Analytical Technologies of Biotechnology
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Module - 2
Radioisotopes Techniques
Lecture - 5
Autoradiography and RIA

In this lecture, we will continue our discussion on liquid scintillation counting. If you could recall in previous lecture, we were discussing about the different various aspects of liquid scintillation counting. We were discussing about how energy transfer takes place from radio isotope to solid solvent from solvent to primary flow, and secondary flow. Then finally electric signal generating through the photo multiplier tube, we have seen through the different advantages and disadvantages of liquid scintillation counting.

If you could recall where we have seen that how the liquid scintillation counting method is very accurate, efficient, and it is a versatile technique. Particularly, for weak beta emitters, it has advantage of counting more than one isotope at a time. If they have sufficient 3 different energy spectrums, so you have lot of advantages of liquid scintillation counting, but there were disadvantages also like quenching was a major problem or photo multiplier noise was a problem, which has been solved to a certain extent. So, even if there are certain disadvantages like cost also, but they could be over looked, because of lot of advantages of liquid scintillation counting.

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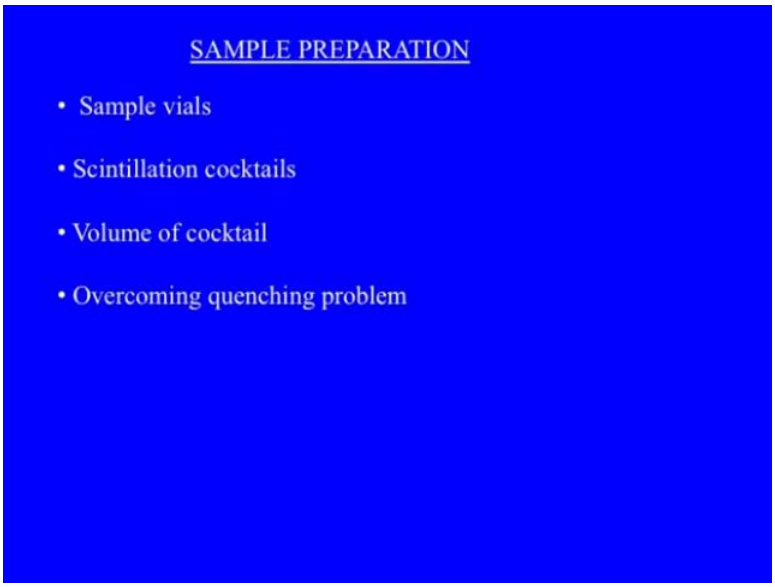

Now, today we are going to discuss about the sample preparation in liquid scintillation counting. So, we will in sample preparation, we require lot of things like sample vials what kind of scintillation cock tail has to be taken, what is the volume of the scintillation cock tail, how sample needs to be prepared for proper counting. All these things, we are going to discuss in this lecture.

Now, let us start with sample vials. Actually, as far as solid scintillation counting is concerned, sample preparation is easy and only involves transferring the sample to a glass or plastic vial or compactable with the counter. As far as liquid scintillation counting is concerned, sample preparation is complex and starts with selection of suitable vial to be used.

Now, types of vials be used as a sample vials, they could be glass, they could be low potassium glass with low levels of 40 k. They could be plastic or poly ethylene vials. Now, glass vials provide unparalleled optical clarity, good visibility. They are chemically inert. So, we are making it suitable for use with aggressive reagents. Many solvents are used actually. Now, glass vials can be reused many times if thoroughly cleaned. Low potassium glass vials could be used where you can reduce the back ground count. So, you have lot of advantages of glass vial in here.

Apart from glass vials, you can use plastic vials. Now, plastic vials exhibits lower back ground label than glass and as compared is more combustible. Therefore, it is easier for

waste disposable. It is shatter proof. Therefore, it is safer in the laboratory. It is cheaper but the problem with this is you cannot reuse it. So, poly ethylene vials give better transfer that is better like transfer and results in higher counting efficiencies.

But, they are inclined to exhibit more phosphoresces than do the glass vials. So, you can choose which vial has to be used, could be plastic vial or could be glass vial. Now, what size of the vials needs to be used here? Now, most of the time, it could range from the size of the vials available, could range from somewhere less than 4 ml up to 20 ml in many photo multiplier tubes. It is kind of 20 ml is the maximum amount, which is fixed due to the dimensions of certain photo multiplier tubes.

Now, mini wise is used to reduce the cost and in terms of environmental issues where scintillation fluids are toxic. So, you can use smaller amount of sample and the scintillation cock tail. Now, some counters are designed except in very small samples in special poly ethylene bags split into array of many compartments. There are more samples in pharmaceutical industry. For doing lot of different kinds of assays, you can use these kinds of sample carriers or vial section.

Then, very important part of sample preparation is scintillation cock tails. Scintillation cock tails are mostly solution of floors. They could be different types of scintillation cock tails. This will involve solvent as well as this will involve primary and secondary flow.

Now, two types of solutions are, one is miscible with aqueous solution and one which are not miscible with aqueous solution. The majority of radioactive species is present in an aqueous form and as such is not miscible with aromatic solvents, which are your scintillators. Now, the most commonly used water accepting cock tail is dioxine. The most common water immiscible ones are dolyvyn based cocktails.

Now, problem with this is it will not take much of the aqueous samples as talwin and water are immiscible. Massive quenching might result a second solvent mixed with talwin. It somewhat solves this problem, but not to a great extent. Now, cock tails based on dioxines that is 1, 4 dioxine and naphthalene can accommodate up to 20 percent volume to volume water and can be used.

They are phased out due to toxicity. For most purposes, less frequently used jay line being cock tails are suitable as they have greater efficiency of detection than talwin and

with lower toxicity. But, talwin is more common because of cost. So, the problem of this is we cannot take lot of water. This could be solved by presence of surfactants in the cock tail. It enables an aqueous sample to come into the intimate contact with the aromatic solvent by forming a stable micro emulsion.

So, this could be one method to accept the aqueous solutions. Therefore, emulsified cock tails are most frequently used for counting aqueous samples. They are composed of solvents scintillators and surfactant, which could be like for example, tritan x 100 could be used as a detergent here.

Now, these can accept up to 50 percent water, but many times problem occurs or which is of phase transition. As water content increases, they might result into two phases appearing. This could hamper the accurate counting actually. So, lots of readymade cock tails are available in market with very precise instructions that it could be used for preparing your samples with particular scintillation cock tails.

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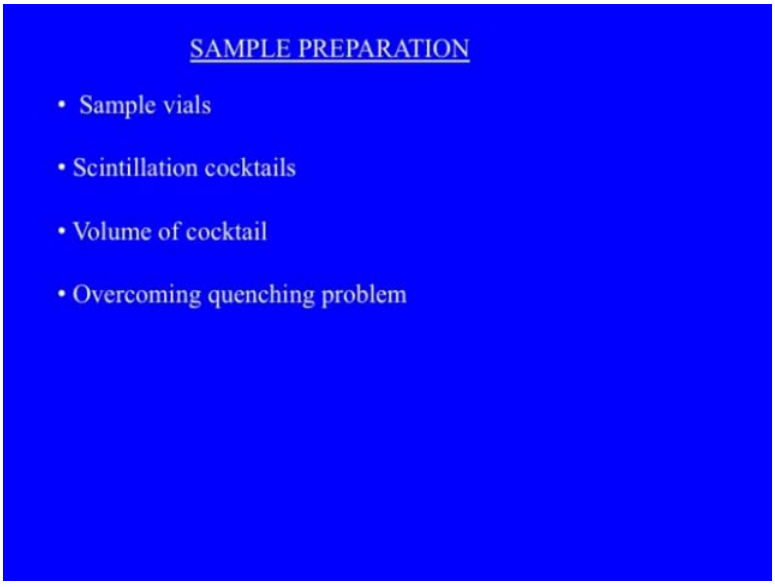

The volume of cock tail is a very critical in measurements or counting efficiencies. One has to use the same amount of volume every time for the sample and instruments. They need to be calibrated with the same value as for the experimental samples. So, this is very important for efficient scintillation counting, which will vary with the sample volume.

So, the volume of the cock tail has to be like same for both standards as well as like instruments needs to be calibrated with the same volume as we use in experimental sample. Now, another problem is when you are preparing samples. How to overcome quenching problem? Now, the quenching problem could be solved by the different methods are there. They have certain advantages and disadvantages. The user has to decide and come to particular way to solve this problem.

Now, samples can be bleached before counting if there is a color quenching. Now, bleaching agents; there are a whole lot of bleaching agents like hydrogen peroxide can be used. But, this gives chemiluminescence. So, care had to be taken while using this hydrogen peroxide. There are solubilization systems.

For example, there are alkaline systems. There are acidic systems in alkaline systems, alkaline hydrolysis and quaternary ammonium hydroxide or Na OH can be used in acidic system, its acidic oxidation. You can use per chloric acid nitric acid or their mixtures could be utilized for soluble addition. There could be lot of other systems also for solubilizations.

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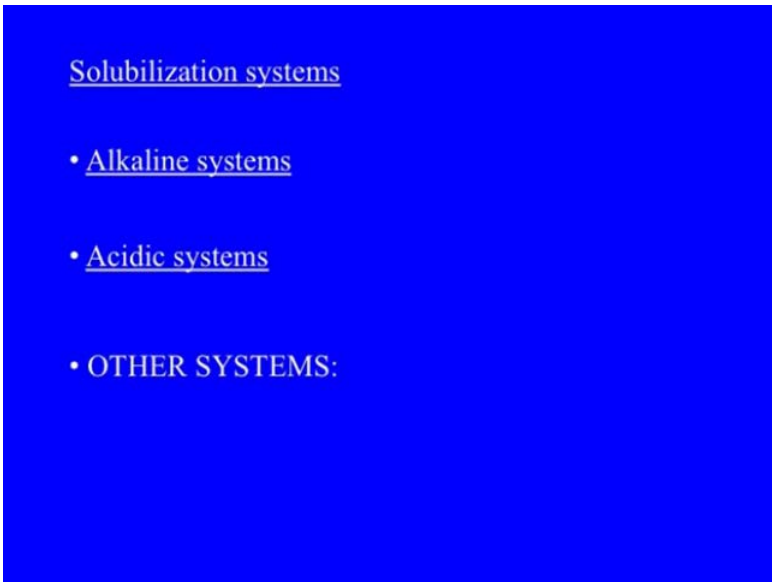

There are tissue solubilisers.

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There are tissue solubilisers like solid samples. Plant and animal tissues are best counted after solubilization quaternary ammines such as NSC solubiliser or solwin. Now, solwin 350 is widely used organic based solubiliser formulated in talwin. It is a classical industry standards solubiliser. It is used for biological and plant samples. It is corrosive and flammable.

So, all these solutions are highly toxic. Great care has to be taken while using them. How do you solubilise? It is simple like you add sample small amount of solubiliser and allow digestion to proceed. After digestion is complete, scintillation cocktail is added and the sample is counted. Problems like chemiluminescence and other has to be needs to be taken care of. There will be because of these materials they might result or show some chemiluminescence. Now, there are combustion methods, which are suitable alternatives to bleaching or solubilization or digestion of tissues.

Now, these combustion methods in how they are used are that samples are combusted in an atmosphere of oxygen in a combustion apparatus. Now, samples say, which are 14 C, will be combusted to 14CO2 or carbon di oxide could be collected in a trapped, agent trapping agent such as NOH. Then counted samples containing say 3 H could be converted to 3 H2O for counting.

So, likewise, you could use lot of different methods where combustion could be an alternative. There could be lot of other methods for preparing sample for lsc, which could

be like cutting of paper chromatogram. It could be membrane filters. It could be used gels, which contain radio isotope or tlc scrapings etc can be used for as a sample actually.

Now, all this sample preparation involves a complex procedure and involves lot of different materials. Now, there is a technique where you do not have to go through the sample preparation. This is called Cerenkov counting. Now, Cerenkov counting is one of like upcoming and it is going to be used and being used in combination quite a lot.

Now, Cerenkov radiation is electromagnetic radiation emitted when a charged particle passes through a di electric medium at a speed greater than the phase velocity of light in that medium. Now, it is useful for same medium high energy emitters in aqueous solution without using cocktails. So, that is a very important part that you really do not have to use cocktail and floors and all those things.

It has decay energy like certain iso radio isotopes or beta particle has decay energy, an excess energy of 0.5 million electron volts. Then this will cause water to emit bluish white light, which is referred to as Cerenkov light after its discovery; this light can be detected using typical liquid scintillation counter. What you see is here really you do not have to do any sample preparation in terms of using scintillation cock tail. It could be directly used for measurement.

Now, what are the advantages of this technique? One is that it is a very simple sample preparation. There is no requirement for organic solvent floors. So, it is relatively cheap technique, no problem of chemical quenching. It can handle large volume of aqueous solution for counting. It allows for analyzing beta emitting isotopes or liquid scintillation counting without using any cock tail.

So, most work on the Cerenkov counting has been done on 32 P, which has its 80 percent of its spectrum above the Cerenkov threshold. It can be detected on 40 percent efficiency sp. As the proportion of the energy spectrum above 0.5 million electron volt increases, the detection efficiency will also increase. So, you look that the table here.

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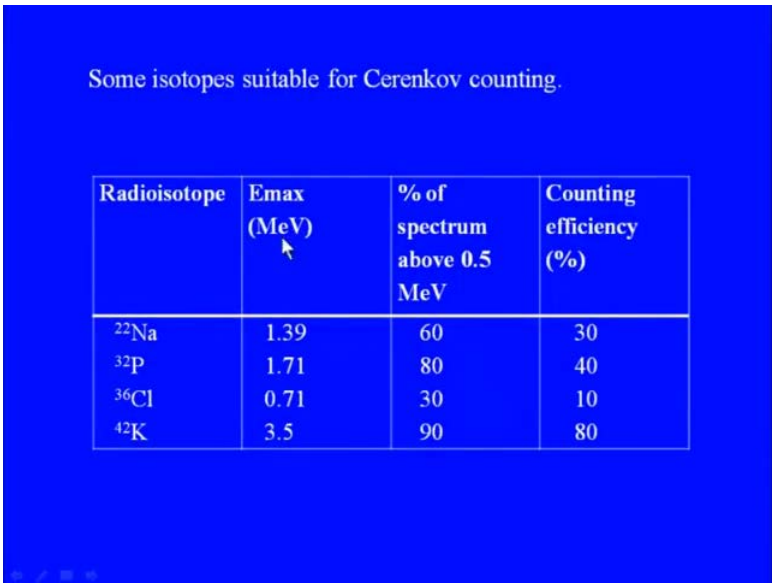

If you see here, there are radio isotopes which have energy spectrum above 0.5 million electron volt. You have percent of spectrum which is 60 above 0.5 million electro volt is 60 percent for 83 percent for 30 P and 30 percent for 30 by C l and 90 for 42 K. They have counting efficiencies, which is 30, 40, 10 and 80 percent respectively. So, what you see here? These isotopes are used for scintillation, liquid scintillation counting or Cerenkov counting in scintillation counters.

This completes our section on the liquid scintillation counting. Now, if we can just go through, we have learnt how liquid scintillation counter works. It is a very versatile technique, very accurate and very efficient technique, particularly for 3 H isotopes, which are very weak. There is a particular way of energy transfer from radio isotope to solvent to primary flow to secondary flow and to multiplier tube.

Then, you have lot of advantages in this sort of technique in terms of efficient counting, which is efficient efficiency is almost 50 percent more in terms in 3 H in terms of measuring more than 2 isotopes at a time through different channels. As we have seen dual labeled sample, this could be measured easily.

So, there are a whole lot of advantages and lot of disadvantages has been partly or completely solved. Also, it is a very versatile, very useful technique. It is much widely used in comparison to say gm counters or gm counting, which are very handy, but

routinely used, mostly for routine purpose and not for like accurate quantification actually. So, we have discussed two methods of detection of radio activity.

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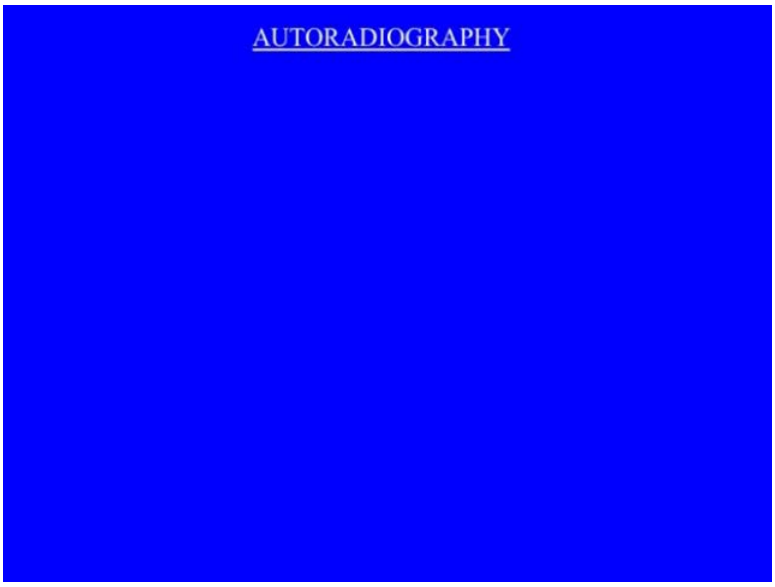

Now, let us move on to the third method of detection that is autoradiography. Now, autoradiography is a technique to localize or to detect or to visualize radio activity, radio actively labeled samples using X ray film or nuclear emulsion. Now, it utilizes the action of ionizing radiation on a photographic emulsion for locating a particular radioactive material in a specimen.

Now, when we say auto, this prefix indicates that radioactive material is within the sample or object, which is going to be imaged here contrast to radiography. Now, recording medium which mixed visible, the resultant image is usually nuclear or photographic emulsion. Now, after chemical development, the resulting pattern of grains shows the distribution of radio activity sometimes in a specimen and microscopic technique. It could be light or electron microscopy could be used for observation. So, this is another technique, which is based on exposure to photographic or nuclear emulsion.

Now, here there are certain differences from standard photographic film emulsion, which is that nuclear emulsion differs from standard one in a high ratio of silver halide to gelatin roughly equal volumes at least in emulsion or may be higher and also in the size of the grain. So, you will have small size of the grains and the nuclear emulsions as compared to photographic emulsion. Now, autoradiography is used to locate rather than quantify.

But, you can use densitometers, which could be used for quantification as they will record the intensity of the image. So, that could be done in biology. This technique has lot of different applications. It may be used to determine tissue localization of a radioactive substance either introduced in to through a metabolic pathway or it is bound to a receptor or hybridized to a nucleic acid. By a lot of other ways, it could be localized or detected.

One example could be like say you want to determine the sample site location of a particular drug, which could be radioactively stable. Then where is the location throughout the body? Then what could be done is that the whole body section of animal could be kept in close contact with a sensitive emulsion, which could be x ray plate or other nuclear emulsion after exposure for certain period of time.

The development of x ray plate will exhibit image of tissue or organs where radio activity was present and location of the drug could be found out. Likewise, radioactive metabolites can be located and recovered on a chromatogram or electro photogram in various metabolic studies.

So, that is how you could do it. It is a very useful technique. Photographic film and autoradiography has come a long way from the time in where 1867, it was observed that uranium salts were responsible for blackening of a photographic film to now where a lot of progress has taken place, where different kinds of emulsions, which is liquid photographic emulsion has been developed for autoradiography. Stripping film has come up. Finally, it is like imaging plate technology has come in 1990s. So, a lot of progress has taken place in this field of autoradiography. Now, let us little bit deal about the principles of autoradiography.

Now, radioactive isotope has an ability to blacken photographic emulsion. Let us see how the whole thing occurs actually. Now, for an autoradiograph radio source that is activity is required, which is emanating from within the material to be imaged that is object or sample. Then you require a sensitive emulsion, which will be exposed here.

Now, emulsion consists of a large number of silver halide crystals as we have discussed which are embedded in a solid phase gelatin. So, what happens is that the beta particles, which are emitted by radio nuclide or a source radioactive source, will penetrate the film

emulsion to a depth, which would be proportional to energy. We are going to discuss that in a little detail.

Now, as particle passes through the film, they will activate the individual silver halide drains into emulsion. Now, this is because of the interaction could be electron interaction with electrons or nuclei. Once these individual silver halide drains are activated, they are rendered or we call them it renders them to susceptible to conversion into a metallic silver. They are exposed and they form a latent heat. Now, they could be then developed photographic developer, which can show them. As blackening of film and fixtures to remove any remaining silver highlights that image could be formed or auto radiographic image could be formed.

Now, each emission converts silver ion to an atom to produce a latent image. When in the development stage, these silver atoms catalyze the reduction of the entire silver halide collection to metallic silver, which produces an autoradiography image. The radio isotope distribution, the distribution of radioactive material may be investigated as a function of time after the injection of the radio labeled compound. For example, in pulse labeling, in place of photographic film, image plate has come with imaging plate scanners, which could be utilized. You can manipulate the film digitally also.

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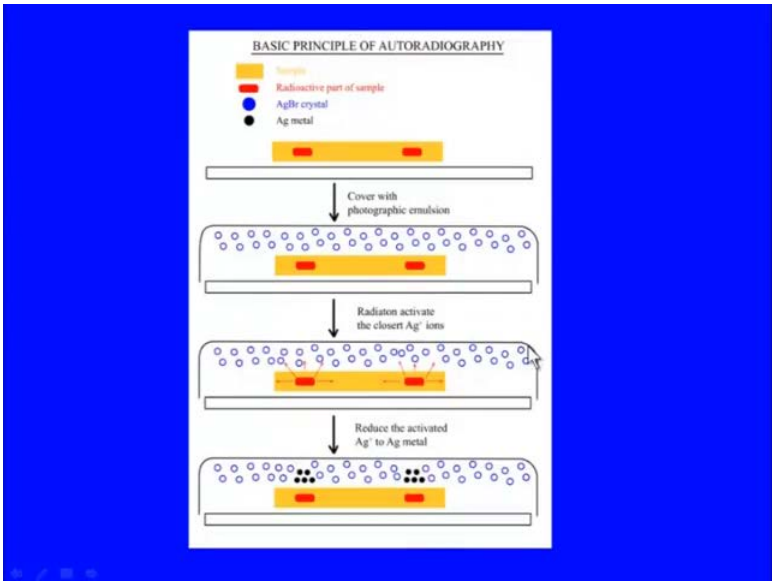

Now, let us little bit see this figure. This figure here kind of explains what we have discussed. Now, if you can see here, there are few things. One is this is your sample. This sample contains the radioactive material. This is your red colored radioactive material.

Now, this sample with radio isotope is what it is done? You have covered it with nuclear or photographic emulsion. As the radioactive source emits the radiation or particles that could be like beta particles, it will strike interact with these grains here, which are shown round grains here.

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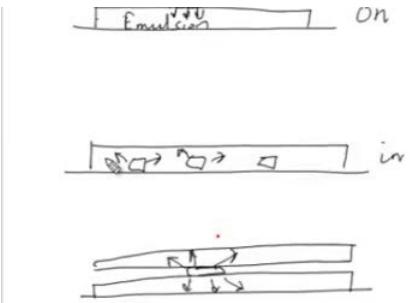

Now, these grains once they interact, they are activated on exposure. They are sensitive. It means they are sensitive for to be developed. Then when you develop, the blackening of the film is seen in here. So, what you see is here seen how from exposure to blackening to development the film where you can see the image occurs.

Now, we call this one is called emitter, which is this and emulsion. This is emitter to emulsion relationship. It is very crucial. Let me show you in this. Let me show you on your screen actually this whole thing. How the emitter and emulsion is important? So, there could be lot of different arrangements like one arrangement I have shown you.

Now, these arrangements could be of many different kinds as you see in your screen. Now, if this is your glass slide, then if your emitter could be this could be your emitter. If this arrangement, this emitter and emulsion arrangement, then that emitter is under the

emitter is under the emulsion. Actually, there could be another arrangement where you have this is your emulsion like; this was your emulsion here.

So, emitter could be above or on surface of the emulsion. So, this will be called condition, which is on the surface of the emulsion. There could be a third condition, where it could be like you have emulsion and your radio isotope is distributed here. We can say this is in actually your emitter and emulsion relationship is that your emitter is in the emulsion. There could be third one where what you could have is you have emulsion here. You have emulsion on the top and your emitter is in between that is it is sandwiched between the emulsions.

So, you could have either of these conditions, which is it could be under, it could be on the surface, it could be in the emulsion and it could be sandwiched between the emulsion. Now, if you see here to go little bit, if it is in the emulsion, which is first place under the emulsion, then half the radiation goes in one direction and half in one . If it is on the surface, again all these half will go. Here, all the emulsions are half and less than 50 percent will reach the emitter emulsion actually here.

Also, that will be like going in all different directions here and this one here will go. It will be exposing on all sides actually. So, you will you can see that depending on the emission and emitter relationship, they will be exposed to a certain extent as not all the particles emitted will be hitting the emulsion. So, this was little bit to show you the relationship between emulsion and the emitter, which is very important in terms of lot of factors. We are going to discuss in a little while.

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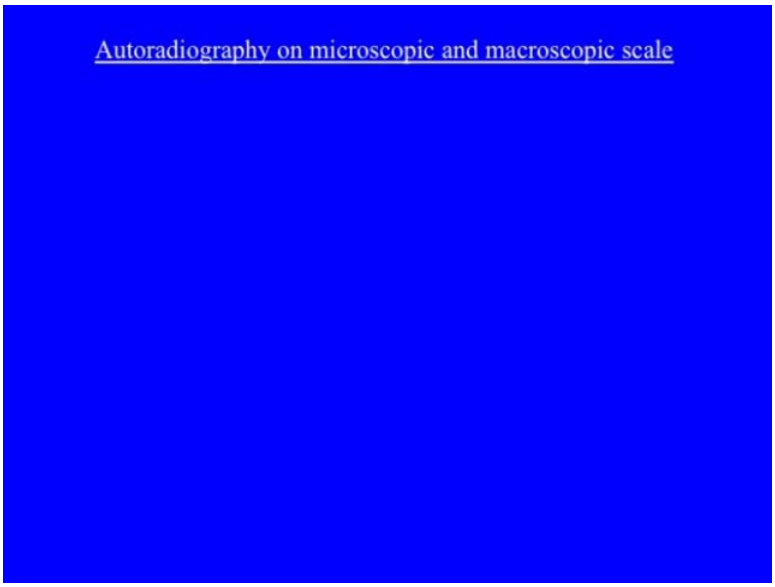

Now, autoradiography can be done on either microscopic scale or macroscopic scale. So, when you say microscopic autoradiography, its resolution is down to 0.5 micrometer or so and then it is done for localization of a tracer at the tissue or cellular label. If it is a macroscopic autoradiography, then resolution could be down to 50 micrometer and localization is at the organ label.

Now, what are the most suitable isotopes, which are used in biological systems in autobiography? The most commonly used radio isotopes are of three types, which could be high energy 32 P. It could be medium energy like 14 C and 35 S beta emitters. It could be low energy like 3 H beta emitters very not so frequently alpha emitters like tellurium and thorium so are used.

So, we are going to discuss about like how they will be exposing a particular emulsion? Now, first thing when we are talking about exposure of a photographic film, there is these are radio particles or emitted particles when. They are exposed. There are certain factors to be considered.

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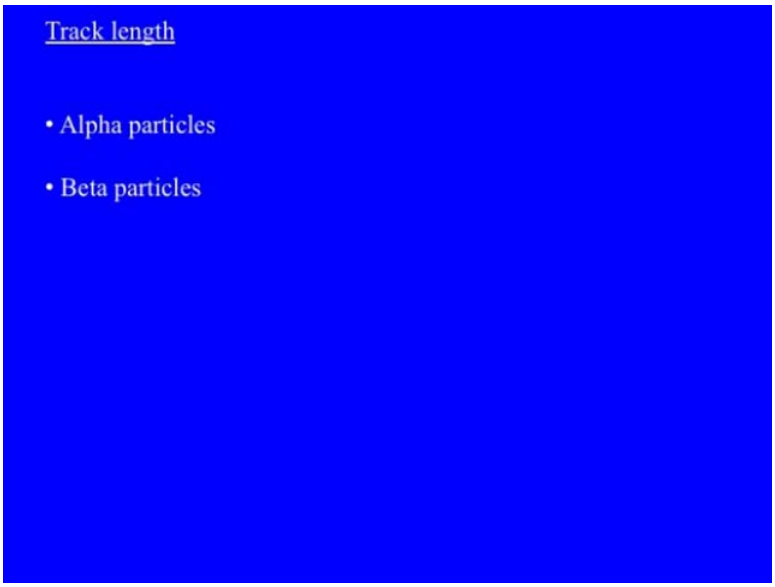

One is that is track length of various emitted particles. Now, when a particle emitted by a radioactive source passes through a nuclear emulsion, it loses energy by collisions with nuclei and other orbital electrons. Now, this energy produces defects in the silver halide crystals and renders them double able as we have discussed earlier. They are exposed.

So, the resulting patterns in the green in the emulsion is called track. It is characterized by three parameters, which is length, which is green density. It is green per unit length or per total track length and shape, which could be linear curved and angled. So, the parameters are determined by the mass energy of the particles emulsion and development of emulsion. Let me show you little bit, what does that means like track length and all those things.

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See this is your emulsion actually, which is server halide gelatin. Now, when we say that radio isotope or beta particle emitted from a radio isotope poses or interacts with the silver halide crystals here, finally, you get a track. What is track? Track is a patron, which is obtained after the exposure, which could be a simple line. It could be a straight line and most of the time, the density of the track as we will discuss is more at the end of the track actually.

If it starts from here, the density will be higher in this particular area. Then it could be like it could have different kinds, it could be straight, it could be curved or it could be very small actually like for 3 H. It does not have so much of energy to have longer tracks. So, you have a track as a particular direction. It has a particular density, which is a grain per unit length exposed. It has particular kind of like how many grains it is going to expose. So, this is like what we were talking about here.

Let us move back here. What we were talking about is that three parameters that is length, grain density and the shape sp length will be higher. If the higher energy is there, the grain density per unit length be mostly again by energy. It is track length will be or density will be higher at the end of the track and the shape, which could be linear, curved. It could be angled as per the particular kind of ammeter actually.

Let us see each of these ammeters and their effect on the particular nuclear emulsion. Let us discuss with alpha particles. Now, alpha particle as we have discussed earlier also, it is

a heavy doubly positively charged and less penetrating. So, these messy particles are not affected by collision with electrons and usually contain a straight path.

So, I have shown you earlier that they will maintain a straight path after collision and will have a tremendous disrupting effect on orbital electrons as they pass through emulsion. Now, these results into excitation of almost every single halide crystals they interact with. Therefore, they generate a very high grain density or and alpha particle will lose energy rapidly as it interacts with very large number of electrons per unit distance. Therefore, it will have short and a straight track of length, which is usually 15 to 40 micrometer.

So, it is like because it loses energy very fast. Though it has lot of energy, but because of its bigger size, it loses energy very fast. It has straight and short and dense breadth length. As far as beta particles are concerned, there are three kinds of beta particles, which is high energy, medium energy and low energy. So, when these beta particles, these are electrons only, these are scattered easily by orbital electrons.

Now, as they collide with other electrons, they rapidly lose energy and sharply deflect at each collision. So, the magnitude will depend on their energy. Now, at a very high energy like for 32 P, particles have a tendency to move in a straight line with minimal deflection due to great momentum. Now, energy will be lost after each collision within orbital electron and probability of deflection and subsequent interaction increases.

This is because you will lose energy. The straight path may not be maintained for short distance. The track remains fairly straight as these deflections are balanced out because of high electron density of metro that is silver halide that is which they are passing.

So, the grain density increases as the particle loses the energy that is the end of the track. The grain density will always be greater at the end of the track than at the beginning. So, that is a very important factor here. If you have three kinds of beta particles that are high energy, medium energy and low energy, we will see how they affect as we discuss.

Now, weak beta emitters like 3 H, 40 C and 35 S are suitable. These ionizing tracks are short and give discrete. This is as compared to 32 P. So, they have short range of the particle. This permits accurate localization; particularly here H is the best radio isotope. It has less energy. It will form short tracks as compared to 14 C, 35 S and particularly 32 P, which will form longer tracks and to really localize, it will be difficult.

Now, microscope can be used for locating to develop film. Now, for localization of DNA bands in an elecrtrophoretic gel, 3 X cannot be used. There 32 P is used. Why? This is because low energy 3 H electrons will disappear; energy within the gel and in the wrapping around the gel. Thus, it is reducing the sensitivity to a very low label. So, more energetic 32 P electrons leave the gel and produce a strong emit. So, it depends on type of application here which we are talking about.

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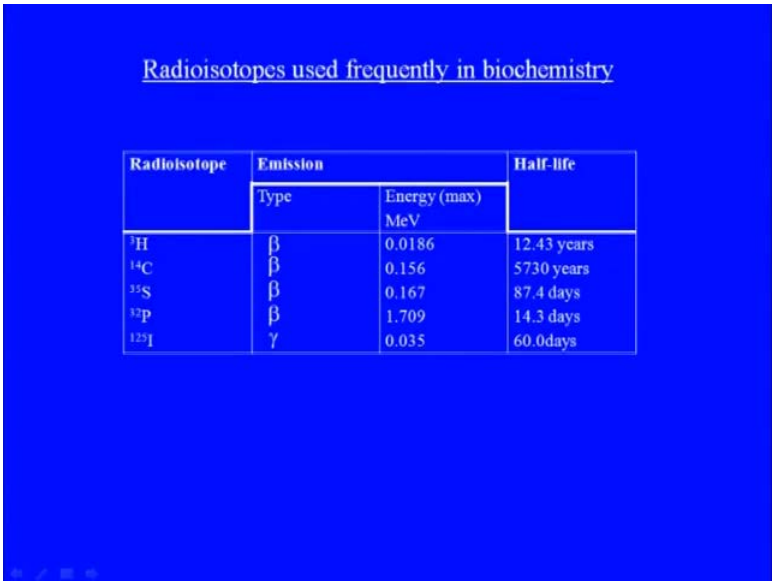

Now, radio isotopes, if you can see this table, they are there. There are whole lot of isotopes could be in here for this particular application, which are 3 H, 14 C, 35 S, 32 P and 125 I. It has mostly beta and 125, I gives gamma radiations. Now, we have talked about types of isotopes or radioactive isotope particles, which are being used in here like mostly beta particles. Now, let us little bit discuss about types of emulsions.

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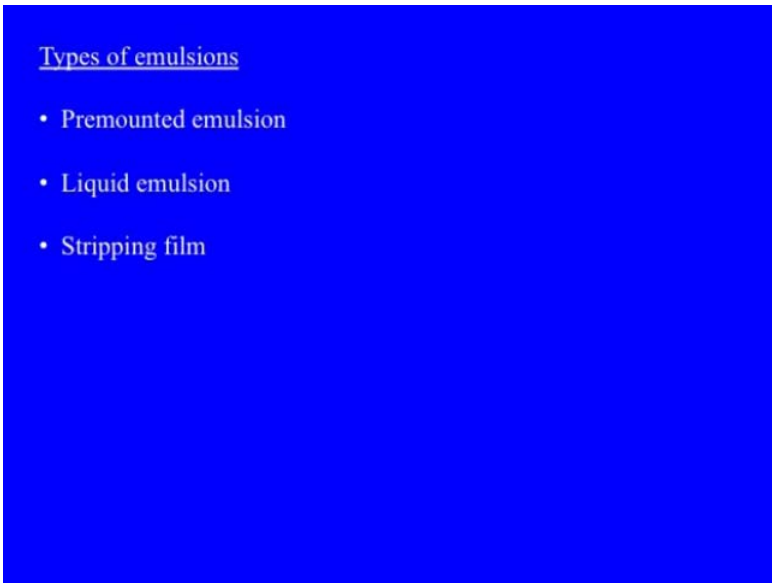

Actually, there are three types of commonly used emulsions. One is called pre mounted emulsion. Another is called liquid emulsion. The third one is stripping film. Now, pre mounted emulsion is relatively thick, may be 50 to 200 micrometer thick layer of emulsion mounted on a glass microscopic slide. As far as liquid emulsion is concerned, it is used in dipping method.

So, what it is done is it is supplied as a shredded gel, which could be melted. A sample mounted on a glass microscopic slide will be dipped into a molten gel and taken out. The emulsion hardens and it forms a film of a particular thickness depending on the concentration of gelatin and liquid.

Now, third one is stripping film. It is supplied as a 5 micro meter filling mounted on a glass and can be removed from the glass with a razor blade. Then it can be placed on the water surface. Now, the pre mounted sample is placed under the floating film and lifted. Then this is allowed to dry and thin emulsion is held tightly to the slide.

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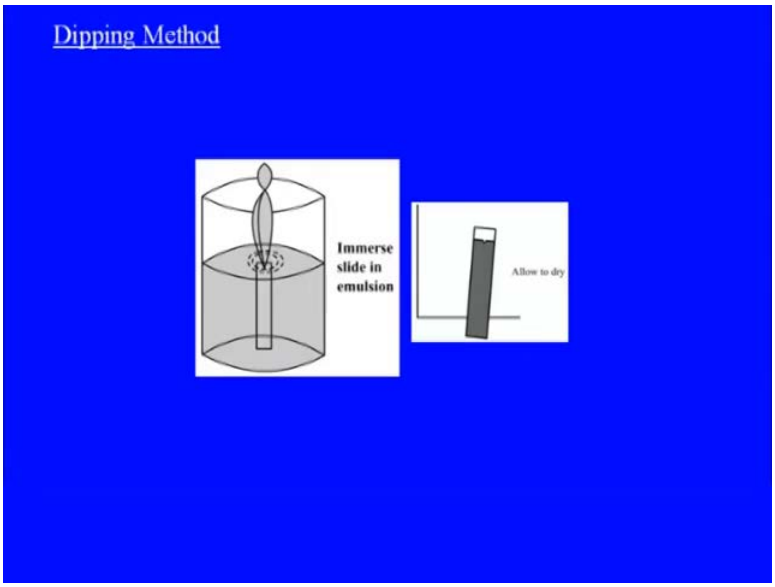

Now, let us see each of them in these figures here. So, if you can see here in dipping method, which is used for liquid emulsions, what is done is there is a liquid emulsion filled in this beaker. You have a slide, which contains the specimen or the sample. You dip the slide into the liquid emulsion, which is melted. Then take it off. Then it is allowed to dry. The liquid emulsion will form a covering around the sample and on the other parts of the slide.

Then, once it is ready, this could be used for or this could be exposing. It could be then finally, used will be allowed to form the radiographic image actually. So, this is how dipping method is performed. Then there is a stripping method. Now, stripping method like as I said, a sheet of commercially available stripping film is utilized, which can be removed with a razor blade. Let us see like how this works out.

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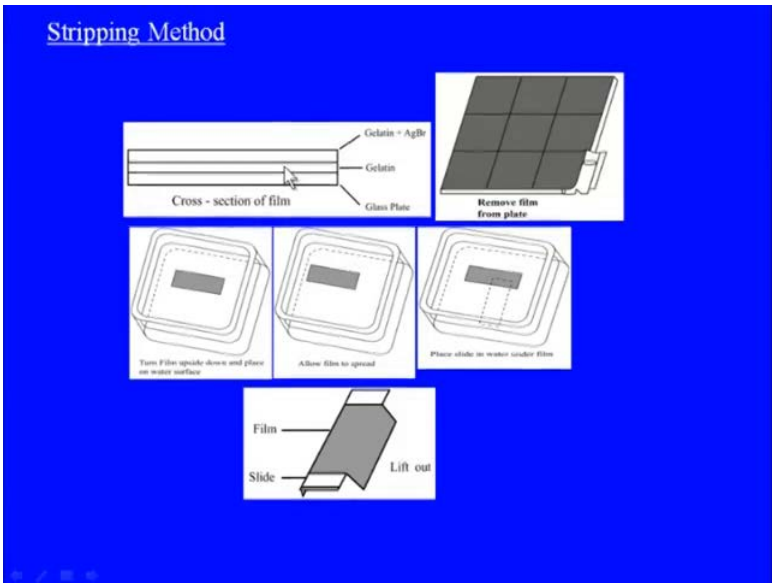

So, what you have is you have a cross section of film here. It looks something like this. There is a rectangular or you can say square strips here. They are on a glass or plastic surface. Now, with a razor, you can remove each of these strips here one by one. They can be used.

What is done is this will be put into the water and it is allowed to swell. Then as it spreads out, what can be done is a slide with a sample could be taken. This could be put under the film. Then it could be lifted like here bit is seen. It is lifted here. The film wraps around the slide. The slide contains the sample under this particular film.

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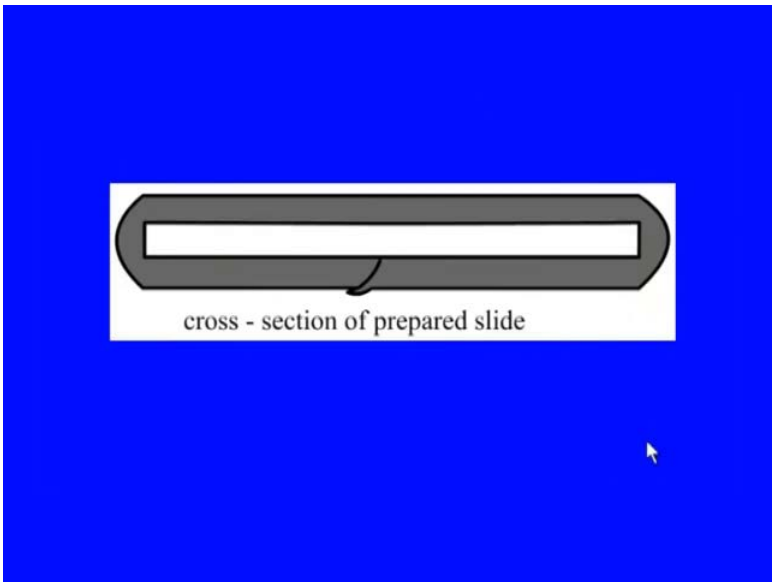

Finally, what you are going to get is adehrn film or which is wrapped around the glass slide. This finally, could be utilized for developing an autoradiography image. So, that is how three kinds of emulsions are utilized. Now, very important part of this method of or any method is what are the resolution, efficiency and the background problems.

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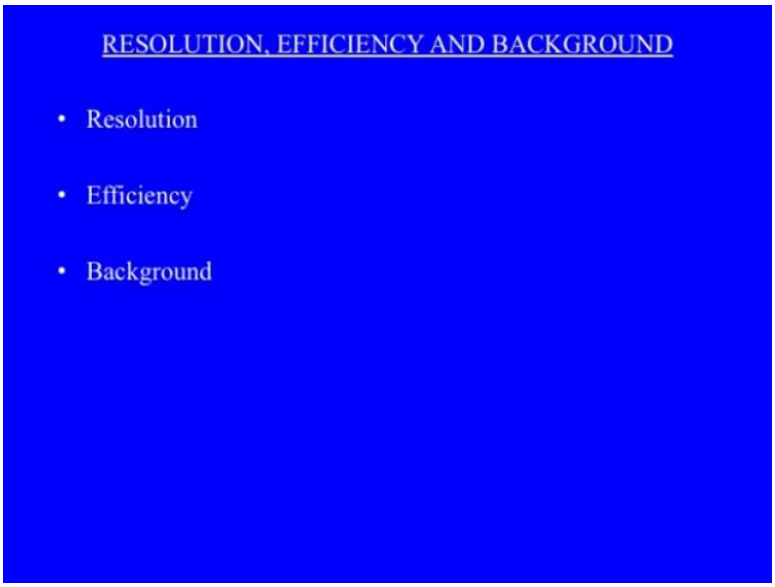

So, high resolution, high efficiency and low background are important factors to be attained in any experiment. So, let us little bit discuss about these factors autoradiography. Now, resolution here is first thing. So, as far as resolution in autoradiography is concerned, it is the ability to determine the position of emitting source to separate the individual grains to get accurate grain count and to separate the emitting sources.

So, that is what resolution is. Can you really like determine the position of emitting source? Can you separate the individual grains to get accurate grain count? That is the most important part. If there are more emitting sources, can you locate them or can you separate both emitting source? This depends on various factors like we have discussed earlier. We will elaborate on that. It will depend on types of radio isotopes that are energy of the radiation. If you consider the energy of the radiation like I said 32 P, if you say we were talking about the track length, now 32 P has higher energy.

So, what will happen? As it travels through the emulsion in beginning, there will not be any track which or if there is a track, it will be not distinguished from the back ground.

So, what will happen? It will travel to a certain distance. The track is seen only when it is has travelled or certain distance in it is very hard to localize it unless it is travelling in all different directions.

Then, all of these tracks could be extrapolated to a origin likewise say, many times 14 C and 30 S gives curved surfaces, curved tracks actually. Now, these curved tracks, again it is hard to localize. If you need a very high resolution images 3 H, if it is a weak 3 H weak beta particle from 3 H, then it might be very difficult to distinguish it from the background. So, all these factors like that are type of radio isotope will determine the resolution. As such, the distance between the radios labeled compo component and the radio sensitive emulsion that is very important.

For example, if there is a very thick emitter, and then may be lot of self absorption occurs. That might be a problem. So, that is the thickness of the object or the thickness of the emulsion exposure period. All these things will be affecting the resolution. Then size of the silver halide grain will certainly affect resolution. If its size is very large, it will be very difficult to count number of grains because smaller grains give higher resolution as compared to larger grains in here.

Now, for example, if there is 3 H, then it may not be able to expose the larger grain or render it developable, rather than small grains, which could be counted as well they could be distinguished from the unexposed grains. Here, sensitivity of the emulsion is also important. For example, if there is a emitter or low energy emitter, then unless there is a sensitive emulsion, it will be very difficult to see the image from that particular resultant of that particular emitter. Certain emitter has a longer track length.

Then, it will only form like a track when it can expose the grain. If the sensitivity of the grain is lower, then you need more energy to expose that. So, again localization will be a problem. Tracks will be appearing only at the end where lot of energy is lost. So, these are very important factors in terms of determining the resolution. Then second factor is efficiency. Now, efficiency means that every decay should produce a track. Now, however if the sample is on the surface of the emulsion, only one half of the decay will enter the emulsion. Now, we say sample is embedded in the emulsion, self absorption of energy by sample.

This is because of finite thickness may not reach or reach with a very least energy and not suitable for producing a track. So, that is another factor, which has to be considered for high energy isotopes like 32 P. Self absorption is not a severe problem as a 5 micro meter thick sample thickness of cell or tissue sections absorbs less than 1 percent energy.

For low energy isotopes like say 40 C or 35 S, it is almost like 82 and 70 percent transmission at the same 5 micro meter thickness. But, for 3 H, if you consider the efficiency is very less. It is like say 16 percent for 0.5 micro meter and 4 percent for 5 micro meters. So, these things have to be considered.

Now, emulsion thickness also affects efficiency. Most grains are produced near end of the track with 32 P having long tracks efficiency is proportional to emulsion thickness to a certain ex extent say for 14 C and 30 S. Maximum efficiency is reached at an emulsion thickness between 3 and 5 micro meter. For 3 H, you have range of 1 micro meter. Nothing is gained by using thicker emulsion. So, if it is a 32 P, you can use a thicker emulsion.

If it is a 3 H, you have to use a very thin emulsion. There is no use of using thicker emulsion for 3 H. So, these factors have to be taken into account to increase the efficiency of the experiment. Then very important factor is back ground. So, developed emulsion has not been exposed to radioactive sample contains the dark grains and these are called background. Now, to identify a track like how to distinguish it from the background a conventional approach is to look for at least 4 grains in a straight line to define a track. In case of 3 H, track may have only 1 or 2 grains.

So, it is important to reduce the background to very low label if such isotopes need to be used. There is another thing is background fog. That is latent image on the developed film can result from say from accidental exposure to light, presence of chemicals or metals in the sample, natural background radioactivity like say from 40 k cosmic rays, mechanical pressure applied and the way film is stored. So, lot of these things can give rise to background.

Now, background can form before the sample is applied or during the exposure in liquid. Emulsion tracks are destroyed if when emulsion is melted. So, that could be one thing in stripping film. The prior background is reduced to a greater ex extent as film contains a

latent image fader. So, that could be another way to reduce the background. Background will always increase during exposure time.

So, one has to shorten the exposure time also. The problem of latent image fading could be another problem because it refers to the fact that exposed grains gradually revert to the unexposed form, hence undevelopable. See all these factors have to be taken into account. They have to be considered when autoradiography is being done.

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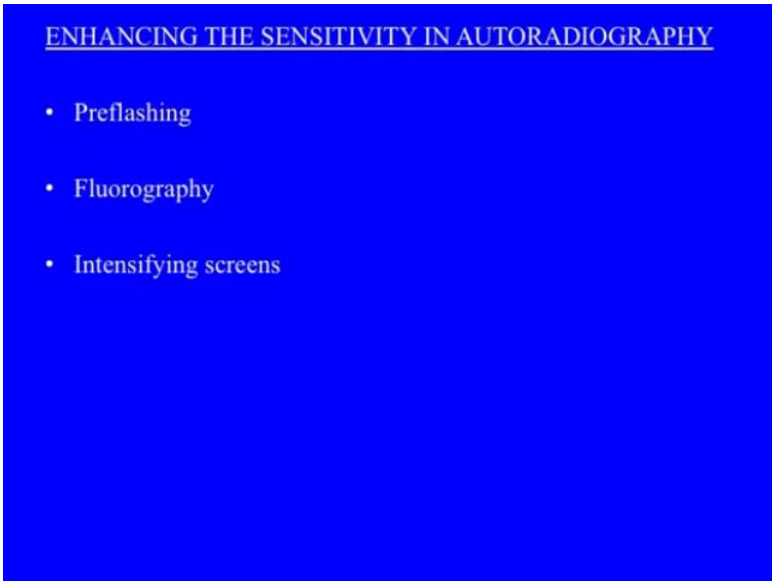

Now, there are certain techniques to enhance the sensitivity factor or sensitivity in autoradiography. We will discuss them one by one. One is pre flashing. Now, pre flashing is like the response of photographic emulsion to radiation or light. It is not linear. It involves a slow lack period or initial phase before a linear phase is obtained. So, the sensitivity of the film may be enhanced by pre flashing. That is it involves exposing the film to a millisecond flash of light prior to placing it on the contact with the sample. So, tech technique is often used in high resolution work.

Then, there is a fluorography that means that the radioactive sample containing soft beta emitters such as 3 H in a chromatogram or electrophoresis gel, they can be located precisely as much of their energy is lost in the gel. So, they cannot be located precisely. However, sensitivity could be enhanced drastically by infiltrating the gel with the scintillator or floor like p p o, and then drying the gel and placing it against a pre flashed x ray film.

So, the negatron emitted from the particular isotope will excite the floor and subsequently emit light. So, thus both excitation and ionization effects are used in fluorography. It great increases greatly the sensitivity with lower energy emitters. Likewise, sensitivity can also be enhanced by adding flows to the emulsion.

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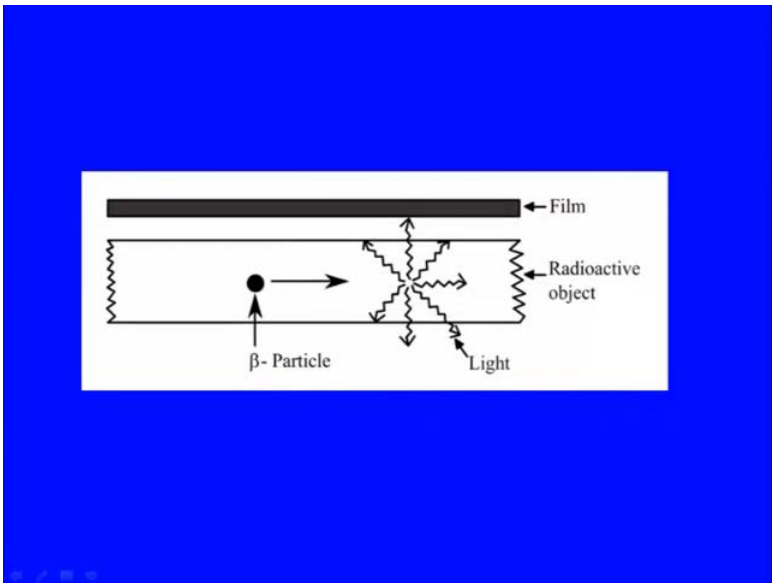

So, this is like shown in this figure that there is a beta particle. There is a flow here, which can increase the sensitivity to many folds. Then the last one is intensifying screens. Now, this is an opposite problem to that of low energy isotopes. So, here radiation from high energy as I told 32 P and gamma isotope labeled samples say for in proteins and other samples.

Now, these are highly penetrating particles or rays. As they penetrate through the film causing little exposure of the film, they produce a very poor image. So, what happens? Substantial improvement in the midst generation can be done by placing on the other side of the film from a sample a thick intensifying screen made up of solid phosphor. So, negatrons are penetrating the film will cause the phosphor to florets and emit light, which super imposes its image on the film exactly. This can enhance the resolution many times.

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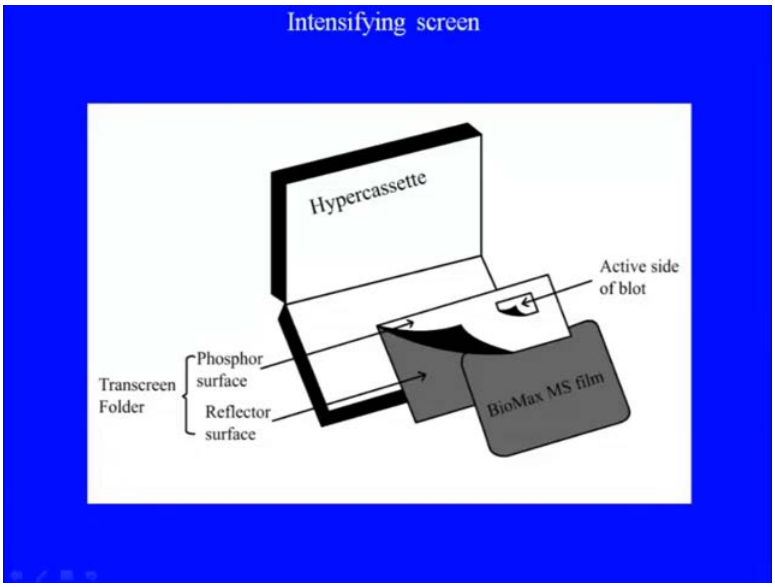

This is shown here. This is a cassette, a hyper cassette. There is like particular kind of film and sample could be placed in here. This is active side of the blot. There is phosphor surface. So, what happens when the high energy particles rays cross over and do not have exposed properly? The x ray film throws the sample. Then image is super imposed made from fluorescence of this phosphor compound.

So, this is one another method, which could be utilized. So, this completes our section on autoradiography. We have discussed three methods in previous lectures, which were based on ionization of gases. Then second method was based on excitation of solutions and solids. Then finally, it was based on exposure of photographic film that is autoradiography. Now, in the next lecture, we are going to discuss one more method that is radioimmunoassay. Also, we will be considering different safety aspects and applications of the radio isotope techniques.

Thank you.