JPCalc for Windows
(JPCalcW)

Junction Potential Calculator

Users' Manual

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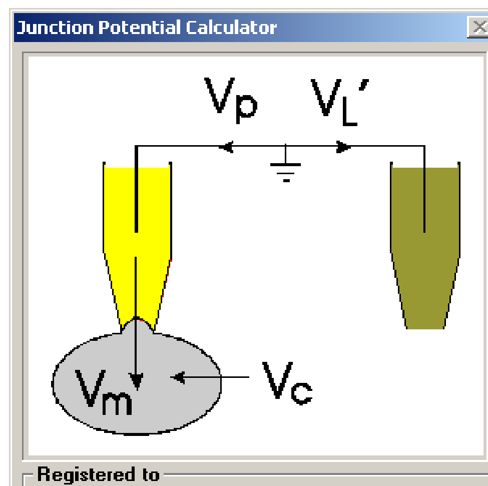

To only be used with a licensed version of JPCalc for Windows or a licensed version of PClamp

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Notes on the new printed and illustrated version of the JPCalc for Windows Users' Manual

Table of Contents for Manual

Section	Page
Program Installation	4
Overview	5
Sign convention used	6
Program Execution	6
Choose Experiment Type Dialog	8
Choose Patch Type Dialog	8
Tutorial Example 1: An outside-out patch calculation	8
Choose Electrode Dialog	9
Choose Temperature Dialog	9
Experimental Procedure	9
Tutorial Example 2: Changing the bath solution	11
Calculation Procedure	11
Choose: New Bath Solution	11
Tutorial Example 3: Free ion concentrations with weak acids and chelating agents like HEPES and EGTA	12
Details of other dialogs	13
Save Dialog	13
Load Dialog	13
Edit Ion Library Dialog	13
Edit Ion Dialog	13
Experimental Procedure Dialog	13
The special characteristics of the different experimental configurations	14
Special Characteristics - Patch-Clamp - Intact Patch	14
Special Characteristics - Excised Inside-Out Patch	14
Special Characteristics - Excised Outside-Out Patch	15
Special Characteristics - Whole-Cell Configuration	15
Special Characteristics - Patch-Clamp - Perforated Patch Configuration	16
Special Characteristics - Intracellular Measurements	16
Special Characteristics - Epithelial Measurements	17
Special Characteristics - Bilayer Measurements	18
Special Characteristics - Measurement of Junction Potential Corrections	18
Ag/AgCl Reference Electrodes	19
Concentrations or Activities?	20
Application of Junction Potential Corrections BEFORE an Experiment	20
References and Further Reading	22
Credits	23
Appendix	24
Table 1: Listing of supplied library file of mobilities with full ion names	25
Tables 2 & 3: Supplementary and Additional listing of mobilities	26
Table 4: Further Listing of ion mobilities added in October 2003	27
Relationship between generalized relative ionic mobility and limiting equivalent conductivity	29

The manual has used some of the On-Line Help Manual content available from within the program, but as indicated in the preceding table of contents, it also includes worked tutorial examples and an appendix. In addition, it includes extensive illustrations of program screens.

For reference, the On-Line Help Manual within the program also has the following contents.

On-Line Help Contents

The following On-line Help Topics are also available from within the program:

Overview

Dialogs

- ***Add Ion Concentration Dialog***
- ***Add Ion Dialog***
- ***Calculate Junction Potentials Dialog***
- ***Choose Electrode Dialog***
- ***Choose Excised Patch Type Dialog***
- ***Choose Experiment Type Dialog***
- ***Choose Patch Type Dialog***
- ***Edit Ion Concentration Dialog***
- ***Edit Ion Library Dialog***
- ***Edit Ion Dialog***
- ***Enter Temperature Value Dialog***
- ***Experimental Procedure Dialog***
- ***Load Dialog***
- ***Save Dialog***

Special Characteristics

- ***Patch-Clamp - Intact Patch***
- ***Excised Inside-Out Patch***
- ***Excised Outside-Out Patch***
- ***Whole-Cell Configuration***
- ***Patch-Clamp - Perforated Patch Configuration***
- ***Intracellular Measurements***
- ***Epithelial Measurements***
- ***Bilayer Measurements***
- ***Measurement of Junction Potential Corrections***

Ag/AgCl Reference Electrodes

Concentrations or Activities?

References and Further Reading

Credits

For on-line information on Help from within program, either click on ***Help*** *or Press* ***F1***

JPCalcW Installation

If the installation program for ***JPCalcW*** does not start automatically when the CD is inserted into the CD ROM drive, then use the set up program ***SETUP.EXE*** in the root directory on the distribution CD (or it may be found on the subdirectory labeled ***Dist_JPCalcW*** if there are other software programs also included on the CD) to install the program fully as a regular Windows program.

Additional Mobility Values

Additional Mobility Values can be obtained from the tables listed in the Appendix of this manual with more up-to-date values in the **Ionic Mobility Listing** (or from other references listed there) from the website:

http://web.med.unsw.edu.au/PHBSoft

July, 2009

Overview

The junction potential calculations and dialog were written by Axon Instruments but based, with permission and consultation, on the program JPCalc by Dr. Peter H. Barry of The University of New South Wales. The dialog has been designed for calculating and indicating the application of liquid junction potential corrections in various electrophysiological situations and it uses graphical illustrations to show how such junction potentials arise in each case. In particular, it is designed to be used for the correction of patch-clamp measurements (for whole-cell, perforated, intact and excised patch configurations), and intracellular, epithelial and bilayer measurements, and also for the correction of direct experimental measurements of junction potentials. Junction potential corrections are particularly important in patch-clamp measurements and failure to apply them can typically result in errors of up to about 10 mV for measurements of membrane potential (Refs 3, 4, 10). It is of course also essential that such corrections be applied in the correct direction. The program enables the appropriate liquid junction potential corrections to be calculated using the generalized Henderson liquid junction potential equation (Refs 1, 2, 3, 4, 9) and makes use of a simple entering and editing routine for typing in solution concentrations and relative mobility data for each ion (Refs 4, 12 and extracting data from Refs 6, 13-15). The program then clearly illustrates the direction in which the junction potentials have to be applied.

The program also enables (1) the calculation of junction potential corrections resulting from additional changes in bathing solution composition and (2) the incorporation of a library of ionic mobilities that will be automatically called up whenever the appropriate abbreviated name of an ion is entered into the program. For N polyvalent ions, the generalized Henderson Equation may be shown to be given by (e.g., Ref. 4):

$$V^S - V^P = (RT/F) \quad S_F \quad \ln\{\sum_{i=1}^{N} z_i^2 u_i a_i^P / \sum_{i=1}^{N} z_i^2 u_i a_i^S\} \qquad (1)$$

where

$$S_F = \sum_{i=1}^{N} [(z_i u_i)(a_i^S - a_i^P)] \quad / \quad \sum_{i=1}^{N} [z_i^2 u_i \quad (a_i^S - a_i^P)]$$

where V^s - V^p represents the potential of the solution (S) with respect to the pipette (P) or electrode and u, a and z represent the mobility, activity and valency (including sign) of each ion species (i); R is the gas constant, T is the temperature in K and F is the Faraday, so that RT/F ln = 58.2 log10 in mV at a temperature of 20°C.

Although the use of the above equation has been checked experimentally with monovalent cations, and found to agree within a fraction of a mV for a number of different solutions (Refs 2, 4, 12), it should still be treated as an estimate, although a good estimate. The use of the Henderson Equation with high concentrations of polyvalent ions has not been so carefully checked and should be considered as an even more approximate estimate at this stage. However, it should be stressed that even carefully measured values of junction potentials still require corrections and are themselves not perfectly accurate.

Two types of reference or measuring electrodes are allowed for: a standard salt bridge/microelectrode type (recommended; with an Ag/AgCl electrode connection remote from the bathing solution) or a silver/silver chloride (Ag/AgCl) type, with an Ag/AgCl wire or pellet directly in contact with the bathing solution (not generally recommended). The user is able to change between these two types. HOWEVER, IN ALMOST EVERY SITUATION THE OPTION TO USE THE DIRECT Ag/AgCl CONNECTION SHOULD NOT BE TAKEN.

This is because the electrode potential is extremely sensitive to the activity of Cl^- ions in the appropriate bathing solution and experimentally it is much more likely to deteriorate or drift when in contact with a flowing bathing solution. Such an Ag/AgCl electrode potential, in fact, is given by:

$$V_{Ag} = V_0 - (RT/F) \ln a_{Cl} \qquad (2)$$

where V_{Ag} refers to the potential of the electrode with respect to the solution; V_0 refers the standard electrode potential for the electrode reaction and a_{Cl} to the activity of the chloride ions in the adjacent bathing solution. The choice of the option to use the direct Ag/AgCl electrode in the bathing solution will be discussed further towards the end of the manual. It will therefore be assumed that the option to use a standard solution type of reference electrode will be chosen.

A screen dialog display also gives some general explanatory comments during use of the program (these will be discussed in more detail later). The first display generally shows the presence of any critical junction potentials and their being balanced by the appropriate amplifier. The location and direction of these critical junction potentials will be shown in the circuit diagram, the potentials being defined as in the direction of the arrow head to the arrow tail.

For the first two major options, pressing the Next button, as prompted, will then simulate either patch-clamping the cell (sealing the pipette against the cell membrane and forming a gigaohm seal) or impaling the cell with the intracellular microelectrode. For the case of the epithelial measurements, the circuit will not need to change. In every case, any unbalanced junction potentials will be displayed in the new configuration and the relationship between the membrane potential and the various other unbalanced potentials in the circuit given. For the case of an intact patch, for example, it can be seen that with the cell patched, the pipette V_L is replaced by V_m, but that the offset value V_L' still remains in the amplifier. The composition of the solutions can then be added to enable calculation of the junction potentials.

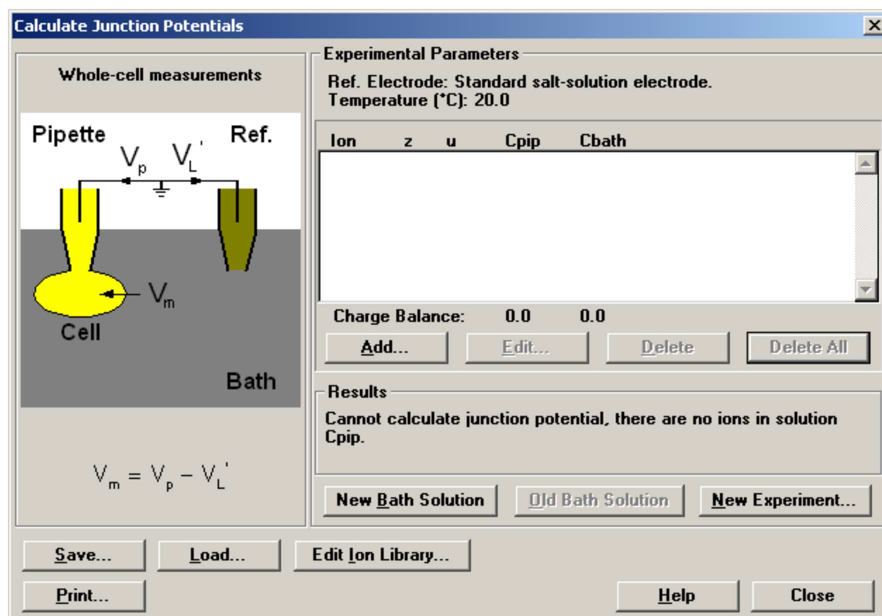
In most biological situations where the overall concentration of ions is the same in each solution, ionic concentration values may generally be used and it is expected that most users of this program will be using concentrations. However, where there are different solutions of pure electrolyte solutions at very different overall ionic strengths, activity values should strictly be used for the greatest accuracy. This is discussed further in the section on ***Concentrations or Activities***. In addition, only the major permeant ions, with concentrations greater than a few mM, need generally be considered to contribute to the junction potential correction.

SIGN CONVENTION USED

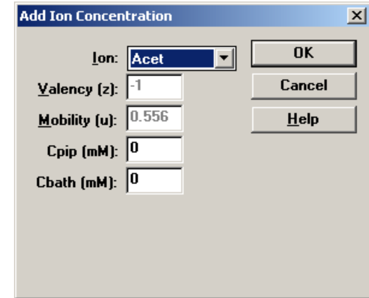
The sign of the potential is as indicated by the arrows in the displays (with the potential of the arrow head being with respect to arrow tail). In each case, the junction potentials are calculated as the potential of the solution with respect to the pipette (Refs 3, 4, 10, 11) and, where appropriate, the potential of the inside surface of the membrane is given with respect to the outside solution.

Program Execution

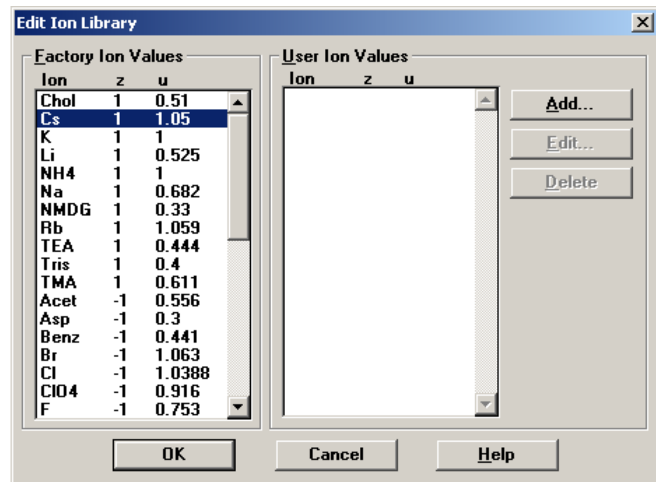
Press OK on initial screen, and depending on the previous use of the program, a screen similar to the one adjacent will appear - the **Calculate Junction Potentials Dialog.** This is the main dialog for the Junction Potential Calculation program. The graphic on the left of the dialog screen shows a pictorial view of the experiment as it is has been previously or currently defined. To define a new



type of experiment, click on the ***New Experiment*** button. In the example, the program has previously been calculating liquid junction potential corrections for the whole-cell patch clamp configuration. However, in that program screen example, all the previous ion values have been deleted.



Use the ***Add…*** button to add the name and concentration of each of the ions, which will be in the pipette and bath solutions. This will bring up a screen like that adjacent. You can then scroll through this alphabetical list of ions, to see if the ion you want is in the Factory List. If it is, then just click on it and add the concentrations in the pipette and bath. When finished, choose ***OK*** to add the ion to the experimental solution setup. Note that you cannot edit the valency and mobility fields for any of the ***Factory Ion Values*** in the ***library of ions.*** If the ion is not in that list, but you know, or wish to estimate its properties, you can add or edit this ion via the ***Edit Ion Library*** button. When you click on this you will get a screen like that adjacent and you can add your own ions into the ***User Ion Values*** Library. Note that the name can only have up to 4 characters. These user-defined ions and their properties can then be edited or deleted. Once added into the ***User Ion Values*** library, the ions will be listed along with the factory ions when you go to the Add... button (for the ***Add Ion Concentration*** Dialog**)** from the main screen in order to add the concentrations of the various solutions. There can be up to four of concentration fields depending on the experiment type, and whether you have changed solutions.



When you are finished and you wish to add the ion to the list of ions in the experimental solution, press the ***OK*** button. To finish the dialog without adding the ion to the list, press the **Cancel** button.

When ions have been added to the solution values, they will show up, with their properties and concentrations in the list on the right of the main program screen. To add a new ion and its concentration, click the ***Add*** button. To edit an existing solution, either double-click on the concentration, or click once to select the concentration and press the ***Edit*** button. You can delete a concentration by selecting the concentration, and then clicking the ***Delete*** button. And you can delete all of the concentrations by clicking on the ***Delete All*** button.

After each change in ion concentrations, the Junction Potential is automatically calculated and placed in the ***Results*** section directly below the list of ion concentrations.

For the majority of experiments, in which the bath solution does not change (or at least the primary ionic constituents do not change), there is no need for the user to enter any details of the reference electrode solution. This is because the junction potential of the reference electrode is constant in these circumstances and under standard experimental practice was zeroed out in the amplifier at the beginning of the experiment. To change the bath solution during the experiment, press the ***New Bath Solution*** button. In addition to being asked to enter the details of the new bath solution you will be asked to enter the details of the reference electrode solution so that the junction potential at the reference electrode can now be taken into account. Once your have changed to a new bath solution you can return to the original bath solution with the ***Old Bath Solution*** button.

To store the experimental settings, including the ionic concentrations, on the hard disk press the ***Save*** button. To retrieve experimental settings press the ***Load*** button.

Choose Experiment Type Dialog

The ***Choose Experiment Type*** is the first of several dialogs that allow you to specify the type of experiment you are performing. The possible options are:

- ***Patch-clamp measurements***
  - ***Intact***
  - Excised
    - ***Inside-out***
    - ***Outside-out***
  - ***Whole cell***
  - ***Perforated***
- ***Intracellular measurements***
- ***Epitihelial measurements***
- ***Bilayer measurements***
- ***Measurement of Junction Potentials***

You can move forward or backward in this series of dialogs by using the ***Next*** and ***Back*** buttons. The ***Cancel*** button will exit you from the dialogs and return you back to your previous experiment.

Choose Patch Type Dialog

The ***Choose Patch Type*** allows you to narrow down what type of patch-clamp experiment you are performing. The possible options are:

Intact, Excised (***Inside-out or Outside-out),*** ***Whole cell,*** ***Perforated***

You can move forward or backward in this series of dialogs by using the ***Next*** and ***Back*** buttons. The ***Cancel*** button will exit you from the dialogs and return you back to your previous experiment.

TUTORIAL EXAMPLE 1: An outside-out patch calculation

Aim: Calculate the liquid junction potential (LJP) correction for such a patch with the following solution compositions**.**

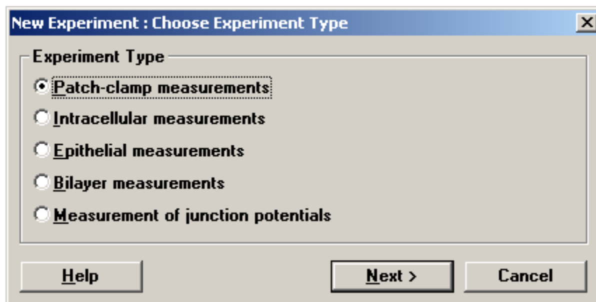
Pipette solution: 120 mM CsF, 20 mM CsCl, 10 mM HEPES, buffered to pH 7.3 with about 5 mM CsOH
Bath solution: 140 mM NaCl, 10 mM HEPES, buffered to pH 7.4 with about 5 mM NaOH,
and 10 mM glucose (since glucose is a non-electrolyte, it will not affect the LJP)

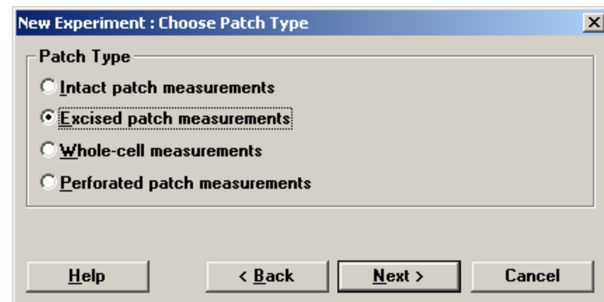
The actual amounts of CsOH and NaOH may vary slightly from these values, but can be measured while making up the solutions. Slight variations will not affect the LJP values significantly.

Note that Cl^- is required in the pipette to give a stable defined Ag/AgCl electrode potential.

Allowing for the approximate concentrations of CsOH and NaOH to buffer HEPES in the two solutions, the free ionic concentrations in mM are:
Pipette: Cs^+ = 145; F^- = 120; Cl^- = 20; $HEPES^-$ = 5
Bath: Na^+ = 145; Cl^- = 140; $HEPES^-$ = 5;

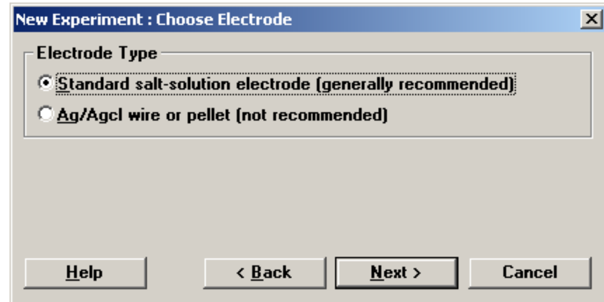
Choose ***New Experiment***, then, ***Patch-clamp measurements*** and ***Excised patch measurements,*** as in the dialogs below, terminating each dialog with ***Next >***.





The ***Choose Excised Patch Type*** button allows you to narrow down what type of excised patch-clamp experiment you are performing. The possible options are: ***Inside-out*** and ***Outside-out.*** You can move forward or backward in this series of dialogs by using the ***Next >*** and ***< Back*** buttons. The ***Cancel*** button will exit you from the dialogs and return you back to your previous experiment.

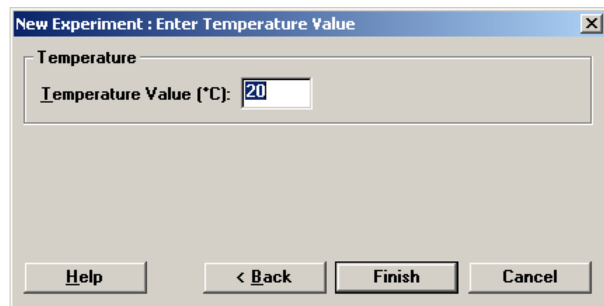
In this case you will choose ***Outside-out patch*** and the go to ***Next.***



Choose Electrode Dialog

The Choose Electrode dialog allows you to choose whether you are using a ***standard*** or a ***silver/silver chloride (Ag/AgCl)*** electrode for the reference electrode in contact with your bath in the experiment. The recommended electrode type is ***standard*** – see discussion later.

You can move forward or backward in this series of dialogs by using the ***Next*** and ***Back*** buttons. The Cancel button will exit you from the dialogs and return you back to your previous experiment.



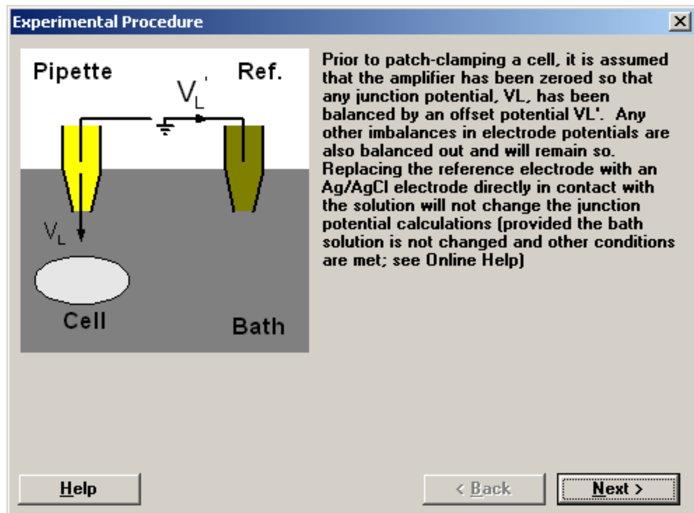
Choose Temperature Dialog

You can then choose the temperature of your bathing solution. This and other values will be retained by the program and will remain constant unless you specifically change them.

Then press ***Finish.***

Experimental Procedure

The next screen shows the situation prior to actually patch-clamping a cell. In this situation when you zero the amplifier, you balance out any offsets in the system from electrodes to liquid junction potentials (LJPs). In particular, the LJP between the pipette and the bathing solution, V_L, (bath with respect to the pipette), will be balanced by an offset, V_L', in the amplifier. Click ***Next*** to continue.

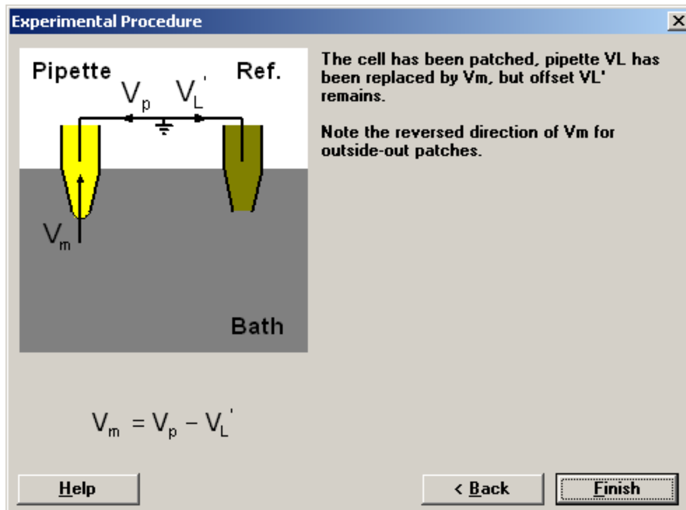


Here,

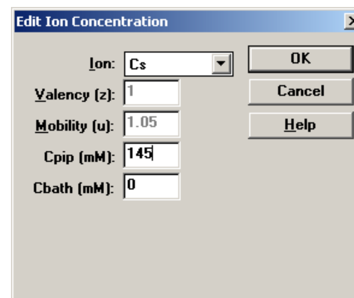
$$V_L = V_L'$$

The next screen shows what happens when the cell is patched and the patch excised with the outside membrane surface facing the outside bathing solution. In this situation the pipette LJP has been replaced by V_m, the membrane potential across the patch, inside surface with respect to outside (this will be in the opposite direction to an inside-out patch). However, the offset in the patch-clamp amplifier still remains. Hence, the membrane potential is now related to both the applied patch potential V_p and the LJP, V_L, by

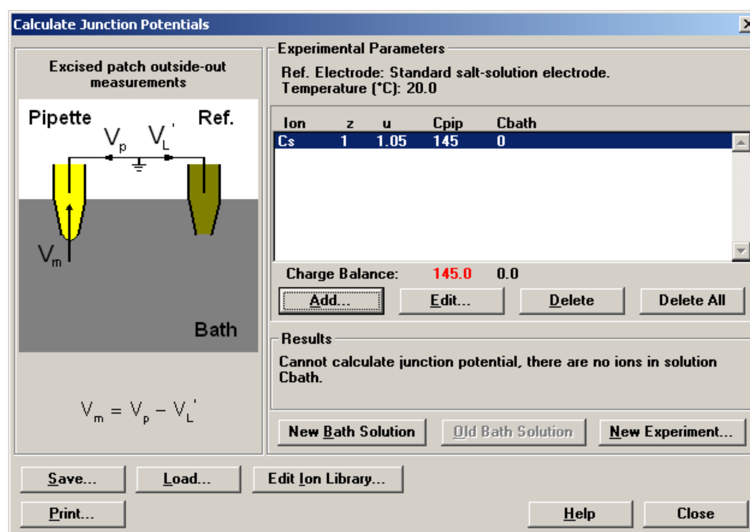
$$V_m = V_p - V_L' = V_p - V_L$$



After pressing the ***Finish*** button, you can use the ***Add…*** button to starting adding the ion concentrations.

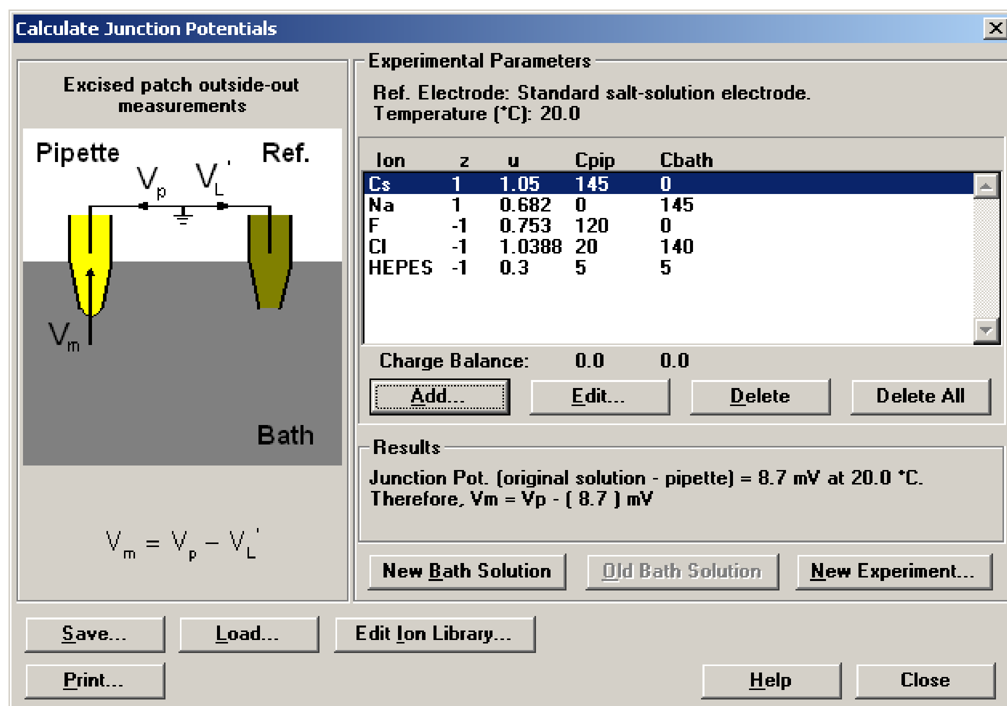


Scroll through the different ions in the built-in ion library to see if the one which you want (e.g., Cs) is there, and if so click on it to choose it and then enter the ion concentrations in both the pipette and bath. Note that you cannot edit the mobility and valency of any ions in the in-built (factory) ion library. If the ion isn't in the library it can be added into the user library using the ***Edit Ion Library …*** button (see P. 5). Press ***OK*** to return to the main program screen. Each added ion is now listed in the right panel of the screen and the net charge balance for each solution is shown. Since there are now ions in the solution bath, their contribution to the LJP cannot yet be shown. All of the other ions in the solution can now be added in the same way.



The result is shown on the right. The LJP has now been calculated and any values of V_m are obtained by subtracting the LJP of 8.7 mV from V_p. That is if V_p is -50 mV, then V_m = -58.7 mV.

Note also that for both the pipette and bath, the charges are balanced. This is one useful, but not foolproof, check that the ion concentrations have been correctly entered. However, if there are large anions,



with very low mobilities, that have not been included, then the charge balance will not be zero. The previous screen values may be saved or printed out with the ***Save*** and ***Print*** buttons.

TUTORIAL EXAMPLE 2: Changing the bath solution

Following on from the same situation as in Tutorial Example 1, assume that the bathing solution is going to be changed to the following composition in mM

New Bathing solution: 140 mM NaGluconate 10 mM HEPES buffered with about 5 mM NaOH to pH 7.4 and again including 10 mM glucose.

Since the bathing solution is being changed after the original zeroing of the patch-clamp amplifier, there will now be an additional contribution to the LJP correction due to changes in the LJP at the reference electrode – bathing solution interface. In order to calculate this contribution, we now need to know the ionic composition of the reference electrode.

Reference electrode solution composition: 140 mM NaCl in 4% agar.
It is generally a good idea for it to have a similar ionic composition to the main bath solution. This minimizes time dependent changes in that LJP (See Ref. 2).

Allowing for the approximate concentration of NaOH in the new bathing solution, the free ionic concentrations in mM are:

New Bath solution:

Na^+ = 145; $Gluconate^-$ = 140; $HEPES^-$ = 5;

Reference electrode solution:
Na^+ = 140; Cl^- = 140;

Calculation Procedure
Choose: *New Bath Solution*

This will bring up the adjacent screen, which shows the contribution of the LJP at the reference electrode. Then choose ***Finish.***

This will bring up the adjacent screen, in which there are no values yet entered for the reference concentrations (**Cref**) or for the new bath solution (**Cbath2**).

Now enter those values in with the ***Add...*** button for new ions or the ***Edit...*** button for existing ions. In the latter case, first click on to the ion to be edited and then on to the ***Edit*** button.

The adjacent screen shows the results when all the free ions have been added to the Cref and Cbath2 columns.

It can be seen that there is now a sizeable LJP contribution at the reference electrode, but in this situation it actually reduces the total LJP from + 8.7 mV to -4.6 mV.

Calculate Junction Potentials

Excised patch outside-out measurements

Pipette V_p V_L' Ref.

V_m V_L^{21}

Bath

$$V_m = V_p - (V_L' + V_L^{21})$$

Experimental Parameters

Ref. Electrode: Standard salt-solution electrode.
Temperature (°C): 20.0

Ion	z	u	Cpip	Cbath	Cref	Cbath2
Cs	1	1.05	145	0	0	0
Na	1	0.682	0	145	140	145
F	-1	0.753	120	0	0	0
Cl	-1	1.0388	20	140	140	0
HEPES	-1	0.3	5	5	0	5
gluc	-1	0.33	0	0	0	140

Charge Balance: 0.0 0.0 0.0 0.0

Add... Edit... Delete Delete All

Results

Junction Pot. (original solution - pipette) = 8.7 mV at 20.0 °C.
VL21[Solution - Ref electrode (new - original)] = -13.3 mV
Therefore, Vm = Vp - (-4.6) mV

New Bath Solution Old Bath Solution New Experiment...

Save... Load... Edit Ion Library...

Print... Help Close

Hence, as may be seen from the above screen, if V_p was -50 mV, V_m would be = -50 – (-4.6) = -45.4 mV

TUTORIAL EXAMPLE 3: Free ion concentrations with weak acids and chelating agents like HEPES and EGTA

Assume a solution had the following composition in mM:

140 NaCl, 2 $CaCl_2$, 5 Na_2EGTA, 10 HEPES buffered with NaOH to pH 7.4.

What would the approximate ionic concentrations be?

Assume again that titrating 10 mM HEPES with NaOH requires about 5 mM NaOH to bring the pH to about 7.4 and that this is not greatly affected by the EGTA, though this can be checked when the solution is being made up. Assume also that 2 mM EGTA will chelate 2 mM Ca^{2+}, then the free ion concentrations (in mM) would be:

$Na^+ \approx 140 + 10 + 5 = 155$; $Cl^- = 144$; $Ca^{2+} \approx 0$; $HEPES^- \approx 5$; $EGTA^{2-} \approx 5 - 2 = 3$

Also, note that at pH 7.4, EGTA, and the free EGTA will mostly be in the $EGTA^{2-}$ form

Details of other dialogs

Save Dialog

This is the standard Windows save file dialog. You can save your results in a file for later use.

Junction Potential Calculation experiment files end with the extension '.jpe'. Pressing the ***Save*** button causes all of the experimental settings to be stored, including the ionic concentrations.

Load Dialog

This is the standard Windows open file dialog.

Junction Potential Calculation experiment files end with the extension '.jpe'. Pressing the ***Load*** button causes all of the experimental settings to be loaded, including the ionic concentrations.

Edit Ion Library Dialog

This dialog allows you to add, edit or remove ions from the ion library. The ion library is used when you are editing solutions to provide a quick way of adding ion information.

When you are adding or editing an ion concentration in the solution, and you type in the name of one of the ions in the library, then it's valency and mobility are automatically filled in.

To add a new ion to the list press the ***Add*** button. To edit an existing ion's valency or mobility, select the ion and press the ***Edit*** button, or double-click on the entry in the list. To remove an ion from the list, select the ion and press the ***Delete*** button.

The list of ions is automatically sorted (by valency and then name) after any addition, change, or deletion.

Edit Ion Dialog

Use the Ion field to change the name of the ion, which can be up to four characters. Use the ***Valency*** and ***Mobility*** fields to change the valency and mobility of the ion.

When you are finished, and you wish to update the ion information in the list, press the ***OK*** button. To finish the dialog, without updating the ion list, press the ***Cancel*** button.

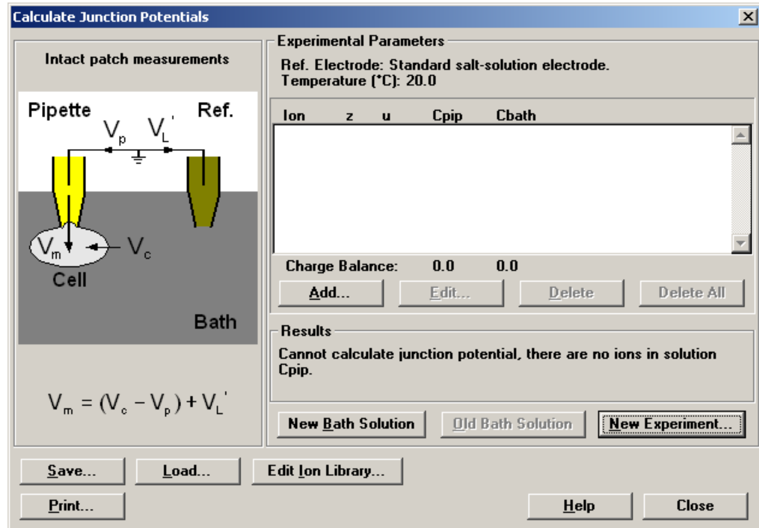
Experimental Procedure Dialog

These dialogs explain the steps in the experiment, and the variables involved in the junction potential calculation. Pressing **< *Back*** will move you backwards in the explanation, pressing ***Next* >** will move you on to the next step.

For most experiment types there are two explanation pages, for epithelial and bilayer there is only one. When you are finished with these, press ***Next* >**, and you will move you on to the next section.

The special characteristics of the different experimental configurations

Special Characteristics - Patch-Clamp - Intact Patch



In the initial situation, after patch-clamping and before any subsequent change in bathing solution composition, the potential across the membrane patch, V_m, is related to the membrane potential of the cell, V_c, the pipette potential, V_p, and the liquid junction potential between the bathing solution and the pipette, V_L, balanced by the offset in the amplifier $V_L'(=V_L)$ and given (Refs 4 & 10) by:

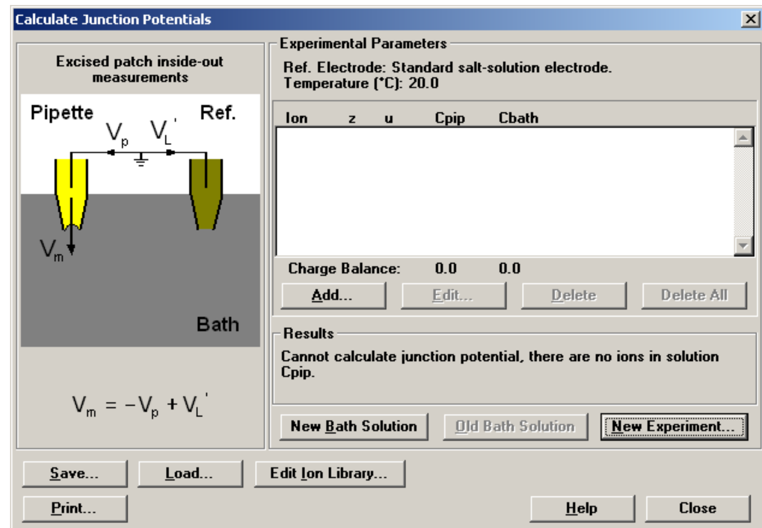
$$V_m = (V_c - V_p) + V_L'$$

If the bathing solution is then subsequently changed, the new potential needs to be further corrected by a liquid junction potential component V_L^{21}, which represents the potential of solution 2 with respect to solution 1, as indicated below:

$$V_m = (V_c - V_p) + (V_L' + V_L^{21})$$

Special Characteristics - Excised Inside-Out Patch

Basically, the situation is similar to the intact patch case above, except that now there is no membrane potential to be considered. The initial situation is as indicated for the intact patch, prior to the formation of a patch. Following satisfactory seal and excision of the patch, as illustrated in the dialog display, V_m, being defined as the potential of the inside membrane surface with respect to the outside one, is now related to V_p and V_L' (e.g., Ref 4) by:



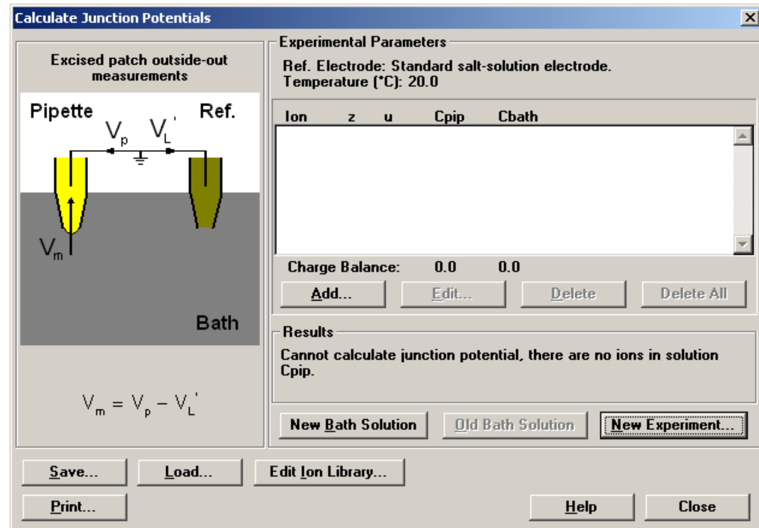
$$V_m = -V_p + V_L'$$

If the bathing solution is then subsequently changed, the new potential needs to be further corrected by a liquid junction potential component V_L^{21}, which represents the potential of solution 2 with respect to solution 1, as indicated below:

$$V_m = -V_p + V_L' + V_L^{21}$$

Special Characteristics - Excised Outside-Out Patch

The situation is very similar to that of the inside-out patch, except that now because of the reversed direction of V_m, still being defined as the potential of the inside membrane surface with respect to the outside one, the equation relating V_m, V_p and V_L', becomes (e.g., Ref 4):



$$V_m = V_p - V_L'$$

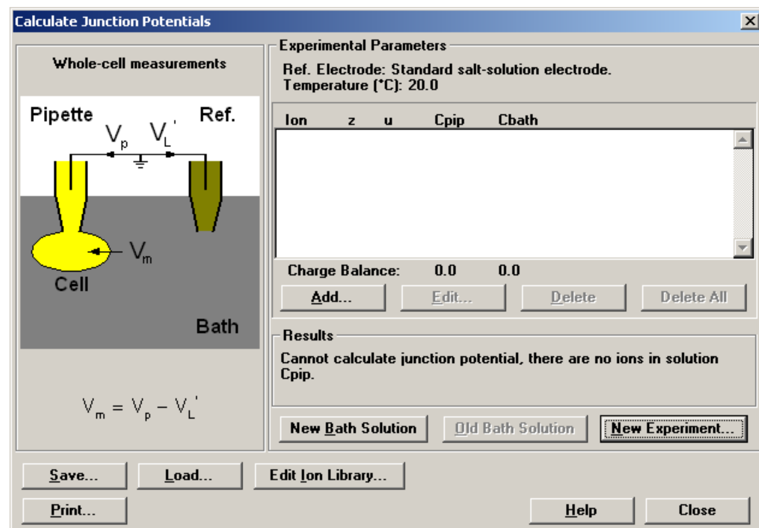
If the bathing solution is then subsequently changed, the new potential needs to be further corrected by a liquid junction potential component V_L^{21}, which represents the potential of solution 2 with respect to solution 1, as indicated below:

$$V_m = -V_p - (V_L' + V_L^{21})$$

Note the reversed direction for V_m in the diagram display

Special Characteristics - Whole-Cell Configuration

The situation is similar to some of the previous configurations, except that now V_m represents the potential of the inside of the cell with respect to the outside solution. Before the cell is patched there will be a junction potential, V_L, representing the potential of the solution with respect to the pipette. If now the cell volume is small in comparison with the pipette volume and exchange between pipette and cell is reasonably fast, the cell contents will soon be dialyzed by the pipette solution. In such a case after the solution exchange is complete, V_m will be related to V_p and V_L' by (e.g., Barry & Lynch, 1991):



$$V_m = V_p - V_L'$$

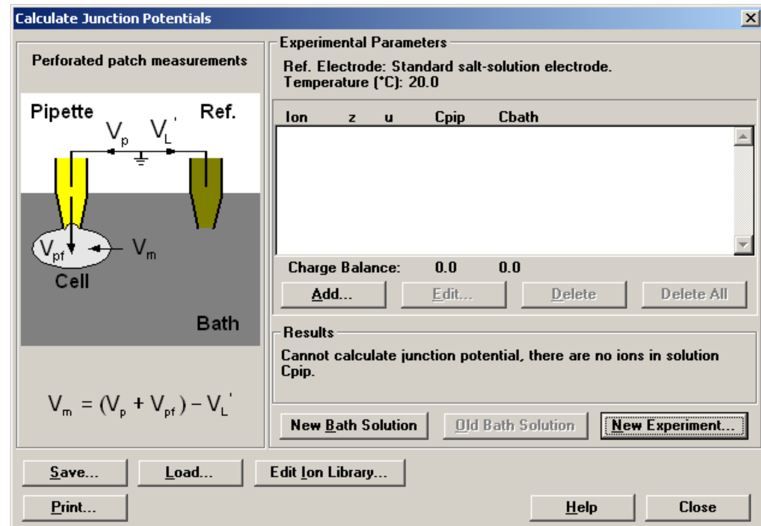
However, before this dialysis occurs, there will also be a junction potential V_L^i between the pipette and cell interior. This is not just a simple liquid junction potential because of the presence of large fairly immobile anions within the cell there is a Donnan potential contribution of about -12 mV to V_L^i (see Refs 7 and 4). This solution exchange generally takes a number of minutes after the formation of a whole-cell configuration, depending on the size of the cell and pipette diameter etc. Therefore, electrical measurements should only be made after the initial drifts in potential have settled down.

If the bathing solution is then subsequently changed, the new potential needs to be further corrected by a liquid junction potential component V_L^{21}, which represents the potential of solution 2 with respect to solution 1, as indicated below:

$$V_m = -V_p - (V_L' + V_L^{21})$$

These measurements can, of course, be carried out as soon as the external solution has been satisfactorily changed.

Special Characteristics - Patch-Clamp - Perforated Patch Configuration



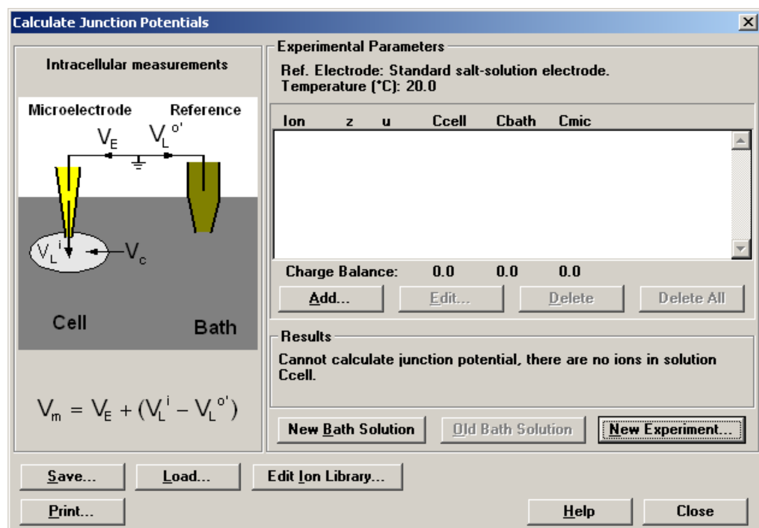
This situation is very similar to the whole cell configuration except that there is no longer complete solution exchange between pipette and cell. The addition of an ionophore (e.g., nystatin) to the pipette solution will now make the membrane permeable to small anions and cations. Hence, only the small permeant ions will exchange between pipette and cell interior and the larger impermeant anions will not be able to do so. Thus there will be a potential difference V_{pf} (perforated patch potential; cell interior with respect to pipette) which will have both a Donnan component due to these impermeant anions and, before complete equilibration, a diffusion potential dependent on the ion selectivity of the ionophore. However, after complete equilibration of the small permeant ions, only the Donnan component of V_{pf} (which will have to be calculated separately) will be left. Vm will again be related to V_p, V_{pf} and V_L' by:

$$V_m = V_p + V_{pf} - V_L'$$

Again, if the external solution is subsequently changed, the new potential across the cell membrane, V_m, will be given by:

$$V_m = V_p + V_{pf} - (V_L' + V_L^{21})$$

Special Characteristics - Intracellular Measurements



Generally, the junction potential corrections for intracellular measurements are not as large as they are in patch-clamp or epithelial measurements. This is because normally high concentration KCl (e.g., 3M KCl) microelectrodes are used, and the high concentration of KCl tends to dominate the other ionic components in the solution and so the two junction potentials (before and after cell impalement) tend to cancel each other out. However, for accurate work or if different lower concentration solutions are used in the microelectrodes, appropriate junction potential corrections should be considered. Before cell impalement, the junction

potential in the external solution, V_L^o, is balanced off in the amplifier with an equal and opposite offset, $V_L^{o\prime}$, as shown in the dialog displays.

Now, when the cell is impaled, there is a new junction potential, V_L^i, between the cell interior and the microelectrode, so that the true membrane potential, V_c is related to the measured value of the potential V_E, and the junction potential difference by:

$$V_c = V_E + (V_L^i - V_L^{o\prime})$$

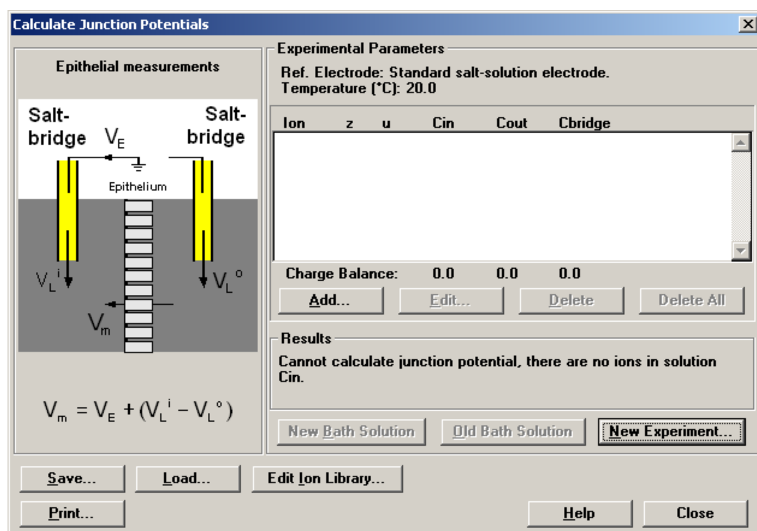
For typical cell interior and extracellular solutions and 3M KCl electrodes, the liquid junction potential correction is normally about 3 mV. It should be noted that in order to minimize history-dependent junction potential effects, such concentrated KCl microelectrodes should have free-flowing junctions (e.g., see Refs 2 & 3). However, the flow of KCl out of the intracellular microelectrode should not be enough to significantly alter the ionic composition of the cells.

During multiple changes in bathing solution, it is preferable to use a reference electrode of similar composition to that of the control solution in order to minimise solution history-dependent effects (see Ref. 2 (Barry and Diamond, 1970), for further details.

Again, as in the previous patch-clamp examples, if the external solution is then changed, there will be an additional junction potential, V_L^{21}, representing the junction potential of solution 2 with respect to solution 1. This value will be calculated by the program and subtracted from the value of V_L^i - V_L^o, as in the equation below:

$$V_c = V_E + (V_L^i - V_L^{o\prime} - V_L^{21})$$

Special Characteristics - Epithelial Measurements



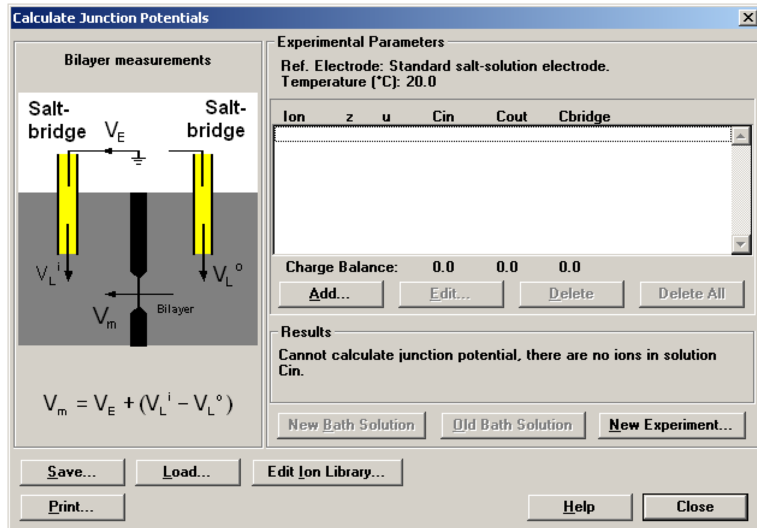
Saline-filled agar-gelled polythene salt bridges are normally used for epithelial measurements. As in the case of patch-clamp measurements, junction potential effects are generally much more significant than they would be in intracellular measurements. This is because of the lower salt concentrations used in the salt bridges and the less mobile ions often used in those salt bridges. Also, in order to minimize history-dependent effects, whereby the junction potential of the salt bridge depends on the previous solutions into which it was placed (see discussion in Ref 1), the composition of the salt bridge is chosen to be of similar composition to one of the solutions bathing the epithelium. For example, if the potential of an epithelium separating 140 mM LiCl and 140 mM NaCl is required, 140 mM NaCl salt bridges can be used. Both solutions can be initially changed to 140 mM NaCl, the amplifier zeroed and then one of the solutions changed to 140 mM LiCl. In this case (see Ref 3), the true epithelial potential difference, V_m, would be related to the measured potential, V_E, and the junction potential difference V_L^i - V_L^o, by:

$$V_m = V_E + (V_L^i - V_L^o)$$

and, for the example above, V_L^o would be zero.

Special Characteristics - Bilayer Measurements

The principles are exactly the same as for epithelial measurements, and it is generally advisable that salt bridges be used rather than Ag/AgCl electrodes directly in contact with the bathing solutions. It should be noted that it is advisable that the composition of the salt bridges be chosen to be of similar composition to that of one of the solutions bathing the bilayer. For example, if the potential of a bilayer separating 140 mM LiCl and 140 mM NaCl is required, 140 mM NaCl salt bridges can be used. The situation considered is one in which either of two conditions pertain:



(1) There is initially no bilayer, the solution on both sides is 140 mM NaCl and the amplifier is zeroed. The bilayer is then formed and the internal solution is changed to 140 mM LiCl without disrupting the bilayer and the potential, V_E, measured.

(2) There is initially no bilayer, the internal solution is 140 mM LiCl and the external solution is 140 mM NaCl. The bilayer is then formed. The internal salt bridge should then be placed into the external solution and the amplifier zeroed. That salt bridge is then replaced into the internal solution and the potential, V_E, measured.

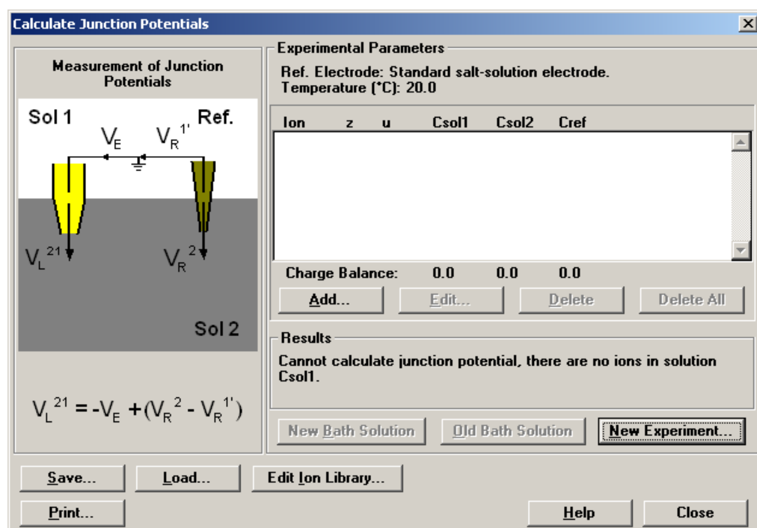
In both cases (1) and (2) (see Ref 5), the true bilayer potential difference, V_m, would be related to the measured potential, V_E, and the junction potential difference V_L^i - V_l^o by:

$$V_m = V_E + (V_L^i - V_L^o)$$

and, for the example above, where the salt bridge has the same composition as that of the external solution, V_L^o would be zero.

Special Characteristics - Measurement of Junction Potential Corrections

An alternative to calculating liquid junction potentials would seem to be to simply measure them. However, in practice, this is really not so simple, because it either involves corrections for other junction potentials in the circuit or for corrections for differences in electrode potentials. However, it is possible to use one of two alternative set-ups to minimize these corrections and with certain assumptions to endeavor to estimate and allow for the corrections. Using the Measurement of Junction Potentials option, the program endeavors to enable these corrections to be made and also calculates the junction potential that is being sought so that its magnitude and sign may be compared with the actual measured value. For the first alternative approach, a free-flowing high concentration (e.g., 2M or 3M) KCl electrode can be used as the reference electrode. An alternative



to a free-flowing high concentration (e.g., 3M) KCl electrode, would be to use a 3M KCl–agar (e.g. 4% agar) salt bridge in polythene tubing, **PROVIDED THE TIP OF THE SALT BRIDGE IN THE TEST SOLUTION IS CUT OFF BY ABOUT 5 mm AFTER EACH TIME THE TEST SOLUTION IS CHANGED TO ONE OF A NEW COMPOSITION,** to ensure that the tip of the salt bridge starts off in that new solution as 3M KCl (see Barry & Diamond, 1970, Ref. 2, for discussion of the problems of high concentration KCl agar salt bridges). The advantage of such a high concentration KCl electrode or salt bridge is that it will tend to minimize the difference between the reference electrode junction potentials, which will then tend to be relatively independent of the bathing solution. The first dialog window shows the basic set-up. It will be assumed that the solution in the main (left) electrode will be the same as in the first bathing solution (Sol 1, in dialog screen). This potential, V_R^1, will be balanced by offset, $V_R^{1'}$, in the recording circuit as indicated.

Moving to the next dialog screen simulates changing the bathing solution to the second solution (Sol 2). The reference junction potential will then change to V_R^2 as indicated. Thus the junction potential to be measured, V_L^{21}, (representing the potential of Solution 2 with respect to Solution 1; as shown in dialog screen), will be related to the measured value, V_E, by:

$$V_L^{21} = -V_E + (V_R^2 - V_R^{1'})$$

The ionic composition and properties of Solution 1, the reference electrode solution and Solution 2 can be entered as for the other junction potential measurements already discussed.

Completing data entry enables the calculation of the final junction potential correction ($V_R^2 - V_R^{1'}$) so that the measured value of V_E can be corrected. It also gives an estimate of the junction potential V_L^{21} for comparison with this corrected value.

The other alternative approach to using a 2M or 3M KCl reference electrode is to use an Ag/AgCl reference electrode directly in contact with the bathing solution. As will be discussed in the next section, which discusses the use of such reference electrodes in general electrophysiological measurements, the changes in the Ag/AgCl reference electrode potentials will be very dependent on the precise values of the Cl^- activity in the two solutions. However, they can be used for the measurement of junction potentials, particularly when the two Cl^- concentrations are very similar (see e.g., Ref 2). With care, In fact, a combination of methods using both types of reference electrodes can be used to validate the measured value of the required junction potential.

Ag/AgCl Reference Electrodes

It is generally inadvisable to use Ag/AgCl reference electrodes directly in contact with the bathing solution, because of the great sensitivity of their electrode potentials to the precise value of the Cl- activity in each solution. Nevertheless, since many research workers do use such a reference electrode system, it was considered of value to incorporate an appropriate option to allow for its use (in all but the epithelial and bilayer measurements), with the warning that it should be used with particular care.

As already noted, PROVIDED THERE IS NO SOLUTION CHANGE during either patch-clamp or intracellular measurements, the use of a good Ag/AgCl electrode (that does not get damaged during the experiment) will result in a constant electrode potential offset that will be balanced off in the measuring circuit. Hence, the use of such an electrode ideally should not affect the junction potential correction in such a situation.

To incorporate the direct Ag/AgCl option, the choice to change temperature/reference electrode should be taken at the beginning of the new experiment option. Immediately, the electrode display will change to that of an Ag/AgCl electrode. When there is a bathing solution change during patch-clamp or intracellular measurements, the Cl^- activity of the bathing solution will be monitored and the change in the Ag/AgCl electrode potential calculated.

Hence, for example for an intact patch, the membrane potential, V_m, will be corrected by the junction potential offset, V_L', and the electrode potential difference V_{Ag}^{21}. This represents the electrode potential for solution 2 (V_{Ag}^{2}) - the electrode potential for solution 1 (V_{Ag}^{1}), where each electrode potential is now measured as the potential of the electrode with respect to the solution (note arrow direction in adjacent diagram).

$$V_m = (V_c - Vp) + (V_L' - V_{Ag}^{21})$$

In this example, Cl^- activities were used rather than Cl^- concentrations. Concentrations are not acceptable for accurate electrode potential corrections. Instead, the proper mean Cl^- activity (see discussion in Refs 2, 3 and 14), taking into account the effects of all the ions in the solution on its activity coefficient. Also, if one of the bathing solutions does not contain any Cl^- ions, a warning message will be flashed up on the screen and an Ag/AgCl correction value will not be displayed. It is because of the difficulty of correctly assessing the precise value of the Cl^- activity and the fact that the Ag/AgCl electrode can readily be damaged, and not be acting as an ideal electrode, that it is strongly recommended that such electrodes should not normally be used in direct contact with bathing solutions, and especially in those situations where the solution composition is being changed.

Concentrations or Activities?

Although strictly, ionic activities rather than ionic concentrations should be used in the generalised Henderson equation, in biological situations such as bi-ionic situations where the total ionic strength is similar in each solution it is acceptable to use concentrations. The Junction Potential Calculator assumes that data are entered as concentrations, but they can as well be entered as activities. However, in situations where one is dealing with **electrolyte solutions at very different overall ionic strengths**, **it is strongly advisable to use activities** to get the most accurate results (see e.g., Ref. 14 for an example, where the difference can be very significant). What also needs to be appreciated, is that in mixtures of different ions the individual ionic activity coefficient depends not just on the concentration of that ion but is rather determined by all the ions present and therefore depends more on the overall ionic strength of the solution. A more detailed discussion of this is given in Refs. 2 and 3. Fortunately, in most biological situations the ionic strength of each solution is approximately the same. In these cases, concentrations, rather than ionic activities, can therefore be used for those solutions with minimal loss of accuracy. However, it should be stressed that where there are radical differences in the overall ionic concentrations of different solution, activity values should really be used for the most accurate junction potential values.

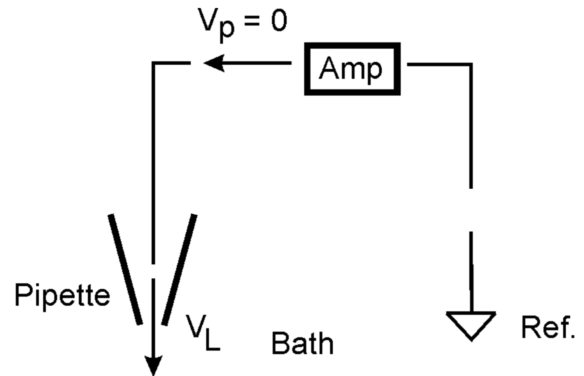
In cases where the user really wants to use activities, they can be readily calculated by multiplying the concentration by the mean activity coefficient for the salt (provided that the latter can be found). Care needs to be exercised by the user when handling activity coefficients. For further discussion of them see Refs (particularly 2, 3 together with 8, 13) and for their values see Refs. (6, 8, 13 and elsewhere in Handbook of Chemistry and Physics, see 16 for reference). Once the activities have been estimated, their values can then simply be entered in place of concentrations.

In addition, for corrections following bathing solution changes, when Ag/AgCl reference electrodes are used, for most purposes Cl^- concentrations will probably be adequate. However, for particularly accurate values of reference potential differences, mean Cl^- activities should rather be used. Again, care should be exercised and the user is referred to the discussion in Refs (2, particularly 3, 8, 13).

Application of Junction Potential Corrections BEFORE an Experiment

To understand how such corrections should be applied, it is helpful to consider the application of liquid junction potential corrections both **after** and **before** an experiment.

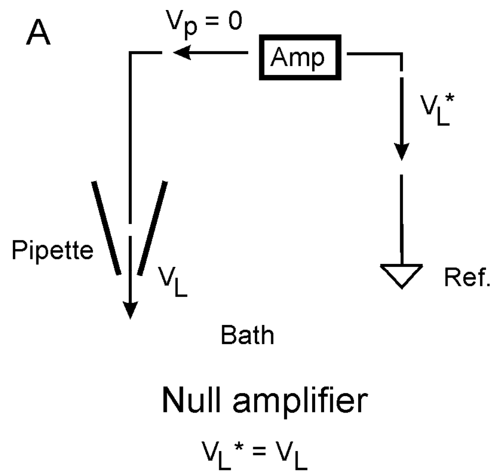
Although we prefer to recommend applying liquid junction potential corrections after an experiment, another option that some people prefer is to do it, *a priori*, or at the beginning of the experiment. This is acceptable **provided there are no solution changes during the experiment** and the correction is applied correctly. The way this is done can be a bit confusing. The following diagrams should clarify how such junction potential corrections need to be made. For comparison, the next figure shows the normal way in which corrections are made **after** an experiment and then how they should be made **before** one.

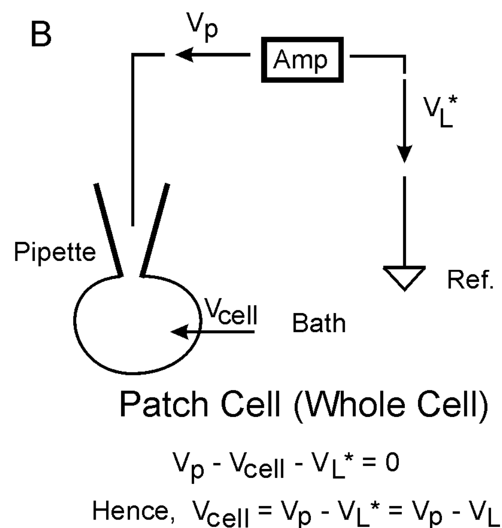


The adjacent diagram shows the initial situation for a patch clamp amplifier and pipette prior to patching on to a cell. In the examples given below all of the other offsets in the circuit, including the liquid junction potential at the reference electrode, which will not change (***assuming no change in the bathing solution***) will be ignored.

Liquid Junction Potential Corrections Applied AFTER an Experiment

The next pair of diagrams show the situation, which we normally recommend, in which the corrections are made **AFTER** the experiment.





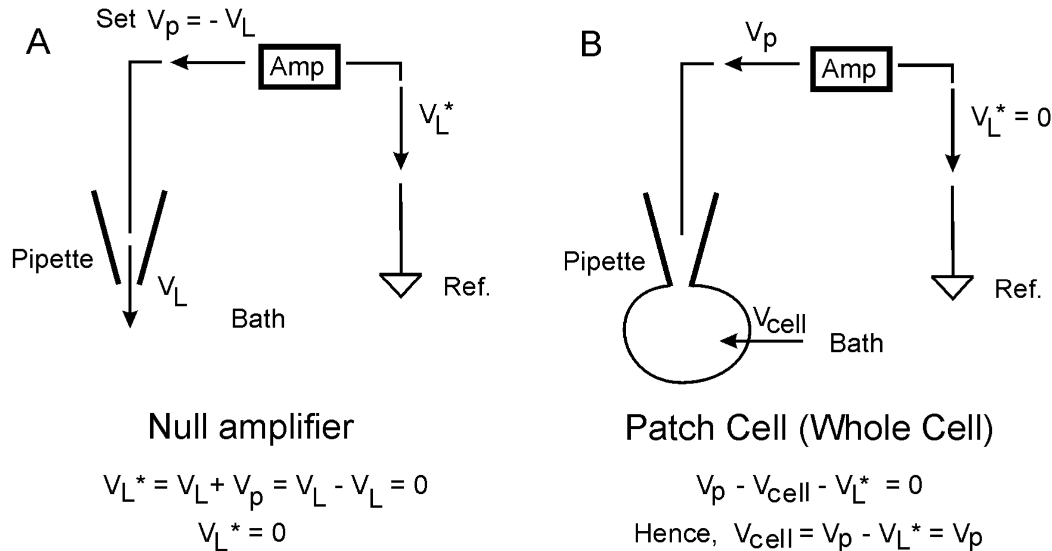
For the example above, in which there is a whole cell configuration, the potential of the cell, V_{cell} or V_m, is as indicated, related to the potential, V_p and liquid junction potential V_L by

$$V_{cell} = V_p - V_L \; .$$

Liquid Junction Potential Corrections Applied BEFORE an Experiment

The next two diagrams show the situation, in which the corrections are made *a priori*, or BEFORE, an experiment.

N.B. **This method should never be used if the bath solution is going to be changed during the experiment**.



In this situation, before the amplifier is zeroed, the calculated (or measured) value of the liquid junction potential, defined to be V_L (bath with respect to pipette) is used to apply a command potential of - V_L, as in Panel A. Then the amplifier is nulled to balance all other sources of potential offsets. It should be noted however, that V_L is no longer balanced with an equal and opposite (non-zero) V_L*. In fact, as indicated, there is now no need for a V_L* offset. That is, V_L* = 0, as indicated in Panel B.
Again, for the example in which there is a whole cell configuration , as shown in Panel B, the potential of the cell, V_m or V_{cell}, will be, as indicated, related to the command potential, V_p simply by:

$$V_{cell} = V_p .$$

N.B. For specific information on the application of such *a priori* corrections to Axon Instruments pClamp software, see the article in *AxoBits* 39, but make sure that this is the *corrected* version of the article available from the website indicated below.

N.B. It should be noted that in the original printed article on Liquid Junction Potential Corrections in AxoBits 39, there was a sign error in our article for some of the examples of the correction BEFORE an experiment. These are now corrected in the downloadable pdf version of the article *(**http://web.med.unsw.edu.au/phbsoft/LJP_article_%20AxoBits%2039.pdf**),* ***downloaded from the PHBSoft website****. To be sure that you don't have a previously cached version, the first line from the bottom of Column 2, P. 8 should be "... holding command of -15.6 mV..".*

References and Further Reading

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Credits

The junction potential calculations and dialog of the JPCalc version for Windows were written by Axon Instruments but based, with permission and consultation, on the DOS program JPCalc by Dr. Peter H. Barry of The University of New South Wales, Sydney 2052, Australia (see Ref 5 in References and Further Reading).

An Appendix follows:

APPENDIX

Please Note: **In the original printed article on Liquid Junction Potential Corrections in AxoBits 39, there was a sign error in our article for some of the examples of the correction BEFORE an experiment. These are now corrected in the downloadable pdf version of the article available on via the PHBSoft website: *http://web.med.unsw.edu.au/PHBSoft/* . Also, see P. 22 of this manual or click on above link (*Application .....*) to see the principles involved.**

IONIC MOBILITY TABLES

N.B. The ionic mobility tables on the website, http://web.med.unsw.edu.au/PHBSoft/ went through a major update in October 2003. There have also been some additional comments added in August 2005.

LISTING OF SUPPLIED IONIC MOBILITIES WITH FULL ION NAMES FOR THE PROGRAM JPCalc/JPCalcW

The following table of relative (generalised) mobility values (relative to K^+; see Appendix below for more information and relationship to limiting equivalent conductivities) was extracted from Table 1 of Barry & Lynch[1], with a slightly amended value for choline following later direct measurements (Ng & Barry[4]). A supplementary list of other ionic mobilities is given in Table 2.

N.B. It should be noted that **only the free ionic concentration (or activity) values** should be used in the program to calculate liquid junction potentials. This is especially important for ions which are not fully ionised in the solution. For example, this may be because they are derived from weak acids like HEPES or are polyvalent chelating agents like EGTA. For example, if titrating 10 mM HEPES with NaOH requires about 5 mM NaOH to bring the pH to about 7.4, then the free $HEPES^-$ concentration will be about 5 mM along with an additional 5 mM of Na^+ (plus any other Na^+ contributions), so it would be these values which should be entered into the program. Similarly, if 10 mM EGTA and 2 mM CaCl2 are added to the solution, then contributions from $EGTA^{2-}$ and Ca^{2+} will be close to about 8 mM and 0 mM at pH 7.4, since virtually all the Ca^{2+} will be chelated by the EGTA, and the free EGTA will mostly be in the $^{2-}$ form. Of course, non-electrolytes do not directly contribute to the liquid junction potential and can generally be ignored.

Note that a number of values in the tables of Lange (2) and CRC (7) have been updated in their most recent editions, currently listed in the references. Where these differ from the values previously listed and incorporated in JPCalc, the new updated values are now listed in Table 1 following in blue and italics. These differences are invariably small.

Tables 1-4 follow.

TABLE 1. These ions are currently included, with their original (not-updated) values, in the JPCalc/JPCalcW and Junction Potential Calculator (in Axon's pClamp) programs

Symbolic Ion Name	Full Ion Name/Formula	Valency	Relative Mobility	Updated value	Ref. for new value
Chol	Choline	1	0.51		
Cs	Cesium	1	1.050		
K	Potassium	1	1.000		
Li	Lithium	1	0.525	0.526	2,7
NH4	Ammonium	1	1.000	1.001	2,7 (avr)
Na	Sodium	1	0.682		
Rb	Rubidium	1	1.059		
TEA	TetraethylAmmonium	1	0.444		
TMA	TetramethylAmmonium	1	0.611		
Acet	Acetate	-1	0.556		
Benz	Benzoate	-1	0.441		
Br	Bromide	-1	1.063		
Cl	Chloride	-1	1.0388	1.0382	2,7
ClO4	Perchlorate	-1	0.916		
F	Fluoride	-1	0.753		
H2PO	H_2PO_4	-1	0.450		
HCO3	HCO_3	-1	0.605		
I	Iodide	-1	1.0450	1.0456	2,7 (avr)
NO3	Nitrate	-1	0.972		
Picr	Picrate	-1	0.411		
Prop	Propionate	-1	0.487		
SCN	Thiocyanate	-1	0.900	0.901	2,7 (avr)
Sulf	Sulfonate	-1	~~0.586~~	now deleted	2
Ba	Barium	2	0.433	0.434	2,7 (avr)
Ca	Calcium	2	0.4048		
Co	Cobalt	2	0.370	0.367	2,7 (avr)
Mg	Magnesium	2	0.361		
Sr	Strontium	2	0.404		
Zn	Zinc	2	0.359		
HPO4	HPO_4	-2	0.390		
SO4	Sulphate	-2	0.544		

For values of some other ions, see Table 1 of Barry & Lynch[1] and Tables 2-4 following and Refs. 2, 6 and 7.

TABLE 2:

SUPPLEMENTARY LISTING OF MOBILITIES WITH FULL ION NAMES FOR THE PROGRAM JPCalc/JPCalcW

The following table of relative mobility values was extracted from Ng and Barry[4] and Keramidas et al.[3].

Symbolic Ion Name	Full Ion Name/Formula	Valency	Relative mobility
NMDG	NMDG	+1	0.33
Tris	Tris	+1	0.40
Asp	Aspartate	-1	0.30
gluc	Gluconate	-1	0.33
Glu	Glutamate	-1	0.26
HEPE	HEPES	-1	0.30
ise	Isethionate	-1	0.52
MES	MES	-1	0.37
MOPS	MOPS	-1	0.35
EGT2	EGTA(2-)	-2	0.24
EGT3	EGTA(3-)	-3	0.25

where the following standard abbreviations apply: NMDG, *N*-methyl-D-glucamine; Tris, tris[hydroxymethyl]aminomethane; HEPES, *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]; MOPS, 3-[*N*-morpholino]propanesulfonic acid; MES, 2-[*N*-morpholino]ethanesulfonic acid. The estimated error in the measurements from Ng and Barry[4] was considered to be less than about 0.005. EGTA(2-) and EGTA(3-) are from Keramidas et al.[3]

TABLE 3. ADDITIONAL LISTING OF MOBILITIES WITH FULL ION NAMES FOR THE PROGRAM JPCalc/JPCalcW

The following table of relative mobility values was calculated from limiting equivalent conductivities in the references below.

Symbolic Ion Name	Full Ion Name/Formula	Valency	Relative mobility	Reference
Tl	Thallium	+1	1.02	7
Butr	Butyrate	-1	0.44	7
Citr	Citrate (3-)	-3	0.318	2
2MAEth	2-(Methyl-Amino) Ethanol (or N-Methylethanolamine)	+1	0.490 ± 0.018	8

An additional listing of ion mobilities added in October 2003 follows in Table 4

TABLE 4. FURTHER LISTING OF ION MOBILITIES ADDED IN OCTOBER 2003

Symbolic ion name	Full ion name / formula	Valency	Relative mobility	Ref
Ag	Silver	+1	0.842	2,7
	Diethylammonium	+1	0.57	2,7
	Dimethylammonium	+1	0.701, 0.705	2,7
	Ethyltrimethylammonium	+1	0.551	2,7
H	Hydrogen	+1	4.763, 4.757	2,7
	Piperidinium	+1	0.506	2,7
	Tetrabutylammonium	+1	0.265	2,7
	Tetrapropylammonium	+1	0.320, 0.318	2,7
	Triethylammonium	+1	0.467	7
	Trimethylammonium	+1	0.642, 0.643	2,7
	Bromoacetate	-1	0.533	2,7
	Bromobenzoate	-1	0.41	2,7
	Chloroacetate	-1	0.574, 0.541	2,7
CNO	Cyanate	-1	0.879	7
	Cyanoacetate	-1	0.590	2,7
	Dichloroacetate	-1	0.521	2,7
	Ethylsulfate	-1	0.539*	7
	Ethylsulfonate	-1	0.539*	2
	Fluoroacetate	-1	0.604	2,7
	Fluorobenzoate	-1	0.45	2,7
	Formate	-1	0.743	2,7
	Iodoacetate	-1	0.552	2,7
	Lactate	-1	0.528	2,7
	Methylsulfate	-1	0.664*	7
	Methylsulfonate (pseudonym = methanesulfonate)	-1	0.664*	2
OH	Hydroxide	-1	2.69	2,7
ReO4	Rhenate	-1	0.747	2,7
	Salicylate	-1	0.49	2,7
	Trichloroacetate	-1	0.498, 0.476	2,7
Cd	Cadmium	+2	0.37	2,7
Cu	Copper	+2	0.385, 0.365	2,7
Fe	Iron	+2	0.36, 0.37	2,7
Hg	mercury	+2	0.433	2,7
Mn	Manganese	+2	0.364	2,7
Ni	Nickel	+2	0.340, 0.337	2,7
Pb	Lead	+2	0.48	2,7

**Note that both methylsulfate (Ref. 7) and methylsulfonate (Ref. 2) had identical limiting equivalent conductances. The same was also true of the values for ethylsulfate (Ref. 7) and ethylsulfonate (Ref. 2). This may mean that, in each case, one of the values was incorrectly copied from the other and is wrong.*

Table 4 (cont. over)

Table 4 (Cont.)

Symbolic ion name	Full ion name / formula	Valency	Relative mobility	Ref
Sr	Strontium	+2	0.404	2,7
Zn	Zinc	+2	0.359	2,7
	Malate	-2	0.400	2,7
	Maleate	-2	0.421	7
	Oxalate	-2	0.504	2,7
	Succinate	-2	0.400	2,7
Gd	Gadolinium	+3	0.306, 0.305	2,7
Fe	Iron	+3	0.313, 0.308	2,7
La	Lanthanum	+3	0.316	2,7
Citr	Citrate	-3	0.318	2,7
ATP	Adenosine 5'-Triphosphate	-2, -3 or -4**	0.15***	9

***The relative proportions of each valency species depends on pH and the ionic composition of the solution.*

****u_{ATP}/u_K was calculated from the value of 3.0×10^{-6} $cm^2.s^{-1}$ for the diffusion coefficient of ATP in free solution (Ref. 9).*

REFERENCES FOR MOBILITY AND LIMITING EQUIVALENT CONDUCTIVITY DATA

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RELATIONSHIP BETWEEN GENERALISED RELATIVE IONIC MOBILITY AND LIMITING EQUIVALENT CONDUCTIVITY

First of all, it should be noted that the relative mobility of an ion, u, required for calculating liquid junction potentials (as listed in the above tables of mobilities and required in *JPCalc* calculations) represents the generalised mobility of an ion relative to K^+. For example, if u_X, is the relative mobility for ion X, with respect to K^+, it will be given by:

$$u_X = u^*_X / u^*_K$$

where u^*_X and u^*_K represent the absolute values of the generalised mobilities of ions X and K^+ respectively. The units of u are, of course, dimensionless.

The following discussion indicates how the generalised mobilities of ions are in turn related to their limiting equivalent conductivities.

Since the velocity of an ion in solution, v, is related to the generalised absolute mobility, u*, and the generalised force, F_x, acting on it, then:

$$v = u^* F_x$$

The force may be in Newtons/ mole or Newtons, depending on whether it is the force acting on a mole of ions or on a single ion (and whichever is chosen will affect the units of u*). The above generalised mobility is what is required for electrodiffusion flux equations, and would normally be that required for a force acting on a mole of ions.

In contrast, electrochemists, when measuring conductivity, use another definition of mobility, which may be defined as u', the electrical mobility, since they measure the mobility as the velocity/ electric field, E (e.g., in volts/m) as:

$$v = u' E$$

Since the actual force is zFE, we also have v = u*zFE, where z is the magnitude of the valency and F is the Faraday. Hence,

$$u^* = u'/zF$$

Because of the above equation and relationship between generalised mobility and limiting equivalent conductivity, Λ^0 (the conductivity of an electrolyte solution per equivalent, in the limit as the concentration goes to zero), the two will be related by:

$$u^* = \Lambda^0 / (zF^2)$$

Hence relative mobility of ion X of valency z is given by:

$$u_X = [\Lambda^0_X / z] / \Lambda^0_K$$

since, for K^+, z = 1.

For a monovalent ion Y, its relative mobility will simply be given by:

$$u_Y = \Lambda^0_Y / \Lambda^0_K$$

where Λ^0_Y is the limiting equivalent conductivity of Y at the same temperature as for Λ^0_K , normally 25 ^oC.

For reference, $\Lambda^0_K = 73.50$ $S.cm^2.equiv^{-1}$ at 25 ^oC (Robinson & Stokes, 1965).

ADDITIONAL MOBILITY VALUES AND UPDATES

See further values in Mobility Listing on Web Home Page at:

http://web.med.unsw.edu.au/PHBSoft

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