Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

Dr. J R Mudakavi
Department of Chemical Engineering
Indian Institute of Science, Bangalore

Lecture – 29
Method development

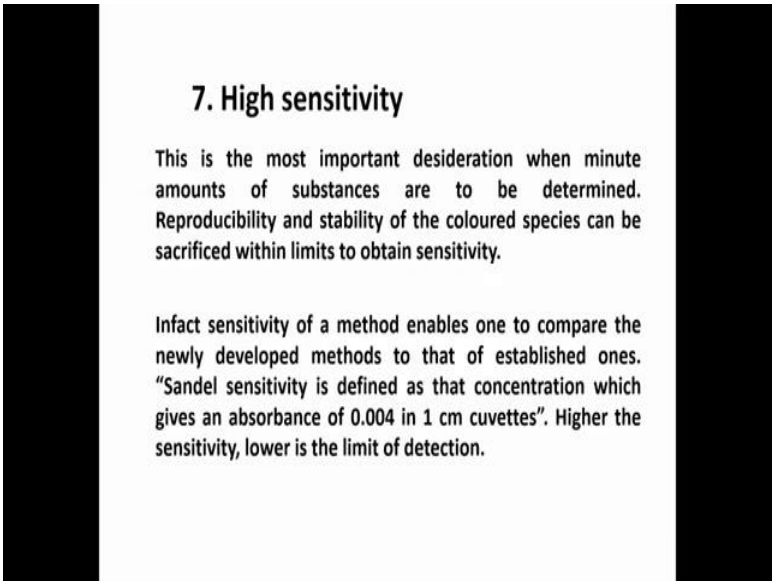
Greetings to you, we continue our discussion on the nuisances of spectrophotometry. Yesterday we had discussed several aspects of how to make a spectrophotometric procedure a specific in presence of other concomitants, we are discussed co precipitation, we have discussed precipitation pH, I just mean redox reactions and then extraction into immiscible solvents collection using a collector and all those things, all these things serve to establish a spectrophotometric method as a leading analytical technique in spite of the presence of other concomitants which may or may not interfere in the spectrophotometric analysis.

So, we are discussing about the extraction in fact, what I wanted to tell you yesterday itself was that the extraction of an molecular complex into a into a non aqueous medium such as benzene, toluene methyl, isobutyl, ketone, zylenes, etcetera, butynol and all these things serve to concentrate the complex in the extracted form where the concentrations of the analyte will be greatly enhanced and the sensitivity also will be enhanced. In fact, as I was about to tell you that extraction into a immiscible solvent has established itself as a great science separate discipline of science in which extraction of the metal ion is a an established technique. For the recovery also in metallurgy as well as it is a means for us increase the sensitivity of the determination. By sensitivity of the determination what we mean is the detectable limit or quantitative limit that can be established using spectrophotometry would be lower, the lower the detection limit the better is the sensitivity.

So, it is always one of the aims of spectrophotometry to achieve lower and lower detection limit, but of course, because it is a relative technique there is always an possibility that there is a bottom limit to which we can go that we have already established earlier in our discussion making the molecular structure into consideration where the molar extinction coefficient should be approximately 10 raised to 5, below that

it is not possible for us to go for lower detection limit now continuing our discussion about the nuisances of spectrophotometry.

(Refer Slide Time: 03:38)



Now, look at this what we look for is the high sensitivity this is the most important desideration when minute amounts of substances are to be determined. Normally what we expect is the sample to be in high concentration. So, that we can take dilute it to the desired level and determine, but it is not always the case quite often we are unless are called upon to determine minute quantities as it is in the given sample, it is like finding out the needles in a haystack what we want to know is whether we find it or not qualitatively, but we have to find out how many needles are there in the haystack that is the beauty of spectrophotometry.

So, the whenever we are supposed to determine minute quantities of an analyte in a given large matrix reproducibility and stability of the colored system can be sacrificed to some extent to obtain higher sensitivity what happens is especially when we are determining very small quantities from a large matrix there will always be certain amount of extraction which we said it may vary from 90 to 99.99 percent which is acceptable. So, if I can get a reproducible result around 90 percent still it is acceptable compared to 99 percent efficiency without so much of reproducibility. So, that extent the reproducibility and stability can be sacrificed a little bit within the limits to obtain higher sensitivity.

In fact, sensitivity of a method enables us to compare different methods suppose I remember still that yesterday I have told you that there are 256 methods for palladium, similar there are hundreds and hundreds of methods for the determination of many metal elements and several other parameters also. So, how do we compare which method is good among them which is sturdier one which is most useful which is very sensitive which is almost specific like that the different parameters are there to compare the spectrophotometers.

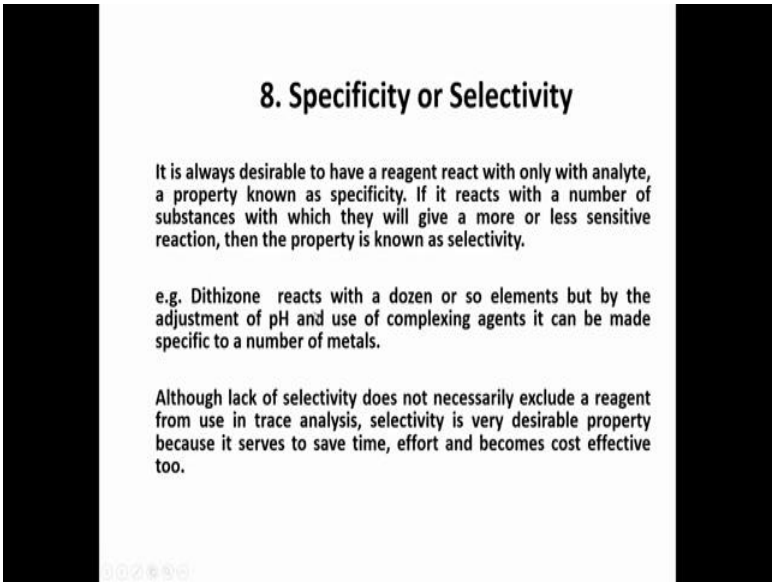
So, this is what we normally aim at whenever we want to take a look at a plethora of experiments plethora of methods and compare among them which one is the best suited for our purpose, so in fact, the sensitivity of a method is has to have certain amount of commonality among all the spectrophotometric methods if I have to compare. So, the unit should be common and then the reproducibility should be there for this purpose we adopt a terminology called as Sandel sensitivity as defined by him, professor Sandel was a very great analytical scientist in the 1960s and his book on colorimetric methods of analysis is one of the most standard book even now. So, Sandel has defined a sensitivity as that concentration which gives you an absorbance of about points of 0.004 in 1 centimeter cuvette. So, higher the sensitivity lower is the limit of detection that is understood.

So, actually what we how we arrive at this Sandel sensitivity, this Sandel sensitivity we have already derived earlier using the instrumental parameters that 0.004 is the 3 sigma 3 times standard deviation of the instrument noise. So, any signal which is more than 3 times the instrumental noise is considered as a definite signal for the presence of an analyte. So, the number 0.004 combines all the parameters of the inconsistencies in the measurement of a particular species and estimates the noise and that should be approximately about 0.001 3 times would be somewhere around 0.004, this is also the sensitivity by which an instrument will show you the difference in the measurement that is why Sandel sensitivity has become a very important parameter for the comparison of spectrophotometric methods.

Now, another parameter that is important for us is specificity or selectivity, it is always desirable to have a reagent react with only the analyte this property is known as specificity. So, if it reacts with a number of substances with which they give more or less sensitive reaction then this property is known as selectivity. Selectivity is that property

which permits a single substance organic reagent to react with number of analyte matrix elements. So, suppose I take a hydroxide (Refer Time: 09:54) as a reagent and then I know that it will react with at least about 10 elements copper, chromium, cobalt, nickel, like that and that is the selectivity. So, the specificity refers to only one parameter that can be determined under the given circumstances then we say the reagent is specific for the analysis of such and such reagent, but if it is not the selectivity we say it is selective towards this group of metals.

(Refer Slide Time: 10:34)



So, I want to give you an example look at this slide the reagent dithizone it reacts with a dozen or so, elements by the adjustment of pH only; that means, at different pH the reagent dithizone reacts with several metals at different pH is giving you a different types of complexes and the use of complexing agents also makes it specific to a number of metals provided they are removed or by adjustment of the pH, I can make the method specific also. So, what is important to understand in these circumstances is although the lack of sensitivity does not necessarily exclude a reagent from using the trace analysis. Selectivity is a very desirable property in all spectrophotometric methods because it serves to save the time effort and becomes cost effective.

Also how does it happen because we use different ph conditions different temperatures extraction coefficients and then extraction into immiscible solvents precipitation, co precipitation collection, as a collector and all these things can make a an selectivity also

a desirable property because with the same reagent I can determine number of elements in the given complex just by adjusting the parameters physical parameters of color development as well as extraction and estimation methods.

Suppose the concentration is very low below the detection limit then I always have a recourse to take the sample in a 5 centimeter cell or a 10 centimeter cell that is why Sandel sensitivity is defined as corresponding to one centimeter cell quiet, but if the concentration is below that level always go for higher cuvette level and if the concentration is very high we can go for lower cuvette possibility also. Now another aspect that is very important in spectrophotometric methods is the reproducibility and stability of the colour. Normally whenever we develop a new method for the estimation of a particular analyte parameter or metal ion what we do is we form the complex study the variation of pH reagent concentration and then stability study the stability and study the reproducibility. So, all the parameters most of them are studied around this level around this around these parameters.

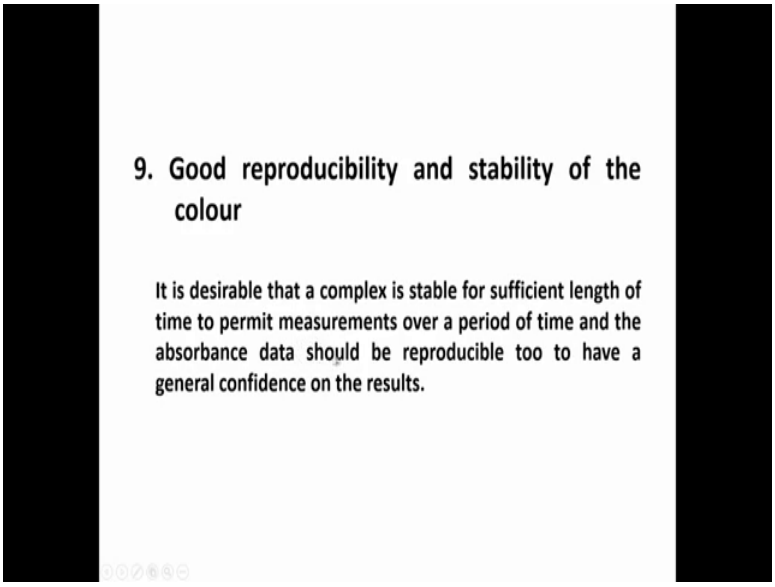
So, it is desirable that a complex should be stable for sufficient length of time, if the complex forms and immediately starts decomposing then it serves no purpose because it will not allow me to time to develop the color go to the spectrophotometer make the measurement come back I do not know what is the safe limit for the measurement of absorbance therefore, it is always preferable to study the stability of a complex whenever we are studying the spectrophotometric finish for a particular parameter. Sometimes it is some complexes are stable for half an hour, some complexes may be stable for one or 2 hours etcetera.

So, if the substance sometimes the reaction raids also very making the development of the color itself another parameter to measure and optimize. Normally some complexes take some time to form the colored species. So, in several spectrophotometric methods you will come across procedures where they say that you add these reagents adjust the pH and add your metal analyte and then wait for 20 minutes or half an hour or one hour before the color is stabilized and then you can make the measurements once the color is stabilized even then it is possible for us to make the measurement over a sufficient length of time.

So, we want to measure normally from making up the solution to a desired level and then taking measurement it takes about 10 to 15 minutes for any normal routine activity for spectrophotometry. So, it is desirable that the substance or the complex should be stable for at least half an hour to make the measurements there are of course, there are several complexes it will start immediately deteriorating decomposing or dissociating into parent chemicals. Sometimes it is possible to see that the color is stable for half an hour, 1 hour, 2 hours, etcetera that is acceptable some complexes are stable for 24 hours, absolutely no problem and there are some complexes like this potassium, dichromate, diphenyl carbozone, etcetera which are stable for days together.

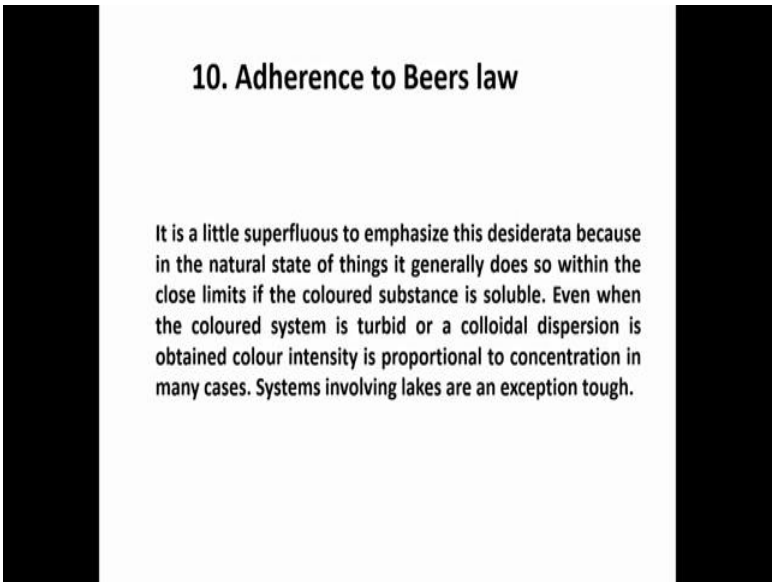
Therefore it is always important for us to determine whether it is what up to what length of time the stability of the color is possible that is stability of the color will permit as to make the measurements at our leisure.

(Refer Slide Time: 16:57)



So, the over a period of time it is important to have a measurement that is important for the stability and absorbance data also should be reproducible this is a very very important aspect otherwise we will not have confidence in the results. So, it is very typical of any spectrophotometric procedure to mention that the complex is stable for about 2 hours, 1 hour like that during which the measurement should be completed.

(Refer Slide Time: 17:31)



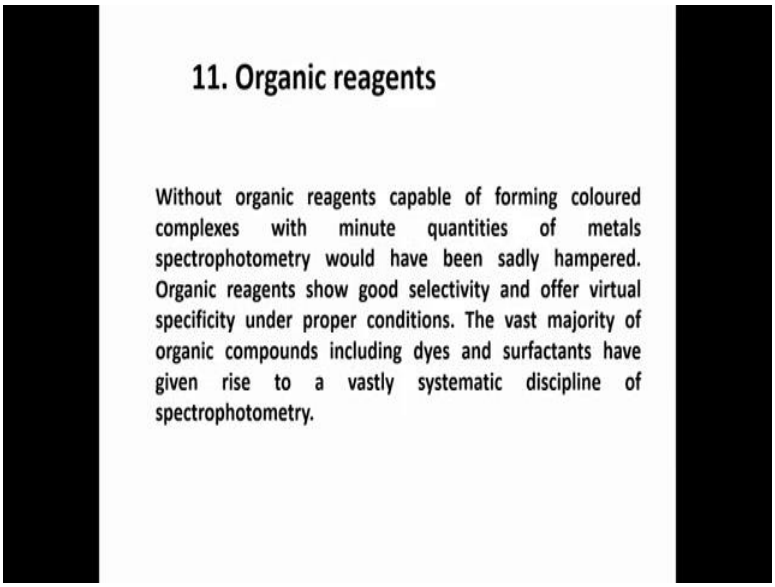
Now, again before I proceed to the different possibilities of the adsorb of the measurements of spectrophotometry I have to again explain to you the requirement for the adherence to the Beer Lambert's law.

Now, I do not have to over emphasize this aspect especially because it is a bit superfluous to state that the system should adhere to Beer Lambert's law otherwise the whole exercise whatever we have been learning or studying all of it will be a waste. So, if it does not either to be a Lambert's law then we do not have a method basically. So, it is in the natural state of things it fits that it generally does. So, within the close limits if the colored substance is soluble solubility of the colored substance is also an important factor whenever there is measurement required because if the substance is not colored it may lead to turbidity and turbidity will lead to scattering and scattering will lead to non adherence to beer Lambert's law.

So, even the when, but suppose the turbidity is very low and reproducible or a colloidal dispersion is obtained the color intensity. So, long as it is proportional to the concentration of the analyte then it is still possible for us to stick to beer Lambert's law and then basically change the measurement method to stick to beer Lambert's law we have to organize we have to organize the concentration of the standards to be always within the range of beer Lambert's law for example, quite often the beer Lambert's law does not pass through the origin.

So, at 0 concentration there should be 0 absorbance quite often it does not happen. So, whenever you see any research paper or something or a publication or a standard method of analysis you will normally come across statements like this method is applicable to 0.1 to 5 ppm of the conium like that. So, you have to prepare the standard concentrations only within that range and not exceed that limit suppose the concentration is higher we know now our tricks how to make it bring it within the measurable limits that is governed by the relative concentration error also and we know several tricks by now how to make it [FL].

(Refer Slide Time: 20:55)



Now, I would also like to emphasize at this point that there are several aspects of spectrophotometry normally we have to look at whenever we want to develop a method for spectrophotometry basically what happens is the spectrophotometric methods usually report the interferences of concomitant metals. For example, if you want to determine iron by orthophinanthrilin method then we know that the basic chemistry of the reaction is orthophinanthrilin will react with iron to form a colored complex 1 is to 2 colored complex which is orange in color soluble in water and it can be determined around pH 3 to ten or something like that in that pH range the color is stable for 24 hours. So, the Beer Lambert's law is applicable from 0.1 to 1 milligram or 1 milligram per liter or 0.1 to 1 p p m.

So, if we know this much of information does it help us to determine whether we can go for a method analysis directly based on this kind of information the answer is still no because we should also know what are the interfering elements in the method. So, it is very important for us to study the interference pattern of a particular method with respect to different metal ions for example, normally we always end up testing whenever we want to develop a method we normally end up testing for different anions and cations nowadays we start the anions and cations at 1,000 times the concentration of the analyte what we normally do is we develop the method add a cation and anion one thousand in concentration 1,000 times then the analyte and check the absorbance if the absorbance remains the same within the experimental possibilities within the experimental error then we say it is not interfering. So, normally we test for interference for sodium potassium chloride sulphates and magnesium all these things are normally normal constituents of water.

So, we also test the based on the availability based on the group reactions for example, iron cobalt nickel they all they are all transition metal elements. So, it is possible that many of these whenever I want to determine iron cobalt and nickel may be interfering it is possible to expect or suspect that such a possibility is there therefore, whenever I develop a particular method I always test for interferences from similar metals, you look at the periodic table, you will see that iron, cobalt, nickel are all in the transition metals and then there are triads whenever there is platinum I R iridium (Refer Time: 24:32) palladium gold silver etcetera all noble metals may interfere in the determination of one another using organic reagents.

So, sometimes up to the general screening normally is done for 1,000 times then if there is interference we normally reduce it by about 200 times and then if even if you find interference even at 100 times then you can reduce it to ten times and then finally, to equal value and if there is still interference at the equal concentration level then you have to find out probably the how to eliminate the interference.

So, in all spectrophotometric methods you will see come across tables that this method can tolerate one thousand times concentration of sodium potassium magnesium etcetera barium etcetera and 100 times so much concentration, so, much of chlorides etcetera sulphates other nitrates and other anions they are also present in the given sample and sometimes normally what happens is we test for the normal constituents of a river water

or, or a flowing water for example, in most of our river waters they are all contaminated nowadays with (Refer Time: 26:06).

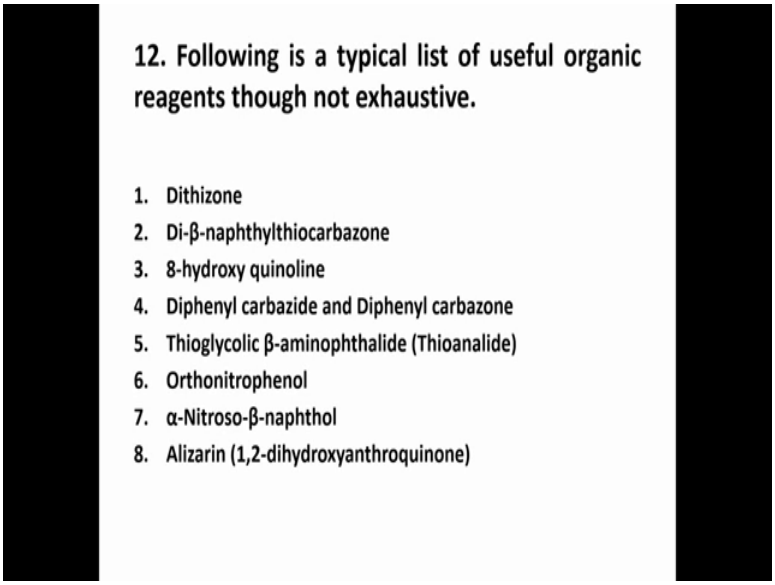
So, soaps and detergents what we use to wash our clothes etcetera normally ends up in sewage along with sewage it enters the river waters. So, most of our river waters are already contaminated with sodium, loreal sulphate and then labsa detergents and orgastirades, etcetera several organic compounds are EDTA for example, that also could be is one of the river water pollutant nowadays. So, the definition of water itself is changing with respect to the pollutants earlier people never used to bother about EDTA as a pollutant. So, they thought they were thinking that EDTA is a penasia for all interfering elements because EDTA forms complex with several metal ions it is a very selective complexing reagent which will form complexes with number of metals for those of you who have studied Vogal, you will see that there are number of methods described using EDTA as a complexing agents these are all described under the head complexometric reagents.

So, what happens is essentially we have to look for potential interference in a given analysis that is very important as far as we are concerned and then potential interference could be anions they could be cations they could be surfactants even among the surfactants there could be anionic surfactant cationic surfactant non ionic surfactants plus in addition to all these storm water storm water contains the fertilizers insecticides herbicides pharmaceutical compounds which we normally take and excrete daily and all these metabolites etcetera, some of them ninety percent of them enter our river water unless they are degraded they are also potential complexing agents present in the river water therefore, it is very very important for us to look at the interference aspects of all spectrophotometric methods before we adopt the method for a particular analysis. So, that brings us to the topic on organic reagents.

Now, I can tell you that look at this slide what I am going to show you now the organic reagents capable of forming colored complexes with minute quantities of metals is possible without that without the organic reagent spectrophotometry as a science would have been poorer it would have been sadly hampered. So, organic reagents normally show very good selectivity and offer virtual specificity under proper conditions the vast majority of organic compounds including dyes and surfactants have given rise to a vastly

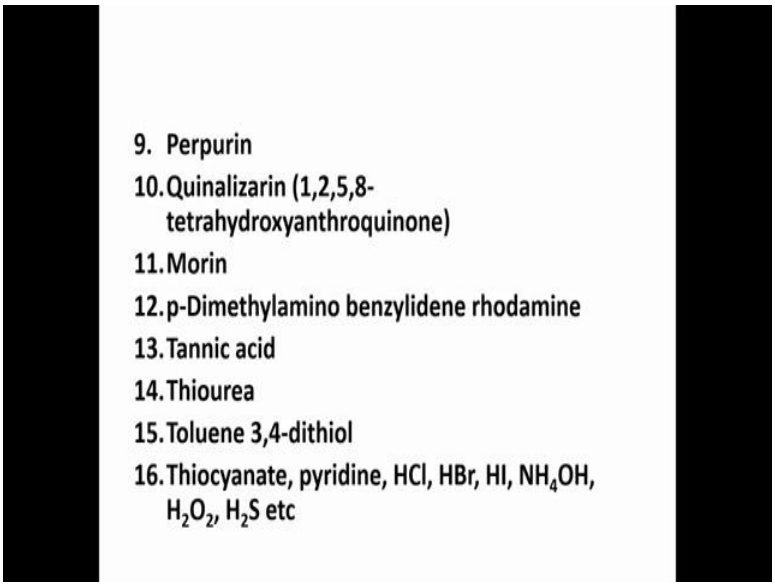
systematic discipline of spectrophotometry this is very important for us to understand the role of organic reagents in all our spectrophotometric procedures.

(Refer Slide Time: 29:48)



So, there are number I am presenting before you a number of organic reagents useful the list is not exhaustive, but these are some of the compounds which you normally come across for spectrophotometric determinations these include dithizone, di beta naphthylthiocarbonate, 8 hydroxy quinoline, is a wonderful medical compound as well as a medicine as well as a compelxing reagent diphenyl carabzide and diphenyl carbazone are the reagents for chromium and orthonitrophenol alpha Nitroso beta naphthol, alizarin and these are all basically 1 or 2 membered aromatic rings with functional groups.

(Refer Slide Time: 30:38)



We can see that there are number of other substances like perpurin, quinalizarin, morin, dimethylamino benzylidene rhodamine and then tannic acid, thiourea, toluene 3-4 dithiol, there are several may be hundreds and hundreds of organic reagents. In fact, you can think of lot of research gets done to prepare synthetic organic reagents which can be specific which are directed to be specific to for a particular metal ion to develop specifically for spectrophotometry as a spectrophotometric reagent. You will come across number of research papers which will describe the synthesis characterization and application of several organic chemicals new organic chemicals aimed at developing as a spectrophotometric procedure for different elements.

So, now that brings us to the realization that whenever we want to do any organic chemical analysis what we wish to do is we have to look for a specific standard methods of method of analysis which will give you the details about the chemical about the complex about the stability lambda max where the substance absorbs maximum etcetera and then it should also give you information about the interference data, as well as sensitivity and all these things will be required. Now this course emphasizes our aim towards in water analysis especially drinking water and effluents.

So, it is better for us to know a little bit about the standard drinking water specifications. So, normally what we come across if you take AIS standard or WHO standard or any other standard for drinking water we will come across number of parameters that are

described which a drinking water should adhere to with respect to the concentration of the pollutants. Look at the slide now.

(Refer Slide Time: 33:09)

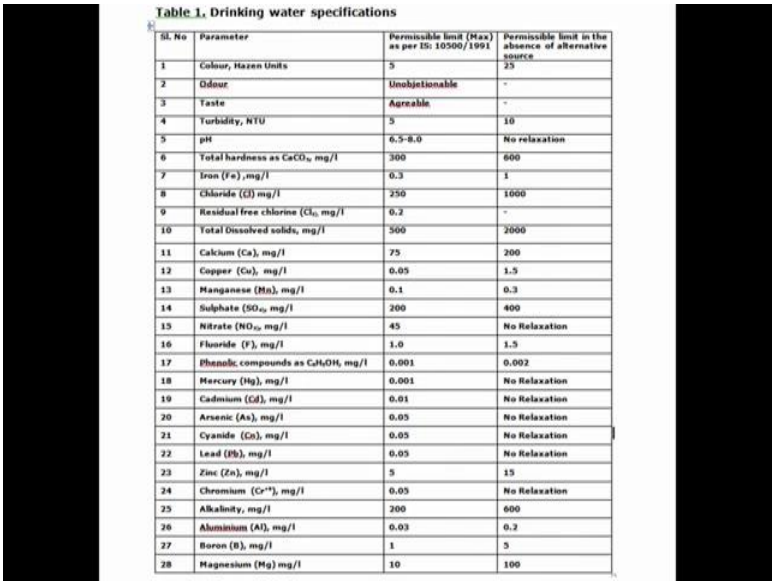


Table 1. Drinking water specifications

Sl. No	Parameter	Permissible limit (Max) as per IS: 10500/1991	Permissible limit in the absence of alternative source
1	Colour, Hazen Units	5	25
2	Odour	Unobjetionable	-
3	Taste	Agreeable	-
4	Turbidity, NTU	5	10
5	pH	6.5-8.0	No relaxation
6	Total hardness as $CaCO_3$, mg/l	300	600
7	Iron (Fe) ,mg/l	0.3	1
8	Chloride (Cl) mg/l	250	1000
9	Residual free chlorine (Cl_2, mg/l	0.2	-
10	Total Dissolved solids, mg/l	500	2000
11	Calcium (Ca), mg/l	75	200
12	Copper (Cu), mg/l	0.05	1.5
13	Manganese (Mn), mg/l	0.1	0.3
14	Sulphate (SO_4, mg/l	200	400
15	Nitrate (NO_3, mg/l	45	No Relaxation
16	Fluoride (F), mg/l	1.0	1.5
17	Phenolic compounds as C_6H_5OH, mg/l	0.001	0.002
18	Mercury (Hg), mg/l	0.001	No Relaxation
19	Cadmium (Cd), mg/l	0.01	No Relaxation
20	Arsenic (As), mg/l	0.05	No Relaxation
21	Cyanide (Cn), mg/l	0.05	No Relaxation
22	Lead (Pb), mg/l	0.05	No Relaxation
23	Zinc (Zn), mg/l	5	15
24	Chromium (Cr^{++}), mg/l	0.05	No Relaxation
25	Alkalinity, mg/l	200	600
26	Aluminium (Al), mg/l	0.03	0.2
27	Boron (B), mg/l	1	5
28	Magnesium (Mg) mg/l	10	100

Now, here the slides parameters include color odor taste turbidity and pH these are the normal parameters required for this thing and you can see that many of them are empirical also for example, color is in unit should be permissible limit is 5, but in the absence of good drinking water it is permitted up to 25 and odor it is a simple parameter it should be unobjectionable taste should be agreeable turbidity should be 5 and pH should be between 6.5 to 8 and then come the chemical parameters that includes total hardness iron chloride residual free chlorine total dissolved solids and then calcium, magnesium, copper, manganese sulphate, nitrate, fluoride, phenolic compounds mercury, cadmium, arsenic, the see the point is all these things are nowadays part of our drinking water which are a little scary because many of these metal ions are all carcinogenic.

So, the emphasis on pure water for drinking is cannot be over emphasized. So, we also look at arsenic, cyanide, lead, zinc, chromium, etcetera aluminum, boron, magnesium and several other parameters normally are required for the chemical analysis. And except the empirical parameters I am happy to tell you that most of the parameters can be followed by spectrophotometry and that is very encouraging because spectrophotometry is the one of the cheapest methods of water analysis.

(Refer Slide Time: 35:21)

Table 2. Parameters and selected analytical reagents

1.	Aluminum	Eriochrome cyanine R
2.	Chromium	Diphenyl carbazone
3.	Copper	Bathocuproine disulphonic acid disodium salt
4.	Fluoride	SPADNS - zirconyl oxychloride
5.	Iron	1,10 phenanthroline; Also *Syzygium jabolana*
6.	Lead	Pyridylazo resorcinol (PAR)
7.	Magnesium	Titian yellow
8.	Nitrite	Sulphanilic acid, N-1-naphthylethylene diamine Dihydrochloride
9.	Nitrate	Chromotropic acid
10.	Chloride	Mercury thiocyanate, Ferric sulphate
11.	phosphate	Stannous chloride reduced phosphomolybdate
12.	Phenols	4-amino antipyrene, ferricyanide
13.	Free Chlorine	N, N-diethyl-p-phenylenediamine (DPD)
14.	Cyanide	Pyridine, Barbituric acid, chloramines T
15.	Sulphate	Barium chloride (Turbidimetric)
16.	Manganese	Pyridyl azo naphthol (PAN)
17.	Arsenic	Rhodamine B, molybdate
18.	Cadmium	Cadion
19.	Zinc	Zincon
20.	Total hardness	Calmagon
21.	Nonionic surfactant	Potassium iodide, iodine
22.	Boron	Carmine
23.	Mercury	Rhodamine 6G, iodide

Now, we have prepared a special cook book for water for the examination of water and waste water for this I have listed here aluminum, chromium, copper, fluoride, iron, lead, magnesium, nitrite, nitrate, chloride, etcetera, I have phosphate phenols free chlorine, cyanide, sulphate, manganese, arsenic, they are all in all there are about 23 parameters which will which can be permitted, which can permit as to determine them in the prescribed limits. And in the next 4 or 5 classes what we will do is we will take a look at each of these parameter using specific reagents; that means, we are going to take a look at the method what is a prescribed method, how does it work and what are the typical characteristics etcetera so that we will discuss in the next session.

Thank you very much.