Acidity Alkalinity -

1. Methyl orange indicator: Dissolved .5 gm of methyl orange in 1 L of distilled water.

2. Phenolphtalein indicator - Dissolved 5 gm phenolphthalein in 1 L of 95.08 ethyl alcohol. Neutralize the solution with .02 N NaOH Solution.

3. Sulphuric acid .02N: Prepare stock H₂SO₄ Solution (approx. .1N) by diluting 3ml H₂SO₄ to 1L of distilled water. Dilute 200ml of the .1N stock solution to 1L with...
distilled water. Standardise the .02N acid against .02N sodium carbonate solution.

4. Sodium Carbonate solution can be made by dissolving 1.06 g of Na₂CO₃ oven dried in 1L of distilled water. The standardization is done by titrating exactly like an alkalinity titration using phenolphthalein or methyl orange as indicator.

1. **Alcoholic Grades**:

The alcoholic grades were prepared from rectified spirit (95% Alcohol). The distilled water and rectified spirit were mixed in following proportions, so as to achieve the desired concentration of alcohol.

<table>
<thead>
<tr>
<th>Alcohol %</th>
<th>Rectified Spirit</th>
<th>Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>30%</td>
<td>31.0ml</td>
<td>68.5ml</td>
</tr>
<tr>
<td>50%</td>
<td>52.6ml</td>
<td>47.4ml</td>
</tr>
<tr>
<td>70%</td>
<td>73.6ml</td>
<td>26.4ml</td>
</tr>
<tr>
<td>90%</td>
<td>94.7ml</td>
<td>5.3ml</td>
</tr>
</tbody>
</table>
Anthrone Reagent:
Dissolved 2 gms of anthrone in 100ml concentrated H₂SO₄

Acetylcholine Iodide:
Dissolve 289.18 gm acetylcholine iodide in 100 ml. distilled water.

Amino Acid:
Dissolve 100 mg desired amino acid in 100 ml. distilled water.

Alkaline Reagent C:

a) 2% Na₂CO₃ in .1N NaOH

b) .5% Copper Sulphate Solution in 1% Sodium potassium Tarterate Solution.

The alkaline reagent C is prepared by mixing 50ml of solution A with 1ml. of solution B just before use.

Bovine Albumin Serum:
Add 10gm of bovine serum albumin to a 100ml ethanol and ether (3:1) mixture and stirred for 15 minutes at 0°C. Decant the
solvent and repeat the washing at 0°C, filter the albumin and wash in ether until dry.

Accurately weight .5gm of defated bovine serum albumin in about 80ML of water. Gently swiril the solution to mix it then transfer to 100ML volumetric flask. Rinse out the beaker several times with distilled water and add the washes to the volumetric flask. Carefully invent the flask to mix the content and make up to the mark with more water. Its concentration is 5mg/ml.

**Bovin's fluid (Alcoholic):**

Saturated solution of picric acid in 70% alcohol - 75ml

Formalin (90%) - 25ml

Glacial Acetic Acid - 5ml

mix all the three, shake and store.

**BUFFER**

**Acetate Buffer:**

a) .2M solution of Acetic acid (11.55 ml in 1000 ml) distilled water.
b) .2m solution Sodium Acetate (27.2 gm in 1000ml)

(x ml of A + yml of B diluted to a total of 100ml)

<table>
<thead>
<tr>
<th>pH</th>
<th>x</th>
<th>y</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>46.3</td>
<td>3.7</td>
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<tr>
<td>4.0</td>
<td>41.0</td>
<td>6.0</td>
</tr>
<tr>
<td>4.4</td>
<td>30.0</td>
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<td>5.2</td>
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<td>30.5</td>
</tr>
<tr>
<td>5.6</td>
<td>4.8</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Citrate Buffers :

a) 0.1M solution of Citric Acid (21.0 gm in 1000 ml)

b) 0.1 M solution of sodium citrate (29.41 gm in 1000ml)

(x ml of A + yml of B dilute to total of 100ml)

<table>
<thead>
<tr>
<th>pH</th>
<th>x</th>
<th>y</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>6.2</td>
<td>7.2</td>
<td>42.8</td>
</tr>
</tbody>
</table>

**Cacodylate Buffers:**

a) 0.2M solution of sodium cacodylate (42.89m/1000ml.)

b) 0.2M HCl

(50 ml of A + x ml of B dilute to total of 200ml)

<table>
<thead>
<tr>
<th>pH</th>
<th>x</th>
</tr>
</thead>
<tbody>
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<tr>
<td>5.4</td>
<td>43.0</td>
</tr>
<tr>
<td>5.0</td>
<td>47.0</td>
</tr>
</tbody>
</table>
Glycine NaOH Buffers:

a) 0.2M solution of Glycine (15.01 gm in 1000 ml)

b) 0.2 M NaOH

(50 ml of A + x ml of B dilute to total of 200ml)

<table>
<thead>
<tr>
<th>pH</th>
<th>x</th>
</tr>
</thead>
<tbody>
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<tr>
<td>10.4</td>
<td>38.6</td>
</tr>
<tr>
<td>10.6</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Phosphate Buffers:

a) .2M solution of sodium phosphate monobasic (27.8 gm in 1000 ml) (Sodium dehydrogen Orthophosphate)

b) .2M solution of sodium phosphate dibasic (53.65 gm in 1000ml) (Sodium hexa metaphosphate)

(x ml of A + y ml of B dilute to total of 200ml)
<table>
<thead>
<tr>
<th>pH</th>
<th>x</th>
<th>y</th>
</tr>
</thead>
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<td>91.5</td>
</tr>
<tr>
<td>8.0</td>
<td>5.3</td>
<td>94.7</td>
</tr>
</tbody>
</table>

**BOD (Biological Oxygen Demand):**

1. **Phosphate Buffer**: Dissolve 8.5 g KH$_2$PO$_4$, 21.75 K$_2$HPO$_4$ 33.4 gm Na$_2$HHPO$_4$.7H$_2$O and 1.7 g NH$_3$Cl in distilled water and dilute to 1L.

2. **Magnesium Sulphate Solution**: Dissolve 22.5 gm MgSO$_4$.7H$_2$O in distilled water and dilute to 1L.
3. **Calcium Chloride Solution:** Dissolve 27.5g anhydrous CaCl$_2$ in distilled water and dilute to 1L.

4. **Ferric Chloride Solution:** Dissolve .25gm FeCl$_3$.6H$_2$O in distilled water and dilute to 1L.

5. **Maganous Sulfales Solution:** Dissolve 480g MnSO$_4$.4H$_2$O or 400 of MnSO$_4$.2H$_2$O or 364 gm MnSO$_4$.H$_2$O in distilled water filter and dilute to 1L, The MnSO$_4$ solution should not give a color with starch when added to an acidified potassium iodine solution.

6. **Alkali-iodine azide reagent:** Dissolve 500g NaOH and 135g NaI in distilled water and dilute to 1L. Add 10gNaN$_3$ dissolved in 40 ML distilled water.

**Colour:**

1. Potassium Chloroplatinate K$_2$ptCl$_6$ (equivalent to 500 mg metallic Pt.)$^3$

2. Cobaltous Chloride (equivalent to about 250 mg metallic CO)
Copper Sulphate (.5%) :

Dissolved 500 mg copper sulphate in 100 ml. distilled water.

COD (Chemical Oxygen Demand):

1. Standard Potassium dichromate Solution, .0417M :
   Dissolve 12.25 g K$_2$Cr$_2$O$_7$, dried at 103°C for 2 h in distilled water and dilute to 1L.

2. Sulfuric acid reagent : Add Ag$_2$SO$_4$ crystals to Con. H$_2$SO$_4$ at the rate of 5.5 g Ag$_2$SO$_4$/KgH$_2$SO$_4$. Let stand 1 to 2 disslove Ag$_2$SO$_4$.

3. Ferroin indicator Solution : Dissolve 1.485g, 10 phenanthroline monohydrate and 695 mg FeSO$_4$.7H$_2$O in distilled water and dilute to 100 ML.

4. Standard ferrous ammonium sulphate solution, approx. .25M, Dissolve 98g Fe(NH$_4$)$_2$(SO$_4$) 2.6H$_2$O in distilled water. Add 20 ml con. H$_2$SO$_4$ cool and dilute to 1000 ML.
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Standardize this solution daily against standard $\text{K}_2\text{Cr}_2\text{O}_7$ solution as follows -

Dilute 10ML Standard $\text{K}_2\text{Cr}_2\text{O}_7$ to about 100ML. Add 30 ML Con. $\text{H}_2\text{SO}_4$ and cool. Titrate with ferrous ammonium sulphate solution using .10 to .15 ml ferroin indicator.

**DO (Demand Oxygen)**:

1. **Manganous Sulphate Solution**: Dissolve 480g $\text{MnSO}_4.4\text{H}_2\text{O}$ or 400g $\text{MnSO}_4.2\text{H}_2\text{O}$ or 364g $\text{MnSO}_4.\text{H}_2\text{O}$ in distilled water, filter and dilute to 1L. The $\text{MnSO}_4$ solution should not give a colour with starch when added to an acidified potassium iodide (KI) solution.

2. **Alkali-iodide-azide reagent**: Dissolve 500g NaOH and 135g NaI in distilled water and dilute to 1L. Add 10g $\text{NaN}_3$ dissolved in 40ML distilled water. Potassium and sodium salts may be used interchangeably.
3. **Starch Indicator**: Dissolve 2g laboratory grade soluble starch and .2g salicylic acid as a preservative in 100ML hot distilled water.

4. **Sodium theosulfate solution .025N**: Dissolve 6.205g Na$_2$S$_2$O$_3$.5H$_2$O in boiled cooled distilled water and dilute to 1000ML. Preserve by adding 5ML chloroform per liter.

**DTNB**:

Dissolve 396.36 mg, 5,5 dithio-bis-2-nitro benzoic acid in 1000ml. distilled water. Further concentrations were prepared by serial dilution.

**Eosin (Alcoholic)**:

Eosin Powder - 1gm

Alcohol 70% or 90% - 100ml.

**Effluent Concentration**:

Effluent in desired concentration was prepared as per formula given below
Required concentration = \( \times \) required volume

Emulcified concentration of effluent

Required concentration = \( (.1, .01, .001, .0001, .00001, .0000001) \) ppm

The effluent was prepared as follows:

Because 10,000 ppm = 1%

so \( R = \frac{1 \times 200}{20} \)

Effluent = 10ml dissolved in 190ml water = stock solution

1 = 10,000 ppm diluted by this formula

1ml of stock solution + 9ml of water = 1000 ppm

1ml of A+ 9ml of water = 100 ppm

1 ml of B+ 9ml of water = 10 ppm

1ml of C+ 9ml of water = 1 ppm

1ml of D+ 9ml of water = .1 ppm

**EDTA:**

Dissolve 100mg of Ethylene Diamine Tetra Acetic Acid in 100 ml. distilled water.
Formalin - 3% :

Formalin 40%  -  10ml
Distilled Water  -  90ml

Folin-Ciocalteau Reagent :

Take Sodium tungstate 100gm, sodium molybadate 25 gm into a two liter flask and add 500 ml. distilled water, 50ml 85% phosphoric acid and 100ml HCl concentration. The mixture was refluxed gently for about 10 hrs with a condensor. After cooling 150gm lithium sulphate, 50ml of distilled water and few drops of bromine water were added. The mixture was re-boiled for another 10 minutes. After cooling the volume of the mixture was made upto 1000ml and filtered. The stock reagent thus prepared was diluted with equal volume of water just before use.

Glucose Solution (Standard) :

Dissolved accurately weighted 10mg glucose in 100ml distilled water.
Glucose-6-phosphate:

Dissolve 200 mg of glucose-6-phosphate Na salt in 100 ml. distilled water.

Glycine:

Dissolve 15.01 gm of glycine in 1000 ml. distilled water.

Haematoxyline (Delafield's):

Haematoxylin Crystals - 4 gms
Absolute Alcohol - 25 ml
Saturated solution of Ammonia Alum - 400 ml
Glycerine - 100 ml
Methyl Alcohol - 100 ml

Dissolve 4 gm. of haematoxylin crystal in 25 ml of absolute alcohol and add to it 400 ml of saturated solution of ammonia alum. Leave it exposed to light and air in unstoppered bottle for about a week, filter and add 100 ml. of glycerine and 100 ml. of methyl alcohol. Allow the solution to stand until the colour is sufficiently dark then filter.
Hydrochloric Acid (0.2 m):

Dissolve 7.30 ml of hydrochloric acid in 100 ml. distilled water.

Molarity $M = \frac{n}{v}$

$n$ - number of am molecule of solute

$v$ - volume of solution in liter

Molar Solution:

$\frac{m \times mw \times \text{volume}}{1000}$

$m = \text{molarity}$

$mw = \text{molecular weight}$

Ninhydrin Solution:

Dissolve 200mg of ninhydrin in 100ml of 95% alcohol.

P-Nitrophenol:

Dissolve 100mg of p-nitrophenol in 100 ml. distilled water.
4-Nitrophenyl Phosphate:

Dissolve 600 mg of p-nitrophenyl phosphate Na salt in 1000 ml. distilled water.

Phosphate:

1. **Strong Acid**: Add 300 ML con. H$_2$SO$_4$ to about 600 ML distilled water. When cool add 4 ML con HNO$_3$ and dilute to 1L.

2. **Ammonium Molybdate reagent**: Dissolve 25g (NH$_4$)$_6$No$_7$O$_{24}.4$H$_2$O in 175 ML distilled water cautiously add 280ML Con. H$_2$SO$_4$ to 400ML distilled water. Cool add Molybdate Solution, and dilute to 1L.

3. **Stannous Chloride reagent**: Dissolve 2.5g fresh SnXl$_3.2$H$_2$O in 100 ML glycerol. Heat in a water both and stirr with a glass rod to hasten dissolution.

4. **Standard Phosphate Solution**: Dissolve 219.5mg anhydrous KH$_3$PO$_4$ in distilled water and dilute to 1000ML
**Potassium Hydroxide (10%)**:  
Dissolved 10gm potassium hydroxide in 50ml. distilled water make the volume 100ml. by addition of more distilled water.

**Resorcinal**:
Mix 1gm of resocricinal in 100 ml alcohol and .2N HCl.

**Sulphate**:
1. **Buffer Solution**: Dissolve 30g Magnesium Chloride, MgCl$_2$·6H$_2$O 5g Sodium acetate, CH$_3$COONa·3H$_2$O, 1.0 g potassium nitrate, KNO$_3$ and 209ML acetic acid, CH$_3$COOH (99%) in 500ML distilled water and make ujp to 1000ML.

2. **Barium Chloride Crystals**: 20 to 30 Mesh.

3. **Standard Sulfate Solution**: Dissolve .147g of anhydrous Na$_2$SO$_4$ in distilled water and dilute to 1000ML.
Sodium Bisulphate:

Dissolve 10gm sodium bisulphate (NaHSO₃) in 200 ml. of distilled water and filtered to obtain the clear solution.

Sodium Carbonate (2%):

Dissolve 4 gm NaOH in 50ml distilled water and dilute to a total of 100ml. with distilled water.

N-Sodium Hydroxide:

Dissolve 40 gm NaOH (MW=40.0) in 1000 ml distilled water to achieve .1N Solution.

Sodium Potassium Tarterate (1):

Dissolve 1gm Sodium potassium tarterate exactly in 100ml water.

Sodium Sulphite:

Dissolve 20 gm Sodium Sulphite (Na₂SO₃) in 100ml distilled water, filtered and stored in well sloppered bottle.
Sulphuric Acid (10N) :

Add 200 ml concentrated H2SO4 to 520 ml. of distilled water.

Sodium Cacodylate :

Dissolve 42.8 gm of Sodium Cacodylate in 1000 ml. of distilled water.

Sodium Carbonate (2%) :

Dissolved 4.0 gm NaOH in 50ml distilled water and dilute to a total 100ml with distilled water.

Sodium Citrate :

Dissolve 29.41 gm of sodium citrate in 1000 ml. of distilled water.

Turbidity :

Solution I - Dissolved 1 gm hydrazine sulphate in distilled water and dilute to 100 ml. in a volumetric flask.
Solution II - Dissolved 10 gm hexamethylenetetramine in distilled water and dilute to 100 ml in a volumetric flask.

Standard turbidity suspension - In a 100 ml volumetric flask add 5.0 solution I and 5.0 ml in a volumetric flask.

Standard Turbidity Suspension - In a 100 ml volumetric flask add 5.0 ml solution I and 5.0 ml solution Ii. Let stand for 24h at 25±3°C, dilute to mark and mix again. The turbidity of this suspension is 400 NTU. Dilute portion of standard turbidity suspension with turbidity free water as required.

Trichloro Acetic Acid (TCA) 10% :

Dissolve 10gm of trichloroacetic acid in 100 ml of distilled water and stored in brown bottle.