**Read Me**

**https://github.com/bbassa9824/Prototype-A-1/releases/tag/Prototype-A-1-V.1.1**

**About the author**

The author of this program, Prototype-A, has educational background in chemistry, biochemistry and computer programming with a Ph.D. degree in medical biochemistry. The impetus for creating the program came from the author’s interaction in various laboratories, with medical and basic science graduates who conducted biochemistry and cell biology projects as part of their course work. The author has over 20 years of experience in biochemistry and cell biology laboratory work. This includes key roles in establishing cell culture facilities for two different biotech companies in San Diego. At the bench level, the author has used most of the commonly used isotopes, prepared most of the buffers commonly used in biochemistry work, and cultured a large variety of cell types. This practical laboratory experience undergirds this project. The author has a well-cited record of publications in biochemistry and cell biology. The author is an Adjunct Professor at the Southern University, Baton Rouge.

**The program**

The main goal of the present project is to develop a computer program that performs all the computations related to the biochemistry laboratory work. Prototype-A by and large fulfills that goal. Especially, using Prototype-A the experimenter will not do any computations of his own. The scope of the program is extensive. It covers such simple tasks as diluting a commercial acid or an organic solvent and preparing molar solutions to writing very complex assay and drug testing protocols. Programming of serial dilutions, and making available 22 custom buffer calculators are some of the highlights of Prototype-A. Please review all the examples embedded in the program (under the “Examples” option) to appreciate the scope of the program. Prototype-A features fully functional “Prepare solution” and “Dilute solution” modules. Many other functions are partially available in this pre-release version.

**Preparation of buffers**

There is a stark contrast between the class room learning and laboratory practice when it comes to the preparation of buffers. In the academic training the students almost always deal with the problems of the kind “what is the pH when the weak acid and its conjugate base are mixed at say X and Y proportions?” In the laboratory however the experimenter has to deal with the problem of “In what proportions do the base and acid have to be mixed to achieve a particular pH?” Although the Henderson-Hasselbalch’s equation (HH) is pivotal for discerning the ratio of salt and acid, the equation is not used very often in laboratory practice. The most commonly used method is to weigh out the free acid or base equivalent to the required molarity (grams equal to molarity times molecular weight per liter), dissolve the same in a volume slightly less than the required final volume, adjust the pH with a strong acid or a strong base as appropriate using a pH meter and then make up the volume to the required final volume. Please review the reference (Dennison, C.) (1) for a better understanding of this simple method. Nevertheless, calculating the molar ratios of acid and its conjugate base using the HH opens up at least two more methods of preparing the buffer. The HH takes the variables pKa, pH, volume, and molarity and when solved gives the values for the number of moles of acid and number of moles of the salt required to constitute the buffer. Then in the first method weights equivalent to the number of moles of these components (Number of moles \* molecular weight) can be mixed in the required final volume of the buffer. In the second method volumes equivalent to the number of moles of salt and acid (Number of moles/Molarity) can be drawn from the stock solutions of salt and acid, respectively.  In the latter case the volume is adjusted to the final required volume after mixing the stock solutions.

Prototype-A describes one to three methods of preparing a buffer against each query, depending on the inputs and commercial availability of the buffer components.  The user first selects the buffer type and then enters values for the pH, volume and the molarity of the buffer. The pKa value at 250C, for the selected buffer is provided by the program.

In summary the buffer preparation methods shown by this program are:

1. To weigh out grams of free acid or base equivalent to the molarity of the buffer (molarity times molecular weight grams per liter) and adjusting the pH with a strong base or a strong acid (NaOH or HCl for example).
2. To determine the number of moles of each salt and acid using HH and dissolve weights equivalent to the number of moles (number of moles \* molecular weight) in the required final volume.
3. To determine the number of moles of salt and acid each using HH and draw volumes equivalent to the number of moles from previously made stock solutions (Number of moles / stock molarity = volume in liters). The stock solutions so drawn are mixed and the volume is adjusted to the final volume. The program presents instructions for this method only if the user enters values for the concentrations of the salt and acid stock solutions.

A note on Good’s buffers is in order. Apart from resisting changes in the hydrogen ion concentration, a buffer must also be biochemically inert. Keeping this criterion in mind, Good et al. synthesized 12 zwitterion compounds as buffering agents. Their number subsequently increased to twenty. Good’s buffers are more soluble in water, less cytotoxic, have minimum salt effects, and work in the pH range of 6 to 8 (2). MES, TES, HEPES, MOPS are some of the Good’s buffers. Complete list of Good’s buffers is available on many internet sites.

**About the programming of serial dilutions**

In many cases biologically active compounds are active at pM to µM concentrations. Hormones are present in the human blood in pM to nM molar concentrations. Therefore, investigators often have to prepare biologically active compounds at ultra-low concentrations.  Stock solutions prepared with accurately weighable quantities of the solute do not translate into accurately measurable delivered volumes. Additionally, the volume of the vehicle in which the solute is delivered is severely restrictive because many of the test compounds are insoluble in water and they are generally dissolved in an organic solvent like dimethylsulfoxide (DMSO). Therefore, the stock solution must be diluted depending on the volume of the vehicle delivered to the final volume (the assay buffer, culture medium etc.). The usual practice is to keep the dilutions and volumes at multiples of 10 scale and determine the dilution manually. This method is not amenable to programming of the serial dilution protocols. To facilitate programming of the serial dilutions the author has proposed a modification of the basic dilution equation as “D = C1P/C2V2”, where D is the fold dilution required of the stock solution prior to its addition to the final solution at P volume (made up to the final volume to be precise.). The “D” which is the times dilution can be split into a few steps to serially dilute the stock. More importantly, this equation makes it easy to delineate serial dilution steps either manually or through a computer program. Please review the information on the usefulness of this modified equation (3). The program first processes the input using the basic dilution equation (C1V1 = C2V2) and then if the V1 value is less than 2 microliters, the program prompts the user to enter a value for the pipetted volume following which the program displays serial dilution instructions in the output textbox of the user interface.

**Semicolon is used as the delimiter**

Under the “DiluteSolution” option the user can request up to six final concentrations. These concentrations must be entered in the “FinalConc” field separating each value by a “;”. The program uses the semicolon as an identifier in separating the values from the string. For example the entries must look like “1;2;3;4;5;6; mM”, “1000;2000;5000; Times” etc. The fields accept only numbers, and semicolon, from the key board or the keypad and concentration units from the keypad. The compound name field however, accepts both alphabet and numerical characters. The same convention is used in separating values in the time intervals field under the “Write protocol” option.

**The times dilution as an expression of concentration**

Apart from molar and weight based (mg/ml, etc.) unit expressions of concentration, one other type of concentration expression that is used quite frequently in biochemistry and cell-biology laboratories is the dilution factor or times dilution. Examples include the titration of anti-sera, testing of field samples for biological contamination, and the assay of samples of unknown purity. The times dilution concentration type is thoroughly covered in Prototype-A. The user has to enter “1” as the stock concentration and select “Anything” as the stock concentration unit. For the final concentrations the user has to enter 10; 100; 1000; etc., for the final desired dilutions and select “Times” as the final concentration unit. This actually means 1/10; 1/100; 1/1000; dilutions. The program converts the times notation to decimal notation, performs the required calculations and converts the decimal notation back to the “times” notation. Again, the user does no computations of his own. There is no limit on the times dilution of the sample that the user may request and up to six final dilutions can be requested. As before the program switches between the basic dilution equation and the MDE depending on the inputs and asks for the pipetted volume if the delivery volume is less than two microliters.

In the development of this program dilution factor as a parameter is used in the modification of the basic dilution equation, and in the verification of accuracy of the serial dilution computations. In the latter case the ratio between stock concentration and final concentration is the expected dilution factor and the ratio between final volume and the initial volume is the dilution factor achieved. Both these values are displayed in the output.

**Highly flexible unit selection**

With Prototype-A the user should first enter the values and the corresponding units can be entered in any denominations. For example, molarity is defined as number of moles of a compound present in a liter volume of the solvent. In the query fields the user can enter volume in liter, milliliters, or microliter units and enter concentration in M, mM, or µM units, and there is no need to enter concentration in molar (M) and volume in Liter units. It is recommended that the user does not do any unit conversions on his own. Please enter the information at hand and let the program do all the conversions. Molecular weight is a ratio and has no units. Just enter the numbers only in the molecular weight field. Under the dilution option the user can enter weight based units for the stock and ask the final concentration in molar units and vice versa. In such a case the program prompts the user to enter the molecular weight.

**General instructions and examples are embedded in the program**

Please click the “Instructions” and review the instructions to obtain a better insight on the use of this program. Please also review the examples given under “Examples” option to understand the scope of the program which is very wide. The examples are programmed in such a way that the values are entered into respective fields by the program upon clicking an example. The user then has to click “Enter” and follow the prompts.

**The output is displayed in three sections**

The output of Prototype-A is displayed in three sections. The first section contains the original query for the sake of records. It is important for the user to review this information and make sure that there are no errors. The second section in most cases displays step by step calculations by which the instructions in the next section are generated. The users are encouraged to verify the calculations from time to time. The third section contains instructions with embedded values for volumes and weights.

The users may contact the author at [bassa\_babu@subr.edu](mailto:bassa_babu@subr.edu) with any questions.

**References:**

1. Dennison, C. A Simple and Universal Method for Making up Buffer Solutions. Biochemical Education. 16: 210-211, (1988).
2. Good, Norman E.; Winget, G. Douglas; Winter, Wilhelmina; Connolly, Thomas N.; Izawa, Seikichi; Singh, Raizada M. M. (1966). "Hydrogen Ion Buffers for Biological Research". *Biochemistry*. **5** (2): 467–477. [doi](https://en.wikipedia.org/wiki/Doi_(identifier)):[10.1021/bi00866a011](https://doi.org/10.1021%2Fbi00866a011)
3. Bassa B, Uppu R. Modification of the Basic Dilution Equation for the Programming of Serial Dilutions. ChemRxiv. Cambridge: Cambridge Open Engage; 2021; this content is a preprint and has not been peer-reviewed. <https://doi.org/10.33774/chemrxiv-2021-lrqnx>