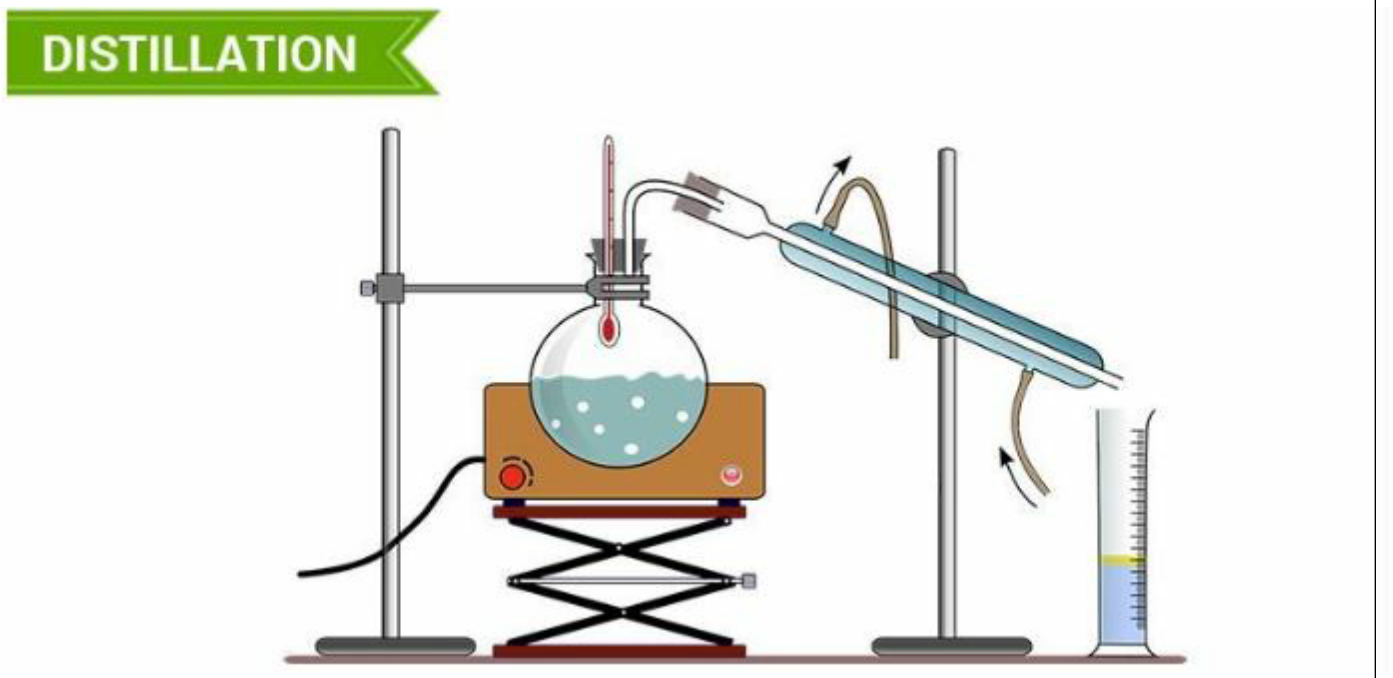
D. Pharm 1st YEAR:

1.1 PHARMACEUTICS I

CHAPTER 11- DISTILLATION

Distillation refers to the selective boiling and subsequent condensation of a component in a liquid mixture. It is a separation technique that can be used to either increase the concentration of a particular component in the mixture or to obtain (almost) pure components from the mixture. The process of distillation exploits the difference in the boiling points of the components in the liquid mixture by forcing one of them into a gaseous state.

It is important to note that distillation is not a chemical reaction but it can be considered as a physical separation process. An illustration describing the laboratory setup that is generally used to execute this process is provided below.



The distillation performed on a laboratory scale often uses batches of the liquid mixture whereas industrial distillation processes are generally continuous, requiring a constant composition of the mixture to be maintained.

Types of Distillation

Some important types of distillation include:

- Simple distillation
- Fractional distillation
- Steam distillation
- Vacuum distillation
- Air-sensitive vacuum distillation
- Short path distillation
- Zone distillation

Simple Distillation

- Simple distillation involves heating the liquid mixture to the boiling point and immediately condensing the resulting vapors.
- This method is only effective for mixtures wherein the boiling points of the liquids are considerably different (a minimum difference of 25^{o}C).
- The purity of the distillate (the purified liquid) is governed by Raoult's law.

Fractional Distillation

Fractional distillation is often used to separate mixtures of liquids that have similar boiling points. It involves several vaporization-condensation steps (which takes place in a fractioning column). This process is also known as rectification. The apparatus required to perform a fractional distillation on a mixture is listed below.

- Round-bottom flask or distilling flask
- A source of heat, which can be a fire or a hot bath.

- Receiving flask to collect the condensed vapors
- Fractioning column
- Thermometer to measure the temperature in the distilling flask
- Condenser
- Standard Glassware.

When heated, the liquid mixture is converted into vapors that rise into the fractioning column. The vapors now cool and condense on the walls of the condenser. The hot vapors emanating from the distilling flask now heat the condensed vapor, creating new vapors.

Many such vaporization-condensation cycles take place and the purity of the distillate improves with every cycle. An illustration depicting a fractional distillation setup is provided below.

Commonly used condensers in laboratories include Liebig condensers and Graham condensers.

Steam Distillation

- Steam distillation is often used to separate heat-sensitive components in a mixture.
- This is done by passing steam through the mixture (which is slightly heated) to vaporize some of it. The process establishes a high heat-transfer rate without the need for high temperatures.
- The resulting vapor is condensed to afford the required distillate.
- The process of steam distillation is used to obtain essential oils and herbal distillates from several aromatic flowers/herbs.

Vacuum Distillation

- Vacuum distillation is ideal for separating mixtures of liquids with very high boiling points.
- In order to boil these compounds, heating to high temperatures is an inefficient method. Therefore, the pressure of the surroundings is

lowered instead.

- The lowering of the pressure enables the component to boil at lower temperatures. Once the vapor pressure of the component is equal to the surrounding pressure, it is converted into a vapor.

- These vapors are then condensed and collected as the distillate. The vacuum distillation method is also used to obtain high-purity samples of compounds that decompose at high temperatures.

Important Applications

- Distillation plays an important role in many water purification techniques. Many desalination plants incorporate this method in order to obtain drinking water from seawater.

- Distilled water has numerous applications, such as in lead-acid batteries and low-volume humidifiers.

- Many fermented products such as alcoholic beverages are purified with the help of this method.

- Many perfumes and food flavorings are obtained from herbs and plants via distillation.

- Oil stabilization is an important type of distillation that reduces the vapor pressure of the crude oil, enabling safe storage and transportation.

- Air can be separated into nitrogen, oxygen, and argon by employing the process of cryogenic distillation.

- Distillation is also employed on an industrial scale to purify the liquid products obtained from chemical synthesis.

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CHAPTER 10: EVAPORATION

Evaporation is known as the process of water changing from liquid to gas or vapor. As in it occurs on the surface of a liquid when it turns into the gaseous phase. It is an endothermic process when the heat is absorbed.

EXAMPLE:

EVAPORATION PAN:

The evaporation pan of this standard set is made of stainless steel and has the dimensions of a “class A“ evaporation pan, namely 54 mm (10 inches) in height and 1206 mm (47.5 inches) in diameter. The evaporation pan is installed on the wooden support, which is set and levelled on the ground in a grassy location, away from bushes, trees and other obstacles which obstruct a natural air flow around the pan, thus representing open water in an open area. Daily the result of evaporation and precipitation is measured within the still well, by means of a high quality evaporation micrometer with a measuring range of 100 mm and an accuracy of 0.02 mm. This accuracy can be obtained because the still well prevents rippling of the water surface. The amount of evaporation is a function of temperature, humidity, wind and other ambient conditions. In order to relate the evaporation to wind current or expected conditions, the maximum and minimum temperature as well as the amount of air passed are recorded with the evaporation. For a more exact use of the evaporation pan it is recommended to use an additional wind path meter. For automatic measurement of the evaporation use can be made of a level sensor. The level sensor consists of a sensitive pressure transducer built in a stainless steel housing. The sensor has a pressure range of 0-20 mbar, accuracy 0,25%. Output signal 0-20 mA, power supply voltage 8-28 V. The sensor is supplied with 5 m cable. The sensor is read-out with a datalogger. To configure and read-out the datalogger and to process the measuring data, use is made of the evaporation pan software.Measuring principle An evaporation pan provides a measurement of the combined effect of temperature, humidity, windspeed and sunshine

on the reference crop evapotranspiration ETo .

The principle of the evaporation pan is the following: the pan is installed in the field. the pan is filled with a known quantity of water (the surface area of the pan is known and the water depth is measured) . the water is allowed to evaporate during a certain period of time (usually 24 hours). For example, each morning at 7 o'clock a measurement is taken. The rainfall, if any, is measured simultaneously ρ after 24 hours, the remaining quantity of water (i.e. water depth) is measured ρ the amount of evaporation per time unit (the difference between the two measured water depths) is calculated; this is the pan evaporation: E pan (in mm/24 hours) ρ the E pan is multiplied by a pan coefficient, K pan, to obtain the ETo. Formula: ETo = K pan × E pan with: ETo : reference crop evapotranspiration K pan : pan coefficient E pan : pan evaporation If the water depth in the pan drops too much (due to lack of rain), water is added and the water depth is measured before and after the water is added. If the water level rises too much (due to rain) water is taken out of the pan and the water depths before and after are measured.

Factors influencing the rate of evaporation

Note: Air used here is a common example; however, the vapor phase can be other gases.

Concentration of the substance evaporating in the air

If the air already has a high concentration of the substance evaporating, then the given substance will evaporate more slowly.

Flow rate of air

This is in part related to the concentration points above. If "fresh" air (i.e., air which is neither already saturated with the substance nor with other substances) is moving over the substance all the time, then the concentration of the substance in the air is less likely to go up with time, thus encouraging faster evaporation. This is the result of the boundary layer at the evaporation surface decreasing with flow velocity, decreasing the diffusion distance in the stagnant layer.

Inter-molecular forces

The stronger the forces keeping the molecules together in the liquid state, the more energy one must get to escape. This is characterized

by the enthalpy of vaporization.

Pressure

Evaporation happens faster if there is less exertion on the surface keeping the molecules from launching themselves.

Surface area

A substance that has a larger surface area will evaporate faster, as there are more surface molecules per unit of volume that are potentially able to escape.

Temperature of the substance

the higher the temperature of the substance the greater the kinetic energy of the molecules at its surface and therefore the faster the rate of their evaporation.

Applications

- Industrial applications include many printing and coating processes; recovering salts from solutions; and drying a variety of materials such as lumber, paper, cloth and chemicals.
- The use of evaporation to dry or concentrate samples is a common preparatory step for many laboratory analyses such as spectroscopy and chromatography. Systems used for this purpose include rotary evaporators and centrifugal evaporators.
- When clothes are hung on a laundry line, even though the ambient temperature is below the boiling point of water, water evaporates. This is accelerated by factors such as low humidity, heat (from the sun), and wind. In a clothes dryer, hot air is blown through the clothes, allowing water to evaporate very rapidly.
- The Matki/Matka, a traditional Indian porous clay container used for storing and cooling water and other liquids.
- The botijo, a traditional Spanish porous clay container designed to cool the contained water by evaporation.
- Evaporative coolers, which can significantly cool a building by simply

blowing dry air over a filter saturated with water.

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16. STUDY OF IMMUNOLOGICAL PRODUCTS LIKE SERA, VACCINE TOXOIDS AND THEIR PREPARATIONS

❖ VACCINES TOXOIDS:

A vaccine is a biological preparation that provides active acquired immunity to a particular infectious disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as a threat, destroy it, and to further recognize and destroy any of the microorganisms associated with that agent that it may encounter in the future.

TOXOID

Toxoid vaccines are made from inactivated toxic compounds that cause illness rather than the micro-organism.[37] Examples of toxoid-based vaccines include tetanus and diphtheria. Toxoid vaccines are known for their efficacy. Not all toxoids are for micro-organisms; for example, *Crotalus atrox* toxoid is used to vaccinate dogs against rattlesnake bites.

PREPARATION:

Two workers make openings in chicken eggs in preparation for production of measles vaccine.

Vaccine production has several stages. First, the antigen itself is generated. Viruses are grown either on primary cells such as chicken eggs (e.g., for influenza) or on continuous cell lines such as cultured human cells (e.g., for hepatitis A).[88] Bacteria are grown in bioreactors (e.g., *Haemophilus influenzae* type b). Likewise, a recombinant protein derived from the viruses or bacteria can be generated in yeast, bacteria, or cell cultures. After the antigen is generated, it is isolated from the cells used to generate it. A virus may need to be inactivated, possibly with no further purification required. Recombinant proteins need many operations involving ultrafiltration and column chromatography. Finally, the vaccine is formulated by adding adjuvant, stabilizers, and

preservatives as needed. The adjuvant enhances the immune response of the antigen, stabilizers increase the storage life, and preservatives allow the use of multidose vials. Combination vaccines are harder to develop and produce, because of potential incompatibilities and interactions among the antigens and other ingredients involved.

Vaccine production techniques are evolving. Cultured mammalian cells are expected to become increasingly important, compared to conventional options such as chicken eggs, due to greater productivity and low incidence of problems with contamination. Recombination technology that produces genetically detoxified vaccine is expected to grow in popularity for the production of bacterial vaccines that use toxoids. Combination vaccines are expected to reduce the quantities of antigens they contain, and thereby decrease undesirable interactions, by using pathogen-associated molecular patterns.[91]

In 2010, India produced 60 percent of the world's vaccine worth about $900 million (€670 million).

EXCIPIENTS

Beside the active vaccine itself, the following excipients and residual manufacturing compounds are present or may be present in vaccine preparations. Aluminum salts or gels are added as adjuvants. Adjuvants are added to promote an earlier, more potent response, and more persistent immune response to the vaccine; they allow for a lower vaccine dosage.

- Antibiotics are added to some vaccines to prevent the growth of bacteria during production and storage of the vaccine.
- Egg protein is present in influenza and yellow fever vaccines as they are prepared using chicken eggs. Other proteins may be present.
- Formaldehyde is used to inactivate bacterial products for toxoid vaccines. Formaldehyde is also used to inactivate unwanted viruses and kill bacteria that might contaminate the vaccine during production.
- Monosodium glutamate (MSG) and 2-phenoxyethanol are used as stabilizers in a few vaccines to help the vaccine remain unchanged when the vaccine is exposed to heat, light, acidity, or humidity.
- Thimerosal is a mercury-containing antimicrobial that is added to vials of vaccine that contain more than one dose to prevent contamination and growth of potentially harmful bacteria. Due to the controversy surrounding thimerosal it has been removed from most vaccines except multi-use influenza, where it was reduced to levels so that a single dose contained less than 1 microgram of mercury, a level similar to eating 10 g of canned tuna.

ROLE OF PRESERVATIVES

Many vaccines need preservatives to prevent serious adverse effects such as *Staphylococcus* infection, which in one 1928 incident killed 12 of 21 children inoculated with a diphtheria vaccine that lacked a preservative.[95] Several preservatives are available, including thiomersal, phenoxyethanol, and formaldehyde. Thiomersal is more effective against bacteria, has a better shelf-life, and improves vaccine stability, potency, and safety; but, in the U.S., the European Union, and a few other affluent countries, it is no longer used as a preservative in childhood vaccines, as a precautionary measure due to its mercury content. Although controversial claims have been made that thiomersal contributes to autism, no convincing scientific evidence supports these claims. Furthermore, a 10–11 year study of 657,461 children found that the MMR vaccine does not cause autism and actually reduced the risk of autism by 7 percent.

Vaccine development has several trends:

- Until recently, most vaccines were aimed at infants and children, but adolescents and adults are increasingly being targeted.
- Combinations of vaccines are becoming more common; vaccines containing five or more components are used in many parts of the world.
- New methods of administering vaccines are being developed, such as skin patches, aerosols via inhalation devices, and eating genetically engineered plants.
- Vaccines are being designed to stimulate innate immune responses, as well as adaptive.
- Attempts are being made to develop vaccines to help cure chronic infections, as opposed to preventing disease.
- Vaccines are being developed to defend against bioterrorist attacks such as anthrax, plague, and smallpox.
- Appreciation for sex and pregnancy differences in vaccine responses "might change the strategies used by public health officials".
- Scientists are now trying to develop synthetic vaccines by reconstructing the outside structure of a virus, this will help prevent vaccine resistance

❖ SERA AND IT'S PREPARATION:

Antiserum is human or nonhuman blood serum containing monoclonal or polyclonal antibodies that is used to spread passive immunity to many diseases via blood donation (plasmaphoresis). For example, convalescent serum, passive antibody transfusion from a previous human survivor, used to be the only known effective treatment for ebola infection with a high success rate of 7 out 8 patients surviving.

Antisera are widely used in diagnostic virology laboratories. The most common use of antiserum in humans is as antitoxin or antivenom to treat envenomation.

Serum therapy, also known as serotherapy, describes the treatment of infectious disease using the serum of animals that have been immunized against the specific organisms or their product, to which the disease is supposedly referable.

Both antisera and antitoxins are means of proactively combating infections. The introduction of compounds to which the immune system responds is an attempt to build up protection against microorganisms or their toxins before the microbes actually invade the body.

The use of antiserum and antitoxin preparations is now a standard avenue of infection control . The beginnings of the strategies dates to the time of Edward Jenner in the late eighteenth century. Then, Jenner used an inoculum of cowpox material to elicit protection against the smallpox virus.

Jenner's strategy of using a live organism to elicit an antibody response led to a "third-party" strategy, whereby serum is obtained from an animal that has been exposed to an antigen or to the microorganism that contains the antigen. This so-called antiserum is injected into the human to introduce the protective antibodies directly, rather than having them manufactured by the person's own immune system.

The same strategy produces antitoxin. In this case, the material injected into the animal would consist of active toxin, but in very low quantities. The intent of the latter is to stimulate antibody production against a toxin that has not been changed by the procedures used to inactivate toxin activity.

The use of antitoxin has been largely supplanted by the injection of a crippled form of the toxin of interest (also known as a toxoid) or a particularly vital fragment of the toxin that is needed for toxic activity. The risk of the use of a toxoid or a fragment of toxin is that the antibody that is produced is sufficiently different from that produced against the real target so as to be ineffective in a person.

Since the time of Jenner, a myriad of anti-sera and antitoxins have been produced against bacterial, viral and protozoan diseases. The results of their use can be dramatic. For example, even in the 1930s, the form of influenza caused by the bacterium *Hemophilus influenzae* was almost always lethal to infants and children. Then,

Elizabeth Hattie, a pediatrician and microbiologist, introduced an anti-influenzal antiserum produced in rabbits. The use of this antiserum reduced *Hemophilus influenzae* influenza-related mortality to less than twenty per cent.

Antiserum can contain just one type of antibody, which is targeted at a single antigen. This is known as monovalent antiserum. Or, the antiserum can contain multiple antibodies, which are directed at different antibody targets. This is known as polyvalent antiserum.

The indirect protective effect of antiserum and antitoxin is passive immunity. That is, a protective response is produced in someone who has not been immunized by direct exposure to the organism. Passive immunity provides immediate but temporary protection.

Antiserum and antitoxin are obtained from the blood of the test animal. The blood is obtained at a pre-determined time following the injection of the antigen, microorganism, or toxoid. The antiserum constitutes part of the plasma, the clear component of the blood that is obtained when the heavier blood cells are separated by spinning the blood in a machine called a centrifuge.

Examples of anti-sera are those against tetanus and rabies. Typically, these anti-sera are administered if someone has been exposed to an environment or, in the case of rabies, an animal, which makes the threat of acquiring the disease real. The anti-sera can boost the chances of successfully combating the infectious organism. After the threat of disease is gone, the protective effect is no longer required.

The advent of antibiotics has largely replaced some types of antiserum. This has been a positive development, for antiserum can cause allergic reactions that in some people are fatal. The allergic nature of antiserum, which is also known as serum shock, arises from the nature of its origin. Because it is derived from an animal, there may be components of the animal present in the antiserum. When introduced into a human, the animal proteins are themselves foreign, and so will produce an immune response. For this reason antiserum is used cautiously today, as in the above examples. The risk of the use of antiserum or antitoxin is more than compensated for by the risk of acquiring a life-threatening malady if treatment is not undertaken.

Serum sickness is a hypersensitive immune reaction to a contaminating animal protein in the antiserum. The antibodies that are produced bind to the antigen to make larger particles called immune complexes. The complexes can become deposited in various tissues, causing a variety of symptoms. The symptoms typically do not appear for a few weeks after the antiserum or antitoxin has been administered.

With the development of sophisticated techniques to examine the genetic material of microorganisms and identify genes that are responsible for the aspects of disease, the use of antiserum and antitoxin may enter a new phase of use. For example, the genetic sequences that are responsible for the protein toxins of the anthrax bacterium are now known. From these sequences the proteins they encode can be manufactured in pure

quantities. These pure proteins can then form the basis of an antitoxin. The antibodies produced in animals can be obtained in very pure form as well, free of contaminating animal proteins. These antibodies will block the binding of the toxin to host tissue, which blocks the toxic effect. In this and other cases, such as an antitoxin being developed to *Escherichia coli*, the use of antitoxin is superior strategy to the use of antibiotics. Antibiotics are capable of killing the anthrax bacterium. They have no effect, however, on action of the toxin that is released by the bacteria.