# CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF CASSAVA STARCH- CHITOSAN BLEND BIODEGRADABLE FILM WITH ADDITION OF BLACK CUMIN OIL

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# ABSTRACT

Biodegradable film made from renewable and naturals polymers such as starch had been in considerable interest. In addition to their undisputed advantage of being obtain from renewable resources, they are required to be biodegradable. In this study chitosan and starch are used as polymer. Chitosan has been found to be nontoxic, biodegradable, biofunctional and biocompatible in addition. Gelatin and polyethylene glycol (PEG) are use as additive to enhance the physical and chemical properties of the biodegradable film. The film preparation process consist of preparation of solution containing chitosan, starch, acetic acid, gelatin , PEG and black cumin oil as materials.. The solution was poured on a glass plate and left at ambient temperature until it completely dry. The film was then peeled off the glass plate. The fabrication films were characterized using fourier transform infrared spectroscopy (FTIR) , differential scanning calorimetry (DSC) , thermogravimetric analysis (TGA) , scanning electron microscope (SEM) and antimicrobial analysis using agar diffusion method (zone inhibition assay) and liquid culture test (optical density measurements). From the experiment result, the film containing of starch, gelatin and black cumin oil have higher thermal stability, compact matrix, slightly higher melting point and also greater antimicrobial properties. Thus, the film may have potential to be use for packaging by having good chemical , and physical properties and also inhibit bacterial growth.

# ABSTRAK

Filem biodegradasi yang diperbuat daripada bahan boleh diperbaharui dan polimer semulajadi seperti kanji menjadi tarikan masa kini. Tambahan lagi, selain daripada kelebihan yang dimiliki ia juga harus biodegradasi. Maka penggunaan kanji boleh dijadikan sebagai jalan penyelesaian kerana bahan ini mudah didapati, murah dan biodegradasi. Chitosan didapati tidak bertoksik dan biodegradasi,.Dengan menggunakan gelatin dan Polyethylene glycol (PEG) sebagai bahan aditif dalam filem biodegradasi untuk mengukuhkan filem tersebut dari segi sifat kimia ,fizik. Proses penyediaan filem terdiri daripada penyedian larutan kanji, larutan chitosan dan dikacau bersama pati minyak jintan hitam dan Polyethylene glycol (PEG). Kemudian larutan dituang ke atas kaca dan dibiarkan kering pada suhu bilik sehingga kering. Setelah siap kering, pengupasan filem dilakukan. Pencirian dilakukan menggunakan *fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) thermogravimetric analysis (TGA), scanning electron microscope (SEM )*dan untuk antimikrob analisis menggunakan *agar diffusion method (zone inhibition assay)* dan *liquid culture test (optical density measurements)*. Hasil daripada ujian yang dijalankan, filem mengandungi kanji, gelatin dan pati minyak jintan hitam mempunyai sifat fizikal dan sifat kimia yang bagus dan juga sifat antimikrob yang bagus. Maka. filem tersebut mempunyai potensi untuk pembungkusan kerana mempunyai sifat fizikal dan kimia yang bagus dan boleh menghalang pembiakan bakteria

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| | |
|---|---|
| SEM | - Scanning electron microscope |
| FTIR | - Fourier transform infrared |
| TGA | - Thermo gravimetric analysis |
| DSC | - Differential scanning calorimeter |
| PEG | - Poly(ethylene glycol) |
| %v/v | - weight percentage for chemical per basis |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

In recent times, biodegradable materials have gained importance particularly for the protection of the environment from ever increasing plastic waste. Partially biodegradable polymers obtained by blending biodegradable and non biodegradable commercial polymers can effectively reduce the volume of plastic waste by partial degradation. They are more useful than completely biodegradable ones due to the economic advantages and superior properties, imparted by the commercial polymer used as a blending component. Among the polysaccharides, starch has generated renewed interest in its use as a component in plastic formulations (Thakore *et.al.*, 2000). Blending has acquired importance in improving the performance of polymeric materials. It has become an economical and versatile route to obtain polymers with a wide range of desirable properties. In order to ensure homogeneity in blends at microscopic level, it is necessary to reduce the interfacial tension, so that the characteristics of blend components are improved or at least retained.

Over the past five years packaging suppliers have been introducing various forms of biodegradable plastics. These are made from a variety of plants, in the main corn, in response to projections that consumers and recycling regulations will drive demand for environmentally-friendly packaging. Antimicrobial packaging is gaining interest from researchers and industry due to its potential to provide quality and safety benefits. Interest in antimicrobial packaging films has increased in recent years due to a concern over the risk of foodborne illness, desire for extended food shelf life and advances in the technology of film production. Slowing the growth of spoilage bacteria will reduce the losses of product to spoilage and extend shelf life. Reduction of pathogen growth will reduce the risk of foodborne illness caused by those products (Dawson *et al* , 2002).

## 1.2 Problem statement

The huge population increase coupled with the improved living conditions of the people led to a dramatical increase of the consumption of plastics worldwide. The chief usages of polymeric materials are packaging, household and domestic products, electrical and electronic goods and also in building, construction and automotive industries. It has been estimated that almost 170 million tones of plastics were produced worldwide during the year 2003. Current statistics for Western Europe estimate the annual total consumption of plastic products at 48.8 million tons for 2003 and generating approximately 15 million tons of waste plastics throughout Europe Thus there is huge waste stream creating the environmental problem (Achilia 2007).

Thermoplastic polymers make up a high proportion of waste and this amount is continuously increasing, thus posing a more serious environmental challenge because of their huge quantity and disposal problem as thermoplastics do not biodegrade for very long time. The land filling of sites and incineration of waste plastics have serious drawbacks. Plastics are produced from petroleum derivatives and are composed primarily of hydrocarbons but also contain additives such as antioxidants, colorants and other stabilizers which are undesirable from environmental and pollution point of view (Hamid *et.al* , 1992 ).

Beside that, the pollution by plastic waste is a major threat to marine life and the cause of the loss of many kinds of marine organisms but today we have some solutions to dispose of the plastic. The solution were by using biodegradable polymer or biodegradable plastic. We should regard this solution as an international issue, because it is our duty to protect our environment, and by using this solution it can reduce the quantity of our plastic waste, it won't generate toxic substance and it is good for sustaining our ecosystem (Okada *et al.,* 2002).

Therefore, the use of agricultural biopolymers that are easily biodegradable not only would solve these problems, but would also provide a potential new use for surplus farm production (Okada *et al.,* 2002). A biodegradable polymer, is a film made from renewable and naturals polymers such as starch and become the alternative to traditional petroleum based plastics. This is film can be degraded by microorganisms into carbon dioxide and water. The use of a biopolymer such as starch can be interesting solutions because this polymer is relatively inexpensive, abundant, biodegradable and edible (Lawton and Tiefenbacher, 1999). Because of the environmental concerns and technological problems such as denaturing effects of thermal polymer processing methods, extrusion and injection molding, the incorporation of biopreservatives into biodegradable films is more suitable than incorporation into plastic films (Appendini *et al.,* 2002).

Antimicrobial packaging has been touted as a major focus in the next generation of active packaging. Antimicrobial packaging is the packaging system that is able to kill or inhibit spoilage and pathogenic microorganisms that are contaminating foods. The new antimicrobial function can be achieved by adding antimicrobial agents in the packaging system and/or using antimicrobial polymers that satisfy conventional packaging requirements. When the packaging system acquires antimicrobial activity, the packaging system (or material) limits or prevents microbial growth by extending the lag period and reducing the growth rate or decreases live counts of microorganisms (Han, 2000).

## 1.3 Objective

a. To fabricate cassava starch-chitosan blend biodegradable film with addition of black cumin oil
b. To characterize the films in term of morphology, physical and chemical changes
c. To analyzes antimicrobial resistance of the fabricated films.

## 1.4 Scope of study

a. Fabrication of biodegradable film from cassava starch-chitosan blend with addition of black cumin oil.
b. The characterization of the films using method as following;
    i. Fourier transform infrared spectroscopy (FTIR)
    ii. Differential scanning calorimetry (DSC)
    iii. Thermogravimetric analysis (TGA)
    iv. Scanning electron microscope (SEM)
c. Analysis of antimicrobial resistance of the films by using the method as following:
    i. Agar diffusion method (Zone Inhibition Assay)
    ii. Liquid culture test (Optical Density Measurements)

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Biodegradable film

There is a considerable interest in biodegradable film made from renewable and naturals polymers such as starch. In addition to their undisputed advantage of being obtain from renewable resources, they are required to be biodegradable. The use of a biopolymer such as starch can be interesting solutions because this polymer is relatively inexpensive, abundant, biodegradable and edible (Lawton and Tiefenbacher, 1999). Thus, new biodegradable films made from edible biopolymers from renewable sources could become an important factor in reducing the environmental impact of plastic waste (Tharanathan, 2003).

Edible, biodegradable films and coatings, by acting as barriers to control the transfer of moisture, oxygen, carbon dioxide, lipids, and flavor components, can prevent quality deterioration and increase the shelf life of commercial packaging material (Cuq *et.al*,1998).

They can be effective carriers of many functional ingredients, such as antimicrobial agents to improve safety and stability of foods also antioxidants to prevent lipid oxidation (Ghanbarzadeh *et.al* 2007). The antimicrobial packaging is a rapidly developing technology that can be employed for controlling the microbiological decay of perishable food products, different organic and inorganic active agents can be incorporated into the polymeric structure to prevent the undesirable microbial spoilage (Buonocore, *et.al*, 2003).

## 2.2 Starch

Starches are basically carbohydrates, known as polysaccharides, for example multiple molecules of sugar. For commercial use, they are derived from a variety of cereals like rice, wheat, sorghum, corn and tubers like potato, tapioca and sweet potato. Internationally popular forms of starch are mostly derived from corn and tapioca/cassava due to their easy availability. Due to a peculiar phenomenon called gelatinization an irreversible swelling of starch granules when treated with hot water, starch turns into a thick paste. When cooled and with certain additives, it forms a gel. This gives it high viscosity which forms the basis of its many uses.

### 2.2.1 Cassava Starch

Cassava starch is a root starch which is produced from the roots of manioc or cassava plant. Cassavas belong to the family *Euphorbiaceae*. Both bitter and sweet cassava are classified as Manihot esculenta or Manihot utilissima or Manihot Aipi. They are produced extensively in Thailand, Malaysia, Indonesia and some parts of Africa.

#### 2.2.1.1 Cassava Starch proximate composition

Starches from different sources are known to differ in their physical and chemical properties. These differences are believed to arise from differences in the amylose/amylopectin ratio in the starch granule, the characteristics of each fraction in terms of molecular weight, length/degree of branching and the physical manner in which these constituents are arranged within the starch granules, and the presence of naturally occurring non carbohydrate impurities (Salehuzzman *et. al,* 1992). The moisture contents for cassava starch are within the 10 to 20% .The ash contents of cassava were 0.29%. Cassava and other starches differed in their amylose content.

Differences in amylose content have been reported to result in differences in starch physicochemical properties The granules of cassava were intermediate sizes. The granules of cassava starch are round or kettledrum shaped with smooth surfaces as shown in figure 1. The granules of cassava starch ranged from 2.81 to 14.03 µm with an average size of 8.42 ± 3.35 µm (Wickramasinghe *et al*, 2009).

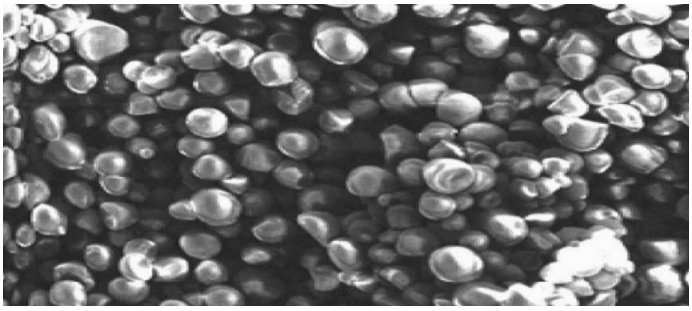

**Figure. 2.1**. SEM micrographs of cassava starch granules

According to the Food and Agriculture Organization (FAO) 2004, one important source of starch in South America is tapioca/ cassava . Therefore, the use of tapioca starch to develop edible films and coatings has been considered (Fama *et.al*, 2005). In order to improve the physical and functional properties of starch films, blending with other biopolymers, hydrophobic substances and/or antimicrobial compounds has been proposed (Anker *et.al*, 2001).

## 2.3 Chitosan

Chitosan is a polymer derived from chitin and is used in applications from health care to agriculture to dyes for fabrics. There are even medical applications. Chitosan itself is the major source of the nutritional supplement glucosamine. Chitosan is, at the pH of the gastrointestinal tract, a positively charged polymer and can bind to negatively charged substances. Chitosan might bind to some dietary lipids. It may also bind to the fat-soluble vitamins A, D, E, and K, as well as flavonoids, carotenoids and some minerals such as zinc, found in foods (Koide, 1998). Chitosan has been found to be nontoxic, biodegradable, biofunctional, biocompatible in addition Chitosan is a natural carbohydrate polymer obtained by the deacetylation of chitin, a major component of shells of crustaceans such as crab, shrimp and crawfish. Its obtention is a way to profit from seafood wastes. Today, chitosan is produced with different deacetylation degrees and molecular weights (No *et.al* , 2007).

**2.4 Gelatin**

Proteins are biopolymers that can be used successfully in food packaging formulations. Therefore they are an attractive alternative to synthetic plastic materials made from non-renewable resources that can be a hazard to the environment. Gelatin obtained by partial degradation of collagen has gained more attention as edible films for its abundance and biodegradability. Gelatin has relatively low cost and excellent functional and filmogenic properties (Cao *et.al* , 2007).

Gelatin film itself, as most protein films, does not have ideal water vapor barrier properties. Thus, some chemical treatments can be applied to modify the polymer network through cross-linking of the polymer chains to improve the hydrocolloid film functionality (Cao *et.al* , 2007).Gelatin is one of the natural polymers ,it is a high molecular weight polypeptide composing of amino acids mainly glycine (27%), hydroxyproline and proline (25%). Because its molecules are tightly bound with hydrogen bonds, pure gelatin films are normally brittle. Furthermore, the polar groups present in its structure cause a gelatin film to have high moisture absorption. Due to these disadvantages, pure gelatin films are not suitable for many applications. Therefore, gelatin is generally blended or copolymerised with other synthetic polymers (Chiellini *et.al,* 2001)

## 2.5 Antimicrobial analysis

There has been a growing interest in recent times to develop materials with film-forming capacity and having antimicrobial properties which help improve food safety and shelf life. Antimicrobial packaging is one of the most promising active packaging systems that have been found highly effective in killing or inhibiting spoilage and pathogenic microorganisms that contaminate foods (Salleh *et.al*, 2007). The new antimicrobial function can be achieved by adding antimicrobial agents in the packaging system and/or using antimicrobial polymers that satisfy conventional packaging requirements. When the packaging system acquires antimicrobial activity, the packaging system (or material) limits or prevents microbial growth by extending the lag period and reducing the growth rate or decreases live counts of microorganisms (Xu *et.al*,2005).

### 2.5.1 Antimicrobial agent black cumin oil

The genus *Nigella*, belonging to the family *Ranunculaceae*, is represented by species of Mediteranean–western Asian origin. These are generally short-lived annuals, typical of disturbed soils or natural communities of semi-arid areas, with a dominance of therophytes. In the natural forms, flowers are bluish, with a variable number of sepals, and characterised by the presence of nectaries. The gynoecium is composed of a variable number of multi-ovule carpels, developing into a follicle after pollination, with single fruits partially connected to form a capsule-like structure.Seeds, of generally small size (1 to 5 mg), dark grey or black colour and with corrugated integuments, represent the useful product. They are known in Arabia as ‘Al-Habba Al-Sawdaa’ or ‘Al-Kammoon Al-Aswad’ (Al-Gaby, 1998), ‘Habbet el Baraka’ and ‘Shunez’ (Burits and Bucar, 2000). This seed oil has been reported to possess on antibacterial activity (Morsi, 2000).

### 2.5.2 Black cumin oil characterization.

Characterization of black seed oil composition by gas chromatography-mass spectrometry analysis (Burtis and Bucar, 2000) has revealed the presence of a variety of compounds possessing antimicrobial properties, including carvacrol (Ultee, *et.al* 2002), thymol (Rasooli and Mirmostafa, 2003), thymohydroquinone, thymoquinone (Burtis and Bucar, 2000), limonene, carvone (Oumzil *et al*., 2002), p-cymene and c-terpinene (Gulluce *et al*., 2003).

### 2.5.3 Proximate analysis

Proximate analysis of the seeds showed a composition of 20.85% protein,38.20% fat, 4.64% moisture, 4.37% ash, 7.94% crude fibre and 31.94% total carbohydrate. No trace of lead, cadmium and arsenic were found in the seeds. The predominant elements present were potassium, phosphorus, sodium and iron while zinc, calcium, magnesium, manganese and copper were found at lower levels (Hosseinzadeh *et.al* ,2004). The seeds may potentially be an important nutritional source as the content of essential amino acids contributes to about 30% of the total protein content while about 84% of the fatty acids is composed of unsaturated fatty acids, predominantly linoleic and oleic acids (Hosseinzadeh *et.al,* 2004). Oil extracts of the seeds also contain significant amounts of sterols. β-Sitosterol was the dominant sterol (69%); while campesterol and stigmasterol constitute 12% and 19%, respectively of the total sterols .

The seed oil was found to be rich in polyphenols (1,744 µg/g) and tocopherols (340 µg/g of total α-,β- and γ-isomers) . *N. sativa* seeds contain 36% to 38% fixed oils, proteins, alkaloids, saponins and 0.4% to 2.5 % essential oil . The fixed oil is composed mainly of fatty acids, namely, linoleic (C18:2), oleic (C18:1) and palmitic acids (C16:0) (Atta, 2003).Many components were characterized from the essential oil, but the major ones were thymoquinone (27.8% to 57.0%), ρ-cymene (7.1% to 15.5%), carvacrol (5.8% to 11.6%), t-anethole (0.25% to 2.3%), 4-terpineol (2.0% to 6.6%) and longifoline (1.0% to 8.0%) (Atta, 2003).

Thymoquinone is the main active constituent of the volatile oil extracted from *Nigella sativa* (Gazzar *et al* , 2006). Good quality control methods were used for quantifying the pharmacological actives thymoquinone, dithymoquinone, thymohydroquinone and thymol, in both the seed oils and extracts of *N.sativa*. Other active principles were nigellone, which was isolated from the volatile oil fraction and was found useful in the treatment of bronchial athma , and nigellidine which contains an indazole nucleus .Three flavonoid glycosides and triterpene saponins were also identified from *Nigella sativa* (Gazzar *et al*,2006).

## 2.6 Characterization

### 2.6.1 Scanning Electron Microscope ( SEM)

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm) (Amit and Arthur, 2009 ).

The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using EDS), crystalline structure, and crystal orientations (using EBSD). The design and function of the SEM is very similar to the EPMA, and considerable overlap in capabilities exists between the two instruments (Amit and Arthur, 2009 ).

### 2.6.1.1 Fundamental Principles of Scanning Electron Microscopy (SEM)

Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and continuum X-rays), visible light (cathodoluminescence--CL), and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (Egerton, 2005).

X-ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete ortitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays that are of a fixed wavelength (that is related to the difference in energy levels of electrons in different shells for a given element). Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly (Egerton, 2005).

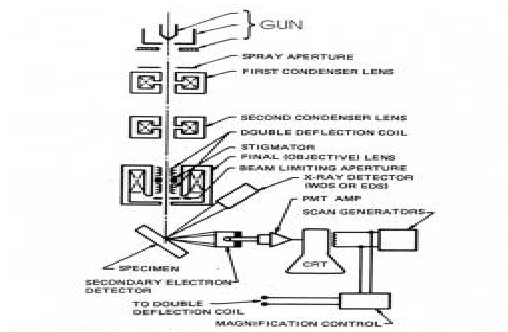


**Figure 2.2** Scanning electron microscopy (SEM) instrumentation

SEMs always have at least one detector (usually a secondary electron detector), and most have additional detectors. The specific capabilities of a particular instrument are critically dependent on which detectors it accommodates (Egerton, 2005).

### 2.6.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR stands for Fourier Transform InfraRed, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum (Du and Hsieh , 2007).

Fourier Transform Infrared Spectroscopy exposes the sample to a single pulse of radiation and measures the response. The resulting signal called free induction decay contains a rapidly decaying composite of all possible frequencies. Due to resonance by the sample, resonant frequencies will be dominant in the signal and by performing a mathematical operation called a Fourier Transform on the signal, the frequency response can be calculated. In this way, Fourier Transform Spectrometer can produce the same kind of spectrum as a conventional spectrometer but in a much shorter time (Du and Hsieh , 2007).

The principles of the Fourier Transform approach can be compared to the behavior of a musical tuning fork. If a tuning fork is exposed to sound waves of varying frequencies, it will vibrate when the sound wave frequencies are in 'tune'. This is similar to conventional spectroscopic techniques, where the radiation frequency is varied and those frequencies where the sample is in 'tune' with the radiation detected. The response from a sample exposed to a pulse of radiation is a signal consisting primarily of the characteristic frequencies for that sample. The Fourier Transform is a mathematical technique for determining these characteristic frequencies from a single composite signal (Du and Hsieh , 2007).

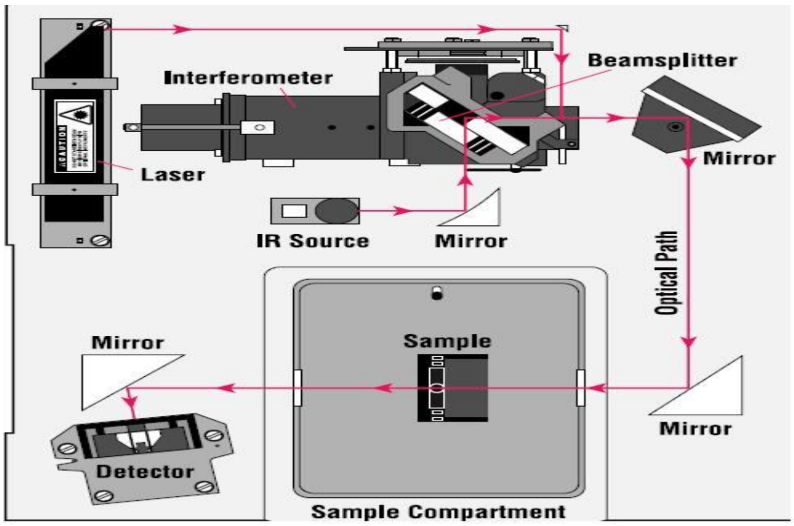


**Figure 2.3:** A simple spectrometer layout

FTIR can identify unknown materials, determine the quality or consistency of a sample and determine the amount of components in a mixture. Fourier transform infrared spectroscopy is preferred over dispersive or filter methods of infrared spectral analysis for several reasons, it is a non-destructive technique, it provides a precise measurement method which requires no external calibration, it can increase speed, collecting a scan every second, it can increase sensitivity – one second scans can be co-added together to ratio out random noise, it has greater optical throughput, and mechanically simple with only one moving part (Du and Hsieh , 2007).

### 2.6.3 Differential Scanning Calorimetry (DSC)

Differential scanning calorimeters (DSCs) provide a widely used and rapid method to determine, as thermometers, temperatures of phase transition or chemical reaction, or, as calorimeters, heat capacities or enthalpies of transition or chemical reaction in the temperature range from about 100 to 1800 K [6±10]. However, the quality of measurements is influenced by instrumental, sample and operator-related parameters, and critically depends on the accuracy of the temperature and caloric calibration of the instrument. DSC instruments are no absolute measuring devices, the measurements are not made in thermal equilibrium and, therefore, the relative data obtained must be related to absolute thermodynamic values by calibration (Plotnikov, 2002).

In principle, three requirements must be fulfilled to obtain correct and reliable DSC data and to guarantee the comparability of published data: a reliable instrument of high reproducibility and sufficiently high resolution, high-precision certified reference materials, traceable to international standards; and metrologically sound and instrument-independent procedures which are directly based on equilibrium thermodynamics, i.e. ITS-90 and traceable heat and heat flow rate standards. The first requirement can be easily fulfilled: Suitable DSC instrumentation is commercially available today from several manufacturers. The second requirement poses a problem: a close examination of the literature reveals serious lack of high-precision and/or certified reference materials, in particular, heat capacity and enthalpy values based on thermal equilibrium data, i.e. adiabatic or drop calorimetry (Plotnikov, 2002).

### 2.6.4 Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) is a technique which monitors changes in the mass of a sample as a function of time and temperature. TGA is commonly employed in research and testing to determine characteristics of materials such as