Analytical Technologies in Biotechnology
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Module - 2
Radioisotopes Techniques
Lecture - 4
Scintillation counting continued

In previous lecture we have started with the methods based upon excitation, that is detection and measurement of radioactivity on the, upon based up on the methods on excitation. We have started with scintillation counting, where we have discussed about solid scintillation counting. In solid scintillation counting you have to keep the sample very close to the solid crystal floor, and there is a photomultiplier tube, put in close to the crystals. So, solid scintillation counters are mostly utilized for strong beta emitters or usually for the radio isotopes, with gamma rays emission, as it is quiet widely utilized, in lot of different biotechnological applications.

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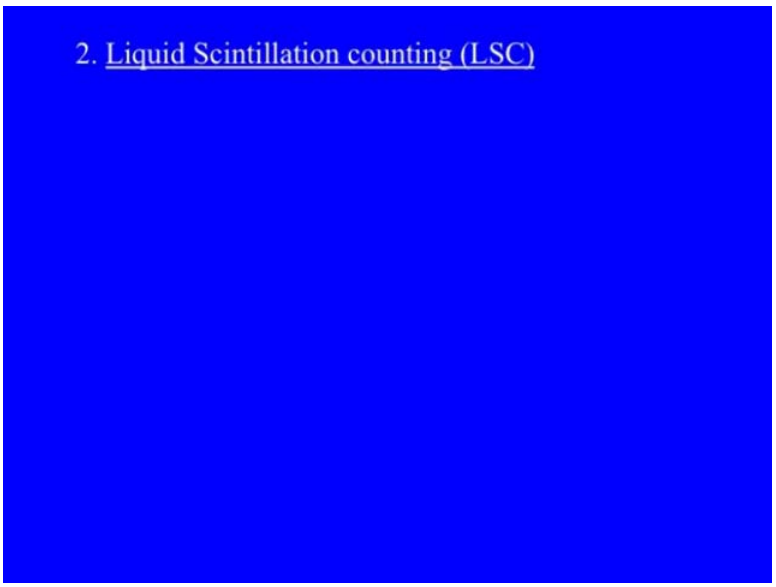

In this lecture, we are going to start with another scintillation counting method, which is the most widely used, that is liquid scintillation counting method. Now GM counters and solid scintillation counters are inefficient for low energy particles, particularly for 3 h, which is widely used in different biotechnological applications. Now two major factors limiting their efficiency are, that not all emitted particles reach the detector and not all

are counted from those, that reach the detector. Now main reason for not reaching the detector is that, the geometry is such that some particles are emitted in a direction, that misses the detector. For example, in a sample placed before GM tube, one half of the particles will be emitted in a direction, away from the detector.

Because, only 50 percent will really enter the tube, 50 percent will go in another direction. So, geometry can be a problem or could be a limiting factor now, reason for not detecting the low energy particles, which reach detectors is that, the particles have insufficient energy to cause ionization of the gas. So, when they reach there, there is, they do not carry sufficient energy to ionize the gas. So, that could be the, another reason, the problem could be solved and efficiency of low energy particles could be increased substantially, if samples are contained within the detector. And problem of self absorption could, can be avoided.

So, a solution is provided by the technique of liquid scintillation counting so what we here, can be done that, those radiations which are lost or those 3 h particles which are lost, could be avoided by putting the sample within the detector. Now liquid scintillation counting is a very standard laboratory method, widely used method like, I said. In which the sample is mixed with a scintillation cocktail, containing a solvent and one or more floors, we will be discussing this in detail. So, what is done is that whichever, radioactive samples you are going to count, they will be put in this cocktail, that is they could be dissolved in this scintillation cocktail or they could be suspended in this particular solution.

Now this cocktail contains an aromatic solvent, which is historically benzene or toluene has been used, but more recently less hazardous solvents has come into favor. And apart from this solvent, they will be containing very small amounts of other additives, known as floors. Now beta particles, emitted from these samples will transfer their energy to the solvent molecule, which in turn will transfer their energy to the floor. So, what is happening, first is very weak beta particles are transferring their energy to the solvent and then that particular energy is being given to the floors. We will see how this is done now, the excited floor molecules will dissipate the energy by emitting light.

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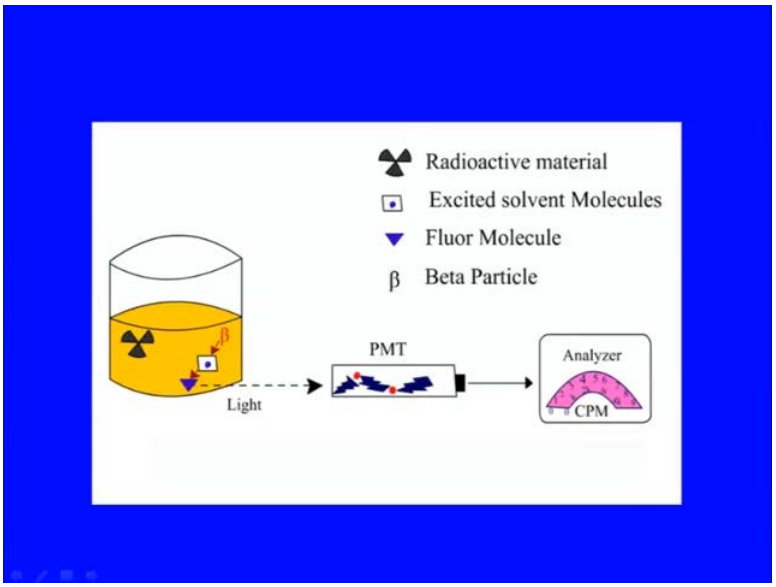

This light will be collected by photomultiplier now, the whole thing is explained quiet well by this diagram here. If you see here there, is a radioactive material here, which is sample, containing radioactive material or radio isotope. Now here, is it is seen that a beta particle is emitted and this beta particle will excite the solvent molecule. Now this excited solvent molecule, on de excitation will emit photons or light and they will be taken up by the floor, which is it could be one floor or it could be two floors, if there is two floors, then one is called, primary floor and another is called secondary floor.

If there are two floors, light will be going to primary floor then from primary floor to secondary floor and then the light emitted by these floors, either primary or secondary will be taken up by the photo multiplier tube and finally, analyzed. So, this is how the whole process in liquid scintillation counting occurs actually, as the radioactive samples are present in the scintillation cocktail, only. And as I have shown you earlier, in previous lecture that how the sample is placed very close to the detector.

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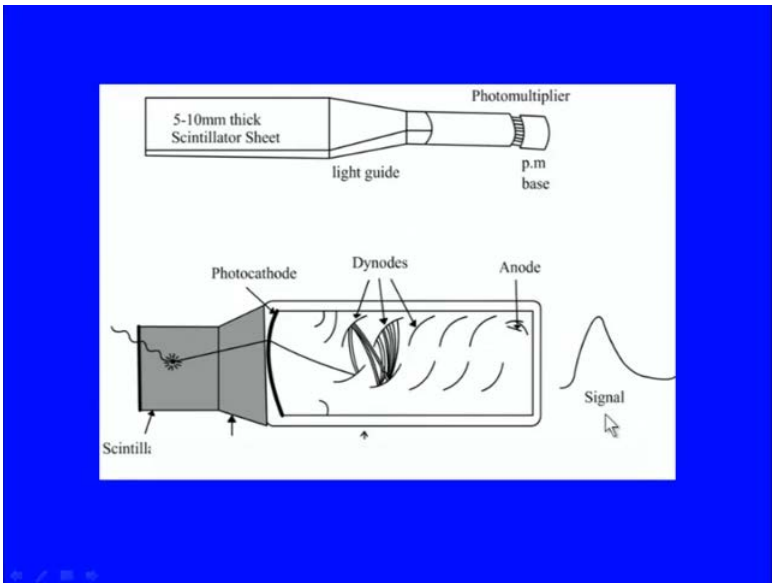

This is the photo multiplier tube, this we have explained earlier in case where, we were discussing stem electron microscope also. So, there is a scintillator which collects this light, these are converted to and through a light guide, they reaches to the tube, there is a photo cathode. These photons are converted to photo electrons, they are amplified and finally, signal is measured.

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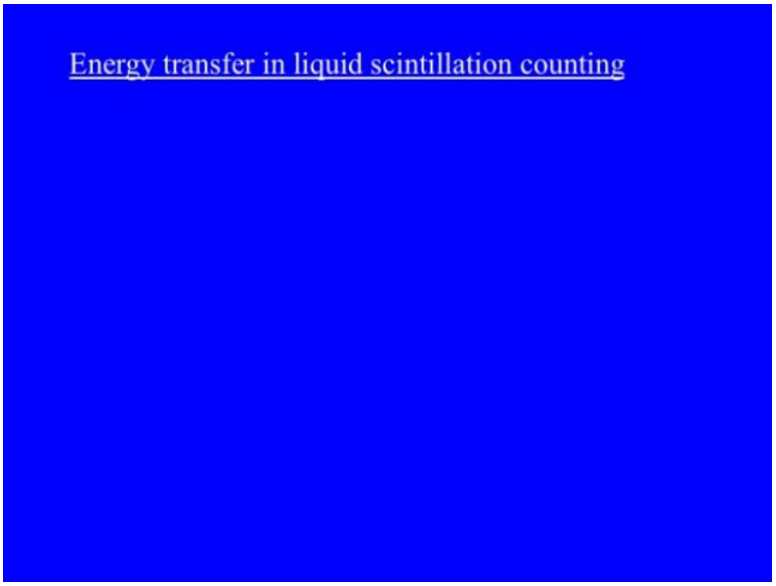

So, what we are looking at is that, how this energy transfer is taking place in liquid scintillation counting. Now, a small number of organic solvent fluorescence bombarded

with radioactivity, like we have seen in the previous figure. Now the light emitted, from this solvent is a very short wavelength so this will not be efficiently detected by photo multipliers, I will show you this is in a little while. However, if a compound that is a floor, at low concentration is dissolved that can accept the energy from the solvent and it ((Refer Time: 07:44)) at a longer wavelength. Then the light can be more efficiently detected now, such a compound is known as a primary floor.

And the most frequently used example is PPO or 25 diphenyloxazole, this is a very common primary floor. Now so what happens the light has, first the solvent is excited then it emits longer wavelength, which is taken by primary floor, it is excited then again it will emit further longer wavelength. Now, it depends on the sensitivity of the photomultiplier tubes whether, it can accept directly that wavelength or not. If wavelength of the photon emitted by the floor, that is primary floor is not in the region of highest sensitivity of the tube then the electric pulse will not be generated.

So, what you require, you require a secondary floor in the vicinity that will absorb the photon emitted by primary floor. Then will re emit them as the, as the fluorosis at a longer wavelength, which can be detected efficiently by photomultiplier. So, little bit let us understand this, on your screen, what is this sensitivity we are talking about and if I could little bit tell you about this.

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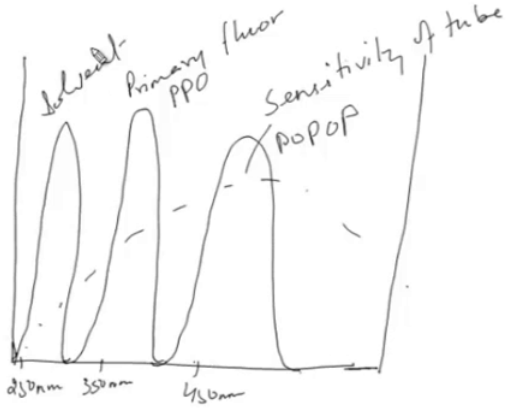

What is we are talking about is, that the whole process of energy transfer has to be detected actually and like I said, beta emitter which excite the solvents, the wavelength is not long enough to be in the region of photomultiplier tube sensitivity. So, what you have is, you have to, have that particular emitted radiation in the sensitive region of the tube. So, if we say this is the sensitive region of the tube, that is the sensitive region or sensitivity of the tube, that is the sensitivity of the tube, that is photomultipliers tube.

Now, let us consider the energy transfer so what happens beta particles, which is emitted from say, 3 h will excite the solvent, got it. So, solvent is excited here which is, could be, whichever solvent is present in here and if we just have to give certain wavelength, say it is around 250 nanometer. Now, if can see this, this particular one is not really in the sensitivity zone of the tube. Now, this solvent will excite the primary floor and the primary floor is excited in here, again and you can see this primary floor, though it is in somewhat in the region. But it is not, it will not be efficiently collected or this wavelength will not be efficient collected by photomultipliers and electric signal will not be generated.

So, this is primary floor or which we were talking about that is PPO. Now so if the light could not be efficiently collected from the primary floor, then it will not be able to generate and the efficient measurements cannot be taken place. So, what you require, you require a second floor which will be called secondary floor. So, then there will be a secondary floor, which is you can see here this is in very much in the, that particular reason that is sensitivity of the tube. And if this is, this particular secondary floor could be one, which could efficiency transfer this light or a particular wave length, which is in this region longer wave length, that could be taken up or which could be read by the photo multiplier tube.

So, this is what we are talking about in terms of the energy transfer or that is the efficient energy transfer, right from the 3 h radioactive material till the second floor and to photo multiplier tube. So, let us return to our discussion so what we are discussing was that you require, you can require two floors rather than, one but it could be that one floor could also work, like in many advanced versions it has, this particular facility. Now so unfortunately the light emitted by PPO like we were talking, is not always detected so you have a secondary floor. And like I said secondary floor is POPOP, which is 14 di 25 phenyloxazole benzene and will call it POPOP.

Now, both floors act here, they are simply acting as wave length shifters actually and they do not observe original energy of beta particle. So, use a primary floor is mandatory because secondary floor can observe, cannot observe, observe the energy that directly from the solvent. So, why not use directly secondary floor, which is in the sensitive region of for photo multiplier tube because this will not be able to take up the wave length, which is emitted by the solvent directly. Therefore, you require a primary floor also, a secondary floors may be unnecessary with modern advanced scintillation counters, where for example, compounds such as butyl PBD could be utilized and do not require a secondary floor.

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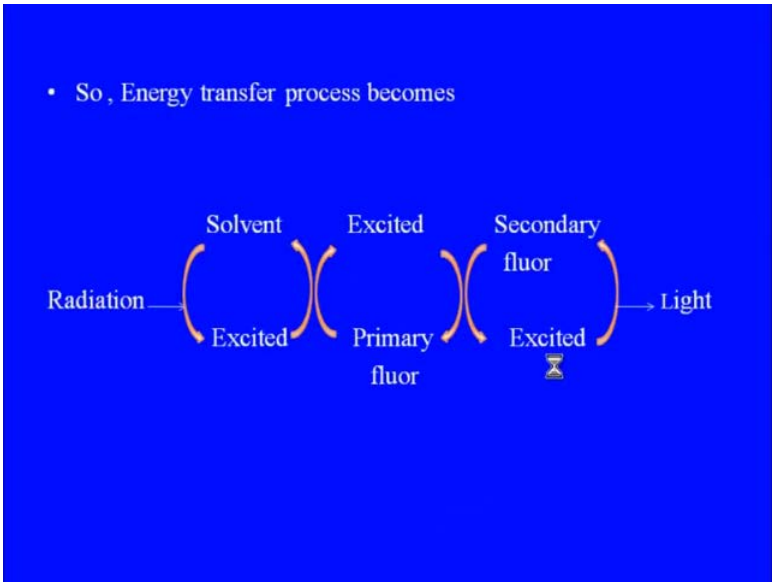

So, essentially what you have is, if we summarize the whole thing that is the energy transferred process and then it becomes something like, shown in here. So, you have radiation in terms of beta particles say, from a very weak radioactive material, that is radio isotope 3 h.

Now this radiation is taken here, there is a solvent, this is taken by the solvent, it is exited, on de excitation it will release some light or particular longer wave length, which will excite primary floor, which is exited again on de excitation, it will emit certain longer wave lengths, which will excite the secondary floor and then on de excitation again it will emit further longer wave length, which will be, which will be read by the photo multiplied tubes. So, that is the whole summary of energy transfer in here.

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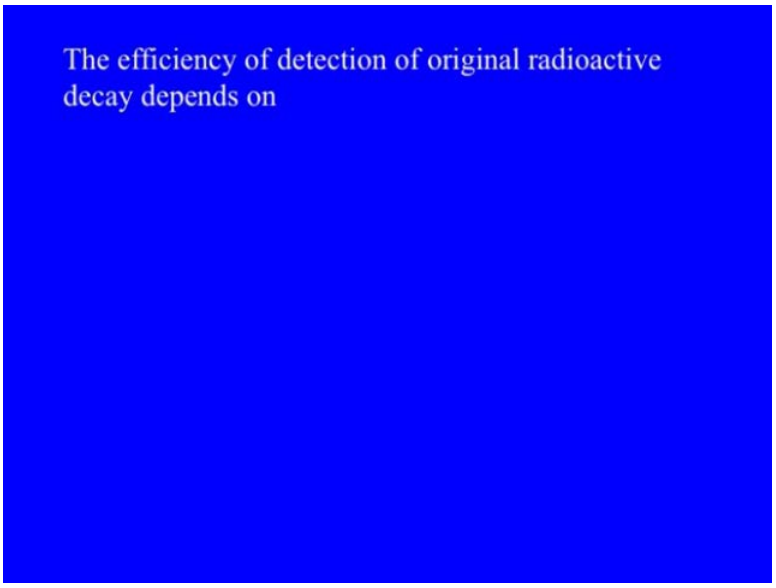

Now the efficiency of detection of original radioactive decay, will depend on many factors. And some of these factors will be, one is the properties of solvent, in that the fraction of the absorbed energy leads to the excitation of solvent molecules. So, properties of solvent would be very important and then the part of the excitation energy is transferred to the primary floor, rather than being dissipated. So, one very important part is, what are the properties of solvent that, in that it could be excited suitably, efficiently and also, then it can transfer the energy to the primary floor, without being wasting, that particular energy being wasted as heat.

Second is the number of photons produced by primary and secondary floors, like how efficiently that energy transfer takes place. Then efficiency of the photo multiplier tube to gather photons, that is also very important and that will depend on, designed or geometry of the tube. So, the design of the photo multiplied tube is also important, the signal to noise ratio of the photo multiplier is another important factor. Finally, efficiency to convert the charge of photo multiplier, into the electric pulse will also determine the efficiency of the detection.

These are some factors, which will be determining the efficiency of detection of original radioactive event. Now scintillation counters have lot of advantages, over GM counters, which we have discussed earlier. Now those advantages, if we count over gas ionization, one they are, one is much higher count rates are possible in scintillation counting, as

compared to GM counting. Because of the rapidity of fluorescence decay, which is almost in the order of 10 leads to the power 9 seconds and when you compare it, to the duct time the GM term which is, 10 leads to the power 9 seconds.

So, the rapidity of or much higher count rates are possible because of the rapidity of fluorescence decay, then much higher counting efficiencies are achieved particularly, for low energy beta emitters. Now, the best efficiency foe 3 h would be 5 percent with GM counters and compared with the scintillation counted, counters like liquid scintillation counters, it is about more than 50 percent efficiency could be achieved routinely. So, you could compare that, the, it is much much efficient than the GM counter. Then ability to accommodate samples of any type like, including liquids, solids, suspensions and gels that is another important part of liquid scintillation counting.

Ease of sample preparation, the ability to count separately different isotopes in the same sample, that is the big big advantage in terms of. If certain sample is containing 2 isotopes, they could be easily distinguished, if the energy spectrum is differing or different for two isotopes. So, that could not be done in GM counters, then scintillation counters are highly automated now and are very good quantitative and qualitative estimations. So, there are lots of advantages of the scintillation counting, particularly liquid scintillation counting, but there are lots of disadvantages also. Now for scintillation counting, most of the enhanced disadvantages have been overcome by improvement in instrument design.

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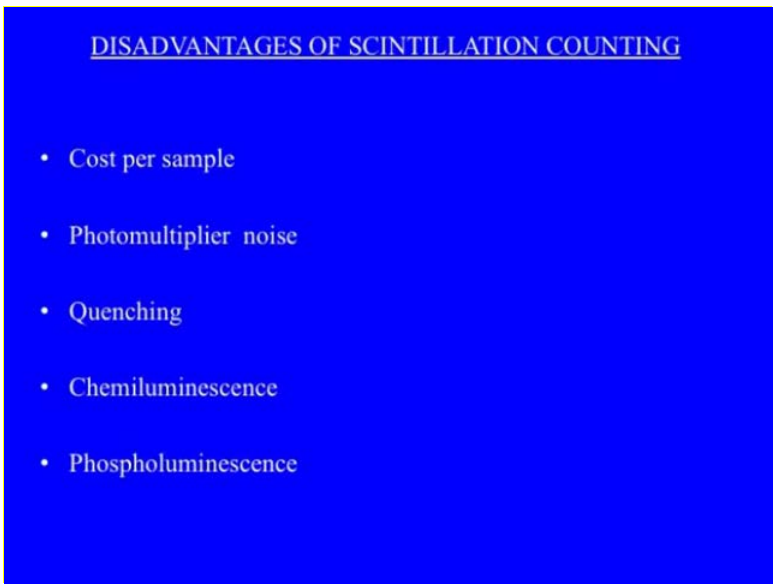

But some of the advantages, which includes, one is cost per sample of scintillation counting, that is very high, but like I said factor could be overload, as it provides versatility, sensitivity, accuracy and ease for most applications. So, it could be overload the cost of the operation could be overload, because of lot of different advantages in here.

Now, there are other important disadvantages, one is photo multiplier noise actually, that is at very high voltage applied to the photo multiplier, the electronic events occur in the system. And these are independent of radio activity, but they will contribute to a high background count, referred to as photo multiplier noise. So, that photo multiplier noise is one problem now, this photo multiplier noise could be partially reduced by cooling the photo multiplier. Apart from cooling, which may be not enough for reducing the noise, there could be, you can put in a pulse height analyzer. Now, pulse height analyzer is set reject most of the noise pulse, which are of low energy, that is a threshold or gate setting is done,

Now this disadvantage could be that, low energy pulse resulting from low energy radioactivity like 3 h, could be discarded. So, what is done is, there is another method is used, which is called coincidence counting, what is done here is, 2 PMT tubes or two photo multiplied tubes are set in coincidence. Such that, only when a pulse is generated in both tubes at the same time, it is allowed to pass two scalar. And this will certainly reduce the noise to a very low level now, a coincidence circuit is set to discriminate between noise and pulse, caused by beta particles. Only in the later case, will light a strike both detectors simultaneously and the coincidence gate will allow or announced that the beta decay has accrued. So, if its noise then possibility is that it will not hit both the tubes.

Now, the chance of noise pulses reaching both photo multiplied tubes simultaneously is very rare or very small. If the coincidence circuit detects a pulse from PMT or photo multiplied tube and not from the other, within 40 nanoseconds, the analyzer will discard the pulse. So, this coincidence circuit ensures that, the background that is liquid scintillation counting background is kept as low as possible here.

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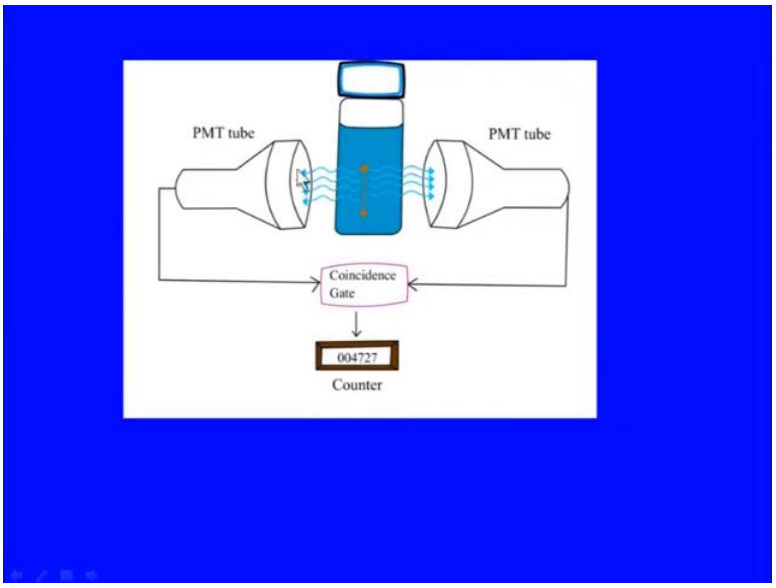

This figure shows, how to photo multiplier tubes have been put in here, you can see there is one photo multiplier tube here, another here and the sample is in between. So, when signal reaches to both, then only it will be counted otherwise, it will not be counted. So, that is the one method to reduce the photo multiplier noise. There are other disadvantages also, one of the greatest disadvantage of scintillation counting is quenching.

Now, this occurs when the energy transfer suffers interference so quenching refers to any process which decreases the fluorescence intensity of a given substance. So, in a sense it is interfering with a quenching, the phenomenon of quenching interfering with the energy transfer, actually. And in like, it will certainly lower the counts now, quenching can be of different kinds and these could be, these kinds could be…

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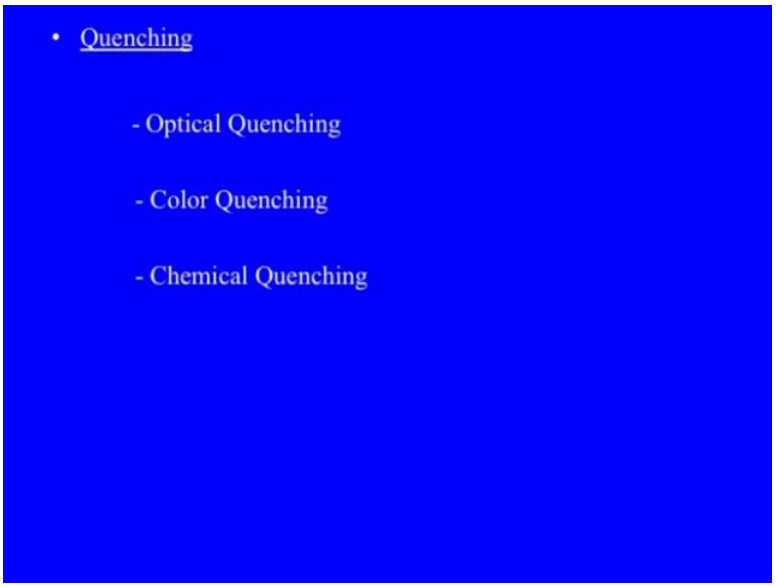

One is optical quenching, now optical quenching occurs when dirty scintillation wires are used, what will happen if these are dirty scintillation wires are used, they will observe some of the light being emitted, before it reaches the photomultiplier. So, what has to be done, that one needs to use clean wire and they should be handled carefully, to avoid any grease or dirt on the wire surface. So, optical quenching is one problem then there could be others like color quenching, the color quenching occurs if the sample is colored and results in light emitted being absorbed.

So, if color is absorbing the emitted light, then again there is a problem with the scintillation counting. And so what has to be done is that, if in some way if color could be reduced or certain like, relative counting could be done, it could be then taken care of. So, color quenching is another problem now, third color quenching problem could be chemical quenching.

Now, this form of quenching occurs, when anything in the sample which transfers with the energy from the solvent to the primary floor or from the primary floor to the secondary floor. It is the most difficult form of quenching, to accommodate now, an appropriate method of standardization, which requires the determining of counting efficiency of each sample. And then conversion of counts per minute to absolute counts has to be done, which is kind of adds to your problem.

The other things like chemiluminescence now, chemiluminescence results from chemical reactions between components of the sample, to be counted and the scintillation cocktail and the produces light emission, unrelated to excitation. So, of the solvent and floor system by radioactivity now, these light emissions are generally low energy events. And they are rejected, by the threshold setting of the photo multiplier, in the same way as a photomultiplier noise. So, chemiluminescence could be the, another problem where, certain chemical reactions are occurring. And they are producing light, which is totally unrelated to excitation and it could be, if it is a weak emissions then they could be easily taken care of by a threshold setting actually.

Like chemiluminescence, there is another phenomenon called phospholuminescence, now this results from the components of the sample, including the wile itself absorbing light emitting it. Now, unlike chemiluminescence, which is once only effect, phospholuminescence will occur on each exposer, of a sample to light. And samples that are pigmented are most likely to give phospholuminescence.

Now, to sort out this problem, samples should be dark adopted prior to counting and sample holder should be kept closed throughout the counting process. Now, despite all the complications described above, which are quenching and other problems, scintillation counters are universal in bio sciences or biological science departments. They are widely used and they are very very useful for different applications and for counting particularly, counting the weak emitters.

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Now, like we said in one of the advantage was, that you can count two samples at a time, that is two isotopes at a time. So, scintillation counting can be used for dual labeled samples, a great advantage of scintillation counting is, ability to determine the ratio of two or more radio isotopes, present in a mixture. Now, this is possible because the size of the electric pulse produced by the conversion of light energy in the photo multiplier is related directly to the energy of the original radioactive event. The resolution of two isotopes is made possible with a pulse height analyzer.

Now, what is pulse height analyzer, it is an electronic equipment that can sort pulses of fluctuation in current voltage, in terms of its magnitude. Now this is equipped with discriminator, that controls, discriminator controls or that determine the voltage levels, defining the voltage range actually. And most of the times two discriminators are usually employed, different beta emitting isotopes have different energy spectra, it is possible to detect and quantify to isotopes, separately in a single chamber, provided their energy spectra are sufficiently different.

If we take some examples of pair of isotopes that have sufficiently different energy spectra, they could be like 3 h and 14 c, 3 h and 35 s, 3 h and 32 p, 35 s and 32 p. If you could recall, I have shown you in previous lectures the beta spectra or beta particle emission spectra, of different isotopes. And they were quite different in all the cases now, like I will show you one figure here, but before that, now this spectra of two isotope, which is, could be there, they will overlap only slightly. What does that mean, that they are sufficiently different now, by setting a pulse height analyzer to reject all pulses of energy, below certain threshold like say, threshold X and it rejects all pulses of energy above certain threshold say, y or window y.

You would be able to set a particular threshold value and also you will have another isotope, you will have another threshold of another threshold say, threshold A, an another window say window B. Now it will be possible to separate to these two isotopes completely so what a pulse height analyzer set with a threshold and window for a particular isotope, this is known as channel actually.

So, what you have done because you would like to detect and quantify, two of the isotopes you have certain threshold values with a pulse height analyzer, set with threshold values for two isotopes. And this particular thing is called channel, most

modern counters operate with say, they are multi-channel analyzers. And so they have, may be they can have more than one channels and they are based on analog digital convertor.

Now, dual labeled counting has proved to be useful in many aspects of molecular biology. Example say, it could be nucleic acid hybridization studies or mechanism of ribosomal sub units, protein synthesis or transcription, metabolism or drug analyses or development, lot of different applications could be there.

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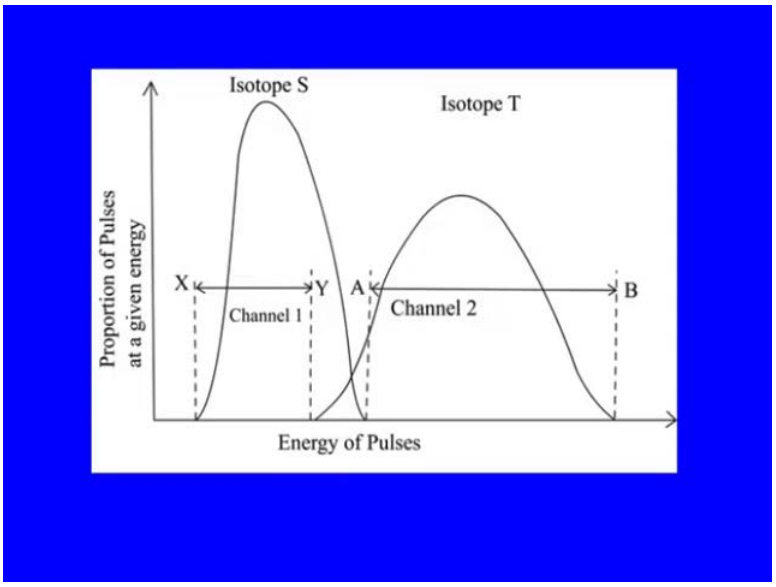

Now, this figure clearly shows what we were discussing here so what you have is you are trying to detect two isotopes, if you can see there is X threshold and there is a Y threshold, which is Y window we call. And this part here, that will be measuring this channel, 1 channel will be measuring isotope S and another channel 2 which is set on another threshold which is A and it will reject all below A all signals below A and there is a window B. So, there will be another channel for second isotope, which we are calling isotope T. So, by putting in two channels, that is multi channel detectors you will be able to detect and quantify the two isotopes, simultaneously in a sample.

So, that is a great advantage of a liquid or scintillation counting over GM counting. Now, let us little bit move into the determination of counting efficiency. Now a major problem and countered in scintillation counting is that of quenching now, this is real problem like we have seen or we have discussed it and it makes it very important, to determine the

counting efficiency. Because, what you are doing is you have to standardize the counting methods so that, you can, you know that every time you are counting correctly. Now this could be done, by one of the several methods of standardization and all of which apply to both solid and liquid scintillation counting.

Now this could be internally standard standardization, that could be external standardization and even that could be a channel ratios taken like, this could be taken in two channels. And it could be ratios could be taken now, very briefly in internal standardization, first sample is counted and it gives a reading of say, a particular count per minute.

Then, it will be removed from the counter and a small amount of standard material of known disintegration will be taken then that will be, sample will be again counted, that will be c counts per minute. And then counting efficiency will be calculated by this formula here, which is 100 in bracket c minus A upon B percent. So, you will be able to, through internal standardization will be able to count the efficiency here through internal standardization.

And it is obviously necessary in this method to use an internal standard, that contains the same isotope as the one being counted and also to ensure that, the standard itself does not act as a quenching agent. So, these are few precautions has to be taken, internal standardization is simple and reliable and corrects adequately for all types of quenching. Now, this method demands calibration of two factors and it demands more accurate pipetting in adding standards. So, these things has to be taken care of, for internal standardization. Then there could be another way channel ratios actually now, when a sample in scintillation counter is quenched, the scintillation process is less efficient.

So, less light is produced for a given quantum energy of radiation. So, what happens then the energy of spectrum for a quenched sample appears to be lower, than for an unquenched sample. So, the higher the degree of quenching, the more pronounced is the resulting decrease in the spectrum. So, the method involves the preparation of a calibration curve based on counting, in two channels that cover different, but overlapping parts of the spectrum. Now, as a sample is quenched and the spectrum shifts to gradually lower apparent energy, the ratio of the counts in sample will be wearing.

To prepare the standard curve, a set of quenched standards is counted. So, the efficiency is plotted against channel ratio to form the standard curve. And once the standard curve has been prepared, the efficiency of the counting experiments, experimental samples can be determined. And samples are counted in the same two channels, the ratio is calculated put into the graphs and the efficiency is read.

So, the multi channel scintillation counters operate on the same principle, but the whole shape and position of the spectrum is analyzed. So, these systems have greater precessions than the two channel approach as the whole of the spectrum is used for analyses. So, here it is shown in here where, you can have count per minute and you can say how the different, due to different quenching effects, the spectrum or a lower energy spectrum is obtained.

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So, this could be through channel ratio it could be done, another is external standardization now, in external standardization, instruments will have a gamma emitting external standard built into the counter. Now under the control of the counter, each sample to be counted is exposed to the external source, which is automatically shifted from a lead sheet to the counting chamber. Now, the gamma radiation penetrates the wile and excites the scintillation floor.

Then, resulting spectrum is unique to the source and significantly different from that, produced by the sample in the wile. So, the gamma source used varies according to the

make of instruments. Now the quenching agents presents in the scintillation fluid will significantly affect the spectrum obtained, the instrument analyses, the instrument analyses this spectrum and ((Refer Time: 36:29)) a quench parameter to it. As for the channel ratio method standard curve is required, that is range of standard is counted and then the external standard spectrum is analyzed, in any each case.

Now resulting data are used of prepare a standard curve, that is held in the instruments computer and unknown samples are then counted in the same way. And the efficiencies read from the standard curved and the sample counts corrected. Now, if you see the advantages and disadvantages, main advantage over channel ratio method being, that it is suited to samples to low count rates. Now, disadvantage could be a standard curve is required for each set of circumstances and user can be killed into a false sense of security. The system is so highly automated that, it is easy to lose sight of the basic principle and a method is not always appropriate. So, these are different methods which are used for standardization that is internal standardization, external standardization and channel ratios.

Now so in this lecture what we have discussed here is, the liquid scintillation counting we have discussed about, how energy transfer is taking place from radio isotope to solvent from solvent to primary floor and from primary floor to the secondary floor and finally, to the photo multiplier tube. So, primary and a secondary floor in a sense work as wave length shifters in this case.

Liquid scintillation counting or scintillation counting is a versatile method, it is widely used in different laboratories for a radioactive measurements of weak emitters. And it is highly efficient method, for both qualitative and quantitative estimations, certainly it needs standardization so that the efficiency of counting can be estimated. And that could be converted to disintegration per minute or per second, that is actual counting. So, in the next lecture we are going to discuss about the sample preparation of liquid scintillation counting. And then will be discussing about the third method, that is auto radiography, for detection and measurement of radioactivity.

Thank you.