31 nuclear magnetic resonance convey to the development of pharmaceutical quantitative analysis using phosphorus -31 nuclear magnetic resonance (^{31}P NMR). As codeine phosphate is not only complicate to be analyzed but also consists of phosphorus, so codeine phosphate is chosen for studying the quantitative analysis by ^{31}P NMR.

The objective of this project is to apply phosphorus -31 nuclear magnetic resonance technique in

Pharmacy. Many factors that effect to the quantitative analysis of codeine phosphate will be studied in order to set up the condition which are giving an high precision and accuracy. Even the method of analysis is more sophisticated due to the using of highly expensive instrument (^{31}P NMR) but the method is much more simple and may cost a little if one intends to do a routine work and using for a long time.