PROTOCOLS FOR DILUTION SERIES BASED ON GEOMETRICAL PROGRESSIONS

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The commonly used dilution series, 1-2, 1-4, 1-8, 1-16, ... is based on a geometrical progression. In comparison with other widely used dilution series, such as 1-10, 1-20, 1-30, 1-40, ... or 1-10, 9-100, 8-100, 7-100, ..., the geometrical progression has the advantages of:

(1) Ease of preparation, mixing one part of the previous dilution with one part of diluent.

(2) The error, that is introduced by the fact that the liminal value of the phenomenon studied lies between the last negative tube and the first positive tube, is constant throughout the dilution range.

The series, 1-2, 1-4, 1-8, 1-16, ... has the disadvantage of:

(1) Too few steps in the decade with a relatively coarse interval between steps.

(2) Awkwardness in the expression of concentrations, especially since the dilution series does not pass through the multiples of ten.

Some years ago, the author[1] published a protocol for a dilution series based on a geometrical progression of the reciprocals of the powers of the eighth root of ten, which yields eight equal logarithmic steps in the decade. It is prepared by mixing 2.9983 parts of the previous dilution with 1 part of water. The cumulative error, introduced by mixing 3 parts of the previous dilution with 1 part of water, is less than 0.1% in the decade, or much smaller than the presumptive error involved in the use of pipettes for making the dilutions. To avoid accumulation of error, it is desirable to make a preliminary series of dilutions of 1-10, 1-100, 1-1000, ... for the start of each decade. Concentrations can be conveniently expressed in a logarithmic notation

pD equals —log of the dilution

[1]Edwin H. Shaw, Jr., Science, 86, 478 (1937).

similar to the commonly used pH. A typical protocol is given in Table I.

TABLE I

PROTOCOL FOR A DILUTION SERIES BASED ON THE RECIPROCALS OF THE POWERS OF THE EIGHTH ROOT OF TEN

Dilution	Concentration grams per 100 ml.	pD	Preparation of Series: ml. of previous dilution	Preparation of Series: ml. of water
1-10	10.000	1.000	stock solution	
1-13.34	7.499	1.125	3	1
1-17.78	5.623	1.250	3	1
1-23.71	4.217	1.375	3	1
1-31.62	3.162	1.500	3	1
1-42.17	2.371	1.625	3	1
1-56.23	1.778	1.750	3	1
1-74.99	1.334	1.875	3	1
1-100	1.000	2.000	1 of 1-10 stock solution	9
1-133.4	0.7499	2.125	3	1
1-177.8	0.5623	2.250	3	1
	and continuing			

Assuming that the actual liminal value of the phenomenon studied lies midway between the last negative tube and the first positive tube, the error of the result is constant over the entire dilution range at 14.3% of this mid-value. In the case of the commonly used dilution series, 1-10, 1-20, 1-30, 1-40, ..., this error varies from 5.26% to 33.33% and averages 12.59%. Should it seem desirable to have the value of pD refer to this probable liminal mid-value, the series can be started with a stock solution that is 1.143-10 instead of 1-10.

Similar protocols, based on the geometrical progression of the reciprocals of the powers of the other roots of ten, may be developed for the production of coarser or finer series of dilutions. In the general case, the series

$$a/x^{0/n},\ a/x^{1/n},\ a/x^{2/n},\ a/x^{3/n},\ \ldots .$$

with the constant ratio, $1/x^{1/n}$, between terms is made by adding p ml. of the previous dilution to q ml. of water, where

$$p = q/(x^{1/n} - 1)$$

The error, due to the fact that the liminal value of the phenomenon studied lies between the last negative tube and the first positive tube, when expressed as percent of the midvalue, equals

$$100\ (x^{1/n} - 1)/(x^{1/n} + 1)$$

For the mid-values of the tubes in the series to be

$$1/x^{0/n},\ 1/x^{1/n},\ 1/x^{2/n},\ 1/x^{3/n},\ \ldots .$$

$$a = zx^{1/n}/(x^{1/n} + 1)$$

When pD is defined as —log Dilution, the pD increment between tubes is (log x) /n).

The data for the preparation of series based on the reciprocals of the powers of the roots of ten are given in Table II.

TABLE II

DATA FOR THE PREPARATION OF GEOMETRICAL PROGRESSION PROTOCOLS

Constant Ratio	ml. of previous dilution per 5 ml. water	Error per cent of midconcentration	Adjusting factor to refer results to midconcentration	Increment of pD
$1/10$	0.555	81.82	1.8182	1
$1/10^{1/2}$	2.31	51.95	1.5195	0.5
$1/10^{1/3}$	4.33	36.60	1.3660	0.333
$1/10^{1/4}$	6.42	28.01	1.2801	0.25
$1/10^{1/5}$	8.55	22.63	1.2263	0.20
$1/10^{1/6}$	10.69	18.96	1.1896	0.1667
$1/10^{1/7}$	12.84	16.30	1.1630	0.1429
$1/10^{1/8}$	14.99	14.29	1.1429	0.125
$1/10^{1/9}$	17.15	12.72	1.1272	0.111
$1/10^{1/10}$	19.31	11.46	1.1146	0.10

It is to be expected that these geometrical dilution series will find increasing use, especially in biological work such as toxicity studies, serological work, etc., where logarithmic plotting is customary. The use of these series yields data which are evenly spaced in a log plot. The series based on the eighth root of ten has recently been applied to the preparation of known samples in an analytical study[2]. In work of this sort, the series has the advantage that the concentrations of the several known samples are not simple multiples and there is less probability of the personal equation influencing the results ni colorimetric work. The author has found the series based on the eighth root of ten very useful in toxicity studies on Paramecium caudatum[3].

With increasing use, the slight disadvantage of non-integral values for the dilutions are more than balanced by the ease of preparation of the series and the constancy of error throughout the dilution range.

[2] P. A. Hansen and V. Nielson, J. Biol. Chem., 131, 309 (1939).
[3] E. H. Shaw, Jr. and L. J. Geppert, Biodynamica, No. 28, 1-11 (1937).
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Leo J. Geppert and Edwin H. Shaw, Jr., Proc. So. Dak. Acad. Sci., 17, 34-38 (1937).