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EVALUATION OF THE SUB-SUPEREQUIVALENCE METHOD
OF ISOTOPE DILUTION ANALYSIS USING
ZINC-DITHIZONE AS A TEST CASE.

THE UNIVERSITY OF OKLAHOMA, PH.D., 1978

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GRADUATE COLLEGE

EVALUATION OF THE SUB-SUPEREQUIVALENCE METHOD OF ISOTOPE DILUTION ANALYSIS USING ZINC-DITHIZONE AS A TEST CASE

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

BY

DARREL RAY CORNELL

Norman, Oklahoma

1978

EVALUATION OF THE SUB-SUPEREQUIVALENCE

METHOD OF ISOTOPE DILUTION ANALYSIS

USING ZINC DITHIZONE AS

A TEST CASE

APPROVED BY

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"There is something fascinating about science. One gets such wholesale returns of conjecture out of such a trifling investment of fact." Mark Twain

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EVALUATION OF THE SUB-SUPEREQUIVALENCE METHOD OF ISOTOPE DILUTION ANALYSIS USING ZINC-DITHIZONE AS A TEST CASE

CHAPTER I

INTRODUCTORY SUMMARY

This paper explored the feasibility, validity, reproducibility and sensitivity of the sub-superequivalence modification of isotope dilution analysis. The classical isotope dilution analysis and its substoichiometric modification have proven to be effective techniques in determining trace quantities of metals in water. Unfortunately, these methods require, respectively, that either the specific activity be known, or that precisely equal quantities of material be separated from the sample and a standard.

Recently, a sub-superequivalence method has been developed. Preliminary work suggested that it might be useful in the study of trace quantities of metals. Zinc was chosen as a test case because it has been investigated using the isotope dilution and substoichiometric methods. Zinc forms a complex with dithizone which is easily extracted with carbon tetrachloride.

The sample to be analyzed was labeled with the appropriate isotope and the solution divided into two series. Experimental conditions were then broken down into four possible cases, depending upon whether the dithizone was either greater than or less than the zinc in the two series.

When the dithizone is substoichiometric in terms of zinc(Cases 2A and 2B), the method proved effective in determining the zinc concentration of a solution.

Results were also encouraging for superstoichiometric quantities of dithizone(Case 1B), provided that such quantities did not become excessive. The results also indicated that primary dithizonates are present when the ratio of zinc to dithizone is high but that with large amounts of dithizone, secondary dithizonates predominate.

A precision and reproducibility study was performed at 10, 1 and 0.1 μg/ml zinc. Accuracy and precision were within 10% at all three concentrations. Extraction became difficult, however, at the 0.1 μg/ml zinc concentration because the extracting dithizone solutions were very dilute and quickly reacted with extraneous metals. For this reason, the solutions had be prepared using pretreated solvents and used as rapidly as possible.

The effects of pH were also investigated. The results showed that the sub-superequivalence method is effective within the pH range of 6-9.

Another study investigated the extraction of zinc from solutions containing up to a 200-fold excess mixture of cadmium, cobalt and nickel. By using the masking agent, diethanoldithiocarbamate, acceptable results were again obtained.

In addition, a comparison was made between the analysis of a solution using the sub-superequivalence method and an atomic absorbance unit. Good agreement was obtained.

In summary, the sub-superequivalence method appears to give accurate results down to 0.1 μg/ml, even in the presence of interfering metals.

CHAPTER II

BACKGROUND AND HISTORICAL REVIEW

Nuclear analytical methods have been developed,[1] but only a few applications have been directed toward the analysis of natural waters. The classical isotope dilution analysis[2] and its substoichiometric modification[3] have proven effective and simple in determing trace quantities of metals in water. These two methods are based upon the conservation of radioactivity, and a comparison of the specific activity of a radiotracer both before and after mixing with the non-radioactive compound to be determined.

In the classical case (Figure 1), two aliquots are taken of a radioactively tagged solution of a known concentration. Each aliquot contains mass, M_y, and activity, A_y. A known volume of the unknown containing mass, M_u, is added to one aliquot. Both aliquots are then reacted, separated, and counted.

By rearranging the classical isotope dilution equation:

$$A_y = S_y M_y = S_x(M_y + M_u) = \frac{R_x M_y}{E_x M_x} + \frac{R_x M_u}{E_x M_x} \qquad 1$$

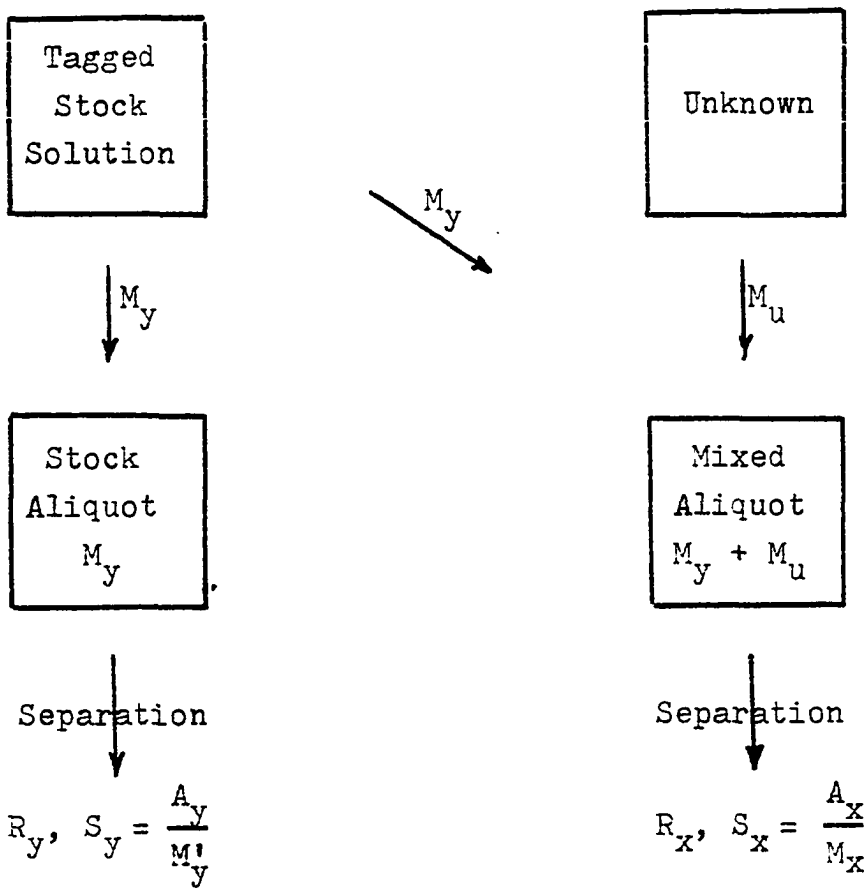


Figure 1. Simple isotope dilution technique

where

A-activity(R/E)
E-efficiency of detection
M-mass
R-counting rate
S-specific activity(R/EM)

and the added subscripts mean

u-unknown sample
x-mixed aliquot
y-tagged stock solution

and converting specific activities into counting rates gives:

$$M_u = M_y \left(\frac{R_y M_x}{R_x M'_y} - 1 \right) \qquad 2$$

Equation 2 assumes that experimental conditions can be used in which $E_y = E_x$. Since R_x/M_x is independent of mass, this method requires only the separation of any pure portion from the original sample. Quantitative separation is thus unnecessary. Additionally, this classical method is highly precise, selective and sensitive. It also is widely applicable, having proven valuable in the analysis of minerals, fatty acids, amino acids and sterols.

This classical method, unfortunately, suffers from limitations which include: the unavailability of labeled compounds, the requirement that the tracers must be radiochemically pure, the added tracer must equilibrate with the sample, and the isolated material must be chemically pure. It also requires a weighing process to determine specific activities.

Determining the weight of the separated species may be eliminated if a substoichiometric modification is used.

By chosing substoichiometric quantities of the separating reagent (SR), equal amounts of material are removed for counting from both the standard and the diluted samples (i.e. $M'_y = M_x$). The classical method is simplified to

$$M_u = M_y \left(\frac{R_y}{R_x} - 1 \right) \qquad 3$$

Some of the methods used to isolate equal quantities of material include:

a) Chelate extraction
b) Electrolysis
c) Ion exchange
d) Precipitation
e) Sorption.

An important advantage of the substoichiometric method is that it allows great selectivity. Interfering species must now compete with the species of interest for limited quantities of the separating reagent. Selectivity may further be enhanced if a masking agent is used, since it helps remove any interfering species.

A major drawback of this modification is that separating equal quantities of material may be impossible, especially if the sample and the standard come from widely different environments (pH, concentrations, or metal interferences). To avoid this difficulty, the sub-superequivalence modification was developed.

Sub-superequivalence Modification

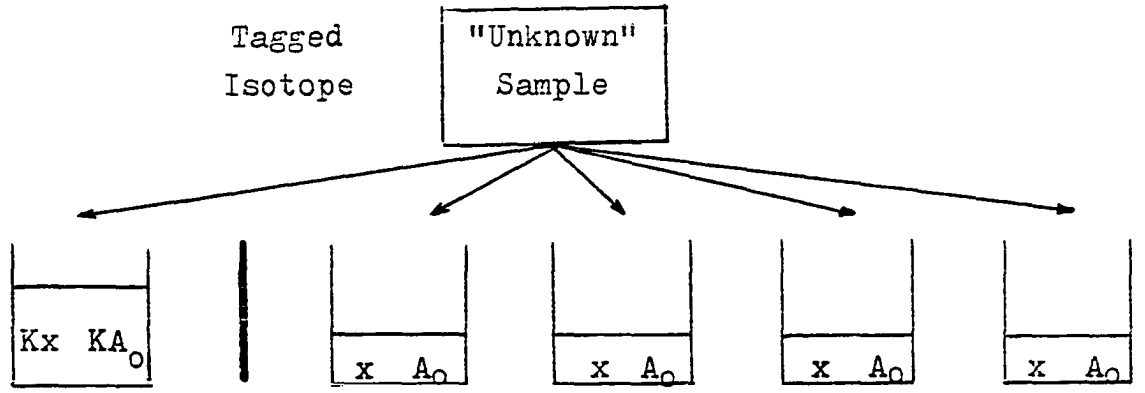
The sub-superequivalence (sub-super-stoichiometric)

technique was proposed by Klas, Tölgyessy and Klehr[4]. The basic concept, along with application-possibilities, were discussed by these three individuals and Lesny[5]. The sample to be analyzed is labeled with an isotope of the element of interest contained in the sample (Figure 2). This solution is then divided into two series, each of whose members consist of duplicate aliquots. Series 1 aliquots have an identical volume, and therefore amount (x) and activity (A_o), of the unknown. Series 2 aliquots are identical to each other (replicates give greater statistical significance), but are K times greater than Series 1 (i.e. Kx and KA_o) in the amount and activity of the unknown. To Series 1, increasing incremental amounts of a known non-radioactive substance ($y_j = y_1, y_2, \ldots, y_n$) are added. Series 1 is thus isotopically diluted with non-radioactive y ($x + y_1$, $x + y_2$, $\ldots, x + y_n$). If all aliquots of both series are brought to the same volume, and if Series 1 consists of an infinite number of members, then one member of Series 1 will contain the same concentration (C_{1k}) of unknown as occurs in Series 2 (C_2):

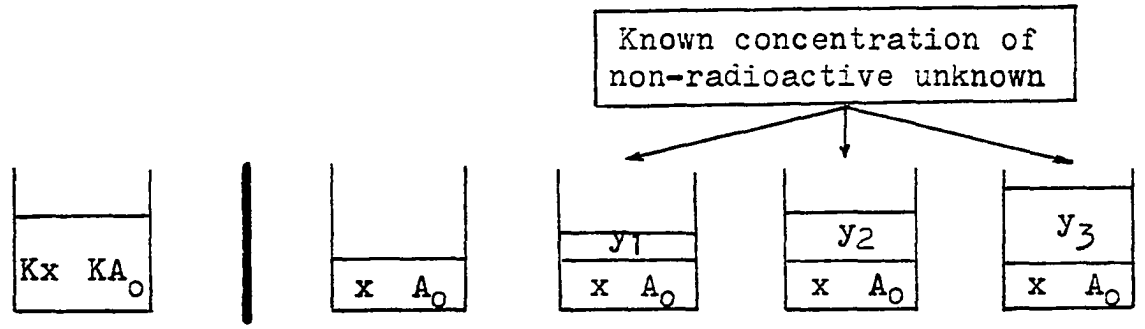
$$C_{1k} = C_2 \qquad 4$$

$$\frac{x + y_k}{V} = \frac{Kx}{V} \; . \qquad 5$$

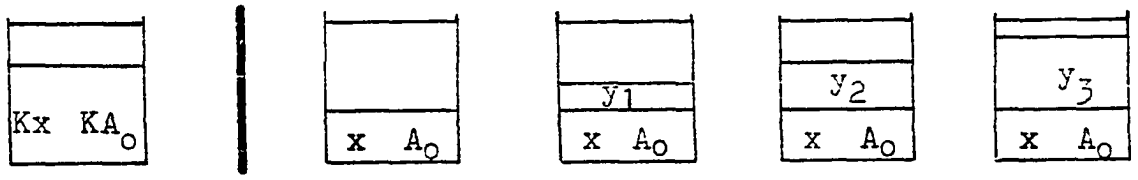
If to all the aliquots we add the same quantity of reacting reagent, the product formed (M_{1k}) in Series 1 for



Step 1- Preparation of two series



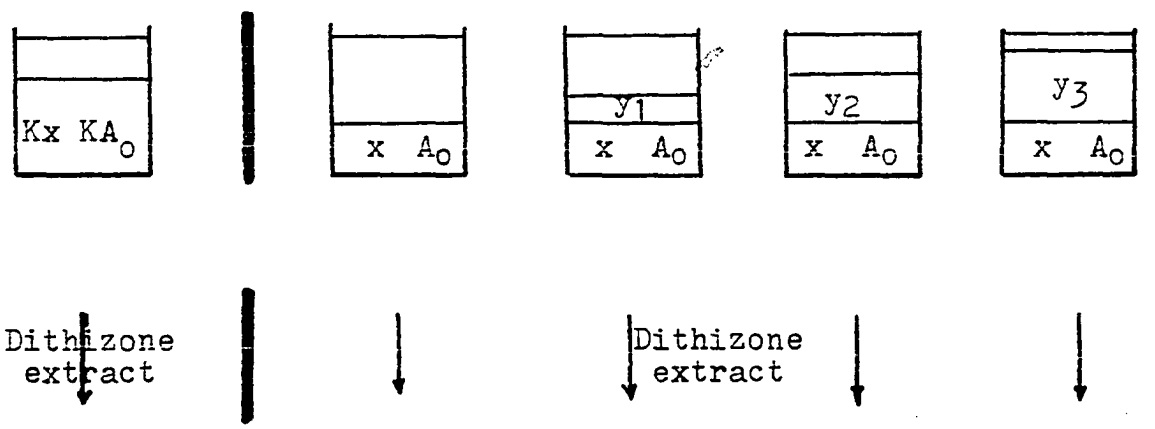
Step 2- Addition of incremental non-radioactive sample



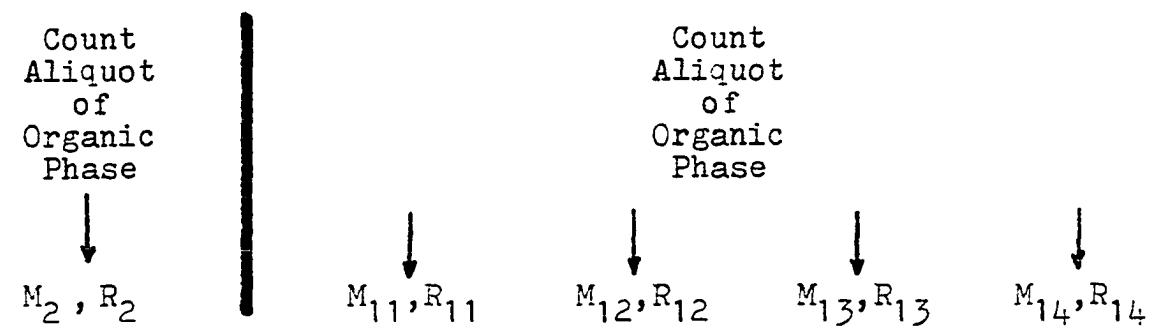
Step 3- Dilution to same volume with deionized water preceded by masking agent addition

SERIES 2 SERIES 1

Figure 2. Sub=superequivalence Modification Technique



Step 4- Extraction with dithizone



Step 5- Counting of organic phase

SERIES 2 SERIES 1

Figure 2(cont). Sub-superequivalence Modification Technique

C_{1k} should have activity A_{1k} and be equal in quantity to the product formed (M_2) in Series 2 having activity A_2. Note that within Series 2, the quantity of product (M_2) and activity (A_2) separated are constant. Isotopically diluted Series 1, however, yields varying quantities of mass (M_{1j}) and activities (A_{1j}).

Writing our conservation of radioactivity:

$$(x + y_j)\frac{A_{1j}}{M_{1j}} = \frac{KxA_2}{KM_2} \qquad 6$$

and defining I_j, we obtain:

$$I_j \equiv \frac{A_2}{A_{1j}} = \frac{M_2(x + y_j)}{M_{1j}x} \qquad 7$$

If counting efficiences are equal (i.e. $E_2 = E_1$), we may substitute counting rates for activities and:

$$I_j = \frac{R_2}{R_{1j}} = \frac{M_2}{M_{1j}}\left(1 + \frac{y_j}{x}\right) \qquad 8$$

Note that I_j is nothing more than the ratio of the activity of Series 2 samples to the activity of Series 1 samples. More importantly, I_j becomes a function of y; all possible curves intersect at $I_k = K$; $y = y_k$ (when $M_{1k} = M_2$). Substituting into Equation 8:

$$K = 1 + \frac{y_k}{x} \qquad 9$$

$$x = \frac{y_k}{K - 1} \; . \qquad 10$$

This equation allows the determination of the unknown x by

finding the intersection of the horizontal line $I_j = K$ with $I_j = f(y)$ and using its projection to the y-axis to evaluate y_k.

Depending upon whether the separating reagent is greater than, equal to , or less than the species of interest in Series 1 and 2, we observe four distinct cases as summarized in Table 1. These cases are plotted in Figure 3.

In the case where $Kx < SR < x + y$, knowing the intercept (y=0) allows the calculation of x:

$$x = \frac{\text{intercept(SR)}}{K} \qquad \text{(Case 1B)} \qquad 11$$

If SR is substoichiometric for both Series 1 and 2 ($SR < x + y$ and $SR < Kx$), the slope of the curve allows us to calculate x:

$$x = \frac{1}{\text{slope}} \; . \qquad \text{(Case 2B)} \qquad 12$$

According to theory, the intercept should equal one.

If SR is substoichiometric for Series 2, but superstoichiometric for Series 1 ($x + y < SR < Kx$), then:

$$x = \frac{SR}{\text{intercept}} \; . \qquad \text{(Case 2A)} \qquad 13$$

Unfortunately, no information can be obtained for those situations in which SR is superstoichiometric in terms of both Series 1 and 2. That is:

x can not be calculated. (Case 1A)

For a more complete discussion of the theoretical

Table 1

Various Case Conditions for
Sub-superequivalence Method

Case	Kx	$x + y_j$	M_{1j}	M_2	I_j	dI/dy	INTERCEPT
1A	$SR \geqslant Kx$	$SR \geqslant x + y_j$	$x + y_j$	Kx	K	0	K
1B	$SR > Kx$	$SR < x + y_j$	SR	Kx	$\frac{K}{SR}(x + y_j)$	K/SR	Kx/SR
2A	$SR < Kx$	$SR \geqslant x + y_j$	$x + y_j$	SR	SR/x	0	SR/x
2B	$SR \leqslant Kx$	$SR < x + y_j$	SR	SR	$1 + \frac{y_j}{x}$	1/x	1

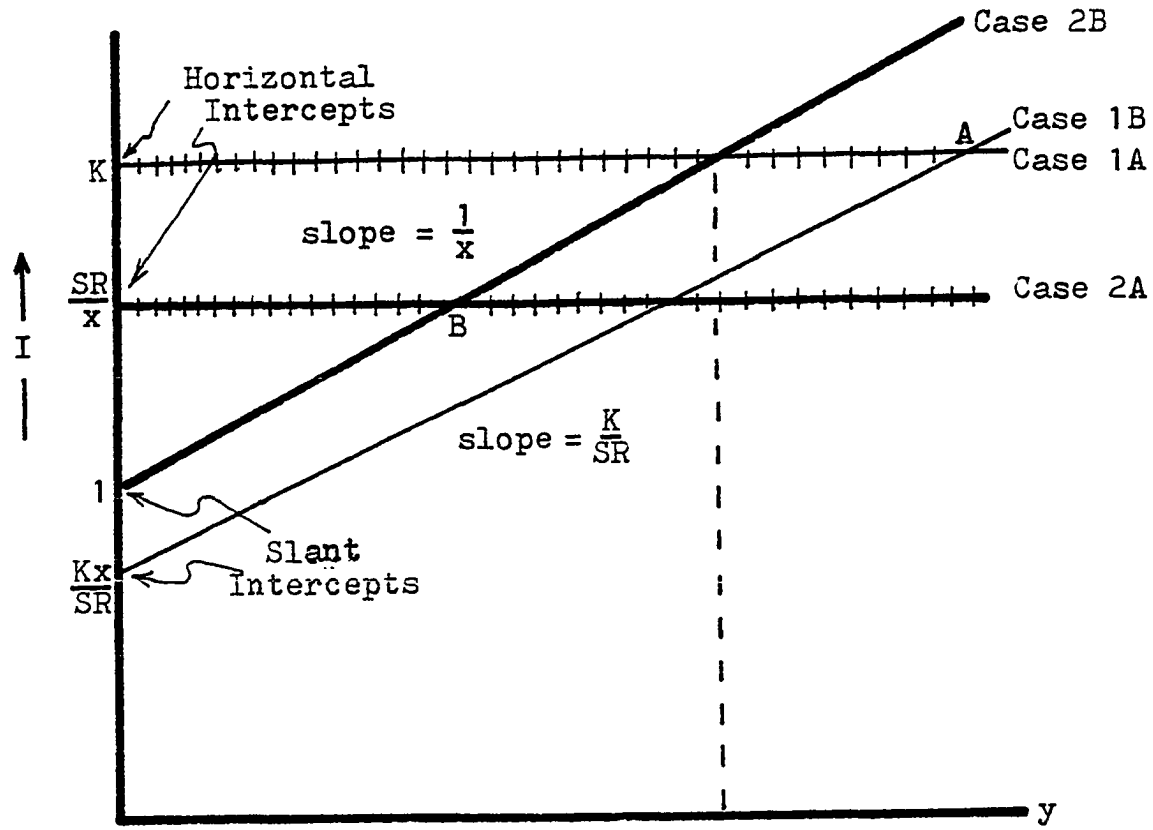


Figure 3. Four Cases of the Sub-superequivalence Approach

implications, see the paper by Klas,et.al.[5]

Prior to the start of this work, three applications of this modification were successfully demonstrated, the first reported in 1974. Tölgyessy[6],et.al., detected 1mg/l cobalt in aqueous samples. Their Series 1 consisted of six beakers, each containing 20ml of ^{60}Co tagged unknown, 1ml of 5% urotropine, 3ml of $10^{-4}M$ EDTA(separating reagent) and 1ml increasing increments of 5µg/ml inactive cobalt. Series 2 consisted of three beakers, each containing 40ml(K=2) of ^{60}Co tagged unknown, 1ml of 5% urotropine and 3ml of $10^{-4}M$ EDTA. Deionized water was added to bring all beakers to the same volume. Each solution was allowed to equilibrate ten minutes before passing through a Dowex 50 x 8 cation exchange column. The exchange column separated the Co^{2+} from complexed cobalt. Washing with water yielded 50ml eluate of which 5.0ml were counted. A typical plot is shown in Figure 4. Their results(summarized in Table 2) confirm the modifications's applicability.

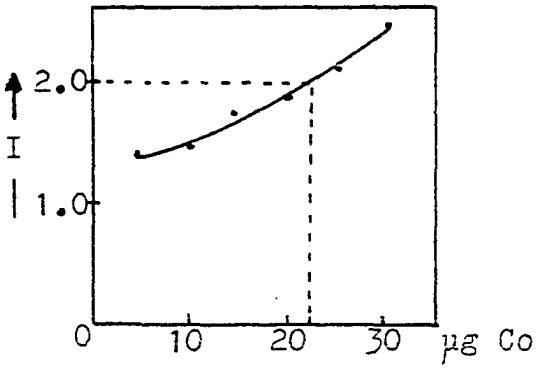


Figure 4. Sub-superequivalence Determination of Cobalt[6].

Table 2

Reproducibility of the Sub-superequivalence Determination of Cobalt[6]

Determination	Concentration(µg/ml)	
	Known	Found
1	1.05	1.17
2		1.12
3		1.05
4		1.02
5		1.10
6		1.07

Average = 1.088
Standard deviation = 0.0534
Standard deviation of average = 0.0218

Table 3

Reproducibility of Cyanocobalamine Determination[7]

Determination	Concentration(µg/ml)	
	Known	Found
1	1.00	0.853
2		0.691
3		0.760
4		0.806
5		1.037
6		0.691

Average = 0.806
Standard deviation = 0.1297
Standard deviation of average = 0.0529

Since cobalt is a component of vitamin B_{12}, the same authors[7] determined B_{12} (cyanocobalamine) by employing an adaptation of the above method. Both the vitamin and the standard were ozonized for sixty minutes. Cobalt was then determined, as before, by diluting with increasing amounts of inactive ozonized cobalt. To all samples, 40 µg of 10^{-3}M EDTA was added. This was followed by passage through the cation column, Table 3 gives the reproducibility they obtained. The authors concluded that the errors are due to incomplete ozonization or adsorption losses during the ozonization.

In one other experiment, Lesny[8], et.al, used sdoium diethydithiocarbamate as the reagent to complex with selenium. Equal 1.0 ml aliquots of Se^{4+} solution (labeled with ^{75}Se) were placed in beakers and incrementally increasing amounts (1, 10,..., 60 µg) of 0.5 µg Se/10 µl inactive selenium were added to form Series 1. Two ml of the tagged solution comprised Series 2. An acetate buffer (pH = 3.6) was added to all the aliquots before they were brought to the same volume with water. To each aliquot, 100 µl of 10^{-3} sodium diethyldithiocarbamate was added, followed by 5.0 ml of CCl_4. All were agitated for two minutes. Although unstable in acid, the selenium-carbamate complex forms quickly, and the reproducibility data obtained from 2.5 ml of the CCl_4 phase is given in Table 4. Comparison of the results obtained polarographically and by

Table 4

Reproducibility of Selenium Determination Using the Sub-superequivalence Method[8]

Determination	Concentration(µg/ml)	
	Known	Found
1	1.94	1.97
2		2.05
3		1.96
4		1.71
5		2.03
6		1.80

Average = 1.92
Standard deviation = 0.1371
Standard deviation of average = 0.0613

Table 5

Determination of Varying Amounts of Selenium Using Sub-superequivalence Method[8]

Determination	Concentration(µg/ml)	
	Known	Found
1	2.49	2.43
2	3.02	3.05
3	3.48	3.35
4	4.01	4.12
5	20.00	20.70

the sub-superequivalence modification at different concentrations are given in Table 5.

Zinc Dithizone System

Given the above successes, the utility of the sub-superequivalence technique in water analysis appears promising. To evaluate this theory, and to study experimental conditions, a zinc dithizone system was studied. This system was chosen because it had been successfully used by Ruzicka and Stary[9] to detect zinc in the range 10^{-3} to 10^{-6} µg/l. Good results were obtained even with the presence of 300-fold excesses of copper, cobalt and nickel.

Dithizone(diphenylthiocarbazone, H_2Dz) is a dark violet powder that is nearly insoluble in water, acid or hydrocarbons. It is fairly soluble in CCl_4(2×10^{-3}M) and $CHCl_3$(7×10^{-2}M); yielding green solutions. In base and some polar solvents, it dissolves yielding a yellow solution. The partition coefficient is high(10^4 for CCl_4 and 2×10^5 for $CHCl_3$).[12]

This partition coefficient, p, is the ratio of the concentration of dithizone present in the organic phase to its concentration in the aqueous phase. As a general rule, p is approximately equal to the ratio of the solubilities in the organic phase to the aqueous phase.

Closely related is q, the ratio of the distribution of the metal concentration between the organic and the

aqueous phase:

$$q = \frac{\text{total conc. of zinc in organic phase}}{\text{total conc. of zinc in aqueous phase}} \qquad 14$$

and E, the percent of zinc extracted:

$$E = \frac{100\,q}{q + \frac{V_{aq}}{V_{org}}} \qquad 15$$

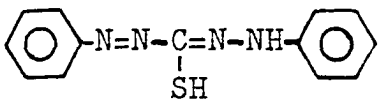
with V_{aq} and V_{org} being the aqueous and organic volumes respectively.

Dithizone and its solutions oxidize readily to diphenylcarbodiazone(DPC). Dithizone is purified in two ways[10]. Recrystallization from $CHCl_3$ is the faster procedure. To ensure complete separation from DPC, dithizone must be dissolved in 0.1% metal-free ammonia water. It is then precipitated with sulfur dioxide, extracted with chloroform and washed with water before being evaporated to dryness.

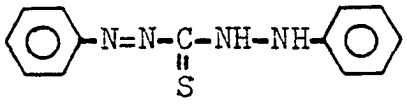
In 1925, H. Fischer[11] began using dithizone to extract heavy metals. Presently, over 15 metals are known to form a metal-dithizone complex. Dithizone's -SH group reacts with metals forming insoluble sulfides or stable complexes. Based upon extraction constants, the extractability of some metal dithizonates is: $Pd^{2+} > Au^{3+} > Hg^{2+} > Ag^{+} > Cu^{2+} > Bi^{3+} > Pt^{2+} > In^{3+} > Zn^{2+} > Cd^{2+} > Co^{2+} > Pb^{2+} > Ni^{2+} > Sn^{2+} > Tl^{+}$. The extraction of the first five or six metal-dithizonates occur from acidic aqueous solutions,

but the remainder require neutral or slightly basic solutions.

Dithizone exists in two forms:



enol



keto

Dithizone reacts with metals preferentially in acid media to yield primary dithizonates (Figure 5). These are more stable and soluble than the secondary dithizonates(Figure 6) which are favored in basic media or when the dithizone is deficient. Apparently, the -SH anionic group of primary dithizonates is capable of displacing coordinated water molecules attached to zinc.

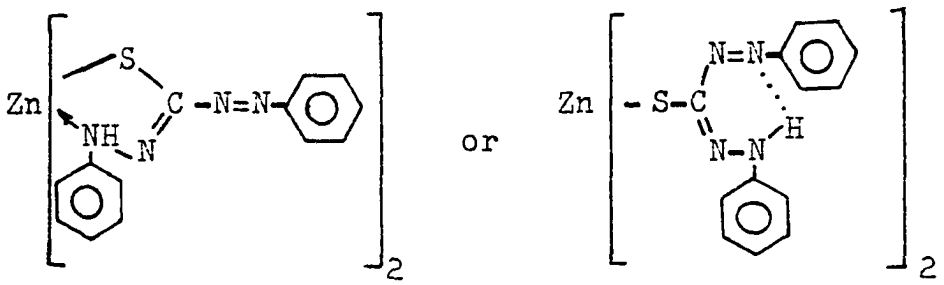


Figure 5. Primary Dithizonates[12]

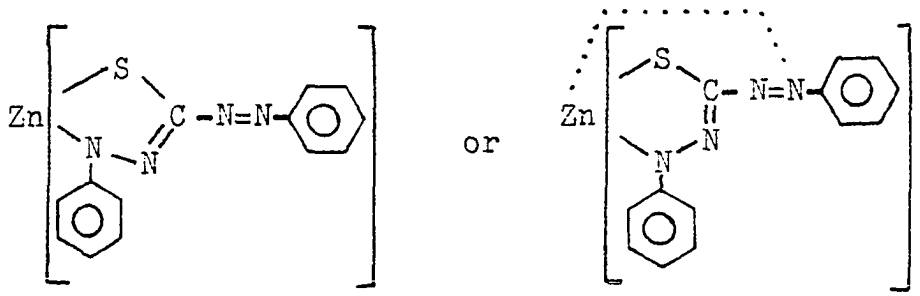


Figure 6. Secondary Dithizonates[12]

Successful extraction requires that both the rate of formation of the zinc-dithiozonate and its rate of transfer between the aqueous and organic phases be rapid. If a dithiozonate has formed, the second factor typically occurs within seconds and can be ignored.

The time to each extraction equilibrium can be measured by extracting identical solutions for different time intervals. This is plotted for bismuth (Figure 7). A similar plot for zinc might be expected.

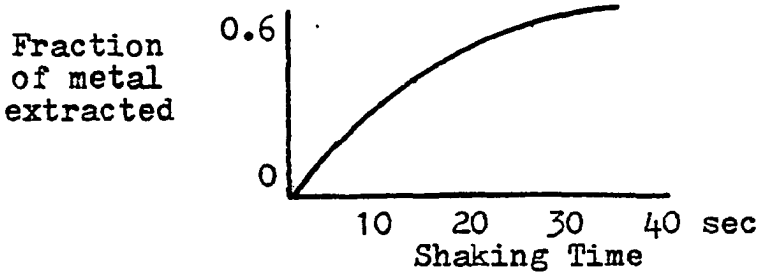


Figure 7. Time Necessary to Reach Extraction Equilibrium of Bismuth (200 ug) using dithizone-CCl_4 [12]

Freiser[13] and Irving[14] studied the extraction of zinc, cobalt and nickel by dithizone and noted a first order extraction in terms of both the metal (M^{2+}) and dithizone, but an inverse first order extraction for H^+. This is shown in Equation 16:

$$-\frac{d(M^{2+})_{org}}{dt} = \frac{k' (M^{2+})(H_2Dz)_{org}}{(H^+)} \qquad 16$$

$$k' = \frac{k\, K_{dithizone}}{P_{dithizone}} \qquad 17$$

with $K_{dithizone}$ being the dissociation constant for H_2Dz (3×10^{-5})[12]. Dithizone extraction is thus more rapid with CCl_4 (log $p_{dithizone}$ = 4) than with $CHCl_3$ (log $p_{dithizone}$ = 5). Experimentally, zinc may be extracted using dithizone dissolved in either carbon tetrachloride for the pH range 6 - 9.5, or in chloroform for the pH range 7 - 10.

Freiser proposed (Equations 18 - 21) that the rate controlling step involves loss of one water molecule from the octahedrally coordinated zinc-water complex. The first dithizone then attaches, forming the Zn-A bond (Equation 19). Expulsion of a second water molecule leads to complete coordination of the dithizone. The metal-dithizone ligands break the bonds between two more water molecules and the metal. Rearrangement (Equation 20) results in a tetrahydral complex which is followed by attachment of the second H_2Dz.

$$H_2Dz_{org} = H_2Dz_{aq} = H^+ + HDz^-_{aq} \qquad 18$$

$$HDz^-_{aq} + Zn(H_2O)_6^{2+} = 2H_2O + Zn(H_2O)_4HDz^+_{aq} \qquad 19$$

$$Zn(H_2O)_4HDz^+_{aq} = 2H_2O + Zn(H_2O)_2HDz^+_{aq} \qquad 20$$

$$Zn(H_2O)_2HDz^+_{aq} + HDz^- = 2H_2O + (ZnH_2Dz_2)_{aq} \qquad 21$$

Irving proposed that Equation 20 was rate determing.

Freiser gives the following second-order rate constants in $CHCl_3$ for the formation of 1:1 dithizonates[15]:

Metals	Typical Rate Constants($mole^{-1}sec^{-1}$)
Zn	6.1×10^6
Co	6.7×10^4
Ni	1.3×10^3

In the neutral covalent complex, the metal is surrounded, and the complex is less hydrophilic; therefore it is more soluble in CCl_4(or $CHCl_3$). Irving[16], studying the x-ray crystallography of both nickel- and zinc-dithizone complexes noted:

> "Two bidentate dithizone residues are tetrahydrally coordinated to zinc through two sulfur and two nitrogen atoms. One phenyl group of each ligand is associated with a chelate ring whereas the other four groups are extended as far as possible from the central atom with two intervening nitrogen atoms that hold it in the trans configuration."

Thus we have two planes(A-Zn-B and Z'-Zn-B', see below) orientated 85°(due to hindrance) to each other:

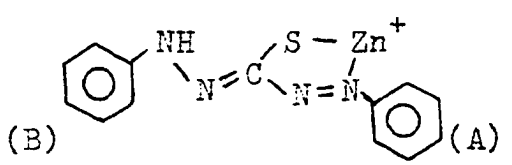


Figure 8. $ZnHDz^+$ Orientation

That this sterically hindered dithizone weakens the water-zinc bond is substantiated by Irving[14,16] who noted an even higher kinetic rate for (di-o-tolyl)- and di(1-naphthyl)-thiocarbazone. This occurs dispite the fact that both these substituted dithizonates are more than fifty times less stable than unsubstituted dithizonate.

Once ZnH_2Dz_2 is formed, more ligands should not react. We thus can ignore covalent ligands which form a complex such as $ZnH_2Dz_2(CCl_4)_x$; but with excess dithizone, any remaining water molecules probably will be displaced to yield an even more hydrophobic complex: $ZnH_2Dz_2(H_2Dz)_x$.

Acids play an important role in solvent extraction. Given:

$$Zn^{2+} + 2H_2Dz_{org} = (ZnH_2Dz_2)_{org} + 2H^+ \qquad 22$$

we can express an extraction constant K:

$$K = \frac{(ZnH_2Dz_2)_{org}\ (H^+)^2}{(Zn^{2+})\ (H_2Dz)^2_{org}} \ . \qquad 23$$

Some typical K values are given in Table 6.

Assuming that $Zn(H_2O)_x(OH)_y$ and $ZnHDz^+$ complexes can be ignored, this simplifies to

$$K = q\ \frac{(H^+)^2}{(H_2Dz)^2_{org}} \ . \qquad 24$$

Therefore, metals having high K values can be separated from those metals having significantly(10^4) lower K values. If the interfering metal is t times more concentrated than the metal of interest, then the ratio of their extraction constants must exceed 10^4t.

Somewhat related is the separation factor, F:

$$F = \frac{q}{q'} = \frac{K\ (H_2Dz)^n_{org}}{K'\ (H^+)^n} \qquad 25$$

Table 6

Selective log K and $pH_{\frac{1}{2}}$ Values for Typical Metals

Metal	log K(in CCl_4)	$pH_{\frac{1}{2}}$
Ag^{+}	7.18	-3.2
Bi^{3+}	9.98	0.7
Cd^{2+}	2.14	2.9
Co^{2+}	1.53	3.2
Cu^{2+}	10.53	-1.3
Ga^{3+}	-1.3 (in $CHCl_3$)	3.6
Hg^{2+}	26.85	-9.4
In^{3+}	4.84	2.4
Ni^{2+}	-1.18	3.4
Pb^{2+}	0.44	3.8
Po^{4+}	2	3
Tl^{+}	-3.3	7.3
Zn^{2+}	2.3	2.8

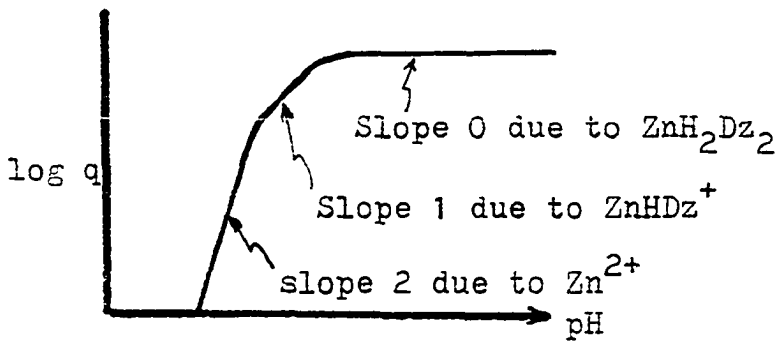


Figure 9. Distribution Ratio as Function of pH

with n being the numeric difference in the ionic charges of the metals associated with q and q'; the later two terms being the ratio of the distribution for the two metals.

Rewriting equations 24 and 15 in logarithmic form:

$$\log q = \log K + 2pH + 2\log(H_2Dz)_{org} \qquad 26$$

$$= \log \frac{EV_{aq}}{V_{org}} - \log(100 - E). \qquad 27$$

We notice from Figure 9 that the metal distriubtion is pH dependent.

Rewriting equation 26 and defining $(pH_{\frac{1}{2}})_{1.0}$ as the pH at which 50% of the zinc is extracted($q = 1$) at 1.0M equilibrium dithizone in the organic phase, we have:

$$(pH_{\frac{1}{2}})_{1.0} = -\frac{\log K}{2}. \qquad 28$$

Equations 26, 27 and 28 define a series of symmetrical sigmoid curves having an initial slope of two which are positioned along the pH axis relative to the value of K. Looking at Figure 9, Stary[12] notes:

> "In this region(i.e. the left portion with slope 2) the metal cation M^{N+} (eg. Zn^{2+}) predominates in the aqueous phase. By forming the complex cation $M_n^{(N-n)+}$ (eg. $ZnHDz^+$) the slope of the curve diminishes and in the region where the slope is equal to zero the uncharged complex MA_n(eg. ZnH_2Dz_2) predominates in both phases and q becomes equal to p_N."

Table 7 compares the effect different pHs have upon

Table 7

Comparison of E and q upon the pH Alterations for the Extraction of Zinc

	$V/V_{org} = 1$		$V/V_{org} = 5$	
	E	q	E	q
$pH_{\frac{1}{2}} - 1$	1.0	0.01	0.2	0.01
$pH_{\frac{1}{2}}$	50	1	16.7	1
$pH_{\frac{1}{2}} + 1$	99	100	95.2	100

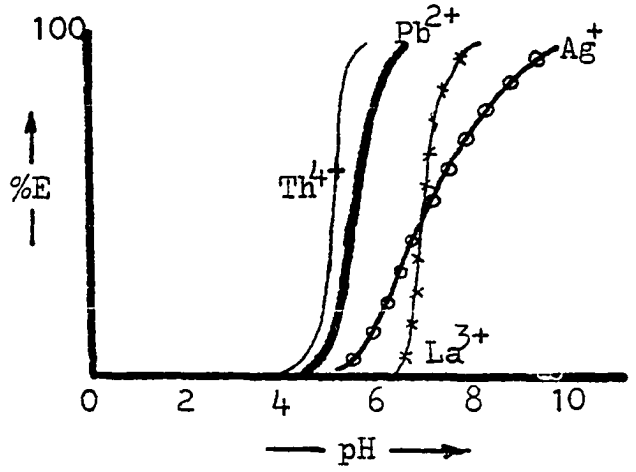


Figure 10. Effect of pH on the Extraction of Ag^{+}, Pb^{2+}, La^{3+} and Th^{4+} with 0.1M 8-hydroxyquinoline in $CHCl_3$[12].

the values of E and q for a divalent metal, such as zinc, for the cases in which $V/V_{org} = 1$ and 5. Similar calculations for polyvalent ions led Stary to observe: "An increase of one unit in the pH will increase the distribution ratio tenfold in the case of univalent ions, but 100-, 1000- and 10,000-fold for divalent, trivalent and tetravalent metal ions respectively".[12] See Figure 10 for the effect of valence and pH upon the percent of metal extracted.

The metal distribution, q, assumes that the hydroxide complexes and dithizone hydrolysis can be ignored. Given, however, the stability constants for $Zn(OH)_3^-$ and $Zn(OH)_4^{2-}$, and the fact that K = 200 for the zinc-dithizone-CCl_4 system, a q of 200 can be calculated(Equation 29) at pH = 4, but it drops to 0.0003 for pH = 14(assuming a total dithizone concentration of 10^{-4}M) using:

$$q = \frac{K\,(H_2Dz)^2_{org}}{\left[1 + \alpha_1(OH) + \alpha_2(OH)^2 + \ldots\right]\,(H^+)^2} \qquad 29$$

with α_n being the respective stability constants of the hydroxy complexes, $Zn(OH)_n^{+2-n}$. With high pHs, ZnHDzOH forms due to hydrolysis. This complex is water soluble, and lowers the extraction coefficient. By increasing the concentration of dithizone in the organic phase, the extraction curves are shifted toward the acid side and such hydroxy complexes are diminished. Thus, a ten-fold increase of dithizone decreases the equilibrium pH by one unit(see Equation 26).

At still higher dithizone concentrations, $ZnH_2Dz_2(H_2Dz)_x$ complexes begin to form and Equation 26 must be amended:

$$-\frac{\log K}{2} = pH - \frac{\log q}{2} + \frac{2+x}{x}\log(H_2Dz)_{org}. \quad 30$$

Substoichiometric Modification

Stary has defined a threshold pH above which 99.9% of the dithizone reacts with a metal. This value may be calculated for the zinc-dithizone complex:

$$pH > \tfrac{1}{2}\left[\log\frac{(H_2Dz)}{2} - \log\left\{(Zn^{2+}) - \frac{(H_2Dz)\,V_{org}}{2\,V_{org}}\right\} - \log K\right] - \log\frac{(H_2Dz)}{1000}. \quad 31$$

Since the extraction is substoichiometric, the rough approximation $(Zn^{2+}) = (H_2Dz)$ may be valid. Insertion into Equation 31 shows that the first two terms contribute little. For the case $V_{org} = V_{aq}$, their sum is zero. If $V_{org} = 10\,V$ or $V_{org} = V/10$, the respective contributions are either $\frac{1}{2}$ or $-\frac{1}{2}$.

Calculations of a threshold pH for Zn^{2+} for different dithizone concentrations are:

Threshold pH	Moles of Dithizone
4.8	10^{-3}
6.8	10^{-5}
8.8	10^{-7}

Unfortunately, the pH can not be increased indefinitely. As mentioned, in basic solution, dithizone dissociates to yield a yellow solution. The maximum pH allowed is:

$$< pK_{dithizone} + \log p_{dithizone} + \log \frac{V_{org}}{V_{aq}}$$

$$pH < 8.5 + \log \frac{V_{org}}{V_{aq}} \quad \text{for } CCl_4 \qquad 32$$

$$< 9.5 + \log \frac{V_{org}}{V_{aq}} \quad \text{for } CHCl_3 .$$

Also, hydroxide and secondary dithizonate formations are encouraged at high pHs.

Facing such restrictions, the substoichiometric method is still more selective than a stoichiometric approach for two reasons:

a) for the case in which the ionic charges (for metals M and M') are equal, the separation is pH independent (Equation 25).

b) if the ionic charges are equal and (M) = (M'), a quantitative separation for which

$$\frac{(MH_2Dz_2)_{org}}{(M'H_2Dz_2)_{org}} > 100 \quad \text{and} \quad \frac{(M)}{(M')} < 0.01$$

requires that $K/K' > 200$ when only half the metal has complexed. As noted, a superstoichiometric approach requires an extraction ratio greater than 10^4.

Substoichiometric sensitivity is limited by:

a) the specific activity of the radioisotope (the higher the specific activity, the smaller is the amount of sample that can be detected).

b) counting efficiency
c) reproducibility
d) the rate of metal complex formation
e) K-value
f) reagent blank correction
g) stability of the organic reagent.

The last two factors are the most serious in dealing with very dilute solutions.

Masking Agent Affects

If a test sample contains interfering metal ions (M^{N+}) whose K values do not differ significantly or which are present in higher concentrations than the element of interest, a masking agent H_gB must be used. Often the addition of a masking or complexing agent aids in preventing metal hydrolysis. The metals are thus held in the aqueous phase as a non-extractable complex MB_s.

The ratio of a metal's concentration to that of the interfering metal in the organic phase is:

$$\frac{(MA_N)_{org}}{(M'A_{N'})_{org}} = \frac{K\,(HA)^n_{org}\,(M')\left[1 + \beta'_s(B)^s\right]}{K'\,(H^+)^n\,(M)\left[1 + \beta_s(B)^s\right]} \quad . \quad 33$$

When using masking agents, Equation 31 must be modified by the addition of the term $\frac{1}{N}\log\left[1 + \beta_u(B)^u\right]$ with N being the metal's charge and β_u the stability constant of the MB_u complex.

One final factor involves the influence a masking agent exerts upon those cases in which low F values exist:

$$F = \frac{q\,[1 + \beta_u(B)^u]}{q'\,[1 + \beta_u(B)^u]} \cdot \qquad 34$$

Since dithizone extracts many metals, masking agents are employed to ensure selective separation. Table 8 lists metals and their masking agents.

Table 8

Conditions and Masking Agents Used with Dithizone to Extract Metals[12]

Masking Agent	Extracted Metals
Basic cyanide	Pb, Bi, Sn, Tl
Dilute acid with thiocyanate	Hg, Au, Cu
Dilute acid with thiocyanate plus cyanide	Hg, Cu
Dilute acid with thiosulfate plus cyanide	Sn, Zn
Dilute acid with EDTA	Pd, Au, Cu
Slightly alkaline diethanolamine dithiocarbamate	Zn
Strong base with tartrate or citrate	Cd, Cu, Ag, Co, Ni, Tl

Precipitation is one method to counteract interfering ions. Diethanoldithiocarbamate tends to react with metals to form crystalline precipitates; zinc is an exception.

Unfortunately, this method can not be used indiscriminately. Many natural waters, such as raw sewage, may contain ligands which form extremely strong complexes with a metal that may make the sub-superequivalence method worthless.

CHAPTER III

EXPERIMENTAL PROCEDURE

This chapter describes the materials, solutions and instrumentation used in this study along with a description of a general experimental procedure.

MATERIALS

Chemicals used and their suppliers were:

Ammonium chloride, Fisher ACS grade
Ammonium hydroxide, Baker reagent grade
Cadmium nitrate, Mallinckrodt analytical grade
Carbon disulfide, Mallinckrodt analytical grade
Carbon tetrachloride, Fisher ACS grade
Chloroform, Mallinckrodt analytical grade
Cobalt nitrate, Mallinckrodt analytical grade
Copper chloride, Mallinckrodt analytical grade
Diethanolamine, Fisher purified grade
Dithizone, Fisher reagent grade
Ethanol, US Industrial Chemical absolute grade
Hydroxyamine hydrochloride, Fisher reagent grade
Lead nitrate, Baker reagent grade
Nickel chloride, Baker reagent grade
Water, deionized
Water, double distilled
Zinc, Baker reagent grade
Zinc 65, New England Nuclear Company.

INSTRUMENTATION

The counting equipment employed included:

High voltage supply, Model 3000 Canberra Indust.
Multiscaler II, Model 132 Baird Atomic Inc.

Nuclear Counter, BNC Portanim Berkeley Nucleonics
pH meter, Digicord Photovolt Corp.
Voltage transformer, Sola Electric Comp.

SOLUTIONS

Standard non-radioactive zinc(Solution 1): 2.5024g zinc was heated slowly for two days in 16ml HCl. Deionized water was added to bring the final volume to 250ml.

Solution Y_{50}: 5.00ml of Solution 1 was diluted to one liter with deionized water. This solution has a concentration of 50mg zinc per liter.

Tagged zinc-65 solution(Solution 0): A solution of about 10 μCi/ml was prepared by adding 1 ml 4N HCl to the 0.15 ml of solution which contained ^{65}Zn(purchased from New England Nuclear Company). The solution was then transferred to 45 ml deionized water. Additional washings of the original container with 4N HCl brought the final volume to 48 ml.

Solution X_{100}: 2.5 ml of Solution 1 was diluted to 250 ml with deionized water.

Solution X_{10}: 1.00 ml of Solution 1 was diluted to one liter with deionized water.

Dithizone: The separating reagent was purified by two methods, both of which appeared to yield stable material. The easiest and cleanest method involved the addition of 5 g Fisher dithizone to 400ml chloroform, followed by gentle heating. The volume of this green solution was soon reduced to 150 ml. Solid crystals formed upon cooling; they

were filtered, air dried, and stored in a cool, dark place.

The supernatant liquid containing some dithizone was further treated[10] by extracting into 500 ml of 1:100 ammonia water. The aqueous phase became an orange-gold color. Two additional ammonia extractions brought the final aqueous volume to 800 ml. Three grams of hydroxyamine hydrochloride (reducing agent) and 90 ml 2N HCl generated a black precipitate which was extracted three times into 100, 75 and 50 ml of $CHCl_3$. The combined solution was evaporated to dryness.

All extracting dithizone solutions were similarly mixed using the appropriate quantity of purified dithizone and a 95/5 mixture of CCl_4 and $CHCl_3$. Chloroform was first added to dissolve the dithizone, and CCl_4 was then added to make the final volume. The solution was filtered through glass wool to insure homogenity by removing flakes of H_2Dz.

Buffer: 5.35 g NH_4Cl was dissolved in one liter of deionized water. Four drops of concentrated ammonia were added to raise the pH to 8.9.

Masking agent: 6.0 g diethanolamine plus 3.5 g CS_2 were dissolved in 120 ml absolute ethanol. This solution was stable for a couple weeks. When needed, 10 ml of it were mixed with 90 ml of the buffer. This mixture was purified by extraction with a dithizone solution until the organic phase remained green. The aqueous phase was back extracted with CCl_4 until both phases were clear.

Cadmium solution: 2.366 g $Cd(NO_3)_2 \cdot 4H_2O$ was dissolved in 125 ml deionized water. This represented 18.9 mg/ml cadmium and 9.45 µg/ml zinc (0.05% zinc impurity).

Cobalt solution: 10.845 g of $Co(NO_3)_2 \cdot 4H_2O$ was dissolved in 125 ml deionized water. We assumed 0.01% zinc impurity (typical of analytical grade material); representing a solution having 86.76 mg/ml cobalt and 8.68 µg/ml zinc.

Copper solution: 6.6199 g $CuCl_2 \cdot 2H_2O$ was added to 125 ml deionized water. Again, no mention of the zinc impurity appeared on the label. Again assuming 0.01% zinc impurity, each milliliter contained 52.96 mg copper and 5.3 µg zinc.

Lead solution: 6.3459 g $Pb(NO_3)_2$ was dissolved in 125 ml deionized water. If we assume 0.01% zinc impurity, then 50.78 mg lead and 5.08 µg zinc were in each milliliter.

Nickel solution: 9.104 g of $NiCl_2 \cdot 6H_2O$ was dissolved in 125 ml deionized water. The listed zinc impurity was 0.003%. Thus each ml contained 72.83 mg nickel and 2.18 µg zinc.

General Experimental Procedure

Duplicate samples were prepared at each concentration in all experiments. Therefore, depending upon the value of K, 10K ml of Solution X was added to the first two 50 ml volumetric flasks for Series 2, while 10ml of this same solution was placed into each of the remaining flasks for Series 1. Since duplicates were run, no Solution Y was

added to any of the first four flasks. To each of the subsequent pairs of flasks, 2 ml incrementally increasing volumes of Solution Y was added; i.e. 2 ml of Y was added to flasks 5 and 6, 4 ml to flasks 7 and 8, etc. In a few runs, either 2.5, 4 or 5 ml incrementally increasing volumes of Solution Y were added. To those samples having interfering metals, 0.5 ml of masking agent solution was added.

In the experiments listed in Table 10, Appendix A.1-A.3 and Appendix J, the x and y solutions were poured directly into the 50 ml volumetric flasks and each sample was crudely adjusted with ammonia and pH paper to the pH values given in each table. Upon reflection, it was decided that results would be more accurate and reproducible if the individual solutions' pHs were identical. In the remaining experiments, sufficient x and y solutions for the entire experiment were poured into beakers, and they were adjusted with ammonia and a pH meter to the listed pHs.

The pH of the deionized and double distilled water used for dilutions was adjusted to 8.0.

Each sample was treated as follows: 9.85 ml of dithizone solution was pipetted into a 125 ml separatory funnel and the sample was added. This mixture was shaken vigorously for two minutes and allowed to settle for about five minutes. The organic phase was withdrawn and 5.00 ml aliquots were transfered to test tubes which previously

had been counted for background. Care was taken to avoid transfering any aqueous phase which might have slipped through the stopcock. The aliquots were counted, corrected for background, and values of I were calculated and tabulated. Values from the tables were plotted as y versus I, and a least-square linear fit was drawn. From this line, $slope^{-1}$, x_{sl}, and x_y were calculated and compared to the known (x_{kn}) value of x.

CHAPTER IV

RESULTS AND DISCUSSION

The first priority was to duplicate the experimental conditions used in the previous work[6,7,8]. This work employed substoichiometric amounts of the separating reagent (dithizone) compared to zinc, both in Series 1 and 2. This represents case 2B conditions. Equal amounts of zinc ($M_2 = M_{1j}$) are separated in both series and the equation $I_j = (M_2/M_{1j})(1 + y_j/x)$ reduces to $I_j = 1 + y_j/x$. A plot of y versus I should yield a straight line having a slant intercept of one, and a slope of 1/x. Thus, two methods exist to determine the "unknown" x: first, x should be the reciprocal of the slope ($x_{sl} = \text{slope}^{-1}$) and second, the vertical projection, y_k, obtained from the horizontal intersection of $I = K$ with the plot of y versus I yields $x_y = y_k/(K - 1)$.

Case 2B

In duplicating the previous work[6], it was decided to also vary the values of K.

$K = 2$: 20 ml of Solution X_{10} for Series 2 and 10 ml for Series 1 were pipetted into the appropriate

flasks and 0, 2, 4, 6, 8 and 10 ml of Solution Y_{50} were added to form Series 1. Two ml of the buffer and 3.0 ml of the masking agent were added to each before adjusting the flasks to a pH of 8.6 and bringing all volumes to 50 ml with water. All samples were extracted with 0.382 µmole dithizone.

Results are given in Table A.1 and Figure A.1. The intercept of 1.08 is a bit high, but well within the range of acceptable values. The values x_y = 9.80 µg/ml and x_{sl} = 10.7 µg/ml are close to the known value of 10.0 µg/ml.

K = 3: This experiment increased the value of K to 3 and used 30 ml of Solution X_{10}. In addition, 2.0 ml of the masking agent and 1.62 µmole dithizone were employed. All the calculated values (intercept = 1.04, x_y = 9.98 µg/ml and x_{sl} = 10.2 µg/ml) are closer to the known value than occurs for K = 2. Results are given in Table A.2 and Figure A.2.

K = 4: This experiment employed the same conditions and quantities of reagents used in the K = 3 experiment except that 40 ml of Solution X_{10} was used instead of 30 ml. The results (intercept = 1.03, x_y = 10.3 µg/ml and x_{sl} = 10.4 µg/ml) are given in Table A.3 and Figure A.3.

Based on the above results for K = 2, 3 and 4 at 10 µg/ml, it appears that the sub-superequivalence method is valid for Case 2B conditions.

One naturally wondered what effect a dilution of both x and y would have upon the results. The experiment was repeated, substituting a 1:1 dilution of Solution X_{10} and a 1:20 dilution of Y_{50}, but omitting the masking agent and the buffer. These last two reagents might inject errors. Instead, the two zinc solutions were initially adjusted with ammonia to the listed pHs and diluted directly to 50 ml. Results are given in Table A.4 and Figure A.4. The intercept is 1.01 and the values of $x_y = 4.99$ µg/ml and $x_{s1} = 5.05$ µg/ml are close to the known value of 5.00 µg/ml. Although such dilutions produced acceptable results, the conditions of having the y solution equal to or less than the x solution concentration should be used with care, since such experiments may not give significantly great variation in values of x + y. Additionally, one can never be certain that the first few samples of Series 1 satisfy the conditions that SR < x + y.

Experimentally, Case 2B results appear to compare favorably with theory; therefore we turned our attention to the other cases.

Case 2A

Case 2A is more involved. Experimentally, we have a situation in which SR is less than Kx, but is greater than x. As more y is added, Case 2A suddenly becomes Case 2B at point B of Figure 3. We thus have three ways to calculate x: first, from the SR/x horizontal intercept

associated with Case 2A and additionally, as in Case 2B, from x_y and x_{sl}.

Duplicate runs 1 and 2 used an amount of dithizone less than Kx and initially greater than x + y. Solution Y was diluted to 12.5 µg/ml. These mixtures were extracted with 2.55 µmole dithizone. If we assume that primary dithizonates occur, then a two to one complex results between SR and zinc (i.e. $Zn(SR)_2$). Since 2.55 µmole of dithizone were used, it can react with 1.27 (i.e. 2.55/2) µmole of zinc. Based upon theory, the horizontal intercept of Case 2A should equal SR/x. The results of runs 1 and 2 are given in Tables B.1 and B.2, and Figures B.1 and B.2 are summarized in Table 9.

A similar run (Table B.3 and Figure B.3) used 1.12 µmole dithizone to extract the samples prepared from x and y solutions, each containing 2.50 µg/ml zinc. This run hopefully removed any ambiguous SR/x intercepts (near 2.0 which might suggest a Case 1A situation) as occurred in the first two runs.

One final fun (Table B.4 and Figure B.4) involving a Case 2A condition used a known x concentration of 2.50 µg/ml zinc and 1.05 µmole of dithizone.

Calculated values for all four experiments are compared in Table 9. The first line of Table 9 lists the slant intercepts of all four runs for Case 2B conditions. We see that with increasing K, this intercept increases

Table 9

Summary of Four Experiments Involving Case 2A and 2B

Line			K = 2	K = 3	K = 4	K = 5	Average
1	Slant	Run 1	1.10	1.16	1.18	1.18	1.16
		Run 2	1.01	1.06	1.10	1.11	1.07
		Run 3	0.953	0.958	0.956		0.956
	Intercept	Run 4	1.05	1.06	1.09		1.07
		Average	1.03	1.06	1.08		1.06
2	Intercept of $\frac{SR}{\bar{x}}$	Run 1	1.88	1.99	2.03	2.03	1.98
		Run 2	1.90	1.99	2.06	2.09	2.01
		Run 3	1.49	1.50	1.50		1.50
		Run 4	1.32	1.33	1.37		1.34
3	x via SR/x in µg/ml (ratio to known x)	Run 1	4.22(0.88)	4.19(0.84)	4.11(0.82)	4.10(0.82)	4.21
		Run 2	4.39(0.88)	4.18(0.84)	4.03(0.81)	3.97(0.80)	4.14
		Run 3	2.46(0.98)	2.44(0.98)	2.44(0.98)		2.44
		Run 4	2.60(1.04)	2.58(1.03)	2.50(1.00)		2.56
4	x via x_y in µg/ml (ratio to known x)	Run 1	5.29(1.06)	4.69(0.94)	4.46(0.89)	4.45(0.89)	4.72
		Run 2	5.48(1.10)	4.94(0.99)	4.58(0.92)	4.43(0.89)	4.86
		Run 3	2.60(1.04)	2.57(1.03)	2.58(1.03)		2.58
		Run 4	2.29(0.92)	2.27(0.91)	2.11(0.84)		2.22
5	x via x_{sl} in µg/ml (ratio to known x)	Run 1	5.87(1.17)	5.57(1.11)	5.46(1.09)	5.45(1.09)	5.59
		Run 2	5.52(1.10)	5.24(1.05)	5.07(1.01)	4.99(1.00)	5.20
		Run 3	2.49(1.00)	2.47(0.99)	2.48(0.99)		2.48
		Run 4	2.41(0.96)	2.41(0.96)	2.32(0.93)		2.38
6	x averaged in µg/ml (ratio to known x)	Run 1	5.19(1.04)	4.82(0.96)	4.68(0.94)	4.67(0.93)	
		Run 2	5.13(1.03)	4.79(0.96)	4.56(0.91)	4.46(0.89)	
		Run 3	2.51(1.00)	2.49(1.00)	2.50(1.00)		
		Run 4	2.43(0.97)	2.42(0.97)	2.31(0.93)		

x known: Runs 1 and 2 = 5.00µg/ml, Runs 3 and 4 = 2.50µg/ml

SR known: Runs 1 and 2 = 2.55µmole, Run 3 = 1.12µmole, Run 4 = 1.05µmole

from the ideal of 1.00. The second line lists the values of the horizontal intercepts for Case 2A conditions. This allows x to be calculated if SR is known (assuming that a $Zn(SR)_2$ complex forms). Line 3 gives these calculated x values and compares (shown in parenthesis) the percent ratio of it to the known values. The next two lines give the calculated x values in terms of x_y and x_{sl}. Finally, an arithmetic average of x is calculated based upon the above three values.

We see that the x values vary between 80-104% of the expected values when calculations based upon SR/x are used. It is interesting, although not necessarily significant, that these calculated x values tend to increase proportionally, compared to the known values, as the known values decrease. Values are more accurate when computed via x_y or x_{sl}; ranging 84-110% for x_y and 93-117% for x_{sl}. It is worth noting that as K increases, the calculated values tend to decrease. This occurs because SR is assumed to remain constant, but the intercept, SR/x, increases with larger values of K. The samples consisting of higher Ks contain greater amounts of radioactivity in Series 2, and some uncomplexed ^{65}Zn probably is carried over by the dithizone. Further, this carry-over might account for the abnormal increases in the calculated x (based upon SR/x) as the known value decreases. This might also cause the slant intercepts to increase with

higher values of K. These intercepts average about 1.06 and vary about it by 0.1 units. Table 9 also gives the values obtained by averaging x_y, x_{sl} and the x value from SR/x. These values appear to yield the best results while also reducing the variance.

Case 1A

Cases 1A and 1B employ superstoichiometric amounts of dithizone. In Case 1A, SR is greater than x until sufficient y is added before it suddenly becomes Case 1A at point A of Figure 3. No information can be obtained from Case 1A conditions except to verify that the line is horizontal and has an intercept of K. One run, illustrated in Table 10 and Figure 11 used 1:100 dilutions of both Solutions X_{10} and Y_{50}. Satisfying our expectations, the horizontal line has an intercept about 2% high, near 2.05.

Case 1B

Unlike Case 2B, we can not obtain information regarding x from x_y (see page 52); rather we use the intercept Kx/SR. Since x and SR were held constant in each run, we should expect to see proportional increases in the intercepts as K increases. Also, proportional increases in the slope, K/SR, should occur.

Four experiments (Appendix C), each using 2.55 µmole dithizone were run. In the first two runs, x = 2.50 µg/ml, while in runs 3 and 4, x = 3.12 µg/ml zinc. The

Table 10

Case 1A - Superstoichiometric with Dithizone

K = 2	SR > Kx	SR = 0.28 µmole
x_{kn}	0.1µg/ml = 0.015µmole/10ml	pH = 8.6
y	0.5µg/ml = 0.077µmole/10ml	pH = 8.6
INTERCEPT = 2.05	x_y = -	x_{sl} = -

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	8828	-
10	0.015	0	0	4414	2.02
10	0.015	2	0.015	4463	1.98
10	0.015	4	0.031	4266	2.07
10	0.015	6	0.046	4328	2.04
10	0.015	8	0.061	4013	2.20
10	0.015	10	0.077	4428	1.99

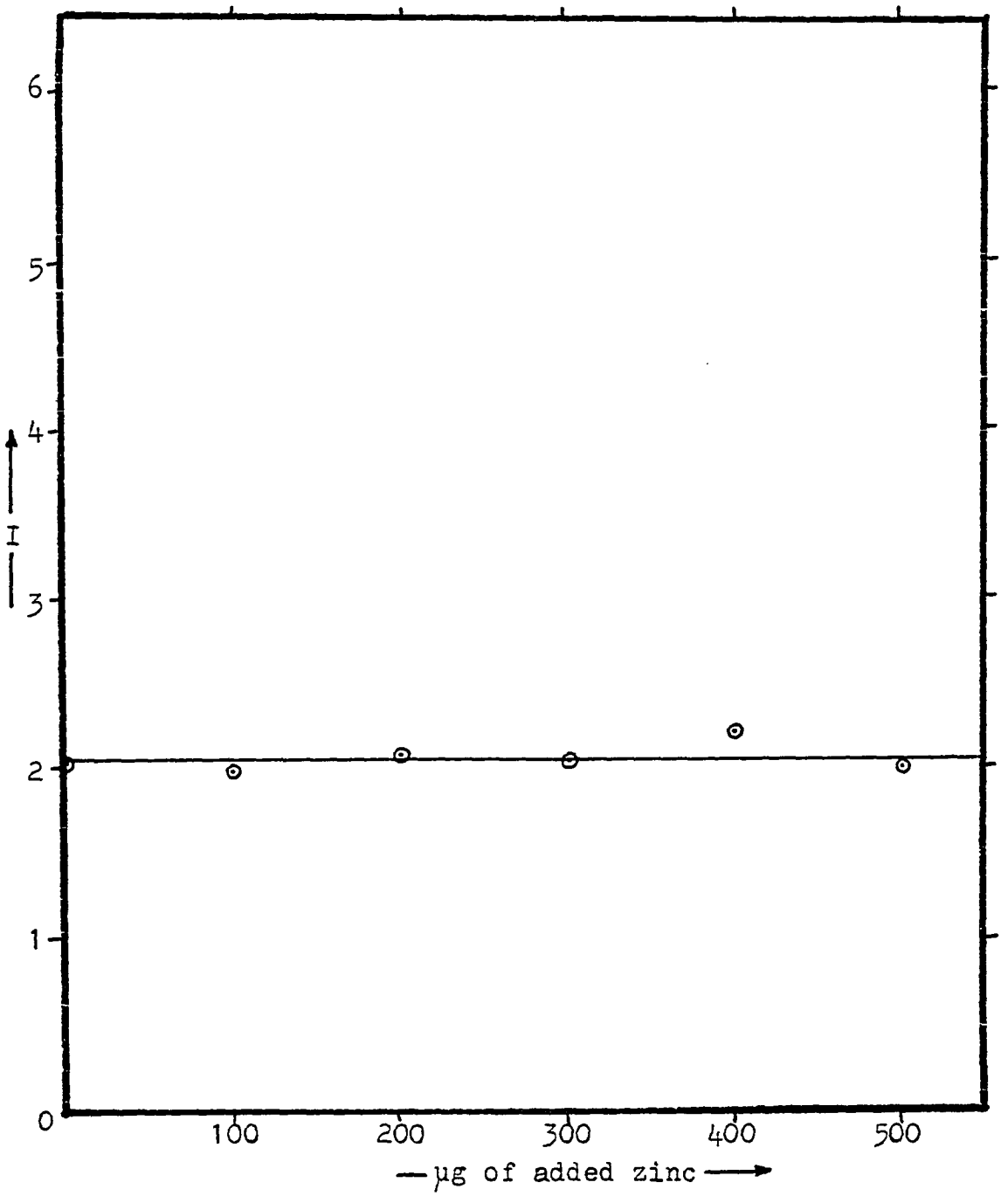


Figure 11. Case 1A - Superstoichiometric with Dithizone

concentration of y in all four runs was 12.5 μg/ml.

If a 2:1 complex exists (i.e. $Zn(SR)_2$) for these superstoichiometric cases, the 2.55 μmole of dithizone can react with 1.27 μmole of zinc. If x = 2.50 μg/ml, Case 1A and 1B conditions should exist for K = 2 and 3 (SR > Kx), but Case 2A and 2B should results when K = 4 and 5 (SR < Kx). The last two runs used 3.12 μg/ml, and we theoretically might expect Case 1A and 1B conditions to exist only for K = 2. The results of these four experiments are given in Appendix C and summarized in Table 11. The values given in Table 11 show that Case 1A and 1B conditions hold for all four runs of K = 2 and 3, but in addition, they also hold for the first two runs of K = 4. The other six results (runs 3 and 4 for K = 4 and all four runs of K = 5) represent Case 2A and 2B conditions. The first line of Table 11 gives the slant intercepts. According to theory, we expect the slant intercepts to be Kx/SR for Case 1B conditions; experimental values are given in Table 11 along with calculated values (shown in parenthesis) based upon $K\bar{x}/SR$. This value of $\bar{x}/SR$ in each run was found by dividing each experimental value (Kx/SR) by its respective K values, summing the first three (runs 1 and 2) or two (runs 3 and 4) values and dividing by the total number of values used. That is:

$$\frac{\bar{x}}{SR} = \frac{1}{m} \sum_{K=2}^{m+1} \left(\frac{Kx}{SR} \cdot \frac{1}{K} \right),$$

Table 11

Summary of Four Experiments Involving
Cases 1A, 1B, 2A and 2B

Line			K = 2	K = 3	K = 4	K = 5
1	Slant Intercept	Run 1 Run 2 Run 3 Run 4	0.607(0.601) 0.749(0.731) 0.766(0.784) 0.744(0.783)	0.919(0.90) 1.12(1.10) 1.19(1.18) 1.18(1.17)	1.18(1.20) 1.42(1.46) 1.31 1.36	1.35 1.48 1.34 1.38
2	Intercept of K or (SR/x)	Run 1 Run 2 Run 3 Run 4	1.91 1.98 1.93 1.86	2.89 2.96 3.01 2.83	3.70 3.74 (3.31) (3.27)	(4.25) (3.92) (3.39) (3.32)
3	x via Kx/SR in µg/ml (ratio to known x)	Run 1 Run 2 Run 3 Run 4	2.53(1.01) 3.12(1.25) 3.19(1.02) 3.22(1.03)	2.50(1.00) 3.12(1.25) 3.31(1.06) 3.28(1.05)	2.45(0.98) 2.95(1.18) - -	- - - -
4	x via SR/x in µg/ml (ratio to known x)	Run 1 Run 2 Run 3 Run 4	- - - -	- - - -	- - 2.52(0.81) 2.55(0.82)	1.96(0.79) 2.12(0.85) 2.46(0.79) 2.51(0.80)
5	x via x_y in µg/ml (ratio to known x)	Run 1 Run 2 Run 3 Run 4	- - - -	- - - -	- - 3.31(1.06) 3.13(1.00)	2.45(0.98) 2.75(1.10) 3.29(1.05) 3.18(1.02)
6	x via x_{sl} in µg/ml (ratio to known x)	Run 1 Run 2 Run 3 Run 4	- - - -	- - - -	- - 3.69(1.18) 3.56(1.14)	2.70(1.08) 3.12(1.25) 3.60(1.15) 3.52(1.13)
7	SR in µmoles at either A or B (SR/Zn ratio)	Run 1 Run 2 Run 3 Run 4	1.58(1.61) 1.54(1.65) 1.60(1.59) 1.52(1.68)	1.58(1.61) 1.54(1.65) 1.61(1.59) 1.52(1.68)	1.58(1.61) 1.54(1.65) 1.63(1.56) 1.52(1.68)	1.58(1.61) 1.54(1.65) 1.61(1.59) 1.52(1.68)
8	SR in µmoles based upon K/SR (SR/Zn ratio)	Run 1 Run 2 Run 3 Run 4	1.84(1.39) 1.89(1.35) 1.93(1.32) 1.92(1.33)	1.82(1.40) 1.90(1.34) 1.86(1.37) 1.89(1.35)	1.90(1.34) 2.00(1.27) - -	- - - -

where m = 3 for runs 1 and 2 and m = 2 for runs 3 and 4. Some interesting results of the slant intercept occur in the first two runs for K = 5 and in the last two runs for K = 4 and 5. Theory tells us that slant intercepts greater than 1.00 are impossible, yet we observe intercepts greater than 1.3. It would be interesting to see what intercept values result if higher K values are used. A possible explanation might involve uncomplexed ^{65}Zn carried over into the organic phase. Another explanation might be an altered complex species.

If Case 1A conditions exist, the horizontal intercept should equal K; otherwise, Case 2A conditions give an intercept equal to SR/x. The horizontal intercepts are listed on line 2. The horizontal K intercepts show slightly low values for all four experiments run at K = 2 and 3. These values do, however, substantiate Case 1A. With K = 4, however, we observe that the first two runs have intercepts around 3.72; a drop of about 7% below the expected 4.00. The other two runs at K = 4 and all runs at K = 5 represent Case 2A situations.

Based upon the actual slant intercepts from the first line, x is calculated for the ten Case 1B conditions and are listed on line three.

For the six experiments (runs 3 and 4 for K = 4 and all four runs for K = 5) representing Cases 2A and 2B, x was calculated on line four as based upon the

horizontal intercept, SR/x, and on lines five and six (the respective x_y and x_{sl} values) from either the intersection of I = K with the least squares linear plot or from slope^{-1}. It is interesting to note that the calcutions based upon the SR/x intercepts (line 4) are about 20% low. Any explanation for this deviation must also account for the accurate ten x values (for Cases 1A and 1B) calculated from Kx/SR shown on line 3. It is of theoretical interest to observe that y_k values calculated for Case 1B conditions are usually meaningless (and are ignored in Table 11). As SR increases, the value of K/SR continues to decline and point A progressses further to the right.

As before, the six x_{sl} values on line 6 are about 15% high.

The values in parenthesis for lines three through six represent the ratio of the calculated x to the known value (i.e. calculated x/known x).

Finally, the number of μmoles of SR is calculated and listed on lines 7 and 8 based upon either: 1) the equivalence point A or B (when SR = x + y) of Figure 3, or 2) the slope, K/SR. The calculated values which are based upon the slope are significantly higher than the SR values calculated at either point A or B. Apparently less of the separating reagent is required to react with the given amount of zinc as y increases.

The values listed in the parenthesis on lines 7 and 8 represent the ratio of the known 2.55 μmole of SR to the above calculated values of SR. The parenthetical values may be interpreted as a number c for $Zn(SR)_c$. If c= 2, then primary dithizonates exist. Secondary dithizonates exist if c = 1. If $1 < c < 2$, then a mixture of primary and secondary dithizonates occur. Assuming, as we did for the above calculations, that c = 2 when none of the non-radioactive zinc has been added (i.e. y = 0), the the calculated values of x based upon Kx/SR from line 3 are in close agreement with the known value of x. The values of x based upon SR/x from line 4, unfortunately, are about 20% low. A possible explanation might be that more uncomplexed ^{65}Zn was incorporated into the organic layers for Series 2 than in Series 1. The calculated value of I would then be higher than if no excess radioactivity had entered the organic layers of Series 1 and 2. A lower value of I thus would give higher calculated values of x. Apparently then, primary dithizonates (c = 2) predominate when y = 0 since SR > Kx.

As more non-radioactive zinc is added, the value of c drops. At the equivalence point A or B, the value of c is approximately 1.63. As more zinc is added we have a situation in which the zinc is superstoichiometric. The average value of c has fallen to 1.35. If the value of c continues to decline as more zinc is added, the question must be asked: "Do we really have a straight line slope?"

Obviously if the contributing values near points A and B of Figure 3 are about 1.6, then the points furthest from the origin require that c must approach 1.0 if an average value of 1.35 can be calculated. With increasingly lower values of c, secondary dithizonates become more predominant and the slope continues to decline. This indicates that secondary dithizonates become more predominant as additional zinc is added. This is understandable since secondary dithizonates are favored in those extractions containing deficient dithizone.

Based upon the above discussion, experimental Cases 1A and 1B conditions should be avoided, since x_y values may be inaccurate and only the values obtained from the Kx/SR intercepts are fairly reliable.

Accuracy and Precision

Since the calculated values of x_y and x_{sl} from the initial study (see Appendix A) are close to the known values, a study of the method's accuracy and precision was undertaken. To check reproducibility, two solutions were prepared by diluting Solution 1 to concentrations of 48.5 µg/ml ($Y_{48.5}$) and 9.7 µg/ml ($X_{9.7}$) zinc. Before each experiment, 75 ml of Solution $Y_{48.5}$ and 190 ml of Solution $X_{9.7}$ were adjusted to their listed (Appendix D) pHs using ammonia. Two different dithizone solutions of 1.97 µmole/9.85 ml (51.6 mg H_2Dz in 960 ml CCl_4 + 35 ml $CHCl_3$) and

1.84 μmole/9.85 ml (26.5 mg H_2Dz in 530 ml CCl_4 + 20 ml $CHCl_3$) were used, but no differences in the results were observed.

The results of each of these ten experiments are given in Appendix D and are summarized in Table 12. The mean of 9.74 μg/ml for x_y and 10.2 μg/ml for x_{sl} represent a 0.4% and 5.2% error respectively. The corresponding standard deviations were 4.1% and 6.5%. That the intercept of 1.04 is 4% high and its standard deviation is 0.058 (5.8%) is illustrative that eight of the ten intercepts lie above 1.00.

A second study (see Appendix E) involved an approximately ten-fold dilution to a known concentration of 1.00 μg/ml. One ml of Solution 1 was diluted with deionized water to 2000 ml. This was further diluted 1:5 to yield Solution X_1 consisting of 1 μg/ml. Table 13 summarizes the results of each experiment. The two values obtained for x, x_y = 1.05 μg/ml and x_{sl} = 1.16 μg/ml are 5.3% and 15.5% high. Their standard deviations, 0.093 and 0.056, are 8.8% and 4.8% of the mean; the former being twice as great as occurred in the first precision runs. The intercept is 1.09 and its standard deviation is 0.055.

A final dilution to 0.1 μg/ml was carried out. Due to fears of contamination at this low concentration, a switch was made from deionized to double distilled water. Five and one-half ml of Solution X_{100} were added to five liters of twice distilled water and this was divided into

Table 12

Reproducibility Studies at 10 µg/ml
Summary of Tables D.1-D.10

RUN	X_y µg/ml	X_{sl} µg/ml	INTERCEPT
1	9.74	10.6	1.08
2	9.70	11.0	1.12
3	9.58	10.2	1.06
4	9.79	10.2	1.04
5	9.63	10.3	1.06
6	9.47	9.44	1.00
7	9.56	10.1	1.05
8	9.14	9.34	1.02
9	10.2	9.32	0.90
10	10.6	11.2	1.06
MEAN	9.74	10.2	1.04
STANDARD DEVIATION	0.40	0.66	0.058

Table 13

Reproducibility Studies at 1 µg/ml
Summary of Tables E.1-E.10

RUN	X_y µg/ml	X_{sl} µg/ml	INTERCEPT
1	1.01	1.12	1.10
2	1.20	1.27	1.06
3	1.05	1.13	1.07
4	0.91	1.14	1.20
5	1.02	1.08	1.05
6	1.04	1.15	1.10
7	1.12	1.15	1.03
8	1.20	1.23	1.03
9	0.98	1.12	1.13
10	1.01	1.16	1.13
MEAN	1.053	1.155	1.089
STANDARD DEVIATION	0.093	0.056	0.055

two portions. The 2600 ml of Solution Y (0.1 μg/ml) was adjusted with ammonia to either pH = 8.15 or 7.90 (to see if slight differences in the threshold pH could be observed) and the 2400 ml Solution x (0.1 μg/ml) was tagged with several drops of ^{65}Zn and adjusted to a pH of 7.80. The pH of the double distilled dilution water was adjusted with ammonia to 8.1 ± 0.2. All solutions were kept covered to avoid contamination and evaporation.

In addition, the dithizone solutions were found to decompose if handled normally. Therefore, the dilution CCl_4 was first treated with diethanoldithiocarbamate and then washed with twice distilled water. This was then stored under helium until just before an experiment was performed. For each run, 180 ml of CCl_4 was withdrawn and the remainder was reflushed and stored under helium. Two ml of the dithizone solution from the first repetition study was added to the withdrawn CCl_4. The results of the ten runs are summarized in Table 14 which consolidates the data presented in Appendix F.

The $\bar{x}$ values, $x_y = 0.0957$ μg/ml and $x_{sl} = 0.114$ μg/ml, represent errors of 4.3% and 14%. Their two standard deviations, 0.00855 and 0.0133, are 8.9% and 11.7% of the means. The intercept of 1.12 has a standard deviation of 0.104 (9.3%).

The mean, standard deviations and their errors for the three dilution studies are compared in Table 15. With

Table 14

Reproducibility Studies at 0.1 µg/ml
Summary of Tables F.1-F.10

RUN	X_y µg/ml	X_{sl} µg/ml	INTERCEPT
1	0.0981	0.116	1.15
2	0.0959	0.134	1.28
3	0.102	0.124	1.18
4	0.0886	0.115	1.23
5	0.0860	0.0926	1.07
6	0.0924	0.105	1.03
7	0.0842	0.126	0.94
8	0.0970	0.0957	1.07
9	0.101	0.122	1.17
10	0.113	0.118	1.04
MEAN	0.0957	0.114	1.12
STANDARD DEVIATION	0.00855	0.0133	0.104

Table 15

Reproducibility Study Summary

	Known(μg/ml)	9.70	0.997	0.100
x_y	Mean	9.74	1.05	0.0957
	Error of Mean	0.4%	5.3%	-4.3%
	Standard Deviation	0.40	0.093	0.0086
	Error of St. Deviation	4.1%	8.8%	8.9%
x_{sl}	Mean	10.2	1.16	0.114
	Error of Mean	5.2%	15.5%	14.0%
	Standard Deviation	0.66	0.056	0.0133
	Error of St. Deviation	6.5%	4.8%	11.7%
INTERCEPT	Mean	1.04	1.09	1.12
	Error of Mean	4.0%	8.9%	12.0%
	Standard Deviation	0.058	0.055	0.104
	Error of St. Deviation	5.8%	5.1%	9.3%

each dilution, no trend is noticed for the mean of x_y but the standard deviation error for the last two is about double the first run. A slight increase also occurs for x_{sl} values as well as its standard deviation error. Finally, the intercept increases about 0.04 units for each ten-fold dilution.

These studies offer no indication that we are reaching the limit of sensitivity. Indeed, Ruzicka[3] reported success down to 3 ppb. One must recall that with dilutions of the separating reagent, the extraction pH is increased. This increase can not occur indefinitely; ultimately, hydroxy complexes and secondary dithizonates become significant. At already low zinc concentrations, one can hardly afford further depletion by hydroxy complexation. The formation of secondary dithizonates, while consistant throughout the experiment, may generate new problems by shifting the extraction from sub- to super-stoichiometric conditions in terms of the separating reagent. Unless the experiment is unambiguous, the results could easily be misinterpreted.

The concentration range of 0.1 μg/ml equals 1.7×10^{-6} M zinc. Based on this, the threshold pH = 7.6 (see page 29). This compares favorably with the maximum pH allowed by Equation 32, namely a pH of 8.5 with CCl_4.

It appears that another ten-fold dilution to 1.7×10^{-7} M will raise the threshold pH to 8.6 and

significant problems could be expected. One alternative might be to substitute $CHCl_3$ as the solvent since its $pK_{dithizone} + \log p_{dithizone}$ value is about one unit higher than for CCl_4. This could allow dilutions down to perhaps 1 ppb. Another alternative might be to dissolve the dithizone in a greater volume of solvent and use proportionally more solution to extract the zinc. This last change increases the value of $\log(V_{org}/V_{aq})$ employed in Equation 32.

Unfortunately, further dilutions, although theoretically acceptable, may require greater care in handling the dithizone. Even at 0.1 μg/ml concentrations, significant adjustments (such as storage under helium and rapid use of the separating reagent after mixing) had to be used. It is not clear whether the dithizone undergoes a chemical breakdown, or if it was reacting with metals in the atmosphere or from the glassware. A definite color change from light green to pink was occurring, however.

Care in handling the glassware was exercised by soaking the washed glassware in 5% HNO_3 solution for ten minutes. This was followed by one soaking in deionized water before twice rinsing in double distilled water. Such treatment should remove interfering metals.

Another source of error was stopcock grease. Experiments in the 0.1 μg/ml range had to be run using minimum amounts of grease; any contact between it and the

dithizone immediately caused a color change to red.

Variations in pH

The problem associated with pH initiated a study in the working range of pH 6 to 10. Solutions X_{10}, Y_{50} and the dilution waters were adjusted to the corresponding pHs. The dithizone concentration consisted of 2.3 µmole per sample. The results of these experiments are given in Appendix G. Excellent agreement occurs for pH 6 - 8, but the points begin to scatter widely at a pH of 9 and no trend is visible for a pH of 10. This is in agreement with theory and previous work.

Conservation of Radioactivity

Another interesting related study investigated the conservation of radioactivity. In order to prove that all the radioactivity is accounted for, an experiment was performed using a procedure similar to that used in the reproducibility study at 10 µg/ml zinc. This time, however, to ensure identical counting efficiencies, 5 ml aliquots from both the 9.85 ml organic phase and 50 ml aqueous phases were counted. The data is presented in Table 16 and Figure 12. Since only one-tenth of the water and 5/9.85 of the organic phase were counted, the figures in Columns A and B must be corrected in the final column. The average of 104989 for Series 1 is 0.504 times the value obtained in Series

Table 16
Conservation of Radioactivity

x_{kn}	9.70µg/ml = 1.48µmole/10ml pH = 8.30	x_y = 11.0µg/ml	INTERCEPT = 1.00
y	48.5 µg/ml = 7.42µmole/10ml pH = 7.45	x_{sl} = 11.0µg/ml	SR = 2.10 µmole

x		y		A	B	I	RADIOACTIVE CONSERVATION
ml	µmole	ml	µmole	CORRECTED 5 MIN H_2O COUNT	CORRECTED 5 MIN CCl_4 COUNT		10A + 1.97B
20	2.97	0	0	13766	35761	-	208109
10	1.48	0	0	3541	36179	0.99	106683
10	1.48	2	1.48	6753	18383	1.95	103745
10	1.48	4	2.97	7962	12811	2.79	104858
10	1.48	6	4.45	8516	9580	3.73	104033
10	1.48	8	5.94	8874	7720	4.63	103948
10	1.48	10	7.42	9393	6464	5.53	106664

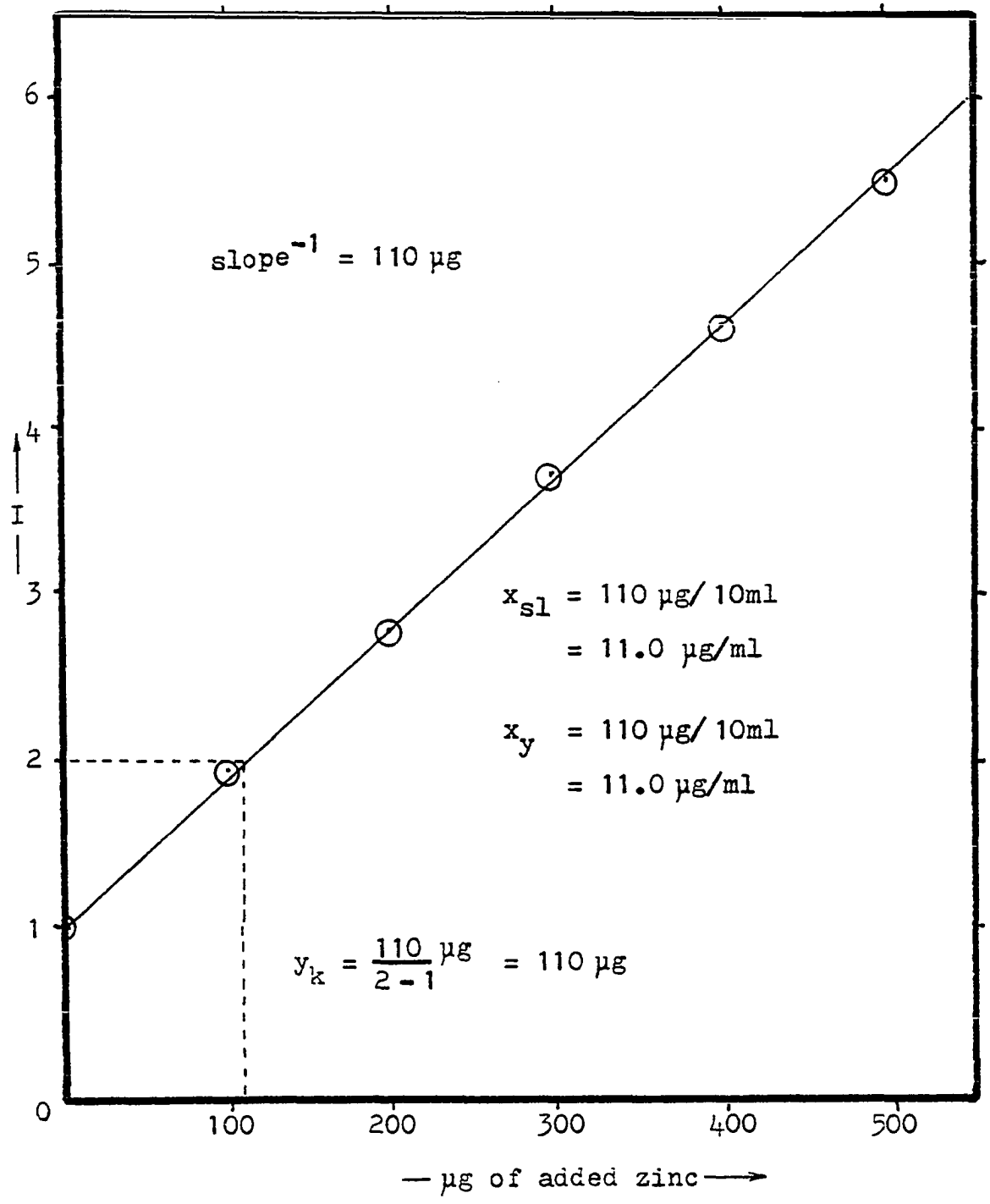


Figure 12. Conservation of Radioactivity

2. Thus all the radioactivity is accounted for and the experimental method is reliable in the sense that all of the added zinc has been accounted for.

Interferences

All the studies up to this point had employed solutions which contained no interfering metals. Unfortunately, natural waters often contain large concentrations of several metals. For this reason, a study of the sub-superequivalence modification using solutions containing zinc mixed with various concentrations of cobalt, copper, lead and nickel was performed. These metals were chosen because they form dithizonates. A points needs to be emphasized in handling the masking agent. The pH had to be maintained above 7: ideally, it would be closer to 8. If this parameter isn't controlled, a dark precipitate occurs upon the addition of dithizone. Speculation is that the metal sulfides may be forming. At low pHs, the normally golden color of the masking agent and metal appears drab green.

Our first effort involved the addition of only 0.1 ml each of the copper (5.3 mg) and cadmium (1.89 mg) solutions to 200 ml of Solution X_{10}. To each flask, 0.5 ml masking agent was added. The total interferences concentration was 36 µg/ml. To the 2000 µg of zinc already present, 3.0 µg of additional zinc was added in the cadmium and copper solutions; therefore the zinc

concentration (after dilution for pH adjustment and the addition of tagged zinc) was 9.93 µg/ml. This was followed by an experiment involving 61 µg/ml lead plus nickel and a known concentration of zinc equal to 9.88 µg/ml. The results of Tables H.1-H.2 and Figures H.1-H.2 show x_y = 10.0 µg/ml and 10.5 µg/ml and x_{sl} = 10.1 µg/ml and 10.3 µg/ml.

These two experiments prompted a run containing cadmium, cobalt, copper and nickel. One-tenth milliliter of each metal solution was added to 415 ml Solution X. The total interference concentration was 56 µg/ml in a solution containing 9.94 µg/ml zinc. The results are given in Table H.3 and Figure H.3. Both of the calculated x_y and x_{sl} values are 10.3 µg/ml.

Although interferences of 40-60 µg/ml are common, one could envision many circumstances in which higher levels would be encountered. Three further experiments looked at individually higher concentrations of cadmium, cobalt and nickel.

To 200 ml of Solution X_{10} mixed with tagged zinc, 2.0 ml of the cadmium solution was added. The interference concentration was 185 µg/ml, while the known zinc concentration was 9.93 µg/ml. Results are given in Table H.4 and Figure H.4.

Next, 3.0 ml of the cobalt solution was mixed with 167 ml of tagged x solution. The cobalt concentration was

1536 µg/ml and the known zinc concentration was 9.92 µg/ml. Here an error of up to 15% was found for the calculated x_y and x_{sl} values of 11.4 and 11.0 µg/ml. Table H.5 and Figure H.5 lists the experimental results.

Finally, 1.0 ml of the nickel solution was added to a similar 167 ml tagged x solution. Nickel concentration was 435 µg/ml in this solution having 9.86 µg/ml zinc. As above with the cobalt experiment, the x_{sl} value of 9.63 µg/ml was closer to x_{kn} than the x_y value of 10.3 µg/ml found in Table H.5 and Figure H.6.

One last experiment raised the interference concentration by the addition of 2.0 ml each of nickel (861 µg/ml), cobalt (1026 µg/ml) and cadmium (223 µg/ml) for a total interference of 2110 µg/ml and a zinc concentration of 9.81 µg/ml. Despite the large contamination, the x_y and x_{sl} values of 10.1 and 10.0 µg/ml from Table H.7 and Figure H.7 compare favorably to the x_{kn} value of 9.81 µg/ml.

Table 17 summarizes the results of these experiments containing interferences.

Comparison with Independent Analysis

In an attempt to examine our accuracy, a comparison was made between the results obtained using the sub-superequivalence modification and the Oklahoma Department of Health's atomic absorption values. Three

Table 17

Summary of Experiments Involving Interference Metals

Run	Interfering Metal	Conc µg/ml	x_{known}	x_y(% error)	x_{sl}(%error)
1	Co Cd	26.5 9.5	9.93 µg/ml	10.0µg/ml (0.5)	10.1µg/ml (1.5)
2	Ni Pb	36 25	9.88 µg/ml	10.5µg/ml (6.3)	10.3µg/ml (4.2)
3	Cd Co Cu Ni	4.6 20.9 12.8 17.6	9.94 µg/ml	10.3 µg/ml (3.2)	10.3 µg/ml (3.2)
4	Cd	185	9.93 µg/ml	10.3 µg/ml (3.7)	10.3 µg/ml (3.7)
5	Co	1536	9.92 µg/ml	11.4 µg/ml (15.3)	11.0 µg/ml (11.2)
6	Ni	435	9.86 µg/ml	10.3 µg/ml (4.5)	9.63 µg/ml (-2.3)
7	Cd Co Ni	223 1026 861	9.81 µg/ml	10.1 µg/ml (3.0)	10.0 µg/ml (1.9)

concentrations of x (as determined by the health agency) were used: 2.9, 1.2 and 0.19 μg/ml. In order to ensure a valid comparison of the two determinations, the value of y used in the sub-superequivalence modification was determined using the absorption unit. This y value was then used to calculate a value for both x_y and x_{sl}. Table 18 summarizes the results of Appendix I and compares our calculated results with those from the state agency. In general, the atomic absorption values are about 13% higher than ours. Such results are encouraging, however, since values below 1 μg/ml are typically difficult to analyze with the atomic absorption approach. Our sub-superequivalence method may, in fact, yield more accurate results.

Additional Factors

During the initial portions of this work, a preliminary study of the effects produced by differing amounts of dithizone was performed. Experimental plots, such as Figure 4, had shown significant deviations in their intercepts (which should have been 1.0). Another set of experiments used an x solution consisting of 10.0 μg/ml and dithizone solutions which increased from 2.55 μmole up to 15.3 μmole. Results are given in Appendix J, the first five of which were carried out in CCl_4 while the last four used $CHCl_3$ as the solvent. Except for experiment J.1, all the experiments involved

Table 18

Comparison Study with Independent Analysis

Concentration of x as determined by:

Health Dept	Sub-superequivalence Method			
	Run	x_y	x_{sl}	Intercept
2.9µg/ml	1A	2.6µg/ml	2.7µg/ml	1.05
	2A	2.4µg/ml	2.7µg/ml	1.09
	3A	2.3µg/ml	2.6µg/ml	1.10
	Average	2.4(-16%)	2.7(-8%)	1.08
1.2µg/ml	1B	1.1µg/ml	1.3µg/ml	1.14
	2B	1.0µg/ml	1.0µg/ml	1.00
	3B	1.1µg/ml	1.3µg/ml	1.13
	Average	1.1(-10%)	1.2(0%)	1.09
0.19µg/ml	1C	0.29µg/ml	0.32µg/ml	1.07
	2C	0.28µg/ml	0.29µg/ml	1.04
	3C	0.26µg/ml	0.34µg/ml	1.23
	Average	0.27(+46%)	0.31(+67%)	1.11

superstoichiometric amounts of dithizone, i.e. SR > 3.06 μmole. In general, both the values of x_y and x_{sl} increase significantly with higher amounts of separating reagent. According to theory, x_{sl} should be an invalid measure because we are using Case 1A and 1B conditions. Several disturbing deviations appear, the first of which is that no relationship can be observed which verifies the Case 1B equation, $slope^{-1} = SR/x$. See Table 19. Secondly, although runs J.2 and J.6 show some 'creeping up' of the intercept, nothing in the theory explains why we should see Case 1A conditions with intercepts greater than a K of two. Finally, experiment J.9 shows an extreme case in which the curve begins with an intercept of 2.7 and declines (as did experiments J.3, J.4, J.5 and J.8) well below the K value expected before rising as more zinc is added. Such a situation may generate a great deal of uncertainity. Does one calculate a y_k value of 115 μg or 540 μg in Figure J.9? In addition, using one-fifth dilutions of our zinc solutions (Table J.10 and Figure J.10) helped to expand the left hand portions of our previous plots. Not only is the y_k value inaccurate, but one could become confused and give up the analysis upon seeing a negative slope.

Based upon this study, a preliminary analysis should be attempted to determine a dithizone concentration which does not greatly exceed the metal's

Table 19

Comparison of the Effects Upon the Use of Increasing Amounts of Dithizone

Run	Solvent	$Slope^{-1}$	SR calculated	SR used(2:1)	Intercept
J.2	CCl_4	119 µg	3.64 µmole	1.66 µmole	1.86
J.3	CCl_4	143 µg	4.38 µmole	1.91 µmole	2.42
J.4	CCl_4	176 µg	5.39 µmole	2.55 µmole	3.72
J.5	CCl_4	1090 µg	33.4 µmole	3.82 µmole	3.67
J.6	$CHCl_3$	102 µg	2.44 µmole	1.91 µmole	1.39
J.7	$CHCl_3$	106 µg	3.24 µmole	2.55 µmole	1.94
J.8	$CHCl_3$	133 µg	4.07 µmole	3.82 µmole	2.38
J.9	$CHCl_3$	-	-	7.65 µmole	2.68

concentration. Apparently, a solvent effect is present, but whether it involves dipolar considerations in uncertain. For a given dithizone concentration, both the slope^{-1} and intercept are less when chloroform rather than carbon tetrachloride is chosen as the solvent.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study examined the sub-superequivalence method which was developed by Klas, Tölgyessy and Klehr.[4] Zinc was chosen as the test metal and dithizone as the separating reagent. Experiments were broken down into the four possible cases in which the separating reagent was either greater than or less than Kx and x + y.

Cases 1A and 1B employed excess dithizone compared to the zinc in Series 2 samples. The concentration of zinc was 10 μg/ml. As expected, experimental results agreed with theory, in that no determination of zinc concentration can be made for Case 1A conditions; rather, only a horizontal line having an intercept of K is observed. Upon the addition of more zinc, samples in Series 2 became representative of Case 1B conditions. Detection of the unknown zinc became possible, since the assumption of a 2:1 ratio for H_2Dz:Zn allowed results to be calculated which were only about 8% higher than the known values. The general thoery proved invalid,

however, when attempting to calculate a meaningful y_k. The study of this case also showed that as more zinc was added to Series 1 samples, a shift occurred in the ratio between dithizone and zinc, decreasing from 2.0 to values less than 1.35. This drop was probably due to the formation of greater amounts of secondary dithizonates. One disturbing result was unexpected; intercepts occurred higher than I = 1.0. Although theory does not allow such situations, these results do allow calculations of x that are no worse than 18% high.

Cases 2A and 2B employed an excess of zinc in Series 2 compared to dithizone. The slant intercepts averaged about 6% higher than the theory predicted. These intercepts appeared to increase in value as K was increased. Case 2A calculations, based upon the SR/x intercepts, yielded x values which were 1-20% low when 5.0 µg/ml zinc and 2.55 µmole dithizone were used but showed better agreement (from 10% low to 3% high) when concentrations were reduced to 2.5 µg/ml zinc and 1.2 µmole dithizone. Calculations based upon x_y and x_{sl} appeared to give higher x values than those obtained from SR/x (and in some runs higher than the known values). The best overall results were obtained by averaging the x values obtained from SR/x, x_y and x_{sl}. This method gave values which were within ± 4% for K = 2 and 3. With higher K values, results deviated

more from the known values.

Experimentally, Case 2B appears best suited in determining zinc concentrations, especially if a low K value of 2 or 3 is used. All remaining experiments in this study therefore used Case 2B conditions.

A precision and reproducibility study using ten experiments each was carried out at 10, 1 and 0.1 µg/ml zinc. Accuracy was within 5% and the precision was within 9% for all three concentrations when x_y was calculated. Deviations became significantly worse in dropping from 10 µg/ml to 1 µg/ml zinc, but they did not appear to become worse with another ten-fold dilution. Of course, more rigid experimental conditions were required. Dithizone had to be freshly prepared from diethanoldithiocarbamate-cleaned CCl_4, which was then stored under helium. The calculations based upon x_{sl} were less accurate than for x_y. Accuracy was within 5% at the 10 µg/ml concentration, but only within 15% at both 1 and 0.1 µg/ml. Precision was within about 6% at 10 µg/ml and 1 µg/ml, but almost doubled at 0.1 µg/ml. Theory predicts that another ten-fold dilution could be made before the threshold pH would be exceeded, but such runs would require extremely clean glassware and freshly prepared dithizone solutions dispensed from reservoirs kept under a helium atmosphere.

The effects of pH were investigated. Excellent

results were obtained for pHs between six and eight, but they began to deteriorate above nine. No runs were made below a pH of six.

Another experiment showed that radioactivity was conserved. This study indicated that most of the radioactivity remains in the water layer, but counting rates were still high enough in the organic layer to give statistically meaningful values within short counting times.

Since natural waters are not pure, a study investigated interferences caused by other metals. Fortunately, the sub-superequivalence approach was effective even with a 200-fold excess mixture of cadmium, cobalt and nickel. Values for x_y and x_{sl} were only 3% high. Since the masking agent, diethanoldithiocarbamate, was used to tie up interferences, extra caution had to be employed to ensure that the pH was maintained close to eight.

Another experiment compared our results obtained from the sub-superequivalence modification with samples analyzed by the Oklahoma Department of Health. Good agreement was obtained.

The last investigation reported involved excessively high concentrations of dithizone. These results showed that x_y, x_{sl} and SR/x values could become worthless if too concentrated a dithizone solution is used.

The conclusion from this study is that very high dithizone concentrations should be avoided.

In summary, the sub-superequivalence method appears useful even in the presence of excess interfering metals. Case 2B conditions employing low K values yield x_y values which are accurate down to 0.1 µg/ml so long as the pH is maintained near eight.

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APPENDIX A

INITIAL STUDIES K =2 AT 10 μg/ml

Table A.1

Initial K = 2 at 10 μg/ml

K = 2	Kx > SR	SR = 0.382 umole
x_{kn}	10.0ug/ml = 1.53umole/10ml	pH = 8.6
y	50.0ug/ml = 7.65umole/10ml	pH = 8.6
INTERCEPT = 1.08	x_y = 9.80ug/ml	x_{sl} = 10.7ug/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	umole	ml	umole		
20	3.06	0	0	4002	-
10	1.53	0	0	3722	1.08
10	1.53	2	1.53	1980	2.01
10	1.53	4	3.06	1330	3.02
10	1.53	6	4.59	1040	3.85
10	1.53	8	6.12	832	4.81
10	1.53	10	7.65	690	5.80

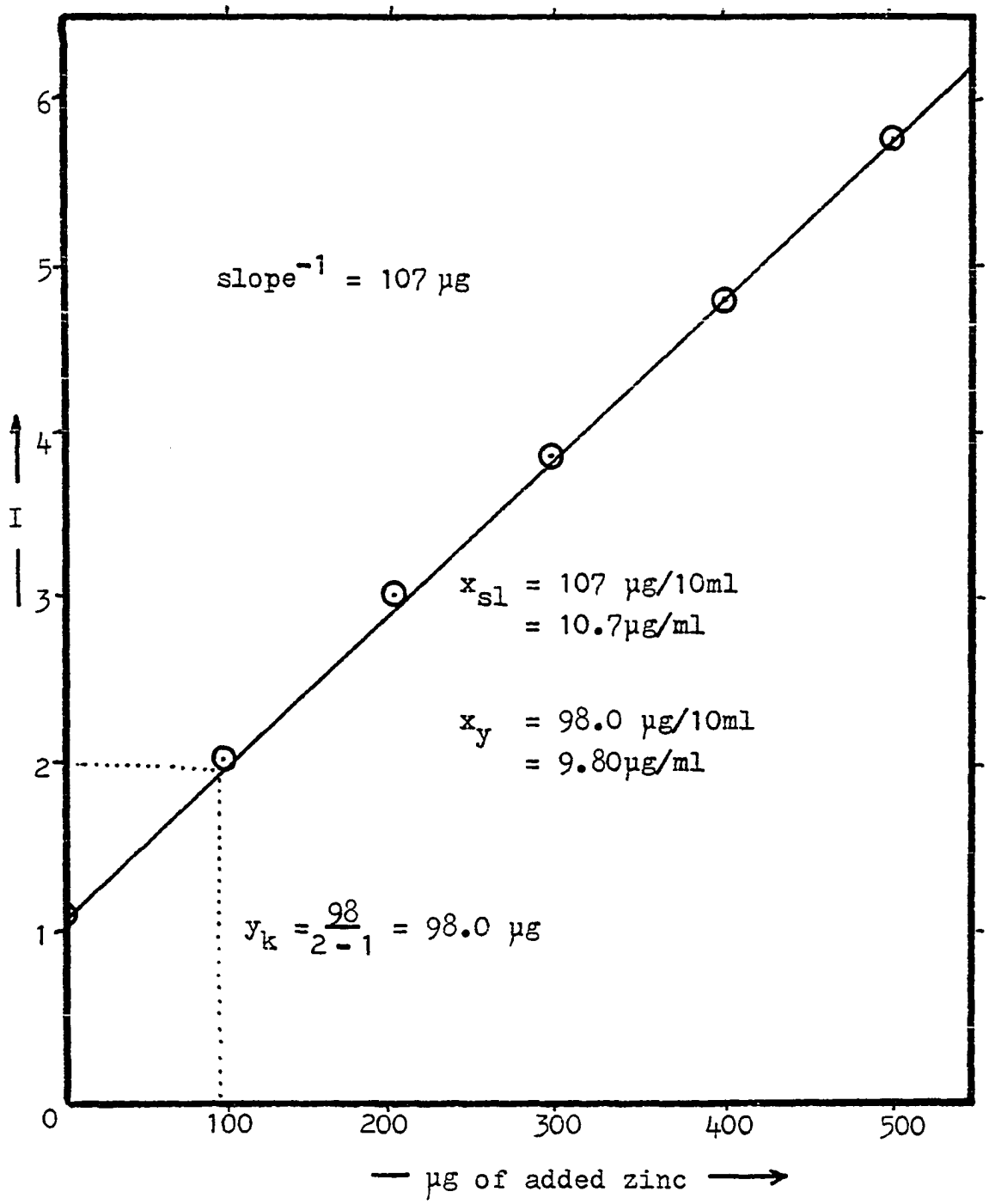

Figure A.1. Initial K = 2 at 10 µg/ml

Table A.2

Initial K = 3 at 10 µg/ml

K = 3	Kx > SR	SR = 1.62 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 1.04	x_y = 9.98µg/ml	x_{sl} = 10.2µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
30	4.59	0	0	5256	-
10	1.53	0	0	4995	1.05
10	1.53	2	1.53	2616	2.01
10	1.53	4	3.06	1748	3.01
10	1.53	6	4.59	1317	3.99
10	1.53	8	6.12	1059	4.96
10	1.53	10	7.65	886	5.94

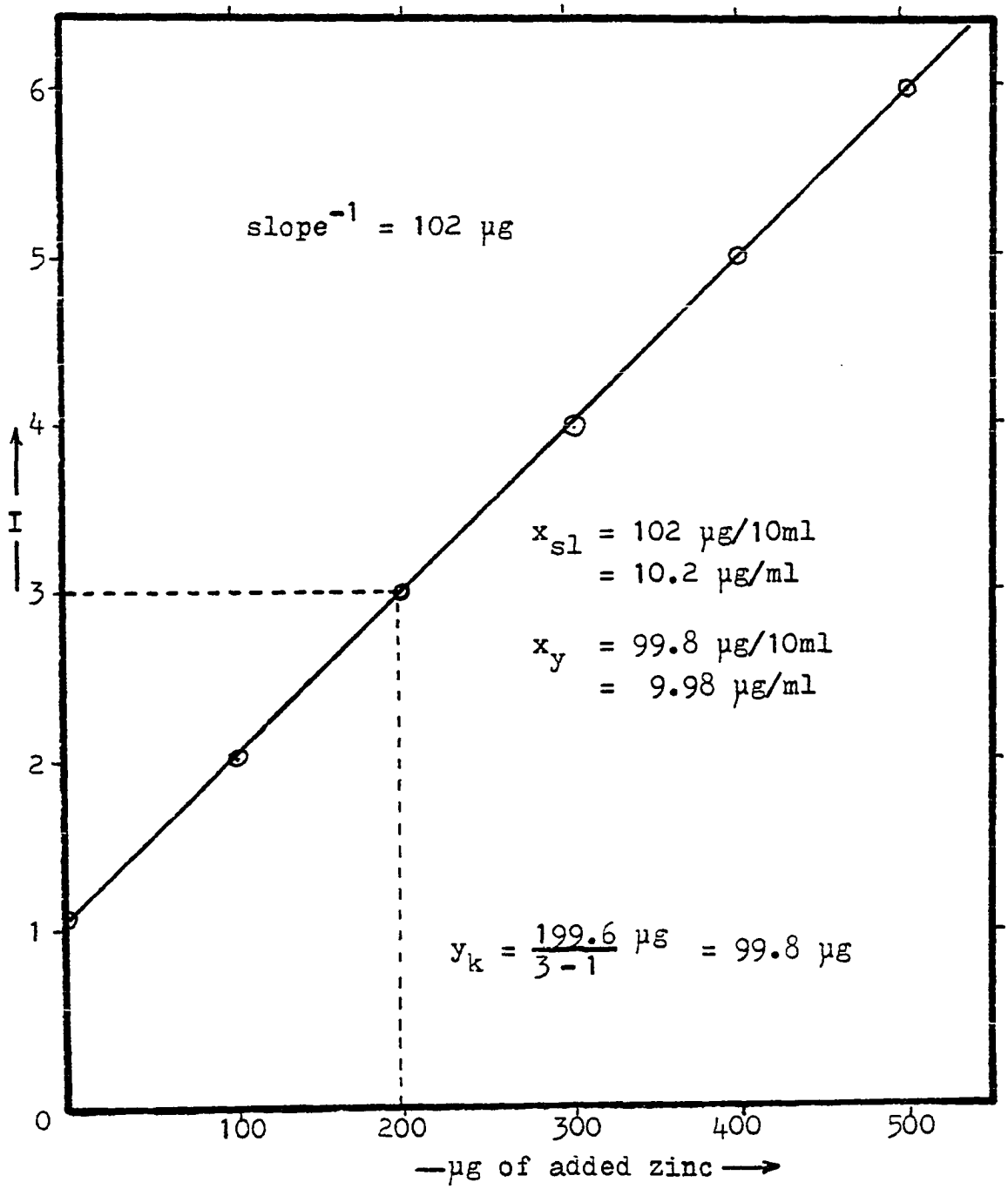

Figure A.2. Initial K = 3 at 10 µg/ml

Table A.3

Initial K = 4 at 10 µg/ml

K = 4	Kx > SR	SR = 1.62 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 1.03	x_y = 10.3µg/ml	x_{sl} = 10.4µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
40	6.12	0	0	5064	-
10	1.53	0	0	5047	1.00
10	1.53	2	1.53	2594	1.95
10	1.53	4	3.06	1706	2.97
10	1.53	6	4.59	1268	4.00
10	1.53	8	6.12	1033	4.90
10	1.53	10	7.65	884	5.73

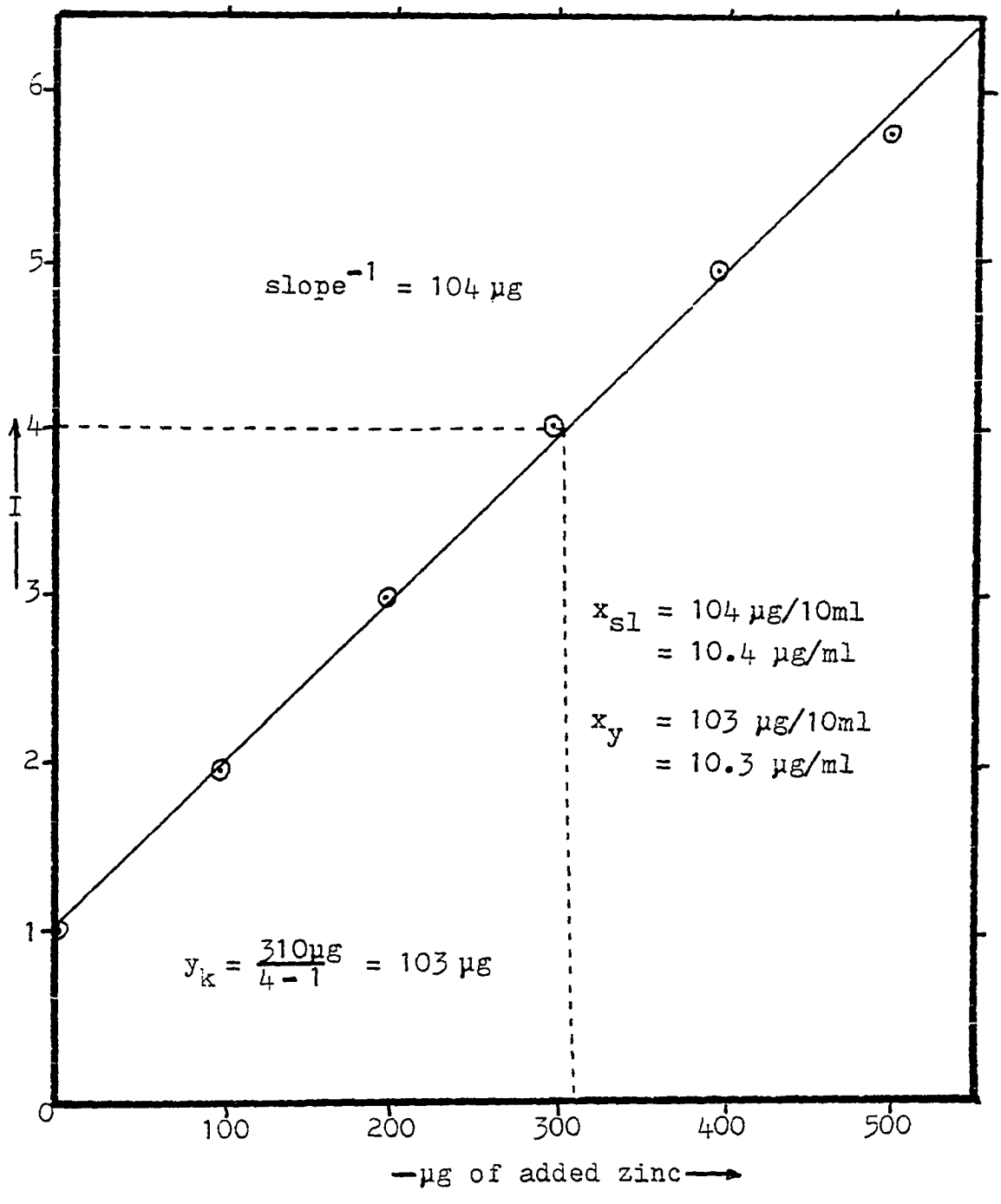

Figure A.3. Initial K = 4 at 10 µg/ml

Table A.4

1:1 Dilution of Solution X_{10} and
1:20 Dilution of Solution Y_{50}

K = 2	Kx > SR	SR = 2.15 µmole
x_{kn}	5.0µg/ml = 0.77µmole/10ml	pH = 8.6
y	2.5µg/ml = 0.38µmole/10ml	pH = 8.6
INTERCEPT = 1.01	x_y = 4.99µg/ml	x_{sl} = 5.05µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	1.53	0	0	3256	-
10	0.77	0	0	3174	1.03
10	0.77	2	0.08	2894	1.13
10	0.77	4	0.15	2884	1.13
10	0.77	6	0.22	2465	1.32
10	0.77	8	0.30	2222	1.47
10	0.77	10	0.38	2199	1.48

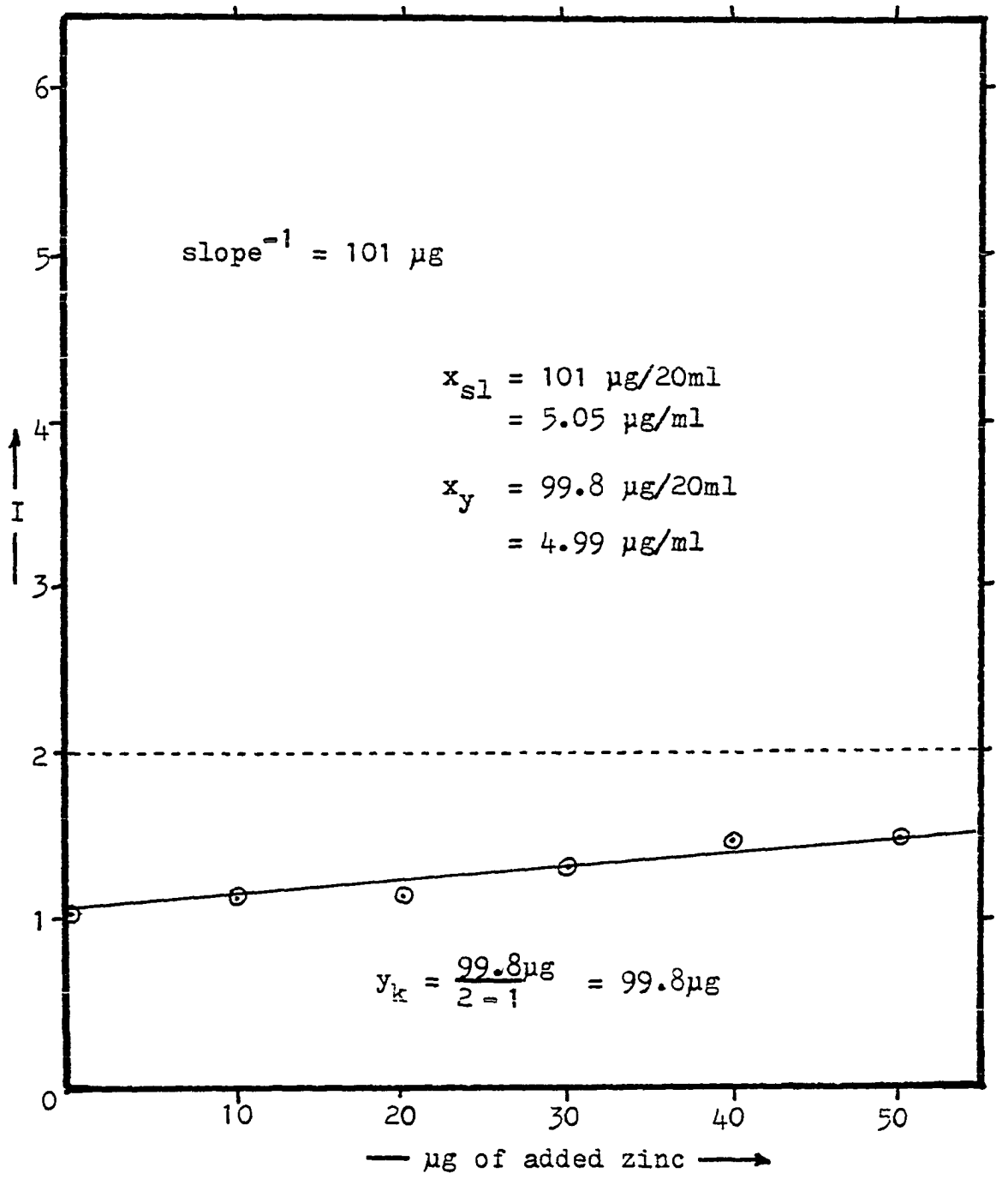

Figure A.4. 1:1 Dilution of Solution X_{10} and 1:20 Dilution of Solution Y_{50}

APPENDIX B

STUDIES INVOLVING CASES 2A AND 2B

Table B.1

Cases 2A and 2B, Run 1

Multiple Ks	SR < Kx SR > x + y → SR < x + y				SR = 2.55umole
x_{kn}	5.00ug/ml = 0.765umole/10ml				pH = 6.85
y	12.5 ug/ml = 1.91 umole/10ml				pH = 7.20
INTERCEPT = 1.10 1.16 1.18 1.18	x_y = 5.29ug/ml 4.69 " 4.46 " 4.45 "				x_{sl} = 5.87ug/ml 5.57 " 5.46 " 5.45 "
x(ml)	y(ml)	CORRECTED MIN.COUNT	I_2	I_3	I_4	I_5
50	0	15305	-	-	-	-
40	0	15278	-	-	-	-
30	0	14982	-	-	-	-
20	0	14207	-	-	-	-
10	0	7572	1.88	1.98	2.02	2.02
10	2½	7504	1.89	2.00	2.04	2.04
10	5	6499	2.19	2.31	2.35	2.36
10	7½	5302	2.68	2.83	2.88	2.89
10	10	4517	3.15	3.32	3.38	3.39
10	12½	3662	3.88	4.09	4.17	4.18
10	15	3345	4.25	4.48	4.57	4.58

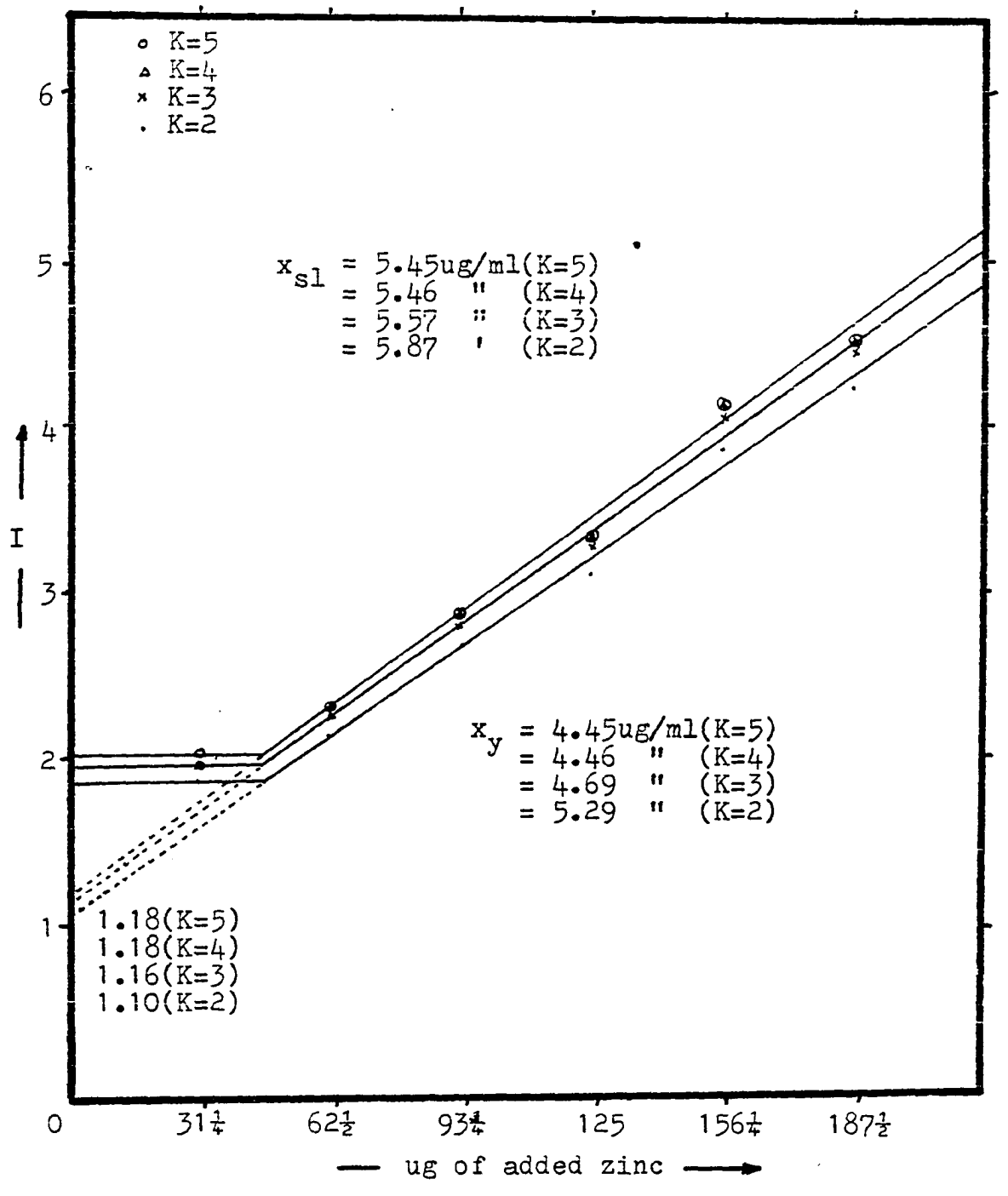

Figure B.1 Cases 2A and 2B, Run 1

Table B.2

Case 2A and 2B, Run 2

Multiple Ks	SR < Kx SR > x + y → SR < x + y				SR = 2.55umole
x_{kn}	5.00ug/ml = 0.765umole/10ml				pH = 6.90
y	12.5 ug/ml = 1.91 umole/10ml				pH = 6.95
INTERCEPT = 1.01 1.06 1.10 1.11	x_y = 5.48ug/ml 4.94 " 4.58 " 4.43 "			x_{sl} = 5.52ug/ml 5.24 " 5.07 " 4.99 "	
x(ml)	y(ml)	CORRECTED MIN.COUNT	I_2	I_3	I_4	I_5
50	0	13478	-	-	-	-
40	0	13284	-	-	-	-
30	0	12827	-	-	-	-
20	0	12196	-	-	-	-
10	0	6483	1.88	1.98	2.05	2.08
10	2½	6387	1.91	2.01	2.08	2.11
10	5	5776	2.11	2.22	2.30	2.33
10	7½	4532	2.69	2.83	2.93	2.97
10	10	3677	3.32	3.49	3.61	3.66
10	12½	3125	3.90	4.10	4.25	4.31
10	15	2812	4.34	4.56	4.72	4.79

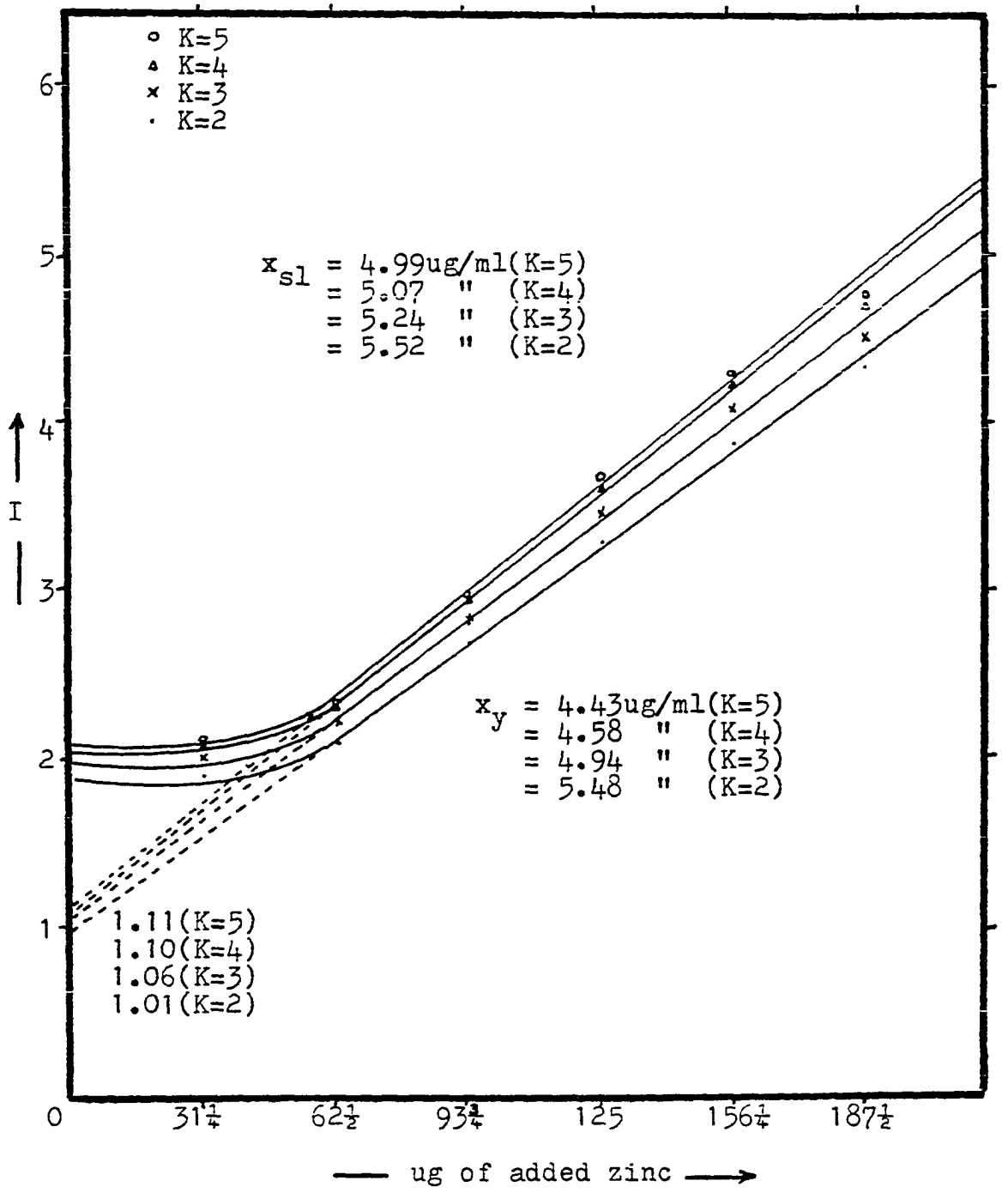

Figure B.2 Cases 2A and 2B, Run 2

Table B.3

Cases 2A and 2B, Run 3

Multiple Ks	SR < Kx SR > x + y → SR < x + y				SR = 1.12umole
x_{kn}	2.50ug/ml = 0.382umole/10ml				pH = 7.00
y	2.50ug/ml = 0.383umole/10ml				pH = 7.40
INTERCEPT = 0.95 0.958 0.956	x_y = 2.60ug/ml 2.57 " 2.58 "			x_{s1} = 2.49ug/ml 2.47 " 2.48 "	
x(ml)	y(ml)	CORRECTED MIN.COUNT	I_2	I_3	I_4	I_5
50	0	-	-	-	-	-
40	0	11000	-	-	-	-
30	0	11060	-	-	-	-
20	0	11041	-	-	-	-
10	0	7421	1.48	1.49	1.49	-
10	2½	7492	1.47	1.48	1.47	-
10	5	7224	1.52	1.53	1.53	-
10	7½	6336	1.74	1.75	1.74	-
10	10	5638	1.95	1.96	1.96	-
10	12½	5117	2.15	2.16	2.16	-
10	15	4351	2.53	2.54	2.54	-
10	17½	4060	2.71	2.72	2.72	-

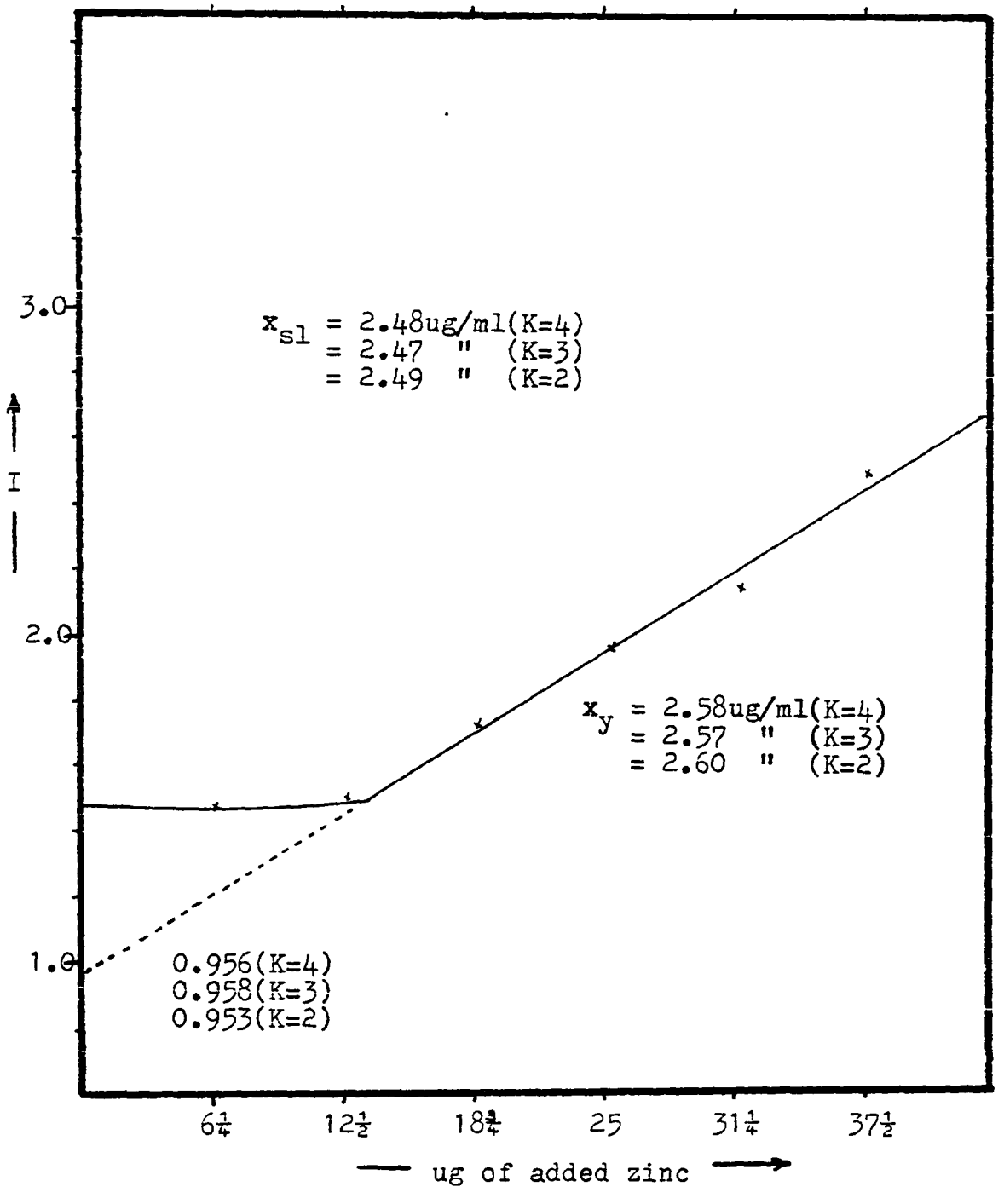

Figure B.3 Cases 2A and 2B, Run 3

Table B.4

Cases 2A and 2B, Run 4

Multiple Ks	SR < Kx SR > x + y → SR < x + y	SR = 1.05umole
x_{kn}	2.5ug/ml = 0.382umole/10ml	pH = 7.70
y	2.5ug/ml = 0.382umole/10ml	pH = 7.55
INTERCEPT = 1.05 1.06 1.09	x_y = 2.29ug/ml 2.27 " 2.11 "	x_{s1} = 2.41ug/ml 2.41 " 2.32 "

x(ml)	y(ml)	CORRECTED MIN. COUNT	I_2	I_3	I_4
40	0	12578	-	-	-
30	0	12155	-	-	-
20	0	12105	-	-	-
10	0	9249	1.309	1.314	1.360
10	½	9429	1.284	1.289	1.334
10	1	8685	1.394	1.400	1.448
10	1½	9760	1.240	1.245	1.289
10	2	9367	1.292	1.298	1.343
10	2½	8752	1.383	1.389	1.437
10	3	8942	1.354	1.359	1.407
10	5	7722	1.568	1.574	1.627
10	10	5794	2.089	2.098	2.171
10	15	4601	2.631	2.642	2.734
10	20	3932	3.079	3.091	3.199
10	25	3323	3.643	3.658	3.785
10	30	2902	4.170	4.188	4.333

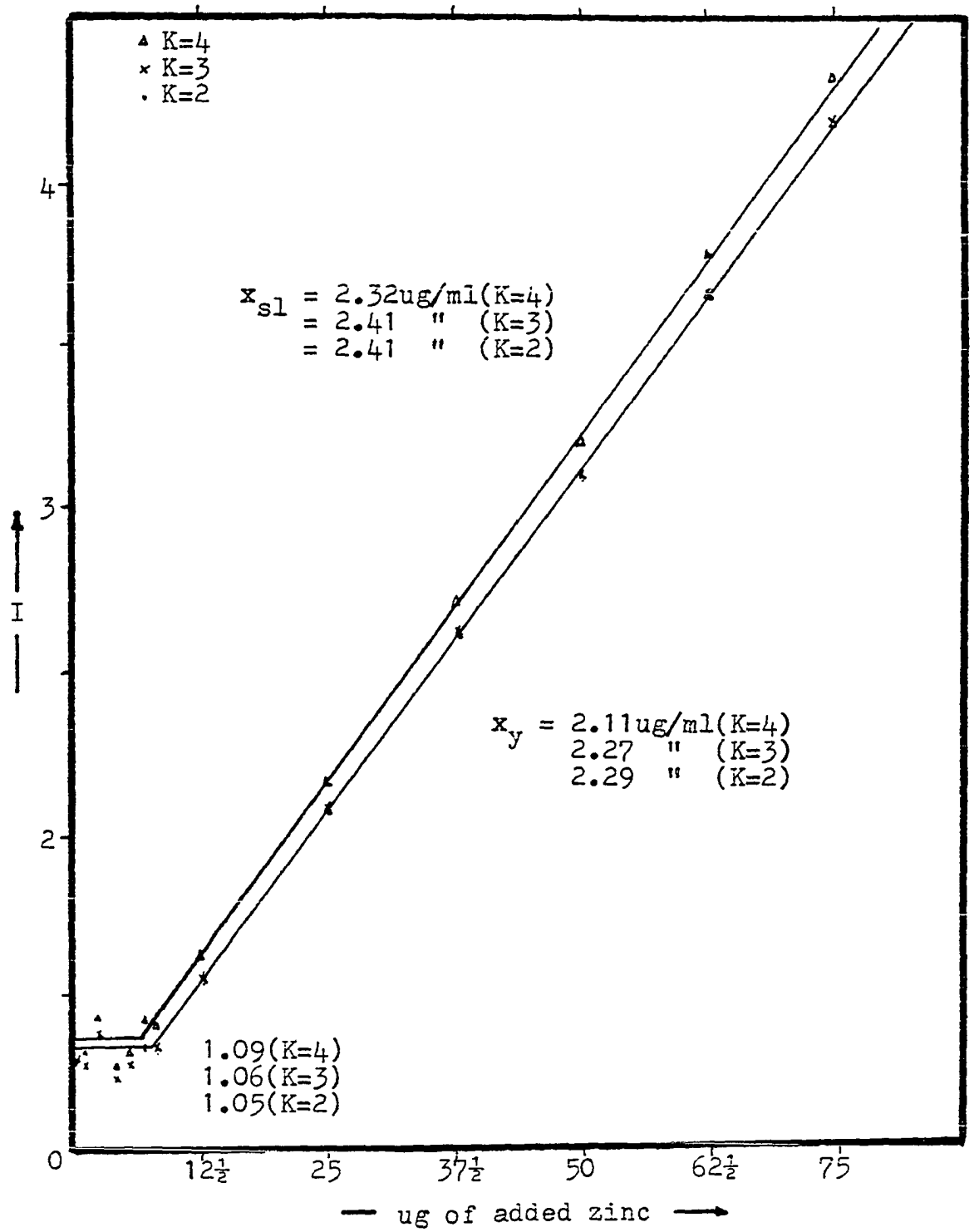

Figure B.4 Cases 2A and 2B, Run 4

APPENDIX C

STUDIES INVOLVING ALL FOUR CASES

Table C.1

Run 1 Involving all 4 Cases

Multiple Ks	SR > Kx → SR < Kx SR > x + y → SR < x + y				SR = 2.55umole	
x_{kn}	2.5ug/ml = 0.382umole/10ml				pH = 7.10	
y	12.5ug/ml = 1.91 umole/10ml				pH = 7.00	
INTERCEPT = 0.61 0.92 1.18 1.35	x_y = - - - 2.45ug/ml				x_{s1} = - - - 2.70ug/ml	
x(ml)	y(ml)	CORRECTED MIN.COUNT	I_2	I_3	I_4	I_5
50	0	25522	-	-	-	-
40	0	22230	-	-	-	-
30	0	17340	-	-	-	-
20	0	11470	-	-	-	-
10	0	5872	1.95	2.95	3.79	4.35
10	2½	6154	1.86	2.82	3.61	4.15
10	5	6154	1.86	2.82	3.61	4.15
10	7½	5285	2.17	3.28	4.21	4.83
10	10	4207	2.73	4.12	5.28	6.07
10	12½	3656	3.14	4.74	6.08	6.98
10	15	3044	3.77	5.70	7.30	8.39

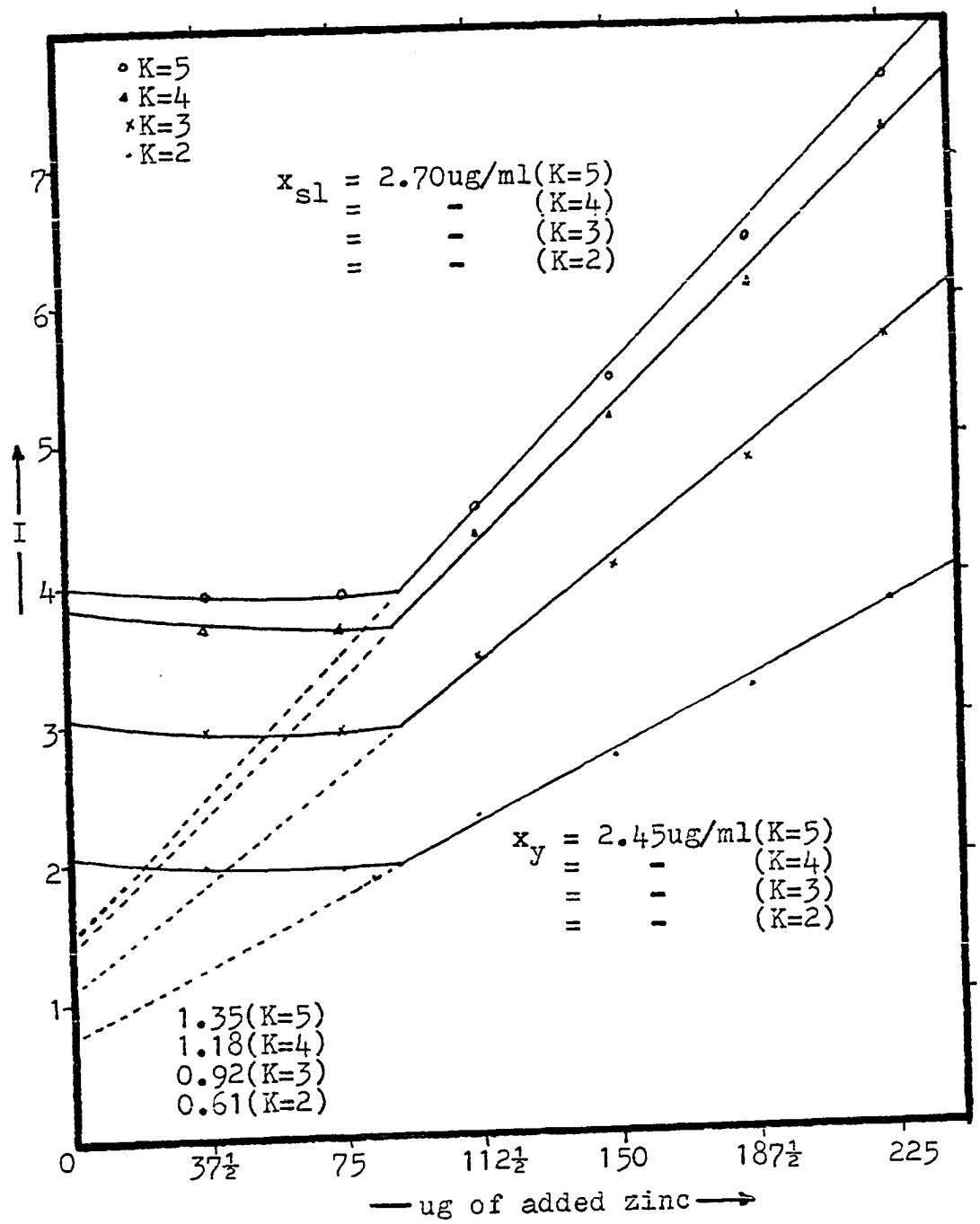

Figure C.1 Run 1 Involving all 4 Cases

Table C.2

Run 2 Involving all 4 Cases

Multiple Ks	SR > Kx → SR < Kx SR > x + y → SR < x + y				SR = 2.55umole	
x_{kn}	2.5ug/ml = 0.382umole/10ml				pH = 7.10	
y	12.5ug/ml = 1.91 umole/10ml				pH = 6.85	
INTERCEPT = 0.75 1.12 1.42 1.48	x_y = - - - 2.75ug/ml				x_{sl} = - - - 3.12ug/ml	
x(ml)	y(ml)	CORRECTED MIN.COUNT	I_2	I_3	I_4	I_5
50	0	29352	-	-	-	-
40	0	28008	-	-	-	-
30	0	22172	-	-	-	-
20	0	14812	-	-	-	-
10	0	7254	2.04	3.06	3.86	4.05
10	2½	7612	1.95	2.91	3.68	3.86
10	5	7624	1.94	2.91	3.67	3.85
10	7½	6448	2.30	3.44	4.34	4.55
10	10	5406	2.74	4.10	5.18	5.43
10	12½	4589	3.23	4.83	6.10	6.40
10	15	3880	3.82	5.71	7.22	7.57

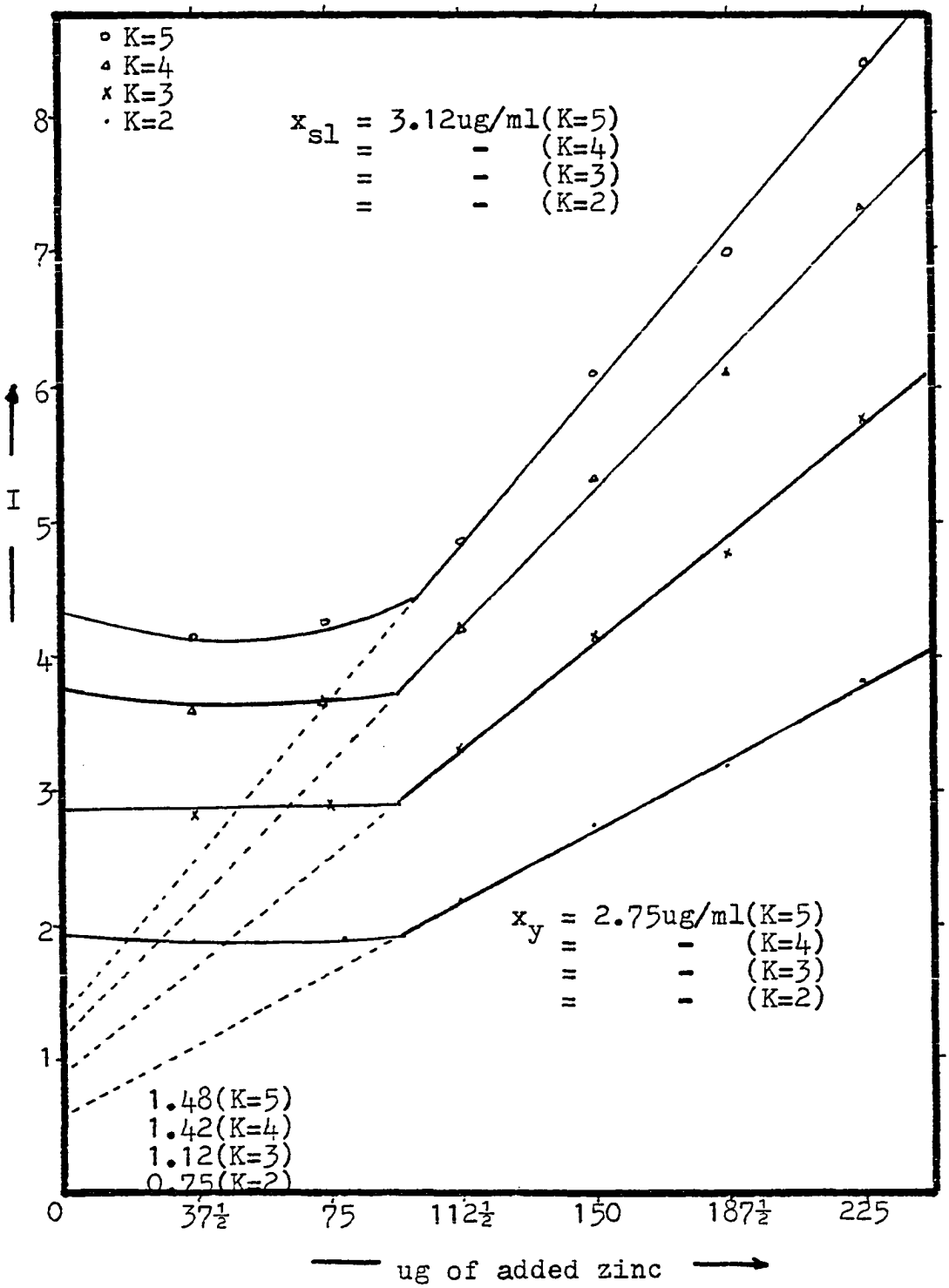

Figure C.2 Run 2 Involving all 4 Cases

Table C.3

Run 3 Involving all 4 Cases

Multiple Ks	SR > Kx → SR < Kx SR > x + y → SR < x + y				SR = 2.55umole	
x_{kn}	3.12ug/ml = 0.478umole/10ml				pH = 6.85	
y	12.5 ug/ml = 1.91 umole/10ml				pH = 7.05	
INTERCEPT = 0.77 1.19 1.31 1.34	x_y = - - 3.31ug/ml 3.29ug/ml			x_{sl} = - - 3.69ug/ml 3.60ug/ml		
x(ml)	y(ml)	CORRECTED MIN.COUNT	I_2	I_3	I_4	I_5
50	0	24997	-	-	-	-
40	0	24376	-	-	-	-
30	0	22202	-	-	-	-
20	0	14246	-	-	-	-
10	0	7298	1.95	3.04	3.34	3.42
10	2½	7448	1.91	2.98	3.27	3.36
10	5	8026	1.78	2.77	3.04	3.11
10	7½	6350	2.24	3.50	3.84	3.94
10	10	5175	2.75	4.29	4.71	4.83
10	12½	4511	3.16	4.92	5.40	5.54
10	15	3759	3.79	5.91	6.48	6.65

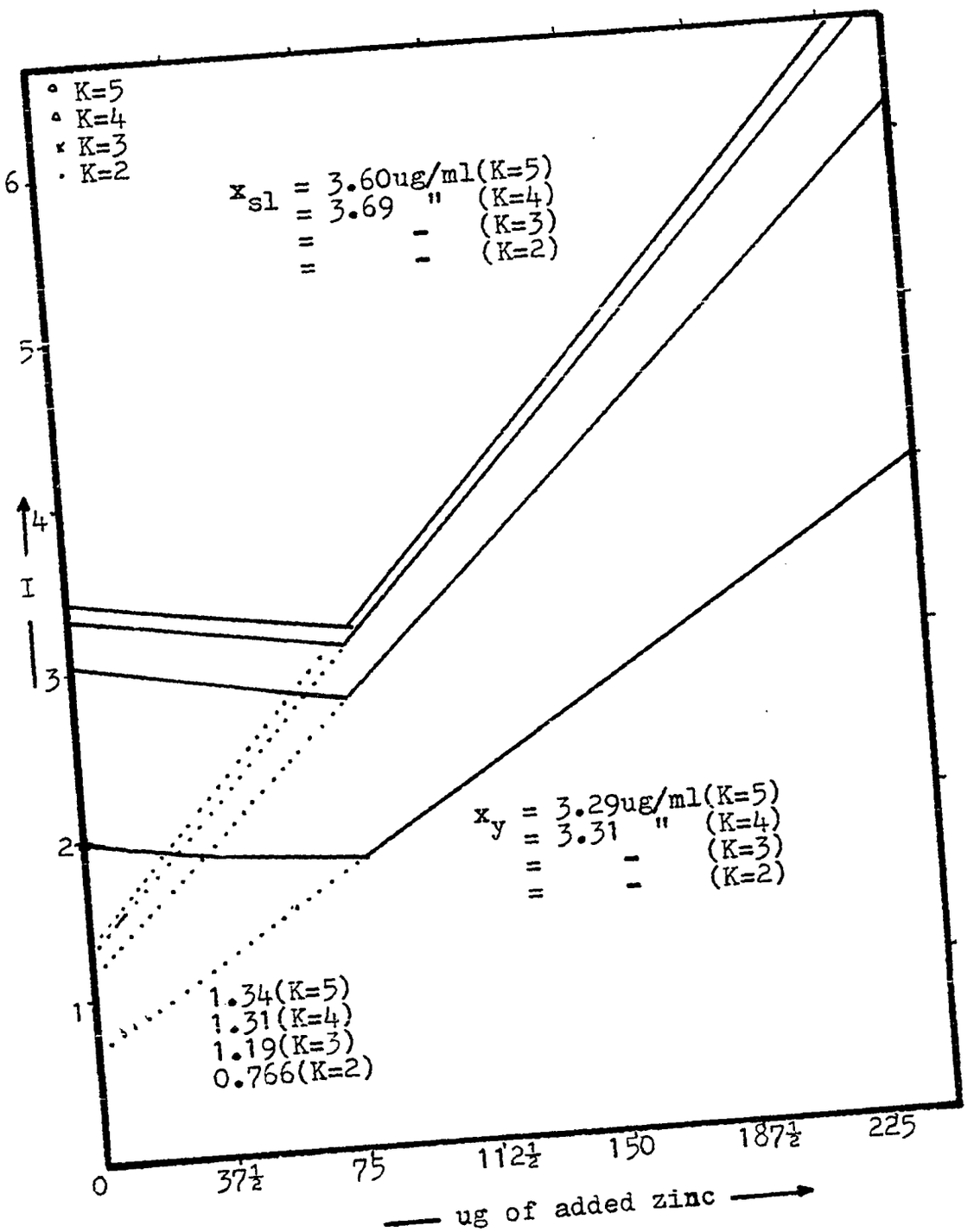

Figure C.3 Run 3 Involving all 4 Cases

Table C.4

Run 4 Involving all 4 Cases

Multiple Ks	SR > Kx ⟶ SR < Kx SR > x + y ⟶ SR < x + y				SR = 2.55umole	
x_{kn}	3.12ug/ml = 0.478umole/10ml				pH = 7.10	
y	12.5 ug/ml = 1.91 umole/10ml				pH = 6.90	
INTERCEPT =	0.77 1.18 1.36 1.38	x_y =	- - 3.13ug/ml 3.18ug/ml	x_{sl} =	- - 3.56ug/ml 3.52ug/ml	
x(ml)	y(ml)	CORRECTED MIN. COUNT	I_2	I_3	I_4	I_5
50	0	16647	-	-	-	-
40	0	16423	-	-	-	-
30	0	14236	-	-	-	-
20	0	9316	-	-	-	-
10	0	5004	1.86	2.84	3.28	3.33
10	2½	5022	1.86	2.83	3.27	3.32
10	5	5040	1.85	2.82	3.26	3.30
10	7½	4080	2.28	3.49	4.02	4.08
10	10	3594	2.59	3.96	4.57	4.63
10	12½	2884	3.23	4.94	5.69	5.77
10	15	2413	3.86	5.90	6.81	6.90

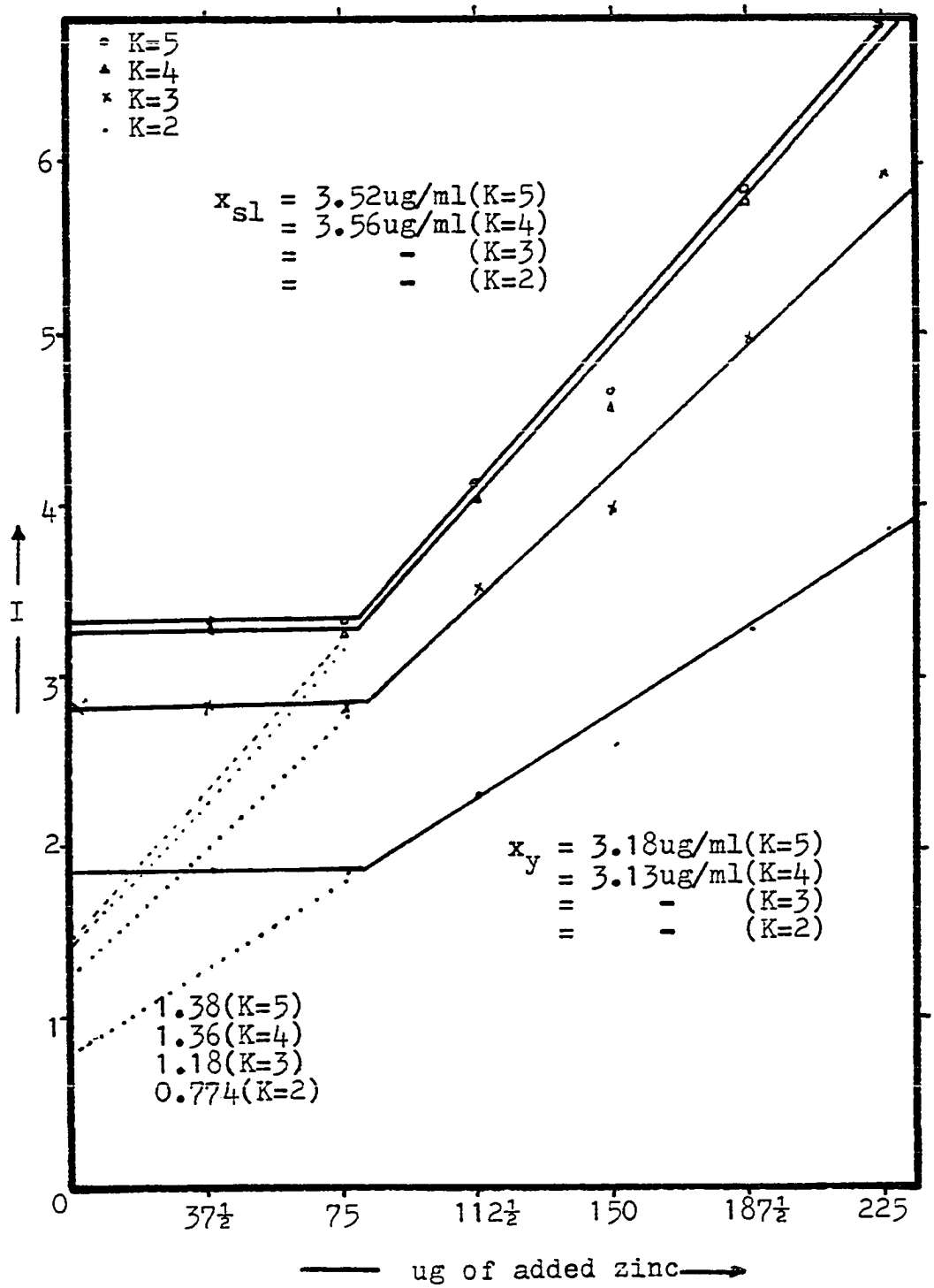

Figure C.4 Run 4 Involving all 4 Cases

APPENDIX D

REPRODUCIBILITY STUDY AT 10 µg/ml

Table D.1

First Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.97 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.50
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.25
INTERCEPT = 1.08	x_y = 9.74µg/ml	x_{sl} = 10.6µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	4276	-
10	1.48	0	0	4144	1.03
10	1.48	2	1.48	2098	2.04
10	1.48	4	2.97	1407	3.04
10	1.48	6	4.45	1111	3.85
10	1.48	8	5.94	861	4.97
10	1.48	10	7.42	745	5.74

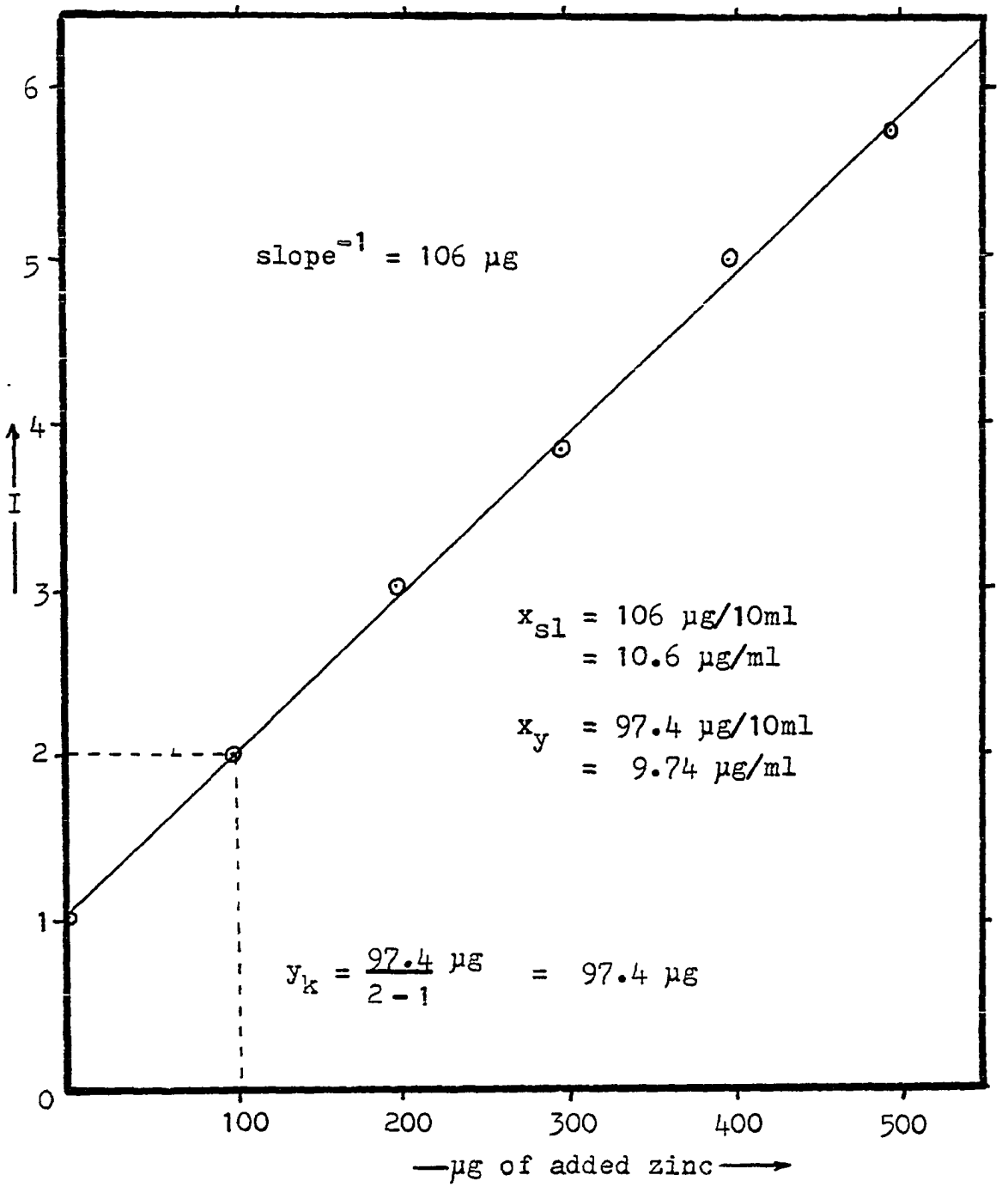

Figure D.1 First Reproducibility Study at 10 µg/ml

Table D.2

Second Reproduciblity Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.97 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.35
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.50

INTERCEPT = 1.12	x_y = 9.70µg/ml	x_{sl} = 11.0µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	5088	-
10	1.48	0	0	4781	1.06
10	1.48	2	1.48	2524	2.02
10	1.48	4	2.97	1684	3.02
10	1.48	6	4.45	1312	3.88
10	1.48	8	5.94	1073	4.74
10	1.48	10	7.42	910	5.59

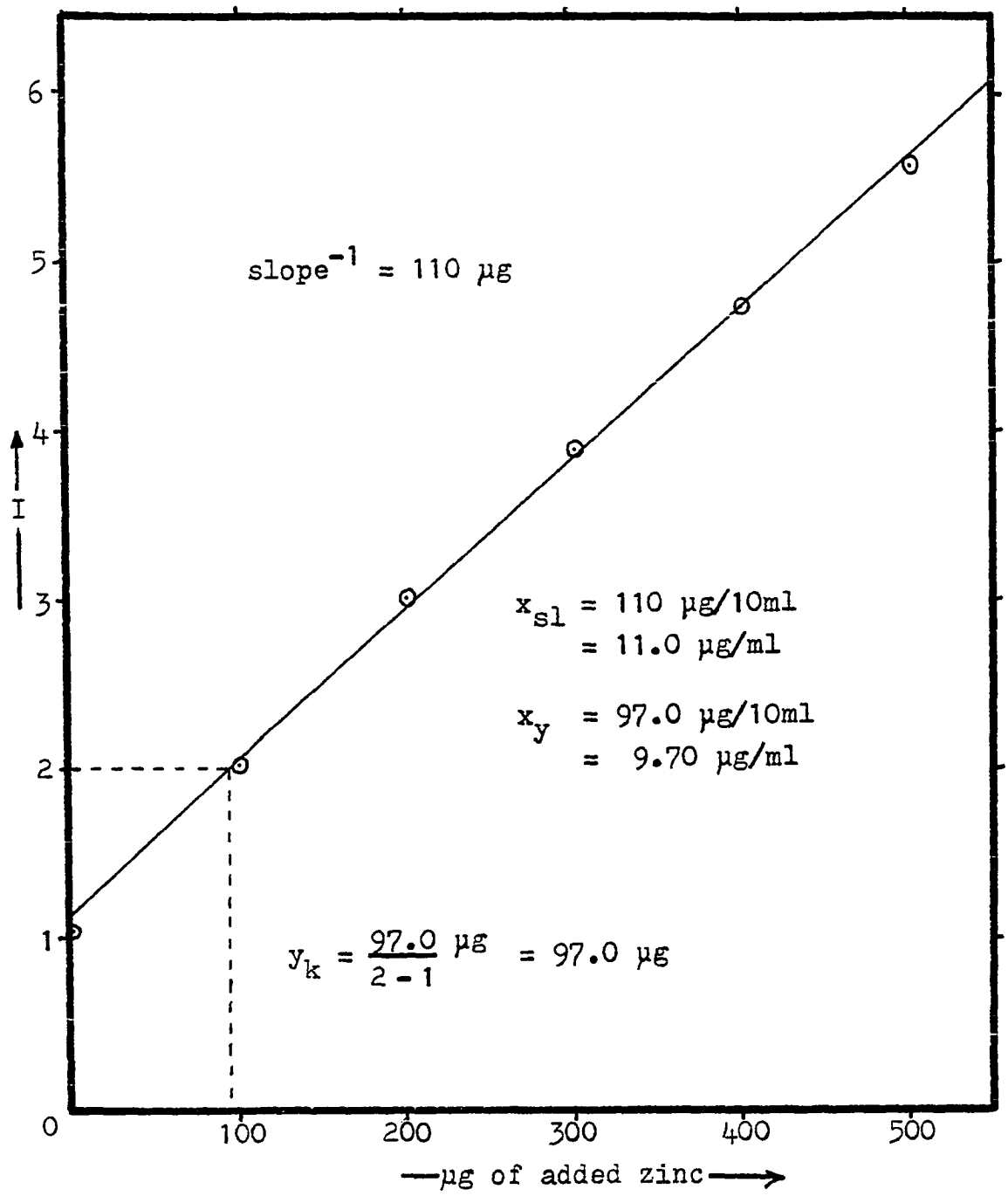

Figure D.2. Second Reproducibility Study at 10 µg/ml

Table D.3

Third Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.97 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 8.05
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.60
INTERCEPT = 1.06	x_y = 9.58µg/ml	x_{sl} = 10.2µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	4984	-
10	1.48	0	0	4808	1.04
10	1.48	2	1.48	2442	2.04
10	1.48	4	2.97	1654	3.01
10	1.48	6	4.45	1228	4.06
10	1.48	8	5.94	994	5.02
10	1.48	10	7.42	844	5.91

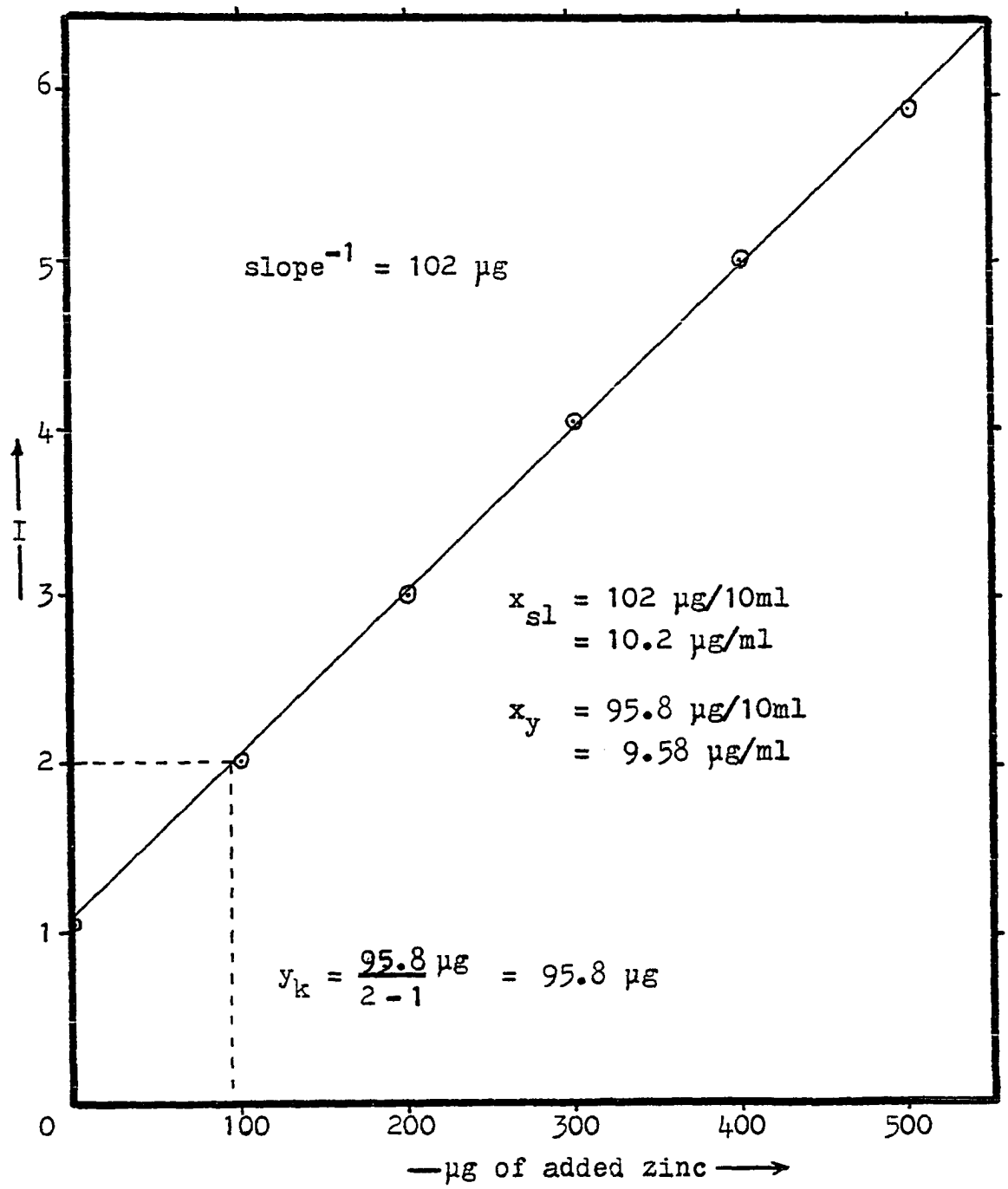

Figure D.3. Third Reproducibility Study at 10 µg/ml

Table D.4

Fourth Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.97 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.45
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.25
INTERCEPT = 1.04	x_y = 9.79µg/ml	x_{sl} = 10.2µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	4723	-
10	1.48	0	0	4742	1.00
10	1.48	2	1.48	2352	2.01
10	1.48	4	2.97	1554	3.04
10	1.48	6	4.45	1166	4.05
10	1.48	8	5.94	952	4.96
10	1.48	10	7.42	805	5.87

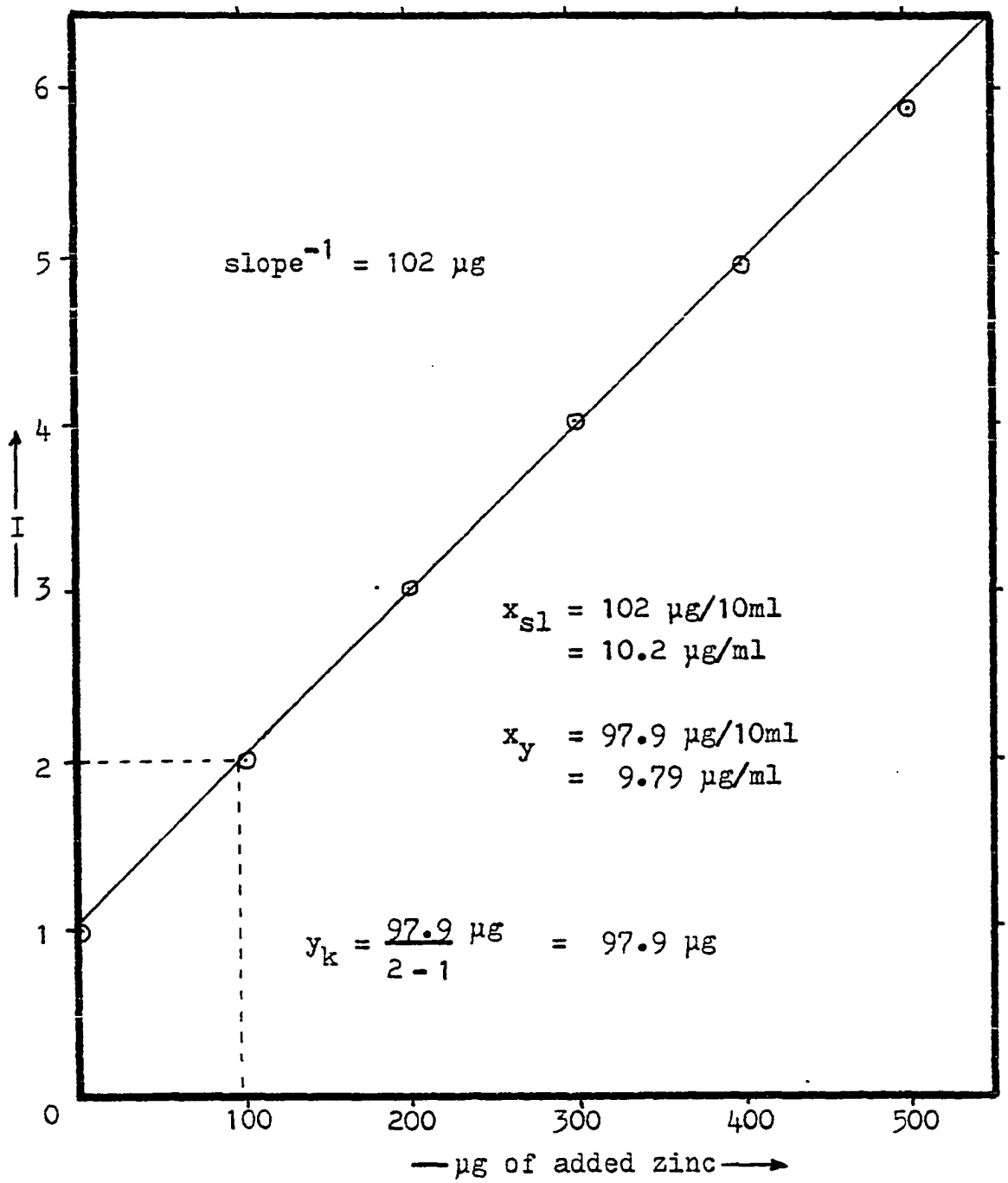

Figure D.4. Fourth Reproducibility Study at 10 µg/ml

Table D.5

Fifth Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.97 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.40
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.40
INTERCEPT = 1.06	x_y = 9.63µg/ml	x_{sl} = 10.3µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	4822	-
10	1.48	0	0	4731	1.02
10	1.48	2	1.48	2318	2.08
10	1.48	4	2.97	1640	2.94
10	1.48	6	4.45	1168	4.13
10	1.48	8	5.94	984	4.90
10	1.48	10	7.42	819	5.89

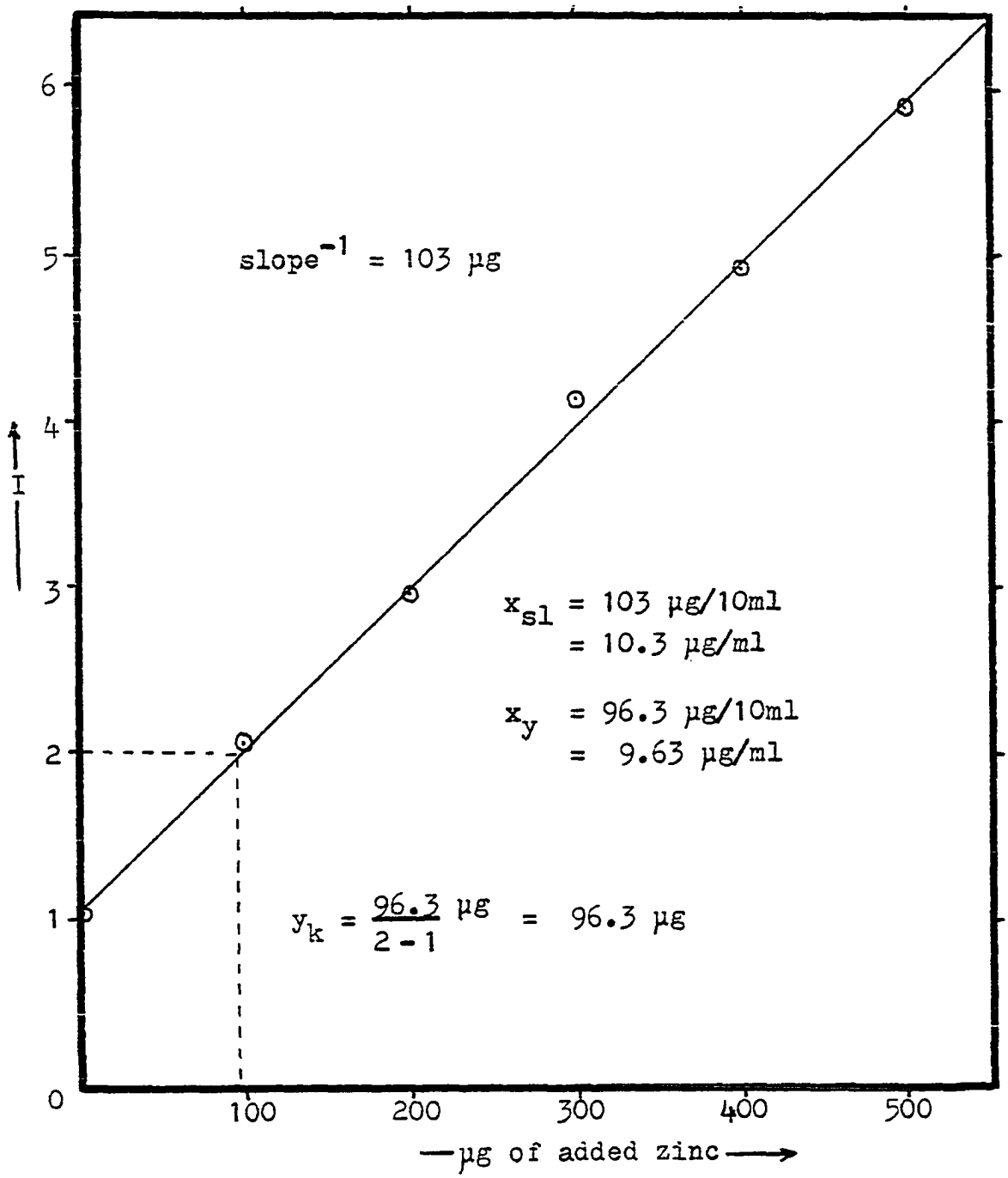

Figure D.5. Fifth Reproducibility Study at 10 µg/ml

Table D.6

Sixth Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.97 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.50
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.25
INTERCEPT = 1.00	x_y = 9.47µg/ml	x_{sl} = 9.44µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	4664	-
10	1.48	0	0	4526	1.03
10	1.48	2	1.48	2255	2.07
10	1.48	4	2.97	1538	3.03
10	1.48	6	4.45	1117	4.18
10	1.48	8	5.94	883	5.28
10	1.48	10	7.42	741	6.29

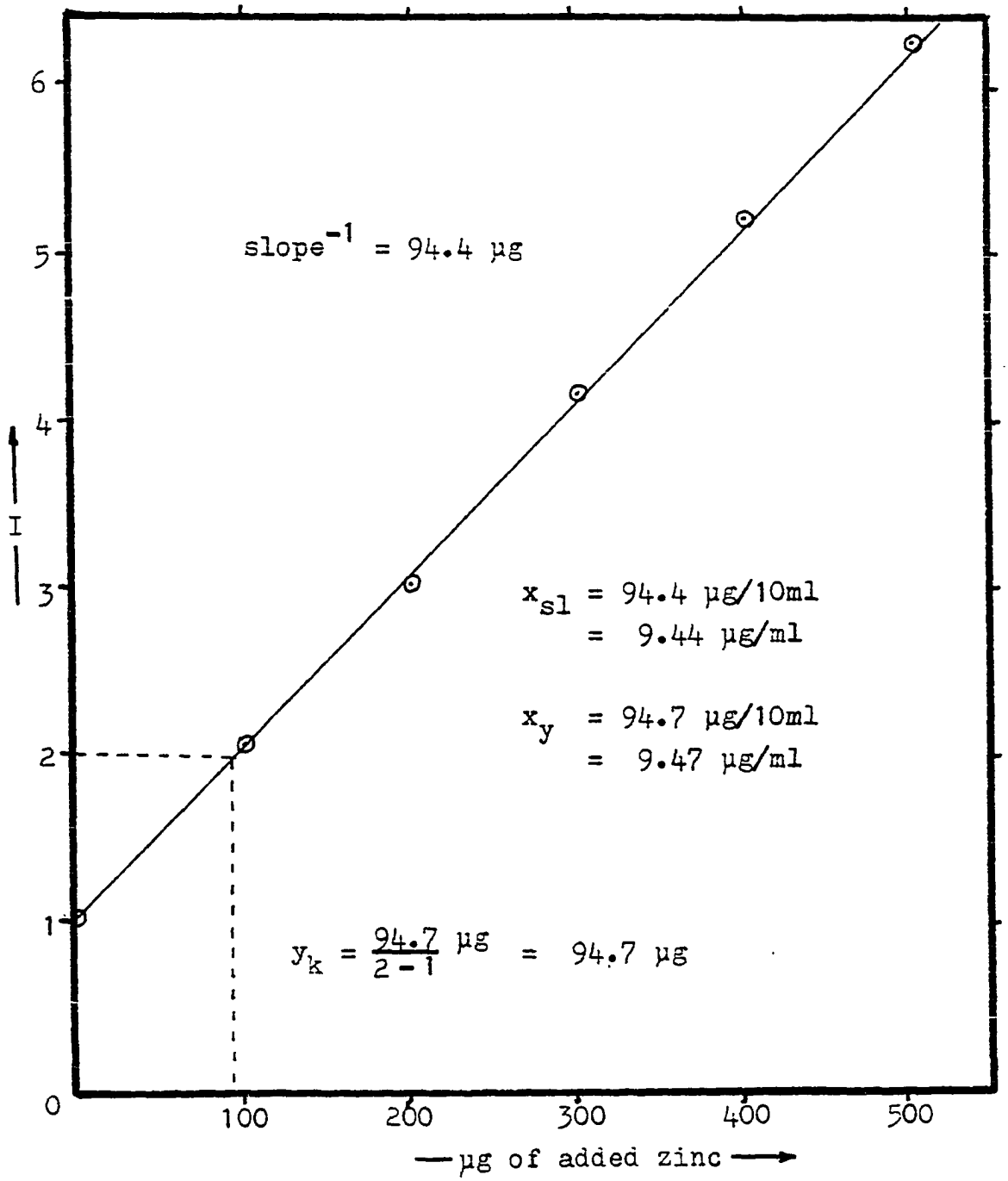

Figure D.6. Sixth Reproducibility Study at 10 µg/ml

Table D.7

Seventh Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.84 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.95
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.60
INTERCEPT = 1.05	x_y = 9.56µg/ml	x_{sl} = 10.1µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	6330	-
10	1.48	0	0	6302	1.00
10	1.48	2	1.48	3064	2.07
10	1.48	4	2.97	2052	3.08
10	1.48	6	4.45	1566	4.04
10	1.48	8	5.94	1264	5.01
10	1.48	10	7.42	1054	6.01

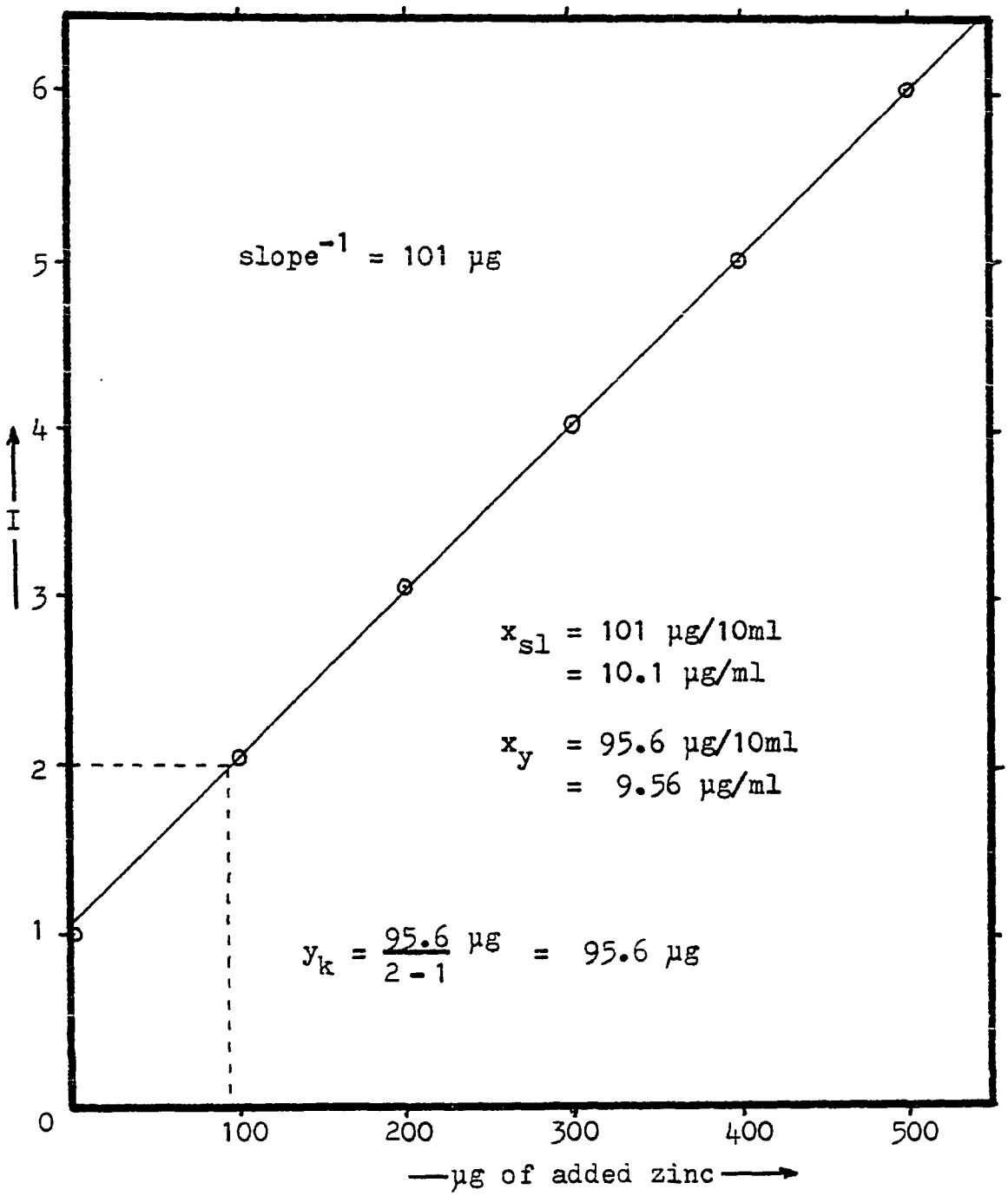

Figure D.7. Seventh Reproducibility Study at 10 µg/ml

Table D.8

Eighth Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.84 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.75
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.45
INTERCEPT = 1.02	x_y = 9.14µg/ml	x_{sl} = 9.34µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	5920	-
10	1.48	0	0	5838	1.01
10	1.48	2	1.48	2819	2.10
10	1.48	4	2.97	1880	3.15
10	1.48	6	4.45	1393	4.25
10	1.48	8	5.94	1115	5.31
10	1.48	10	7.42	930	6.36

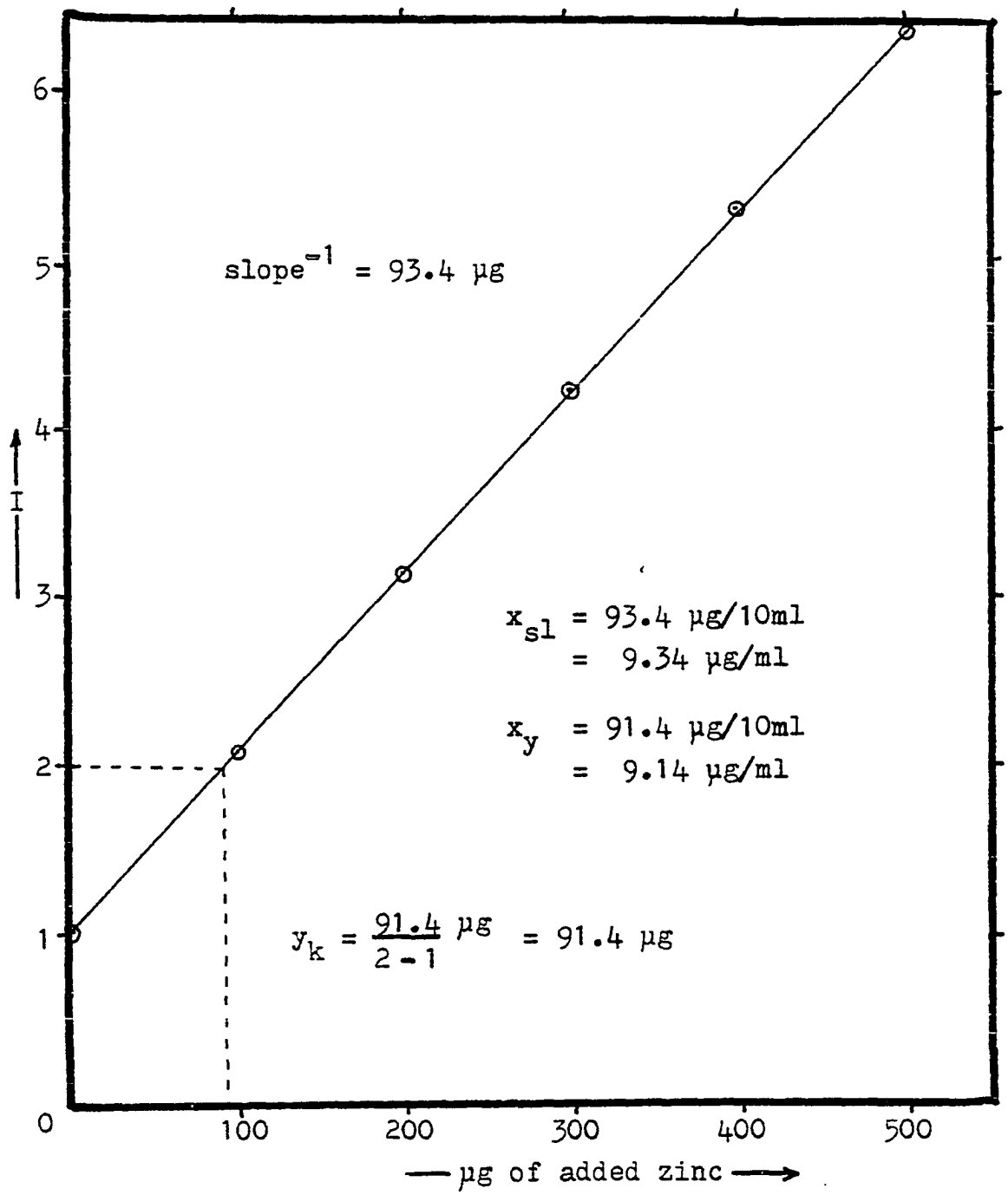

Figure D.8. Eighth Reproducibility Study at 10 µg/ml

Table D.9

Ninth Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.84 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 8.45
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.40
INTERCEPT = 0.90	x_y = 10.2µg/ml	x_{sl} = 9.32µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	5441	-
10	1.48	0	0	5151	0.90
10	1.48	2	1.48	2919	1.86
10	1.48	4	2.97	1761	3.09
10	1.48	6	4.45	1294	4.20
10	1.48	8	5.94	1059	5.14
10	1.48	10	7.42	868	6.27

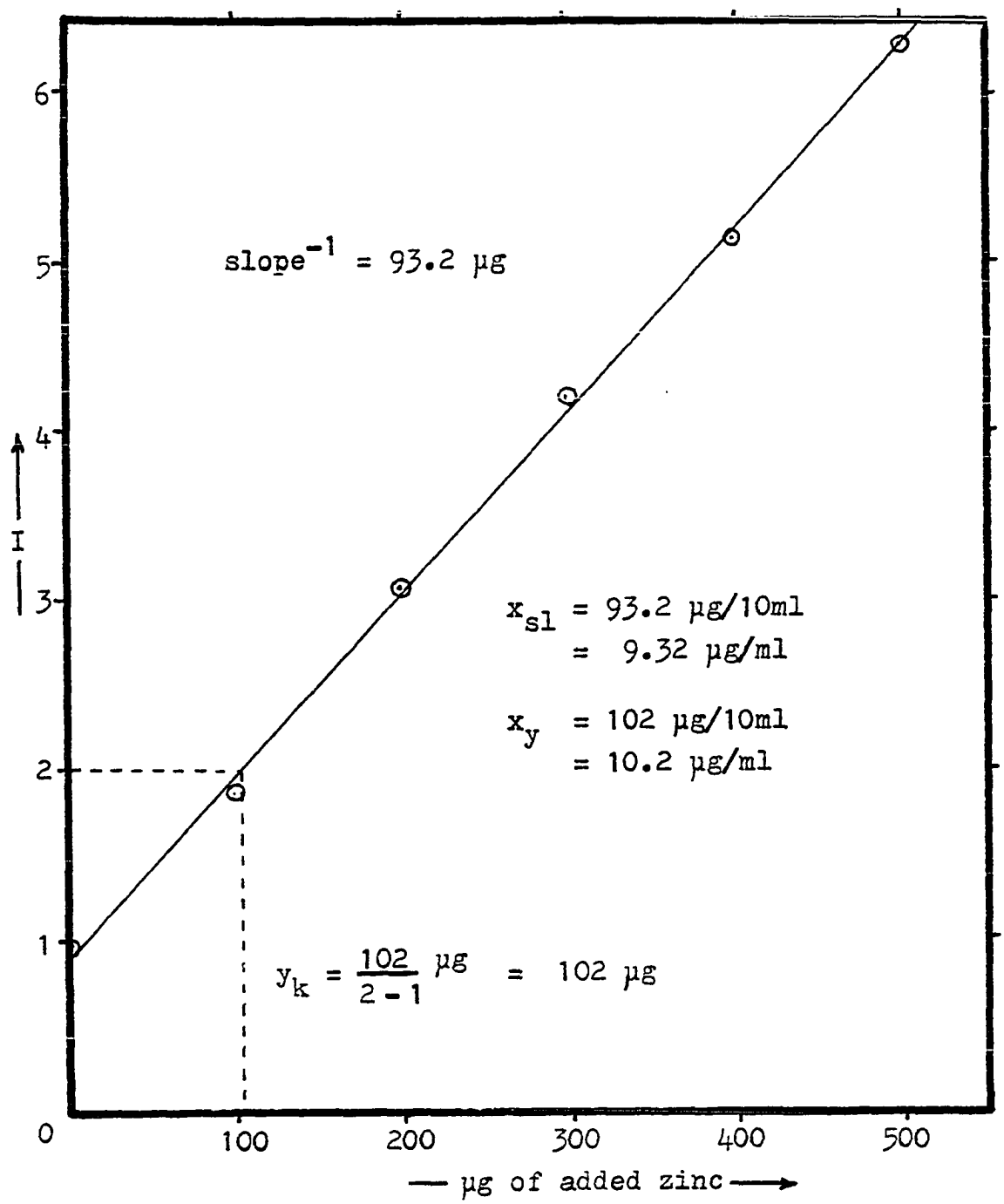

Figure D.9. Ninth Reproducibility Study at 10 µg/ml

Table D.10

Tenth Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 1.84 µmole
x_{kn}	9.70µg/ml = 1.48µmole/10ml	pH = 7.35
y	48.5µg/ml = 7.42µmole/10ml	pH = 7.25
INTERCEPT = 1.06	x_y = 10.6µg/ml	x_{sl} = 11.2µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	2.97	0	0	3677	-
10	1.48	0	0	3689	1.00
10	1.48	2	1.48	1878	1.96
10	1.48	4	2.97	1302	2.82
10	1.48	6	4.45	957	3.84
10	1.48	8	5.94	778	4.73
10	1.48	10	7.42	-	-

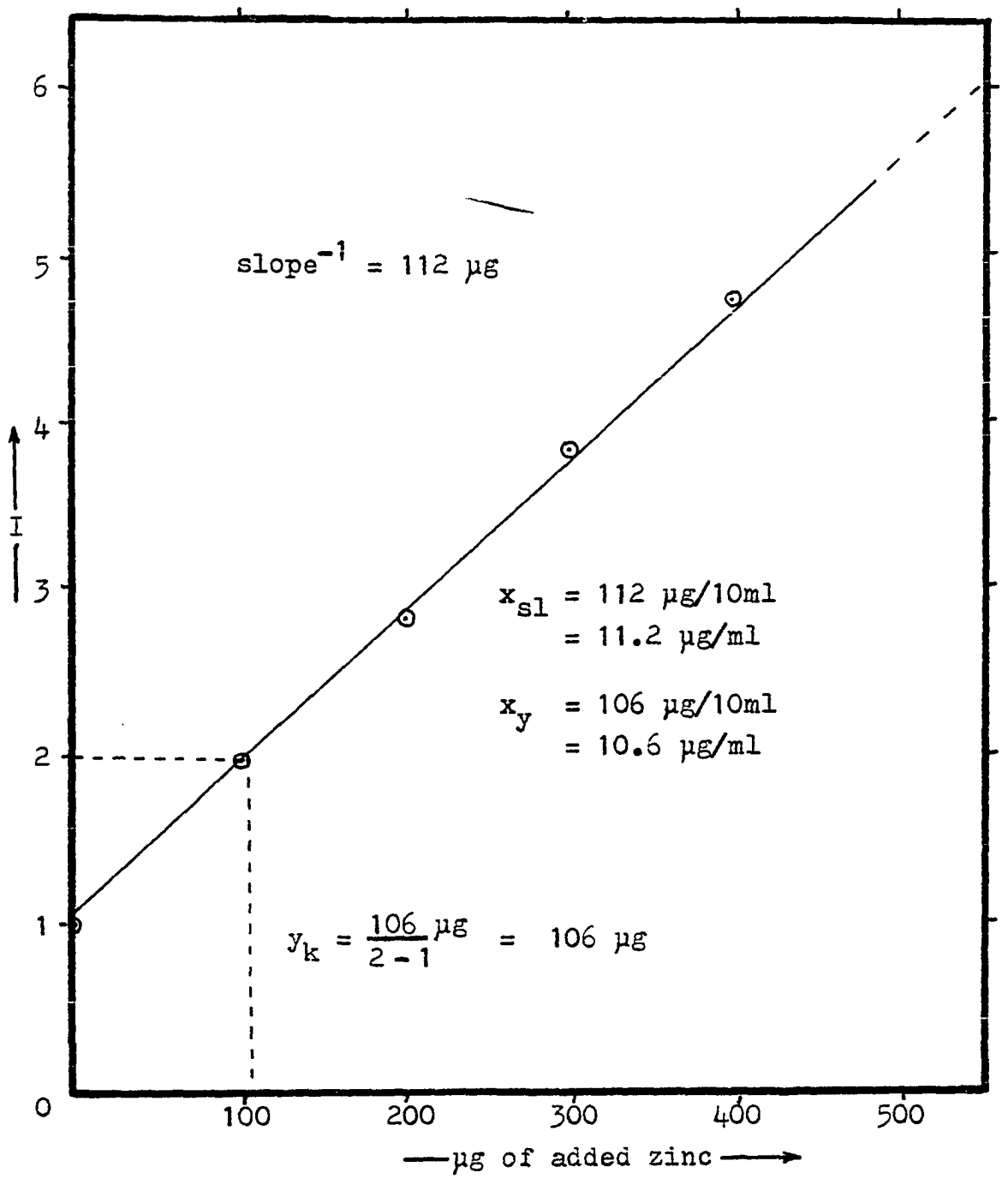

Figure D.10. Tenth Reproducibility Study at 10 µg/ml

APPENDIX E

REPRODUCIBILITY STUDY AT 1 µg/ml

Table E.1

First Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 7.60
y	5.0µg/ml = 0.77µmole/10ml	pH = 6.90
INTERCEPT = 1.10	x_y = 1.01µg/ml	x_{sl} = 1.12µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	7686	-
10	0.15	0	0	7270	1.06
10	0.15	2	0.15	3944	1.95
10	0.15	4	0.31	2539	3.03
10	0.15	6	0.46	1876	3.88
10	0.15	8	0.61	1626	4.47
10	0.15	10	0.77	1291	5.63

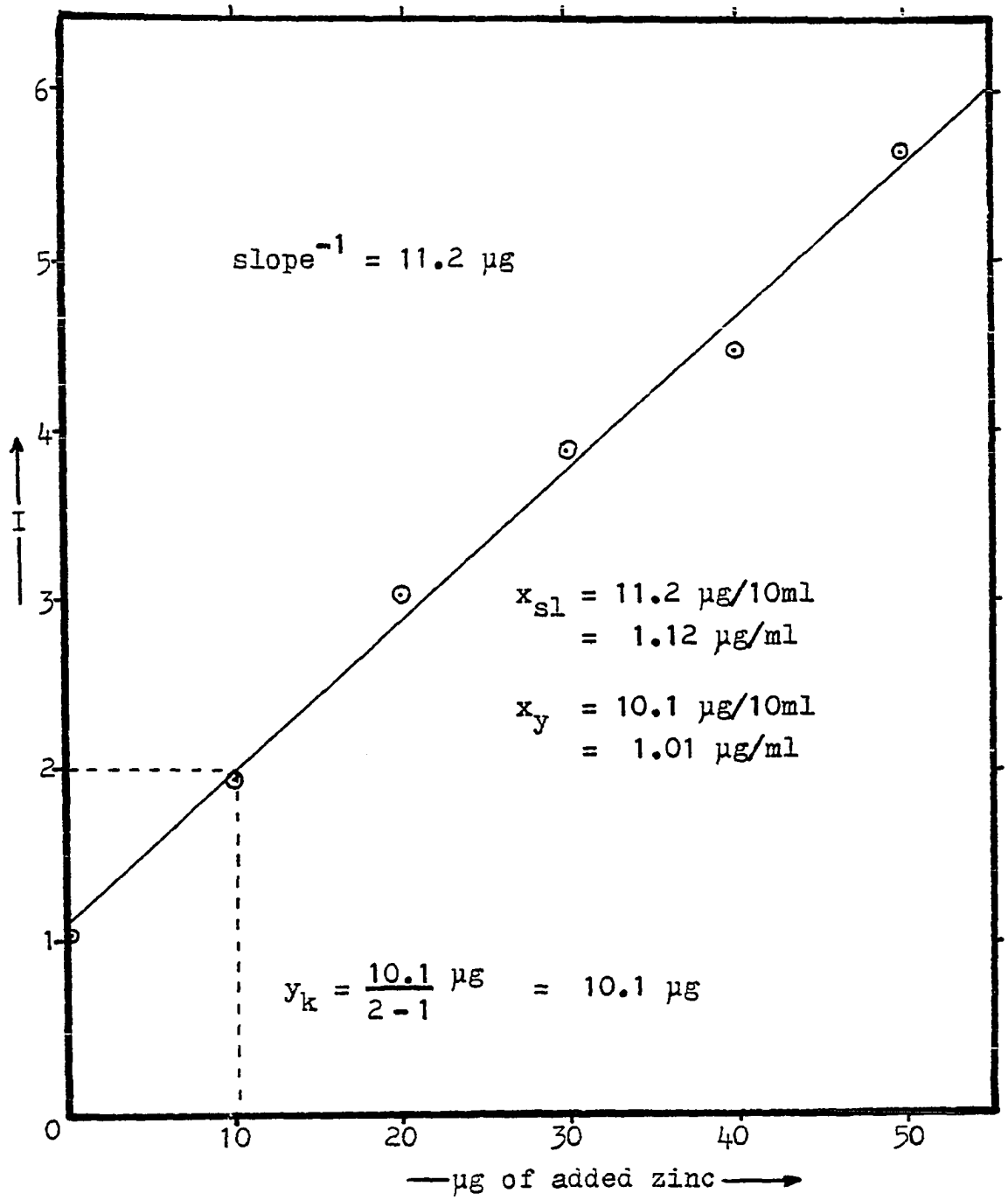

Figure E.1. First Reproducibility Study at 1 µg/ml

Table E.2

Second Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 7.00
y	5.0µg/ml = 0.77µmole/10ml	pH = 7.95

INTERCEPT = 1.06	x_y = 1.20µg/ml	x_{sl} = 1.27µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	5813	-
10	0.15	0	0	5672	1.02
10	0.15	2	0.15	3099	1.88
10	0.15	4	0.31	2202	2.64
10	0.15	6	0.46	1691	3.44
10	0.15	8	0.61	1403	4.14
10	0.15	10	0.77	1162	5.00

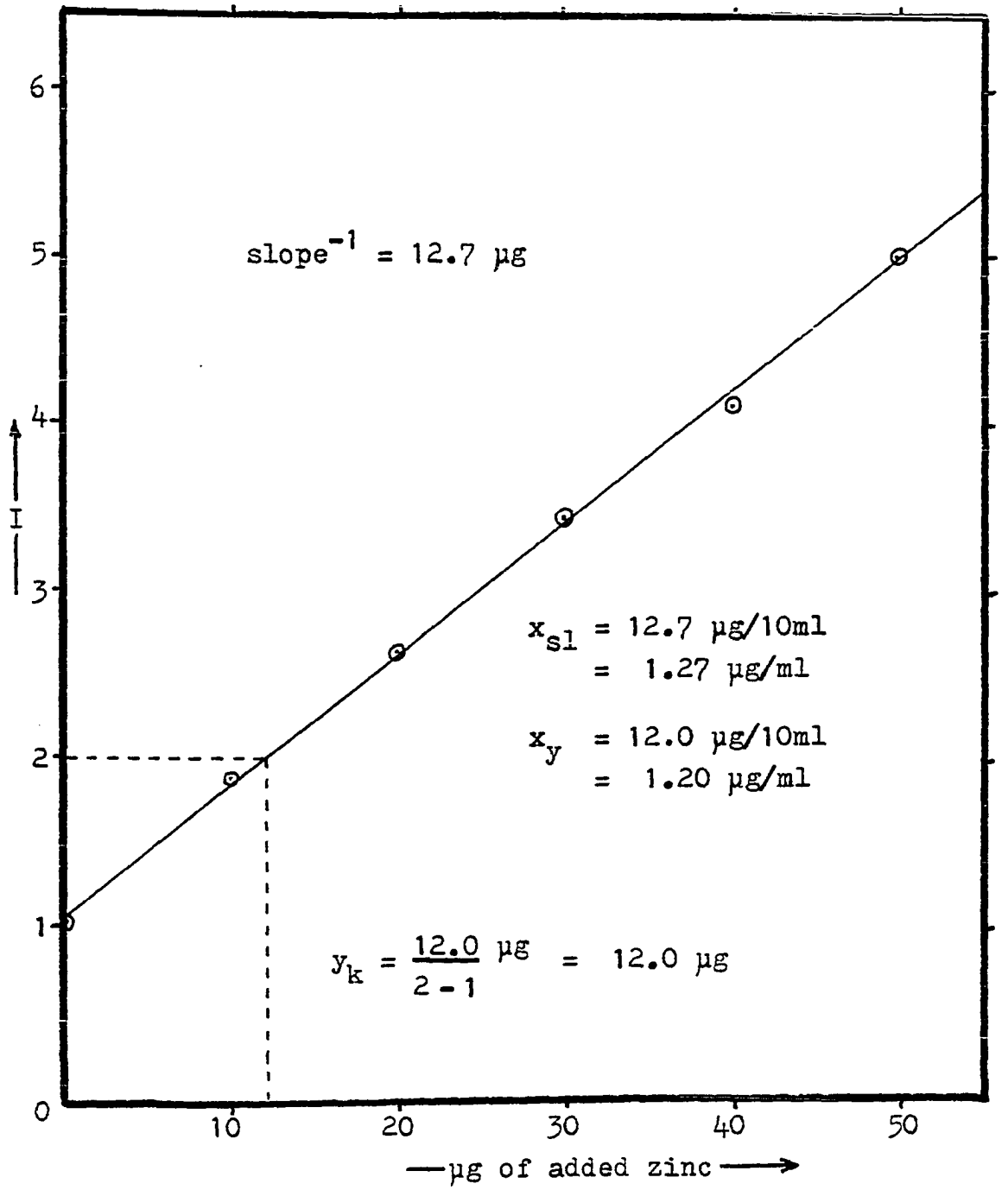

Figure E.2. Second Reproducibility Study at 1 µg/ml

Table E.3

Third Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 7.25
y	5.0µg/ml = 0.77µmole/10ml	pH = 7.20
INTERCEPT = 1.07	x_y = 1.05µg/ml	x_{sl} = 1.13µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	4670	-
10	0.15	0	0	4413	1.06
10	0.15	2	0.15	2401	1.94
10	0.15	4	0.31	1646	2.84
10	0.15	6	0.46	1223	3.82
10	0.15	8	0.61	1038	4.50
10	0.15	10	0.77	845	5.53

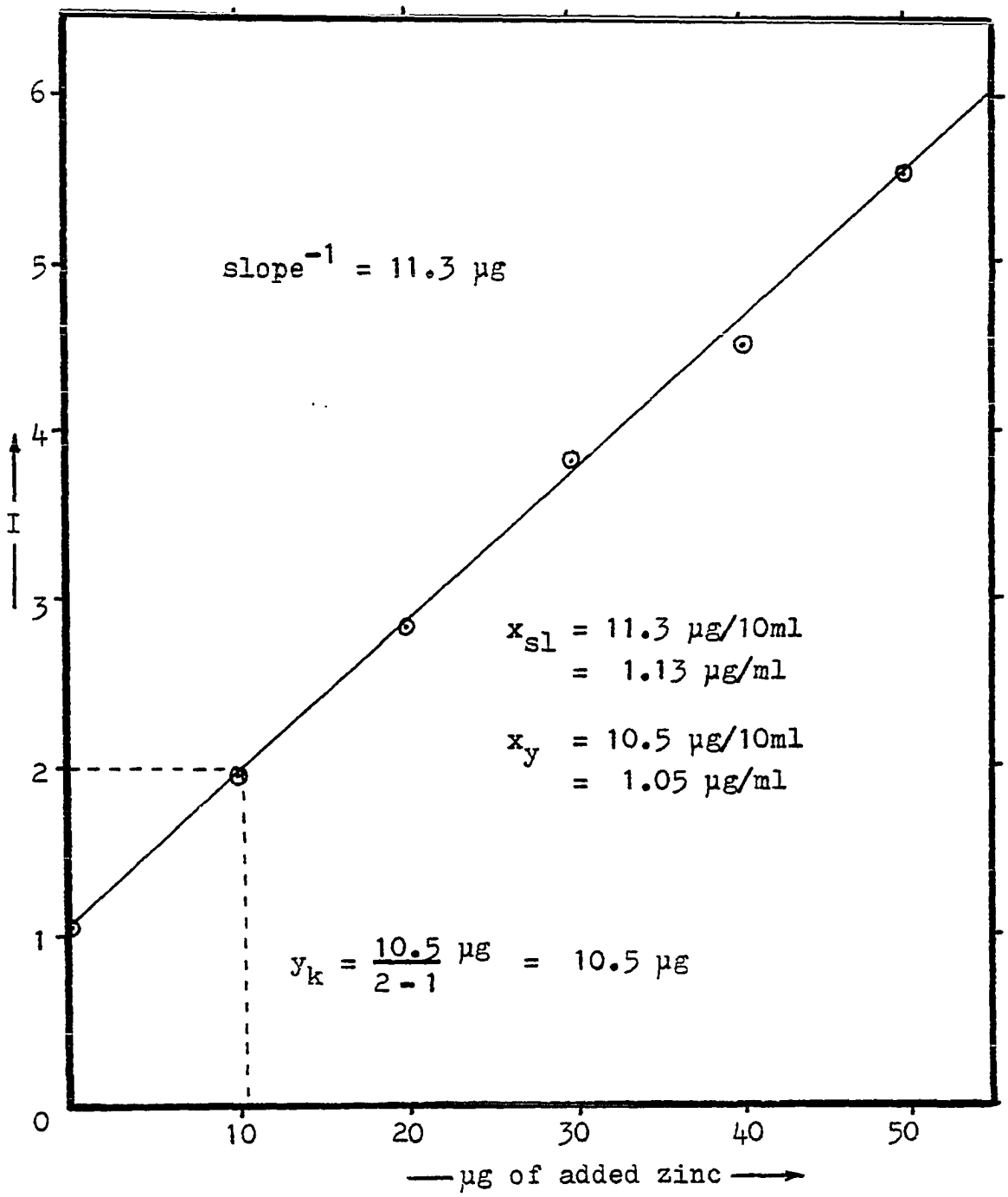

Figure E.3. Third Reproducibility Study at 1 µg/ml

Table E.4

Fourth Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 7.35
y	5.0µg/ml = 0.77µmole/10ml	pH = 6.85
INTERCEPT = 1.20	x_y = 0.91µg/ml	x_{sl} = 1.14µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	5046	-
10	0.15	0	0	4553	1.11
10	0.15	2	0.15	2467	2.04
10	0.15	4	0.31	1686	2.99
10	0.15	6	0.46	1242	4.06
10	0.15	8	0.61	1048	4.81
10	0.15	10	0.77	938	5.39

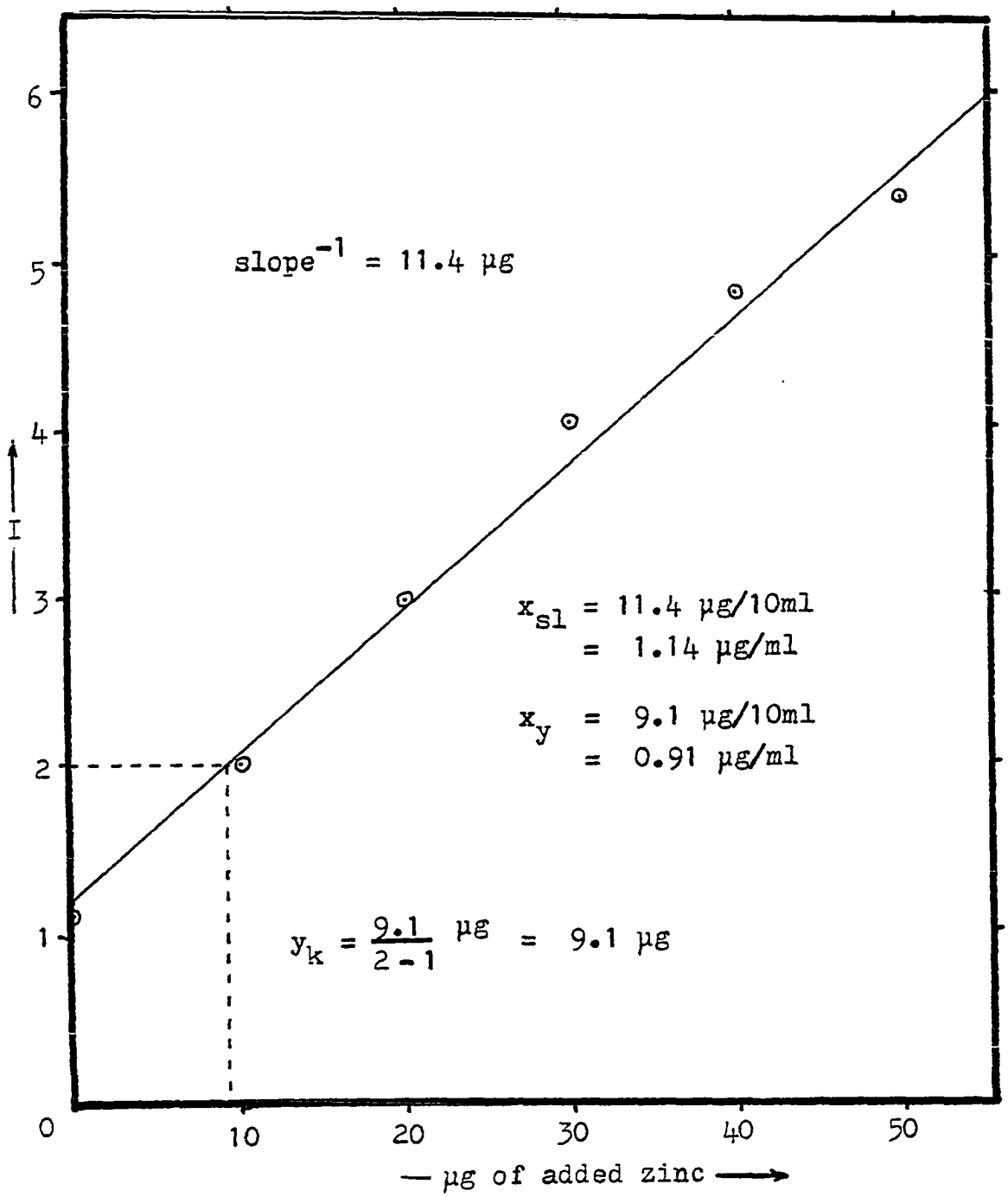

Figure E.4. Fourth Reproducibility Study at 1 µg/ml

Table E.5

Fifth Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 7.80
y	5.0µg/ml = 0.77µmole/10ml	pH = 7.10
INTERCEPT = 1.05	x_y = 1.02µg/ml	x_{sl} = 1.08µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	7522	-
10	0.15	0	0	7475	1.01
10	0.15	2	0.15	3699	2.03
10	0.15	4	0.31	2613	2.88
10	0.15	6	0.49	1992	3.78
10	0.15	8	0.61	1514	4.97
10	0.15	10	0.77	1353	5.56

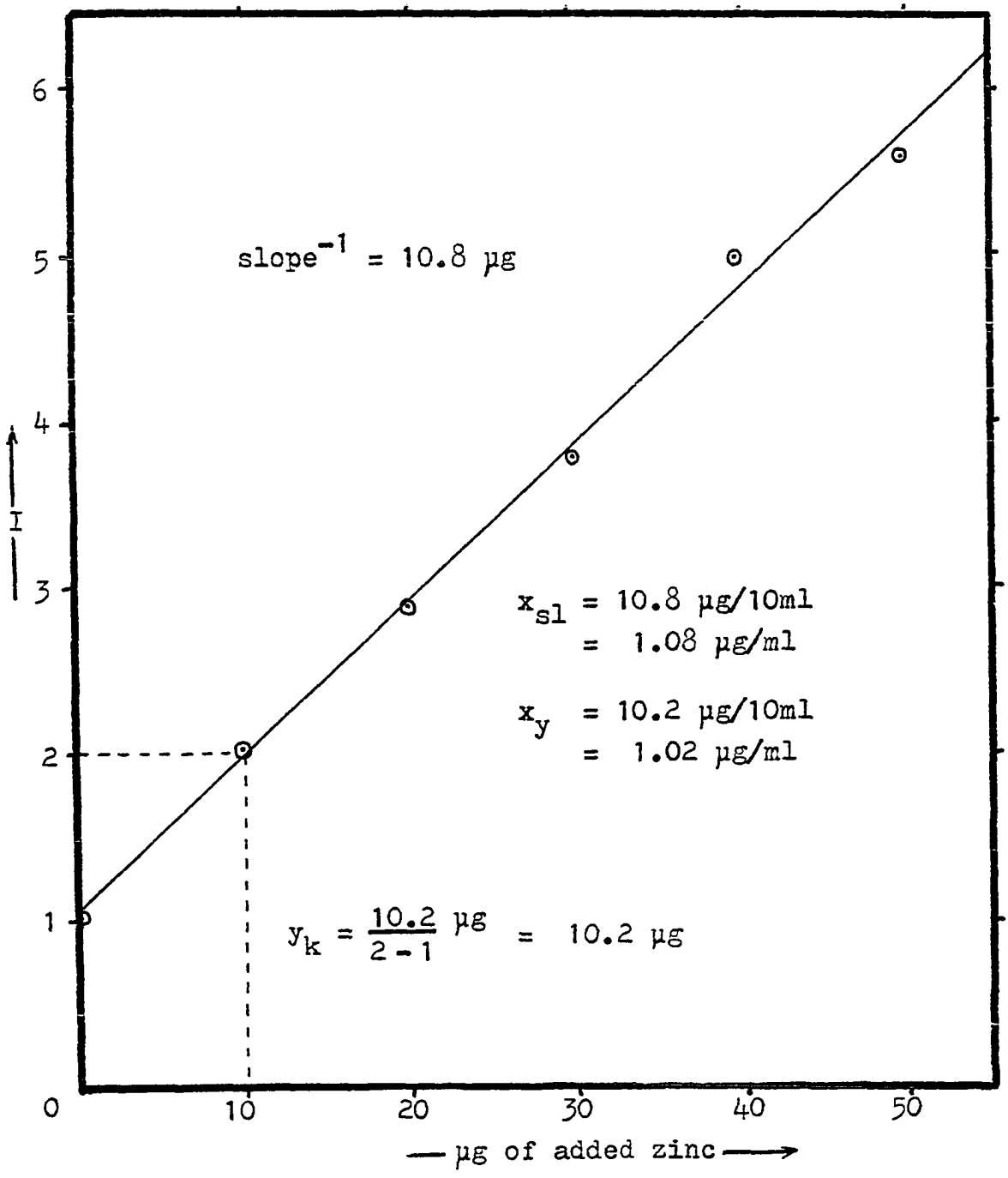

Figure E.5. Fifth Reproducibility Study at 1 µg/ml

Table E.6

Sixth Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 7.20
y	5.0µg/ml = 0.77µmole/10ml	pH = 6.95
INTERCEPT = 1.10	x_y = 1.04µg/ml	x_{sl} = 1.15µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	6306	-
10	0.15	0	0	5736	1.10
10	0.15	2	0.15	3184	1.98
10	0.15	4	0.31	2222	2.84
10	0.15	6	0.46	1670	3.78
10	0.15	8	0.61	1425	4.42
10	0.15	10	0.77	1136	5.55

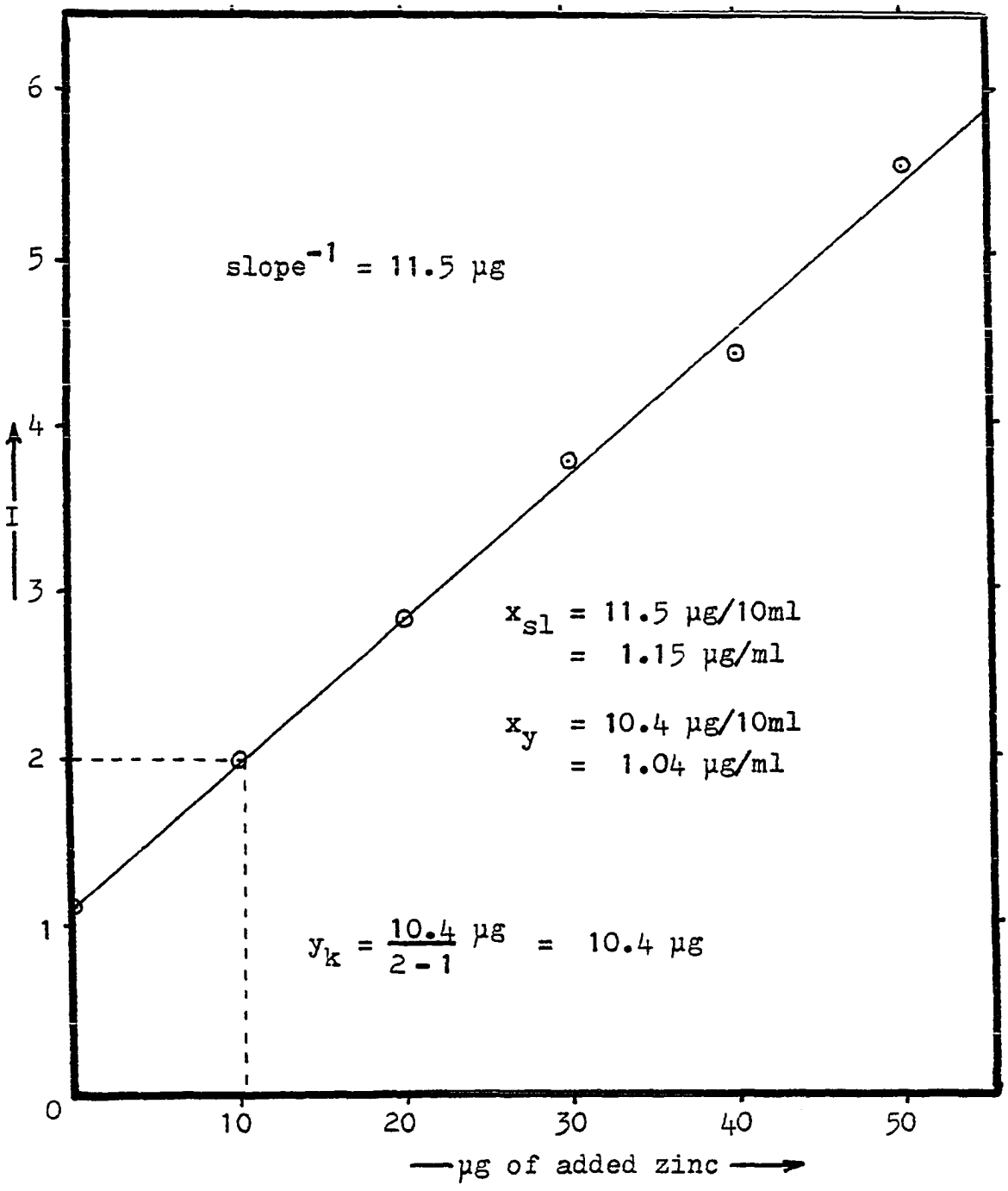

Figure E.6. Sixth Reproducibility Study at 1 µg/ml

Table E.7

Seventh Reproducibility Study at 10 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 6.85
y	5.0µg/ml = 0.77µmole/10ml	pH = 7.05
INTERCEPT = 1.03	x_y = 1.12µg/ml	x_{sl} = 1.15µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	7653	-
10	0.15	0	0	7530	1.02
10	0.15	2	0.15	3939	1.94
10	0.15	4	0.31	2777	2.76
10	0.15	6	0.46	2091	3.66
10	0.15	8	0.61	1738	4.40
10	0.15	10	0.77	1401	5.46

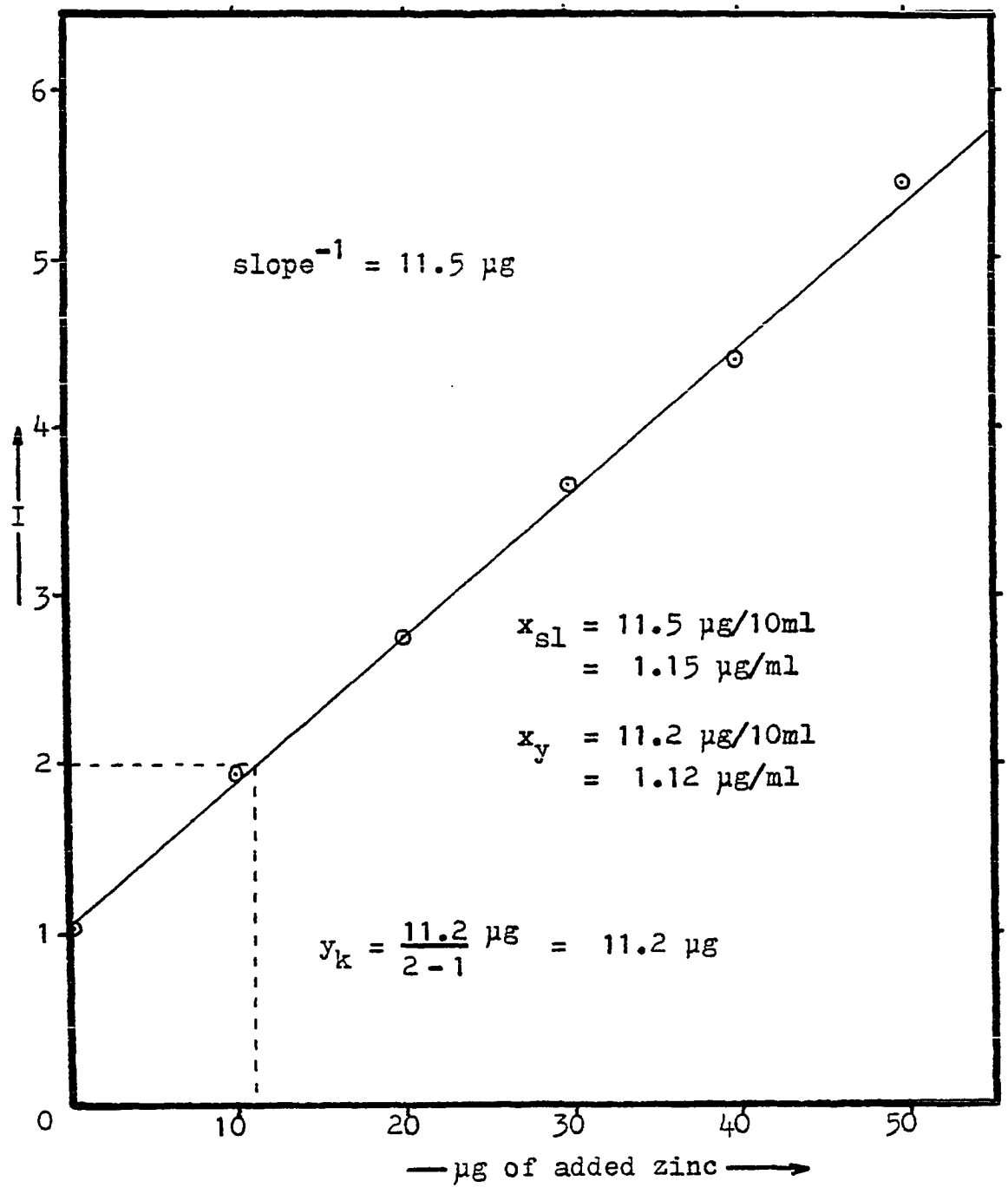

Figure E.7. Seventh Reproducibility Study at 1 µg/ml

Table E.8

Eighth Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 6.90
y	5.0µg/ml = 0.77µmole/10ml	pH = 7.15
INTERCEPT = 1.03	x_y = 1.20µg/ml	x_{sl} = 1.23µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	7189	-
10	0.15	0	0	7026	1.02
10	0.15	2	0.15	4000	1.80
10	0.15	4	0.31	2676	2.69
10	0.15	6	0.46	2056	3.50
10	0.15	8	0.61	1658	4.34
10	0.15	10	0.77	1426	5.04

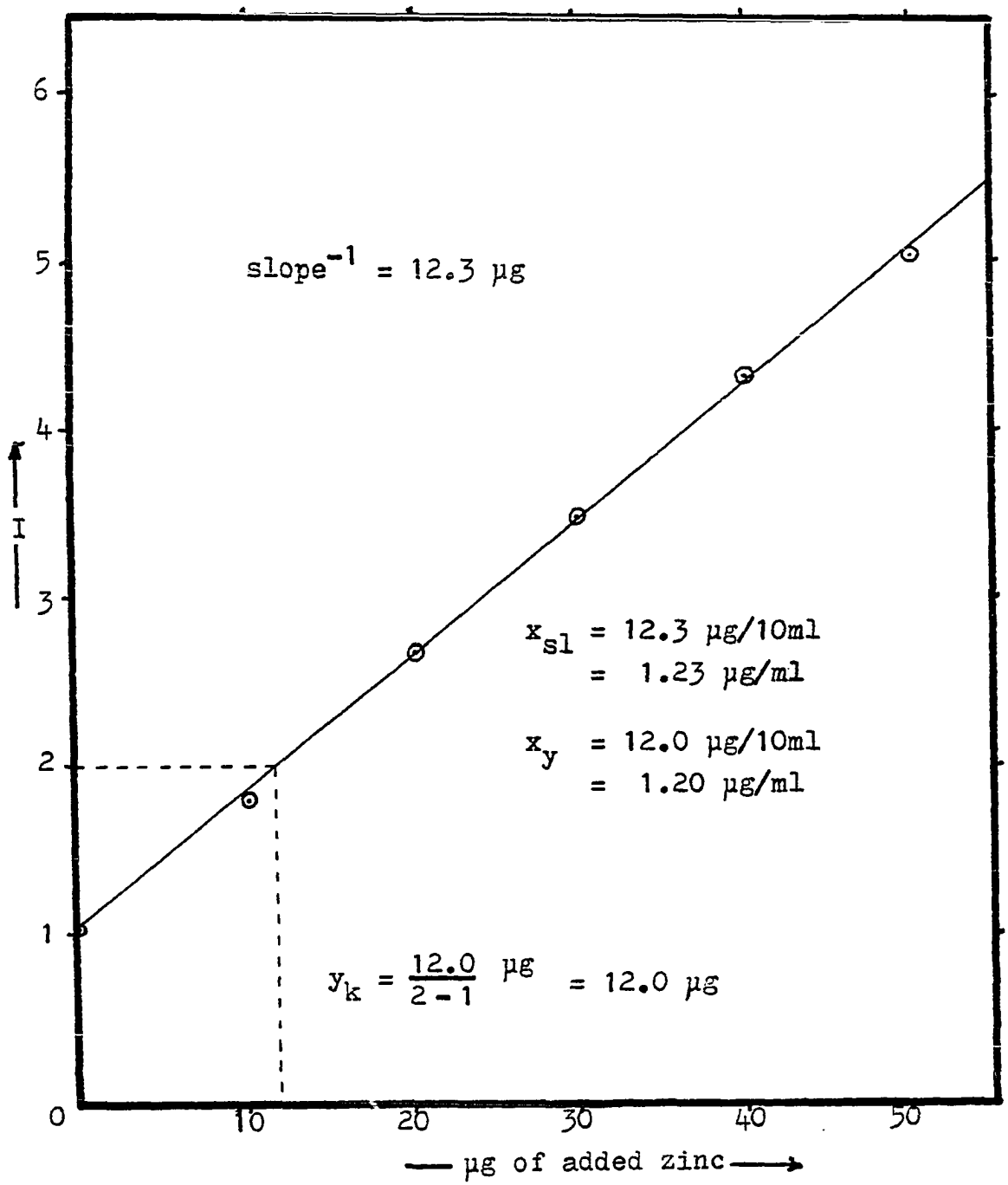

Figure E.8. Eighth Reproducibility Study at 1 µg/ml

Table E.9

Ninth Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 7.10
y	5.0µg/ml = 0.77µmole/10ml	pH = 7.15
INTERCEPT = 1.13	x_y = 0.98µg/ml	x_{sl}= 1.12µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	6218	-
10	0.15	0	0	5691	1.09
10	0.15	2	0.15	3033	2.05
10	0.15	4	0.31	2107	2.95
10	0.15	6	0.46	1654	3.76
10	0.15	8	0.61	1317	4.72
10	0.15	10	0.77	1120	5.55

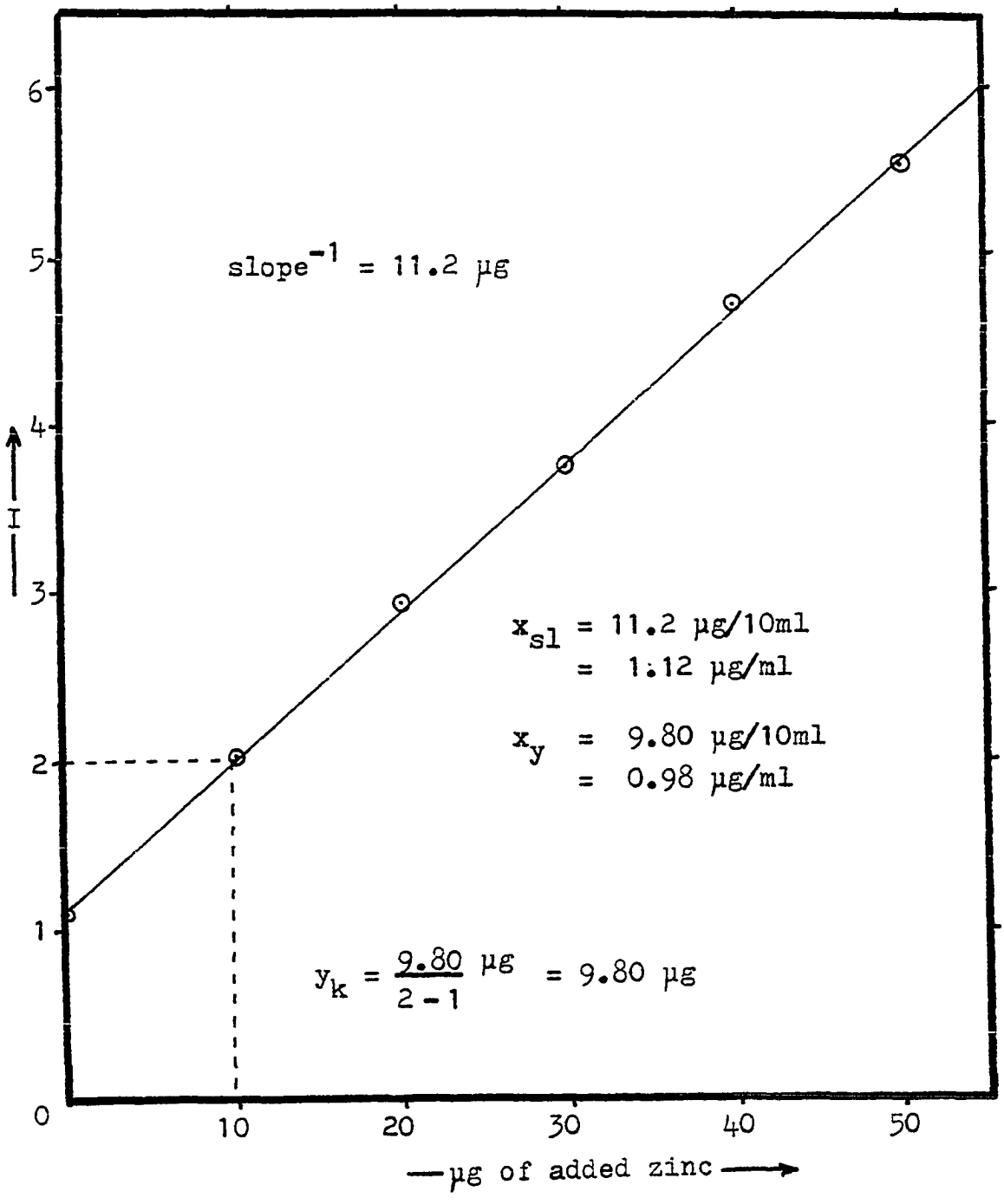

Figure E.9. Ninth Reproducibility Study at 1 µg/ml

Table E.10

Tenth Reproducibility Study at 1 µg/ml

K = 2	Kx > SR	SR = 0.19 µmole
x_{kn}	1.0µg/ml = 0.15µmole/10ml	pH = 6.90
y	5.0µg/ml = 0.77µmole/10ml	pH = 6.90
INTERCEPT = 1.13	x_y = 1.01µg/ml	x_{sl} = 1.16µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.31	0	0	7964	-
10	0.15	0	0	7162	1.11
10	0.15	2	0.15	3946	2.02
10	0.15	4	0.31	2802	2.84
10	0.15	6	0.46	2110	3.78
10	0.15	8	0.61	1806	4.41
10	0.15	10	0.77	1441	5.51

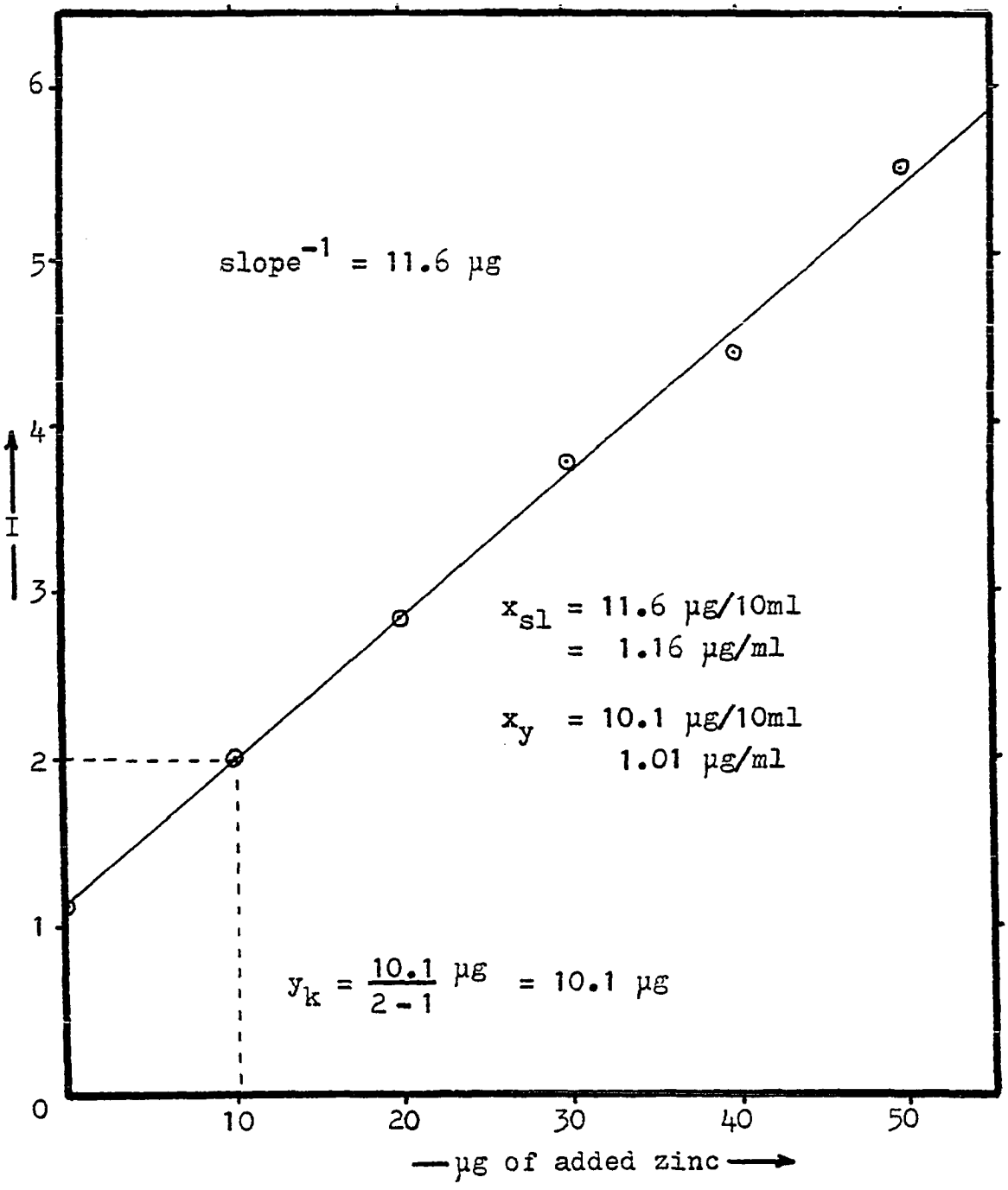

Figure E.10. Tenth Reproducibility Study at 1 µg/ml

APPENDIX F

REPRODUCIBILITY STUDY AT 0.1 µg/ml

Table F.1

First Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 8.15
INTERCEPT = 1.15	x_y = 0.0981µg/ml	x_{sl} = 0.116µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	1536	-
10	0.015	0	0	1356	1.13
10	0.015	4	0.012	1041	1.48
10	0.015	8	0.024	833	1.87
10	0.015	12	0.037	719	2.14
10	0.015	16	0.049	574	2.68
10	0.015	20	0.061	-	-
10	0.015	24	0.073	486	3.16

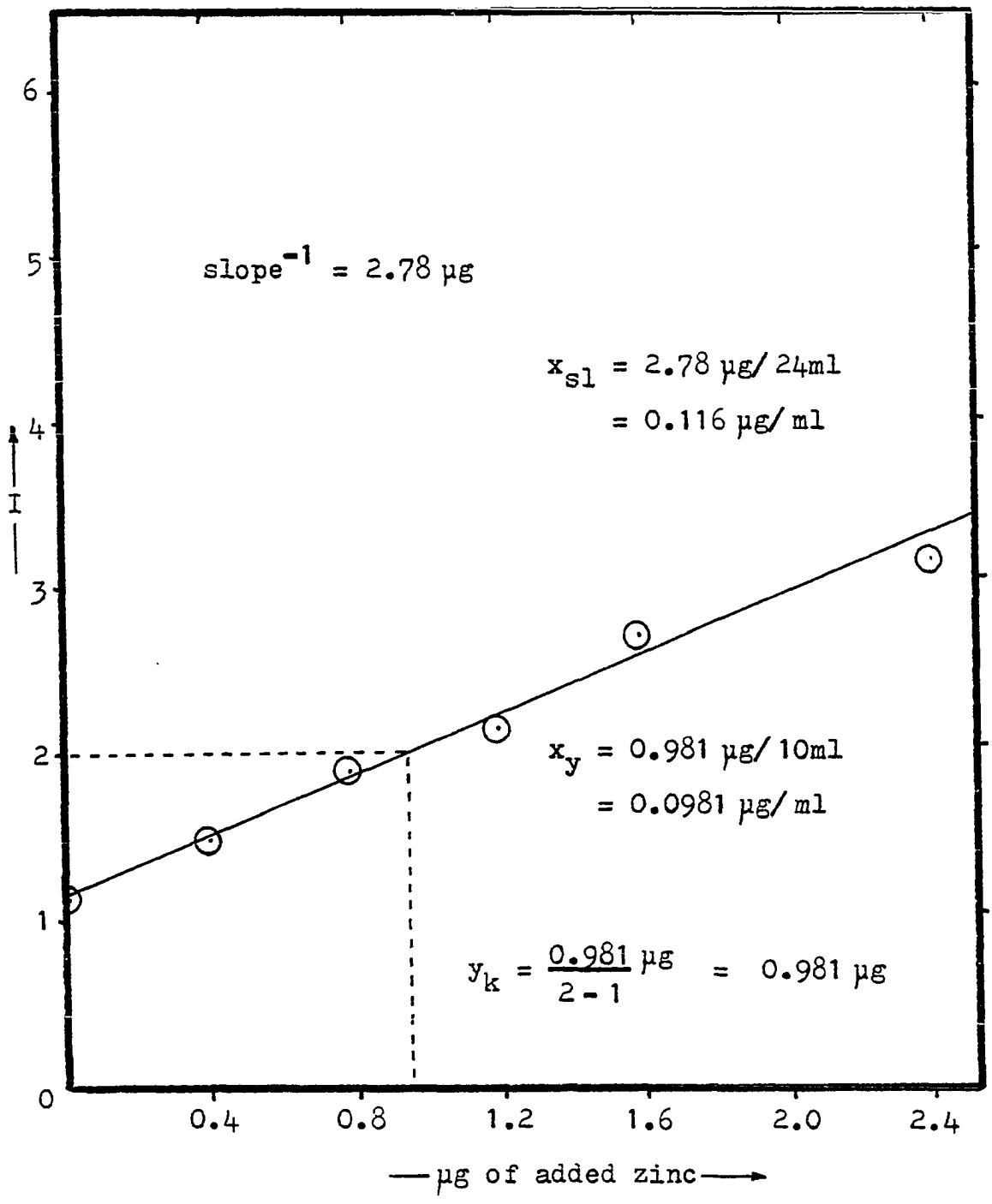

Figure F.1. First Reproducibility Study at 0.1 µg/ml

Table F.2

Second Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 8.15
INTERCEPT = 1.28	x_y = 0.0959µg/ml	x_{sl} = 0.134µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	4335	-
10	0.015	0	0	3368	1.29
10	0.015	4	0.012	2822	1.54
10	0.015	8	0.024	2230	1.94
10	0.015	12	0.037	2024	2.14
10	0.015	16	0.049	1709	2.54
10	0.015	20	0.061	1584	2.74

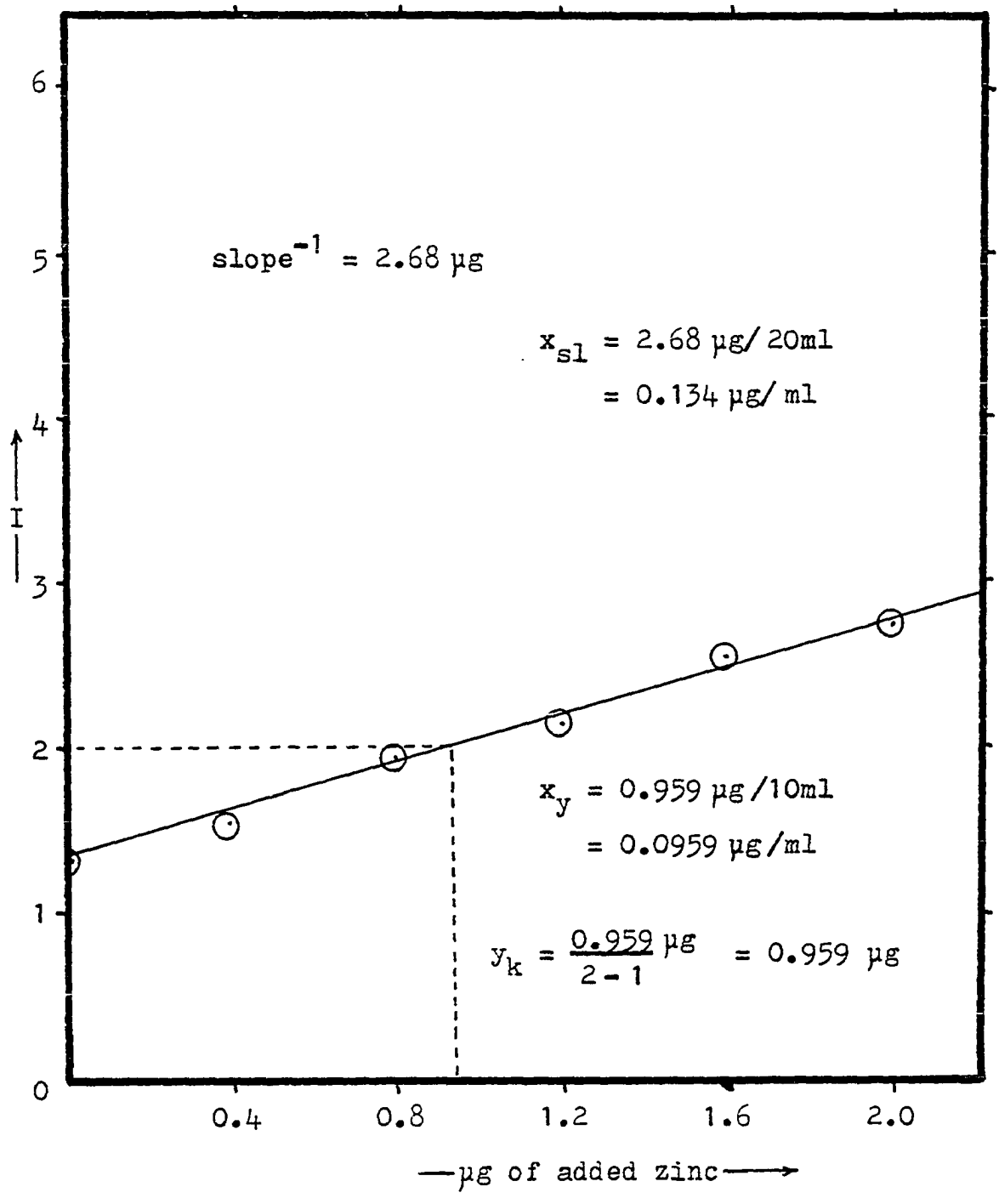

Figure F.2. Second Reproducibility Study at 0.1 µg/ml

Table F.3

Third Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 8.15
INTERCEPT = 1.18	x_y = 0.102µg/ml	x_{sl} = 0.124µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	4162	-
10	0.015	0	0	3843	1.08
10	0.015	4	0.012	2744	1.52
10	0.015	8	0.024	2155	1.93
10	0.015	12	0.037	1901	2.19
10	0.015	16	0.049	1672	2.49
10	0.015	20	0.061	1535	2.71

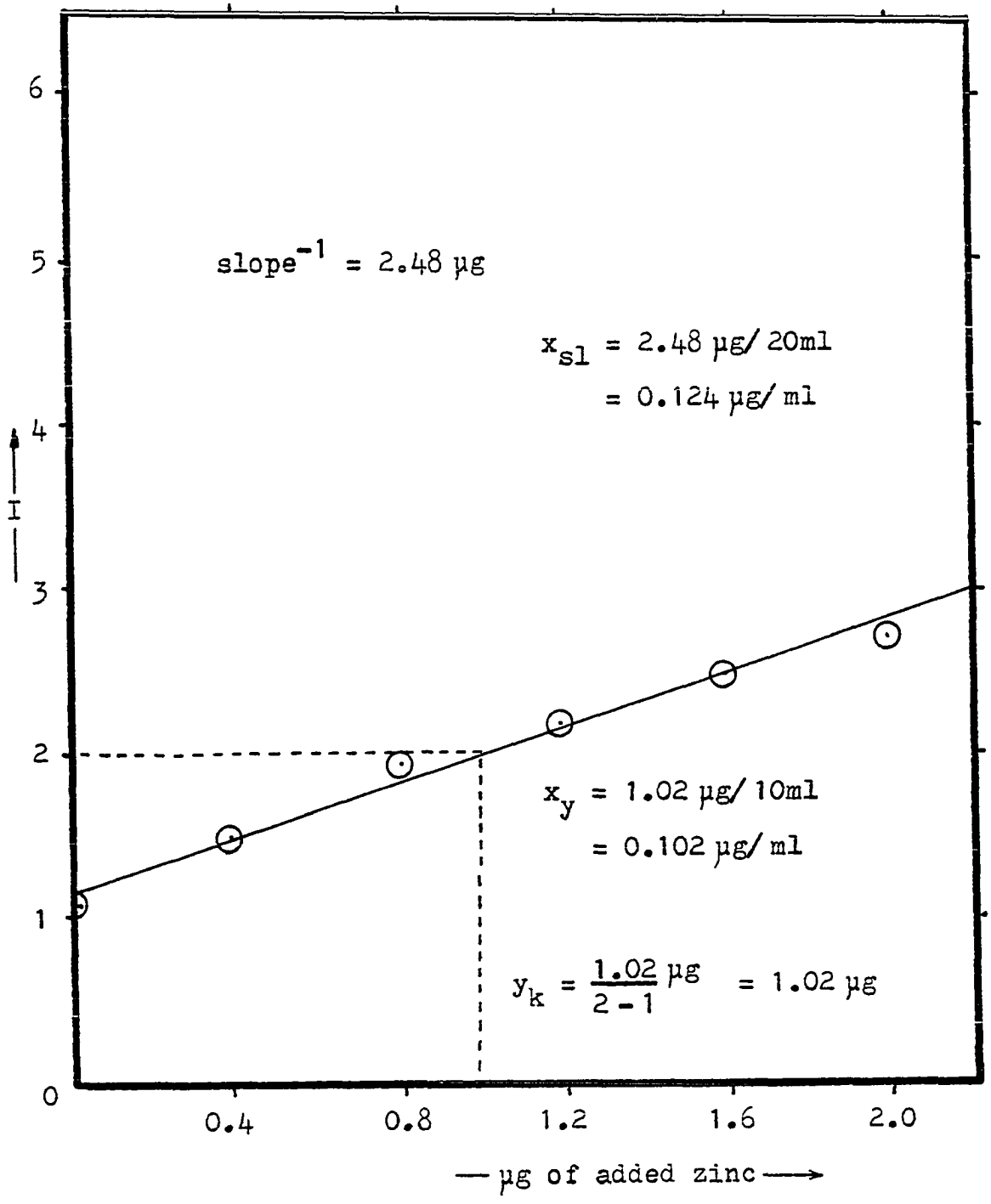

Figure F.3. Third Reproducibility Study at 0.1 µg/ml

Table F.4

Fourth Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 8.15
INTERCEPT = 1.23	x_y = 0.0886µg/ml	x_{sl} = 0.115µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	3757	-
10	0.015	0	0	3110	1.21
10	0.015	4	0.012	2433	1.54
10	0.015	8	0.024	1927	1.95
10	0.015	12	0.037	1588	2.37
10	0.015	16	0.049	1427	2.63
10	0.015	20	0.061	1293	2.91

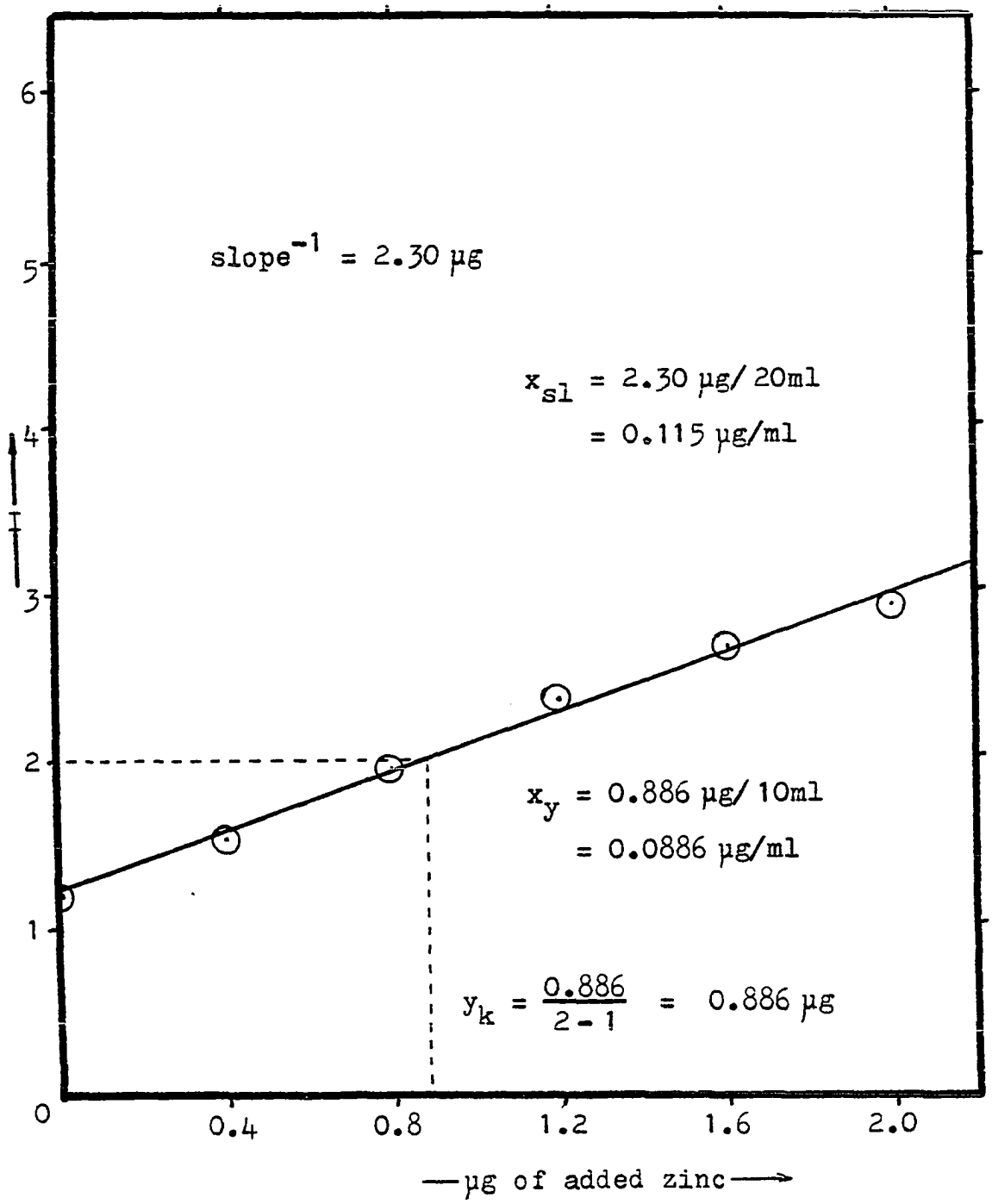

Figure F.4. Fourth Reproducibility Study at 0.1 µg/ml

Table F.5

Fifth Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 8.15
INTERCEPT = 1.07	x_y = 0.0860µg/ml	x_{sl} = 0.0926µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	1574	-
10	0.015	0	0	1402	1.12
10	0.015	4	0.012	1011	1.56
10	0.015	8	0.024	862	1.83
10	0.015	12	0.037	691	2.28
10	0.015	16	0.049	556	2.83
10	0.015	20	0.061	478	3.29

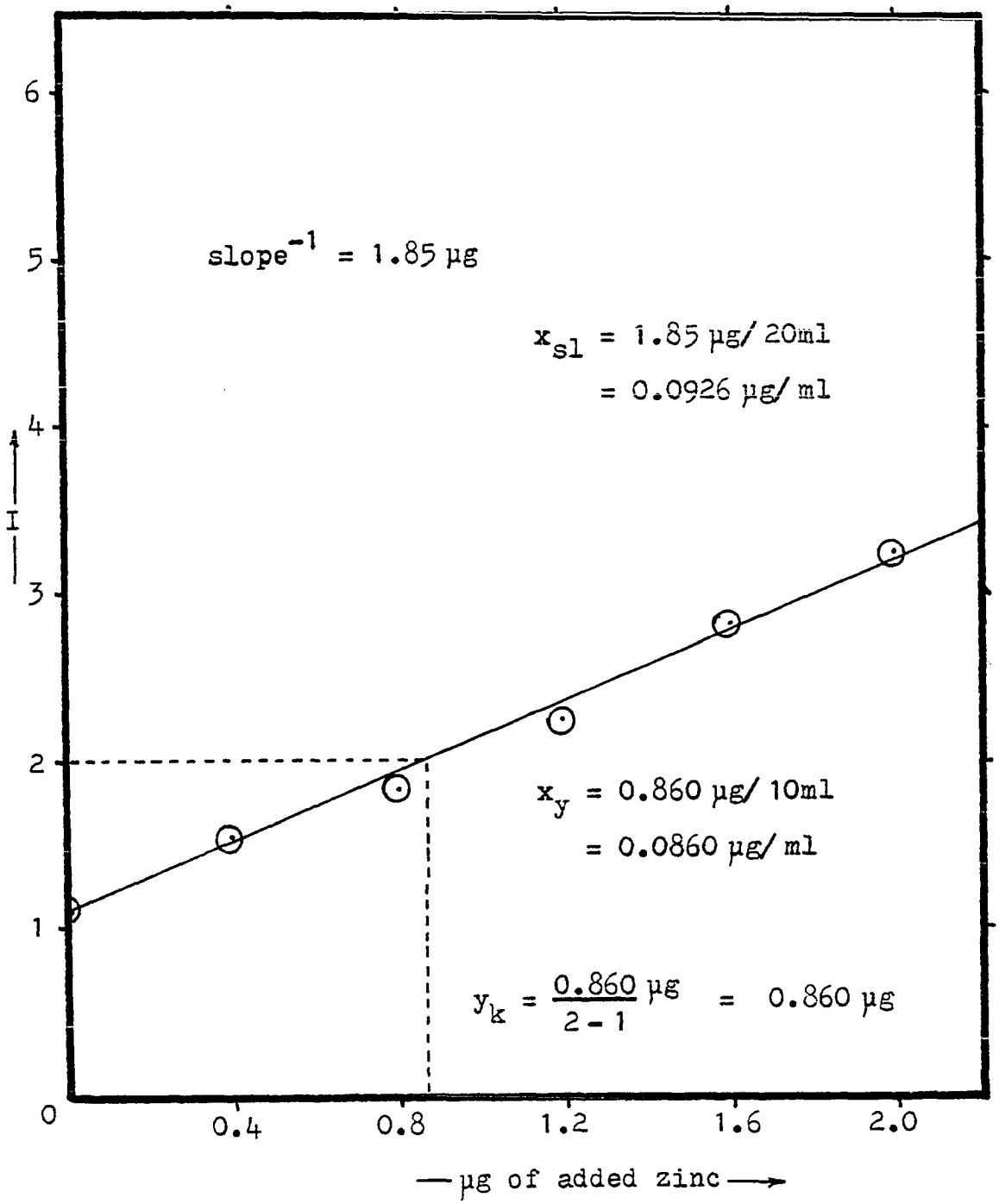

Figure F.5. Fifth Reproducibility Study at 0.1 µg/ml

Table F.6

Sixth Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 7.90
INTERCEPT = 1.03	x_y = 0.0924µg/ml	x_{sl} = 0.105µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	3329	-
10	0.015	0	0	3186	1.05
10	0.015	4	0.012	2305	1.44
10	0.015	8	0.024	1785	1.86
10	0.015	12	0.037	1489	2.24
10	0.015	16	0.049	1182	2.82
10	0.015	20	0.061	1082	3.08

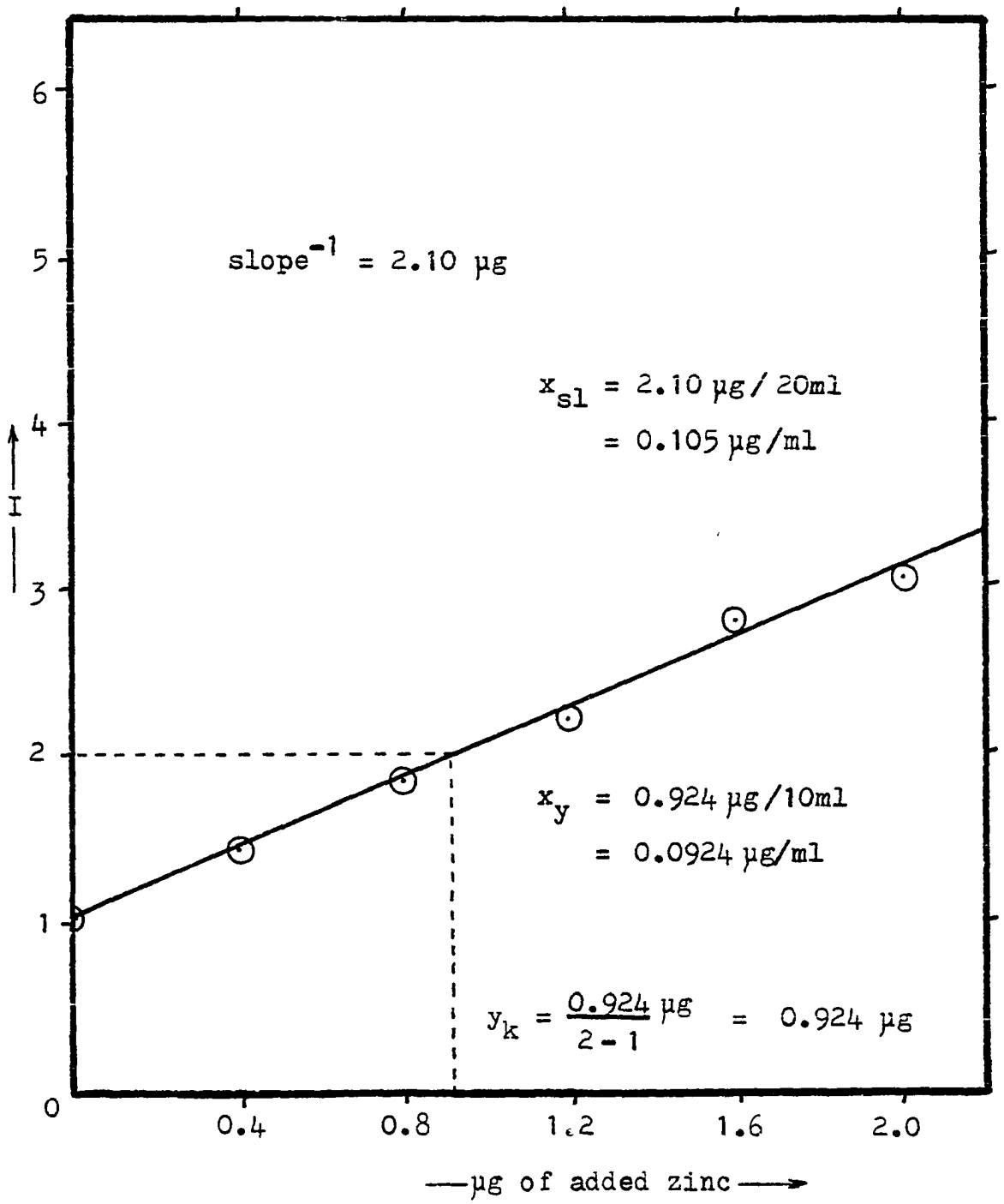

Figure F.6. Sixth Reproducibility Study at 0.1 µg/ml

Table F.7

Seventh Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 7.90
INTERCEPT = 0.94	x_y = 0.0842µg/ml	x_{sl} = 0.126µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	3308	-
10	0.015	0	0	3250	1.02
10	0.015	4	0.012	2342	1.41
10	0.015	8	0.024	1720	1.92
10	0.015	12	0.037	1340	2.47
10	0.015	16	0.049	1209	2.74
10	0.015	20	0.061	910	3.64

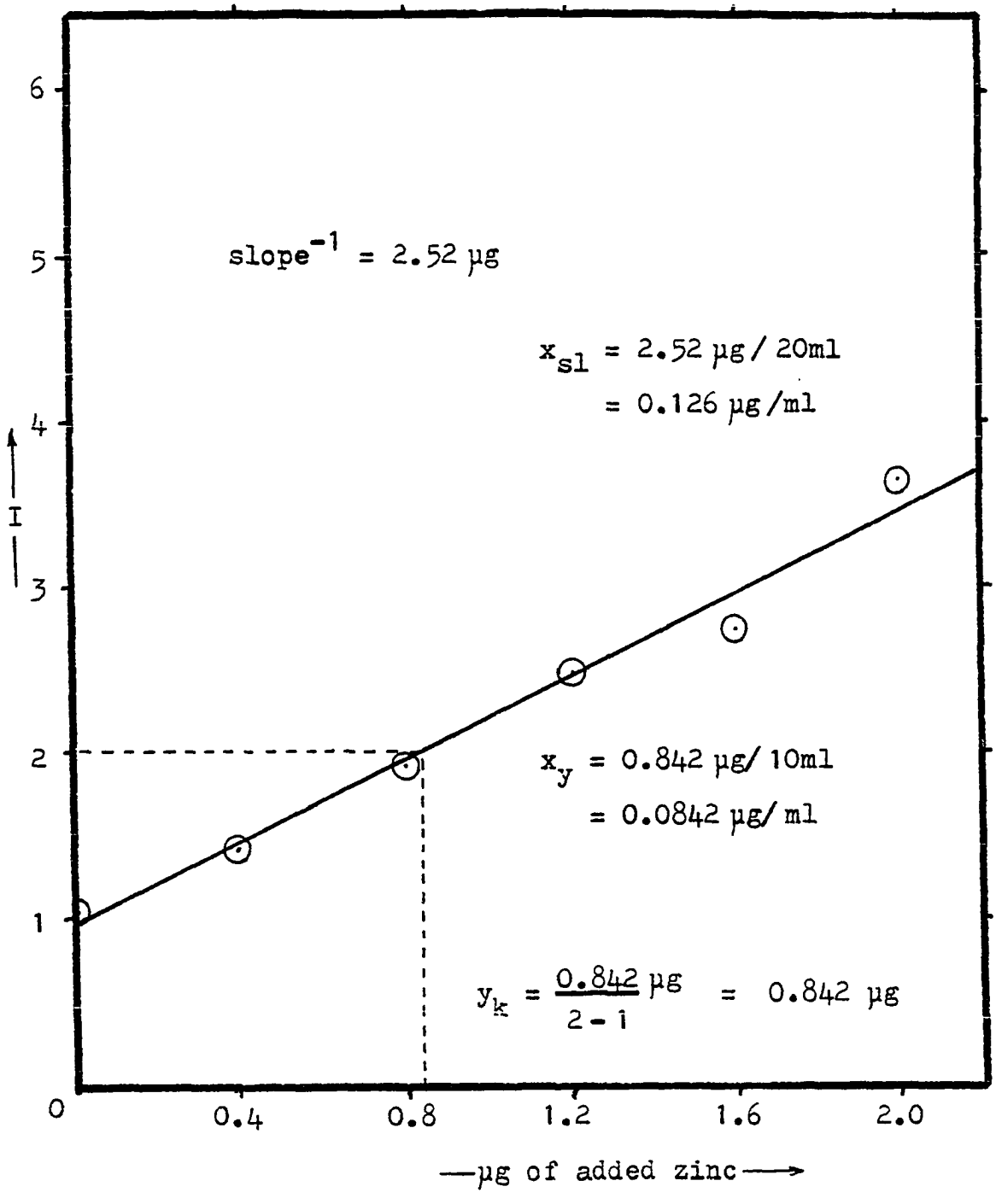

Figure F.7. Seventh Reproducibility Study at 0.1 µg/ml

Table F.8

Eighth Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 7.90
INTERCEPT = 1.07	x_y = 0.0970µg/ml	x_{sl} = 0.0957µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	4247	-
10	0.015	0	0	4146	1.02
10	0.015	4	0.012	2945	1.44
10	0.015	8	0.024	2178	1.95
10	0.015	12	0.037	1921	2.21
10	0.015	16	0.049	1647	2.58
10	0.015	20	0.061	1430	2.97

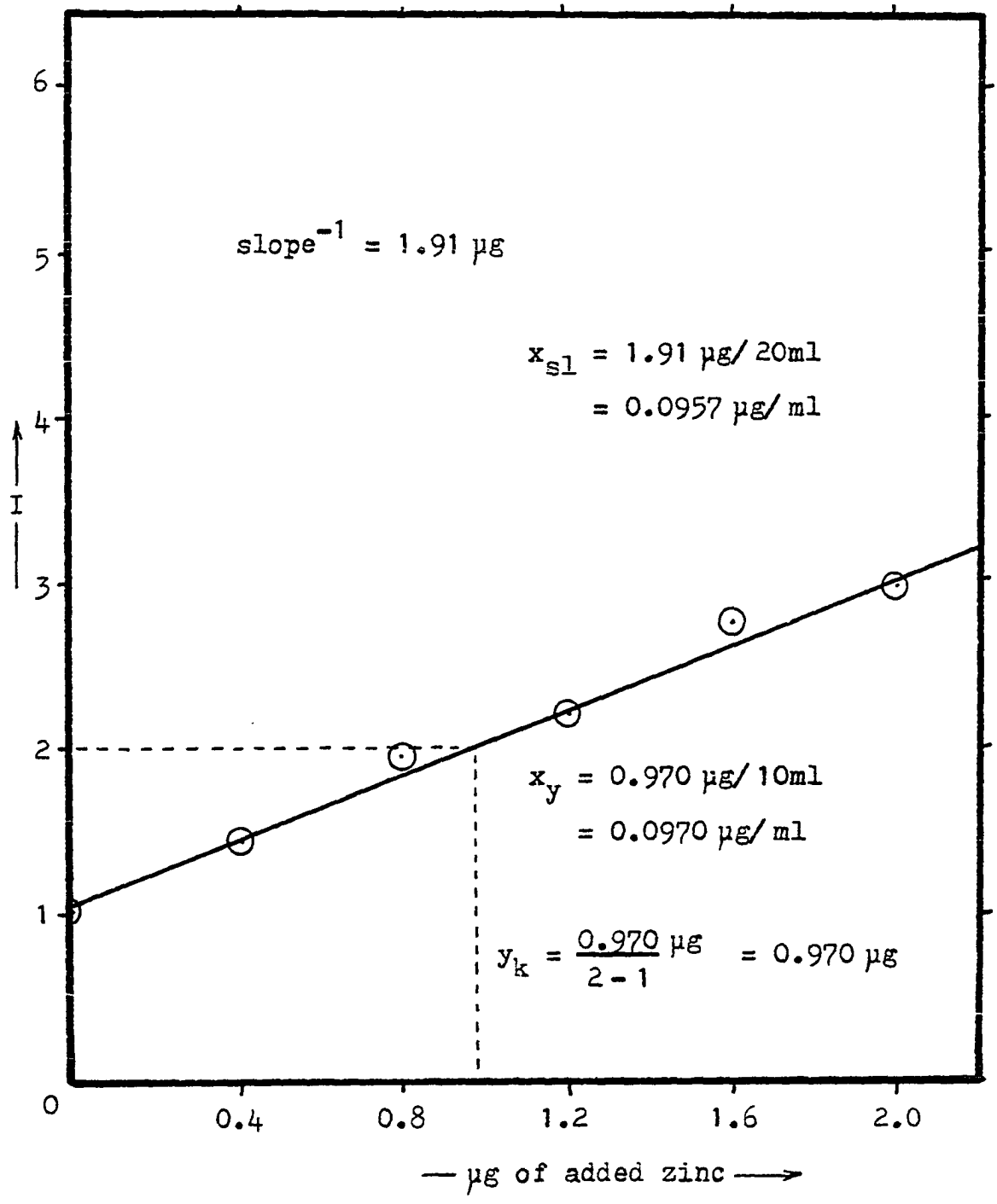

Figure F.8. Eighth Reproducibility Study at 0.1 µg/ml

Table F.9

Ninth Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 7.90
INTERCEPT = 1.17	x_y = 0.101µg/ml	x_{sl} = 0.122µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	6094	-
10	0.015	0	0	5349	1.14
10	0.015	4	0.012	4057	1.50
10	0.015	8	0.024	3218	1.89
10	0.015	12	0.037	2902	2.10
10	0.015	16	0.049	2371	2.57
10	0.015	20	0.061	2213	2.75

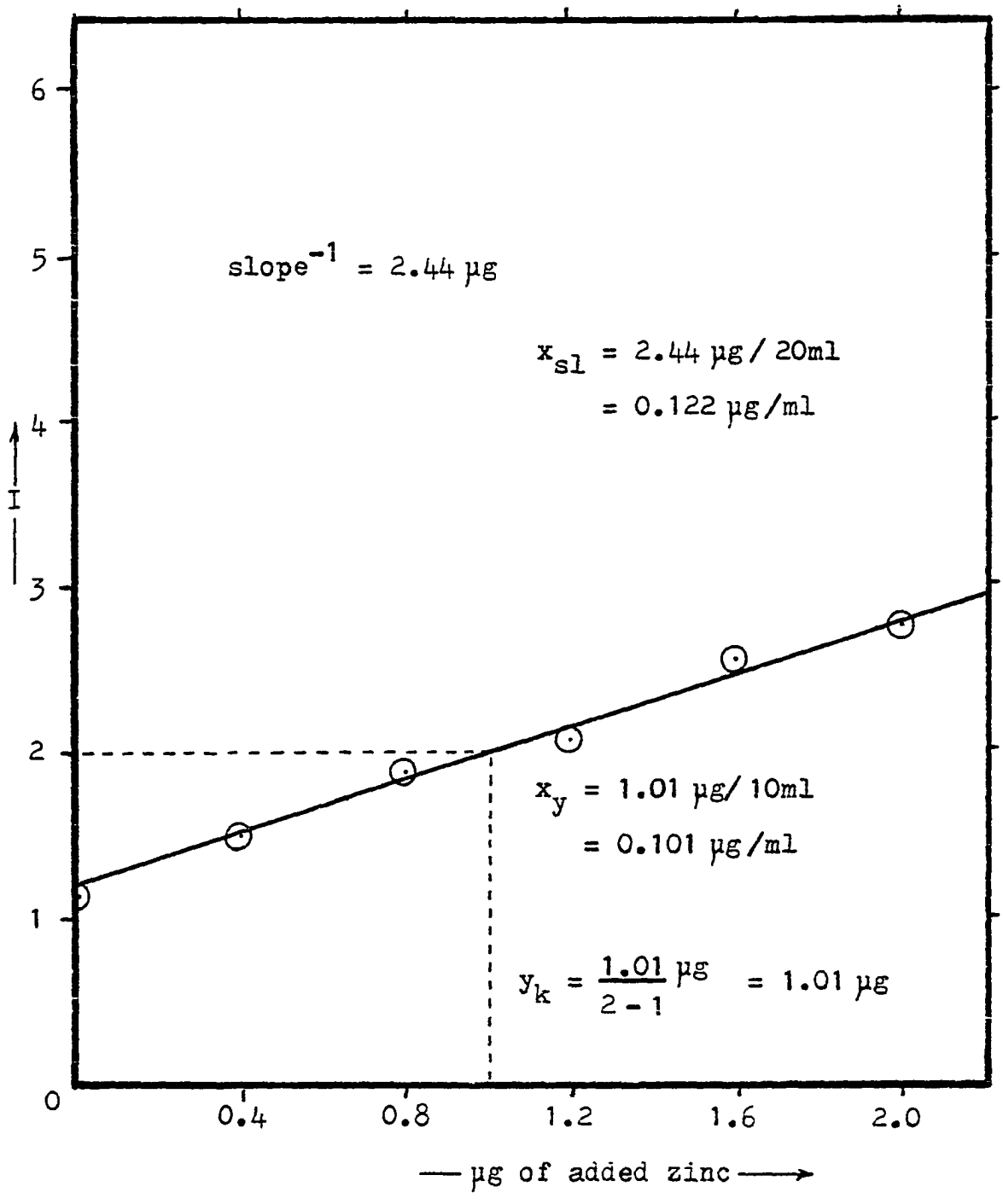

Figure F.9. Ninth Reproducibility Study at 0.1 µg/ml

Table F.10

Tenth Reproducibility Study at 0.1 µg/ml

K = 2	Kx > SR	SR = 0.02 µmole
x_{kn}	0.100µg/ml = 0.015µmole/10ml	pH = 7.80
y	0.100µg/ml = 0.015µmole/10ml	pH = 7.90
INTERCEPT = 1.04	x_y = 0.113µg/ml	x_{sl} = 0.118µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.031	0	0	4178	-
10	0.015	0	0	3990	1.05
10	0.015	4	0.012	2916	1.43
10	0.015	8	0.024	2397	1.74
10	0.015	12	0.037	2225	1.88
10	0.015	16	0.049	1687	2.48
10	0.015	20	0.061	1511	2.76

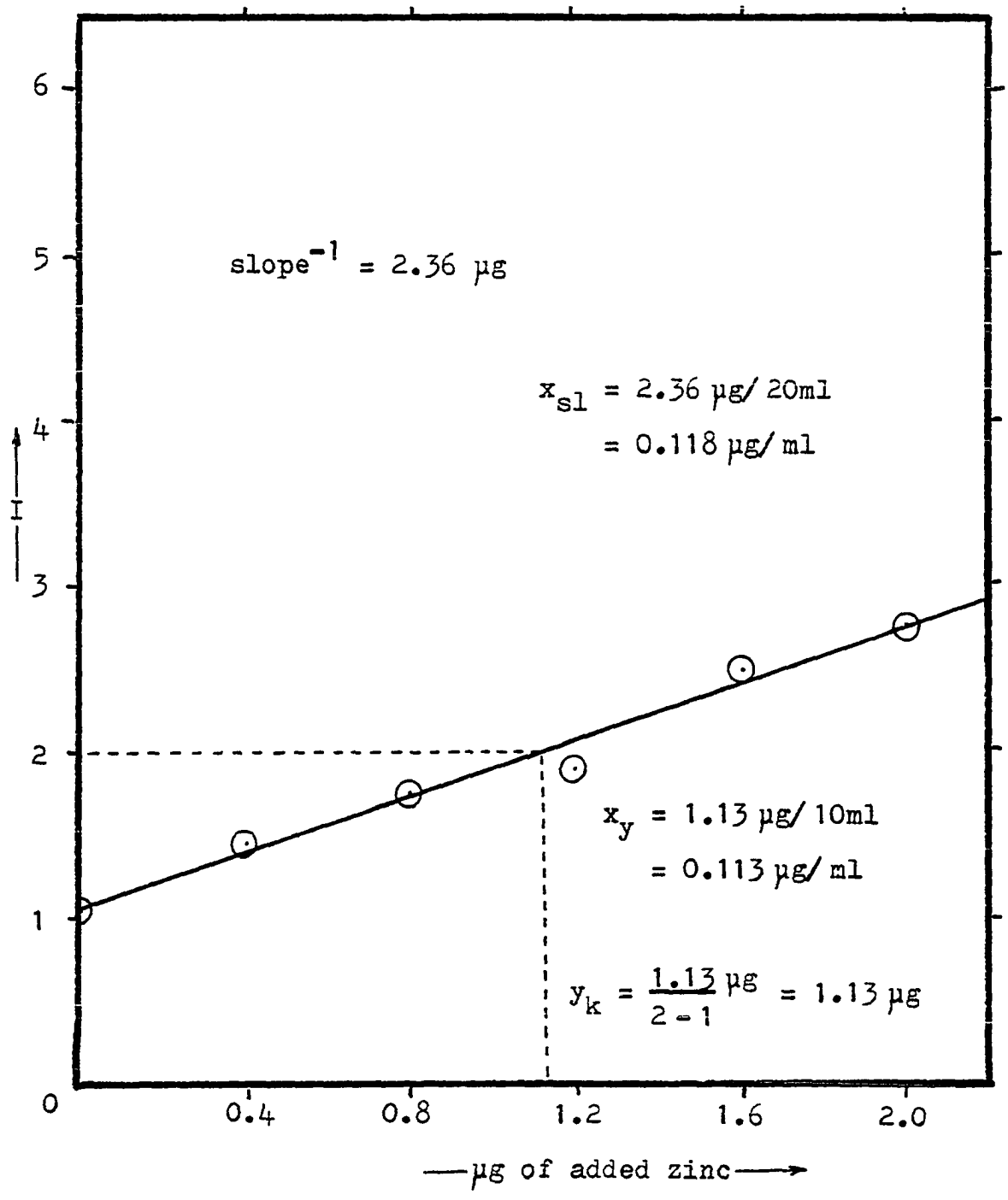

Figure F.10. Tenth Reproducibility Study at 0.1 µg/ml

APPENDIX G

THE EFFECTS PRODUCED BY VARYING pH

Table G.1

Separation Effect at pH 6

K = 2	Kx > SR	SR = 2.30 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 6.10
y	50.0µg/ml = 7.65µmole/10ml	pH = 6.15
INTERCEPT = 0.99	x_y = 10.3µg/ml	x_{sl} = 10.2µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	3532	-
10	1.53	0	0	3472	1.02
10	1.53	2	1.53	1762	2.00
10	1.53	4	3.06	1206	2.93
10	1.53	6	4.59	929	3.80
10	1.53	8	6.12	710	4.97
10	1.53	10	7.65	596	5.92

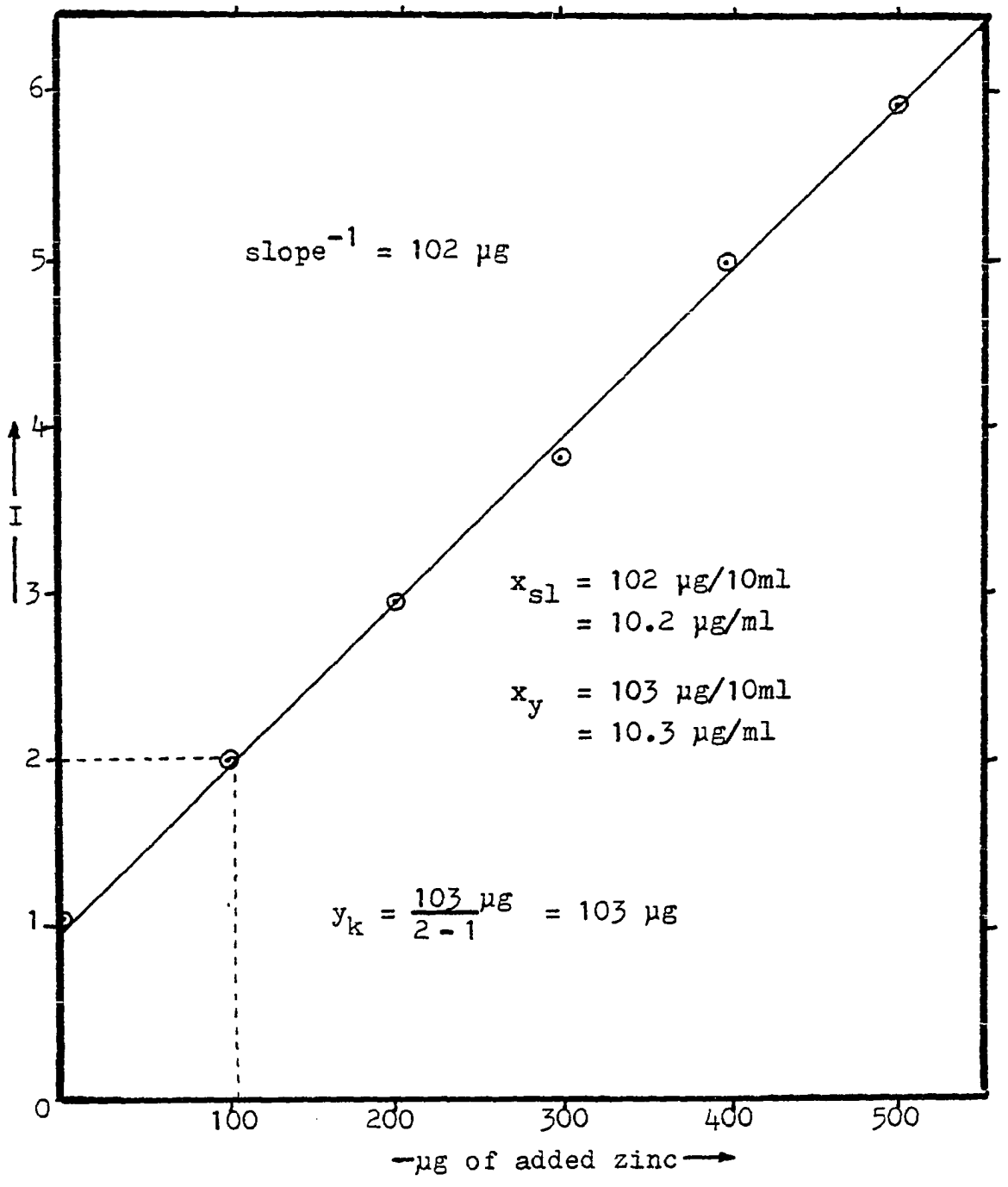

Figure G.1. Separation Effect at pH 6

Table G.2

Separation Effect at pH 7

K = 2	Kx > SR	SR = 2.30 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 6.90
y	50.0µg/ml = 7.65µmole/10ml	pH = 7.06
INTERCEPT = 1.03	x_y = 10.3µg/ml	x_{sl} = 10.6µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	3995	-
10	1.53	0	0	3956	1.01
10	1.53	2	1.53	2011	1.99
10	1.53	4	3.06	1400	2.85
10	1.53	6	4.59	1030	3.88
10	1.53	8	6.12	816	4.90
10	1.53	10	7.65	710	5.62

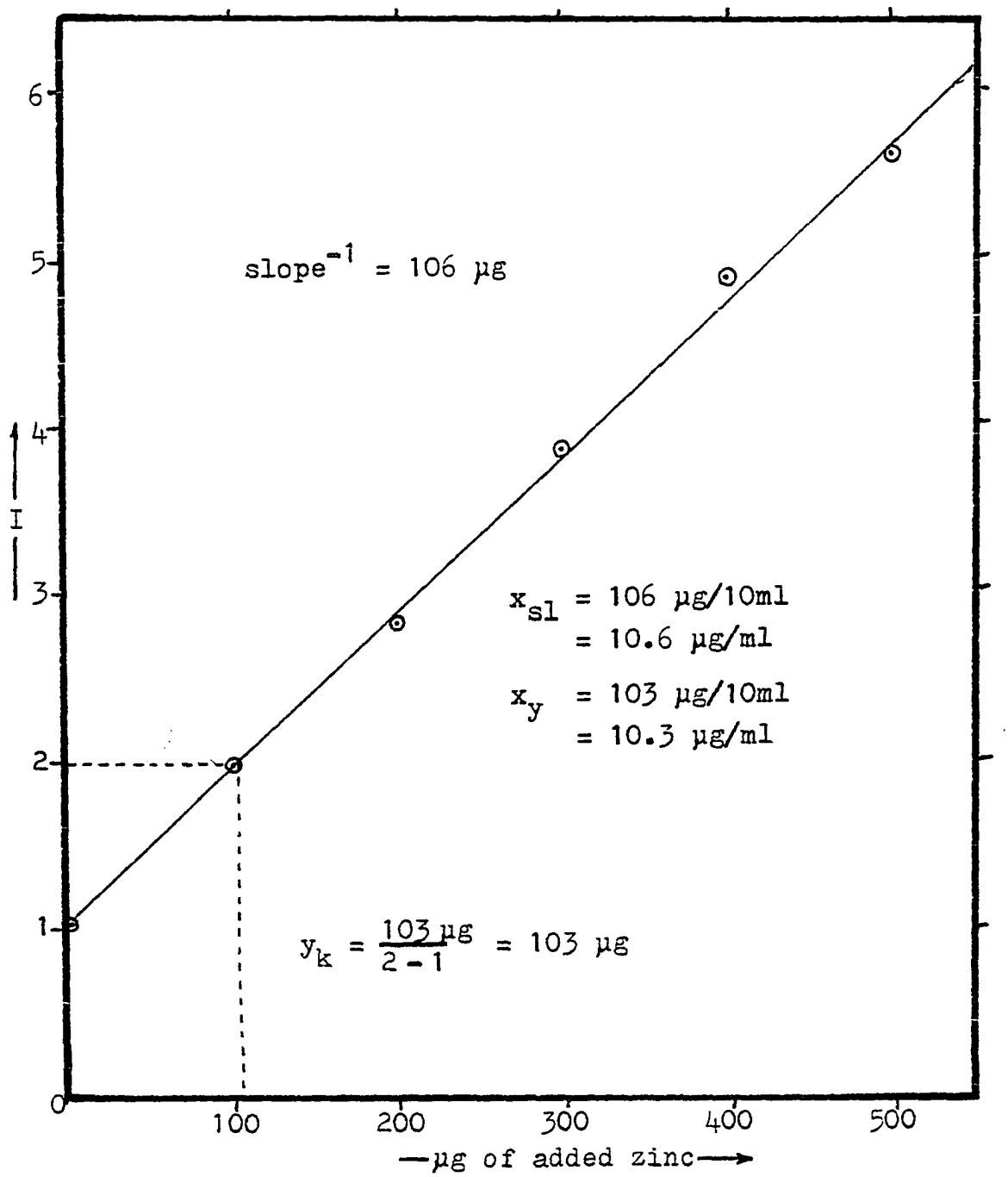

Figure G.2. Separation Effect at pH 7

Table G.3

Separation Effect at pH 8

K = 2	Kx > SR	SR = 2.34 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.07
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.00
INTERCEPT = 0.98	x_y = 10.0µg/ml	x_{sl} = 10.0µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	7048	-
10	1.53	0	0	7066	1.00
10	1.53	2	1.53	3510	2.01
10	1.53	4	3.06	2397	2.94
10	1.53	6	4.59	1806	3.90
10	1.53	8	6.12	1384	5.09
10	1.53	10	7.65	1177	5.99

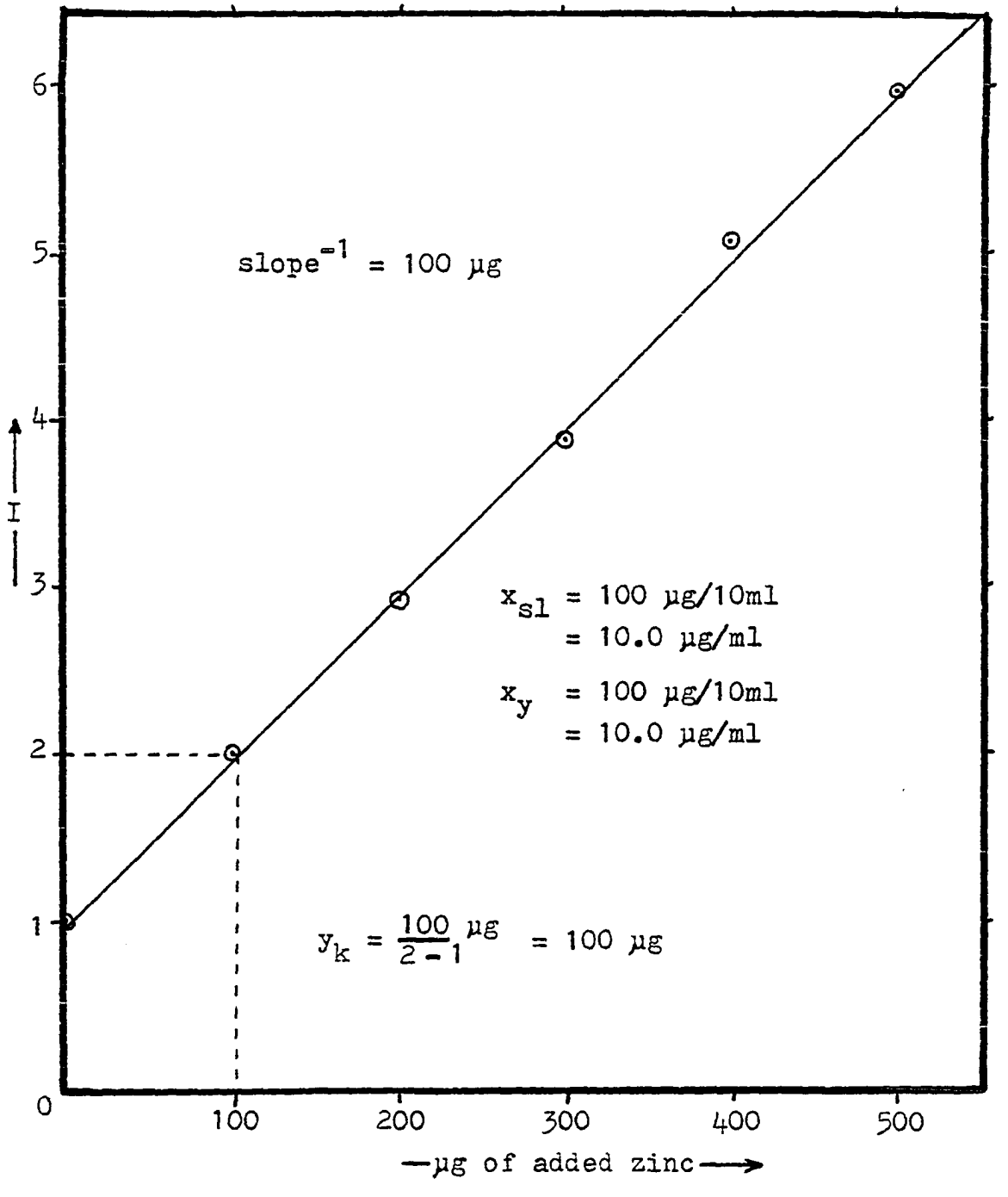

Figure G.3. Separation Effect at pH 8

Table G.4

Separation Effect at pH 9

K = 2	Kx > SR	SR = 2.34 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 9.10
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.98
INTERCEPT = 1.14	x_y = 7.72µg/ml	x_{sl} = 9.01µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	2455	-
10	1.53	0	0	1703	1.44
10	1.53	2	1.53	988	2.49
10	1.53	4	3.06	940	2.61
10	1.53	6	4.59	652	3.77
10	1.53	8	6.12	361	6.80
10	1.53	10	7.65	385	6.38

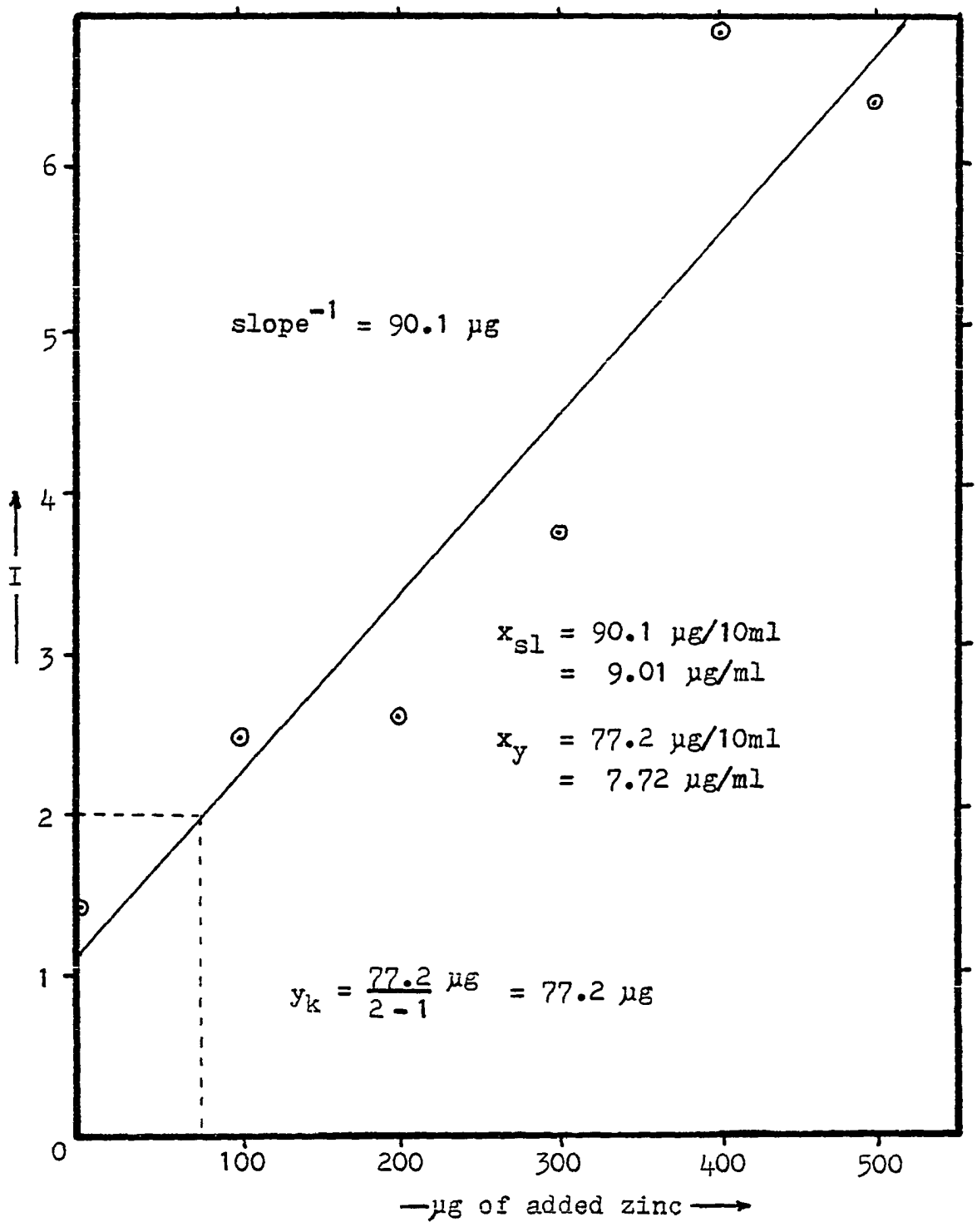

Figure G.4. Separation Effect at pH 9

Table G.5

Separation Effect at pH 10

K = 2	Kx > SR	SR = 2.30 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 9.95
y	50.0µg/ml = 7.65µmole/10ml	pH = 9.92
INTERCEPT = 0.97	x_y > 50µg/ml	x_{sl} > 50µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	1175	-
10	1.53	0	0	1033	1.11
10	1.53	2	1.53	1000	1.18
10	1.53	4	3.06	796	1.48
10	1.53	6	4.59	960	1.22
10	1.53	8	6.12	900	1.31
10	1.53	10	7.65	478	2.46

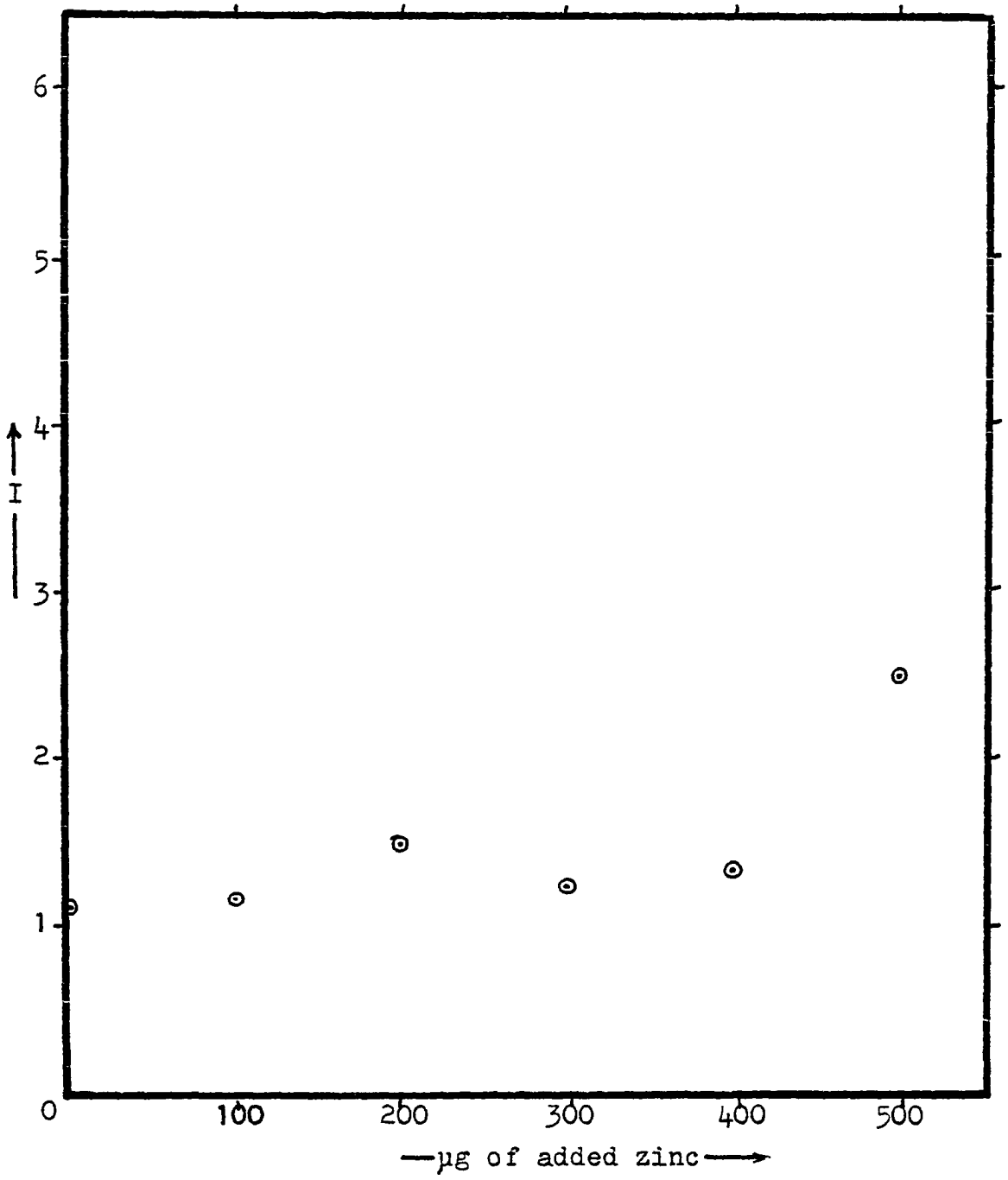

Figure G.5. Separation Effect at pH 10

APPENDIX H

THE EFFECTS PRODUCED BY INTERFERENCES

Table H.1

Extraction Involving 36 µg/ml Cd and Co

K = 2	Kx > SR	SR = 1.41 µmole
x_{kn}	9.93µg/ml = 1.52µmole/10ml	pH = 6.85
y	10.0µg/ml = 1.53µmole/10ml	pH = 7.50
INTERCEPT = 1.01	x_y = 10.0µg/ml	x_{sl} = 10.1µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.04	0	0	3762	-
10	1.52	0	0	3732	1.01
10	1.52	5	0.77	2568	1.46
10	1.52	10	1.53	1871	2.01
10	1.52	15	2.30	1474	2.55
10	1.52	20	3.06	1267	2.97
10	1.52	25	3.82	1082	3.45
10	1.52	30	4.59	938	3.98

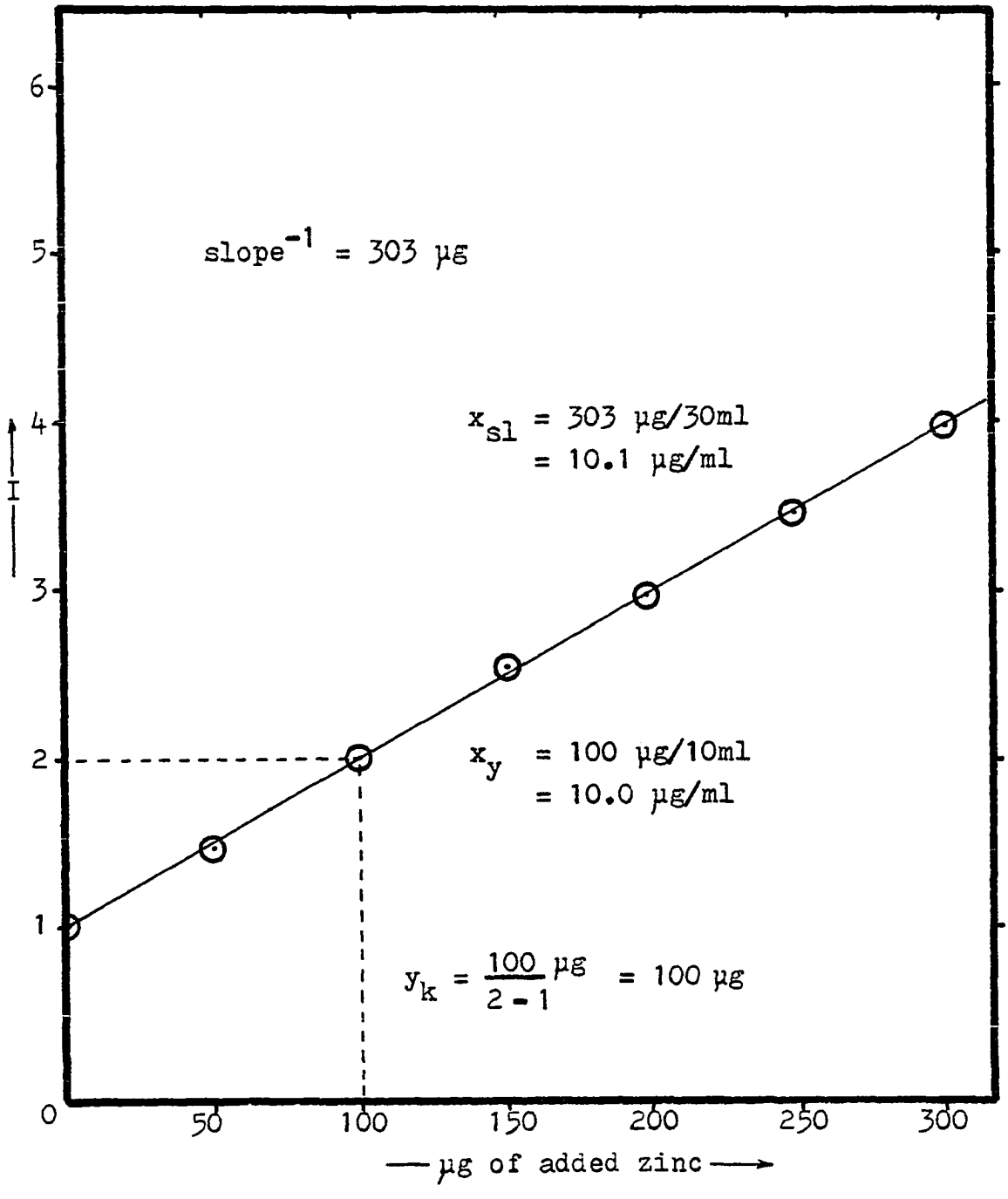

Figure H.1. Extraction Involving 36 µg/ml Cd and Co

Table H.2

Extraction Involving 61 µg/ml Pb and Ni

K = 2	Kx > SR	SR = 1.41 µmole
x_{kn}	9.88µg/ml = 1.51µmole/10ml	pH = 7.15
y	10.0µg/ml = 1.53µmole/10ml	pH = 7.25
INTERCEPT = 0.98	x_y = 10.5µg/ml	x_{sl} = 10.3µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.02	0	0	4526	-
10	1.51	0	0	4576	0.99
10	1.51	5	0.76	3163	1.43
10	1.51	10	1.53	2291	1.98
10	1.51	15	2.30	1832	2.47
10	1.51	20	3.06	1584	2.86
10	1.51	25	3.82	1328	3.41
10	1.51	30	4.59	1163	3.89

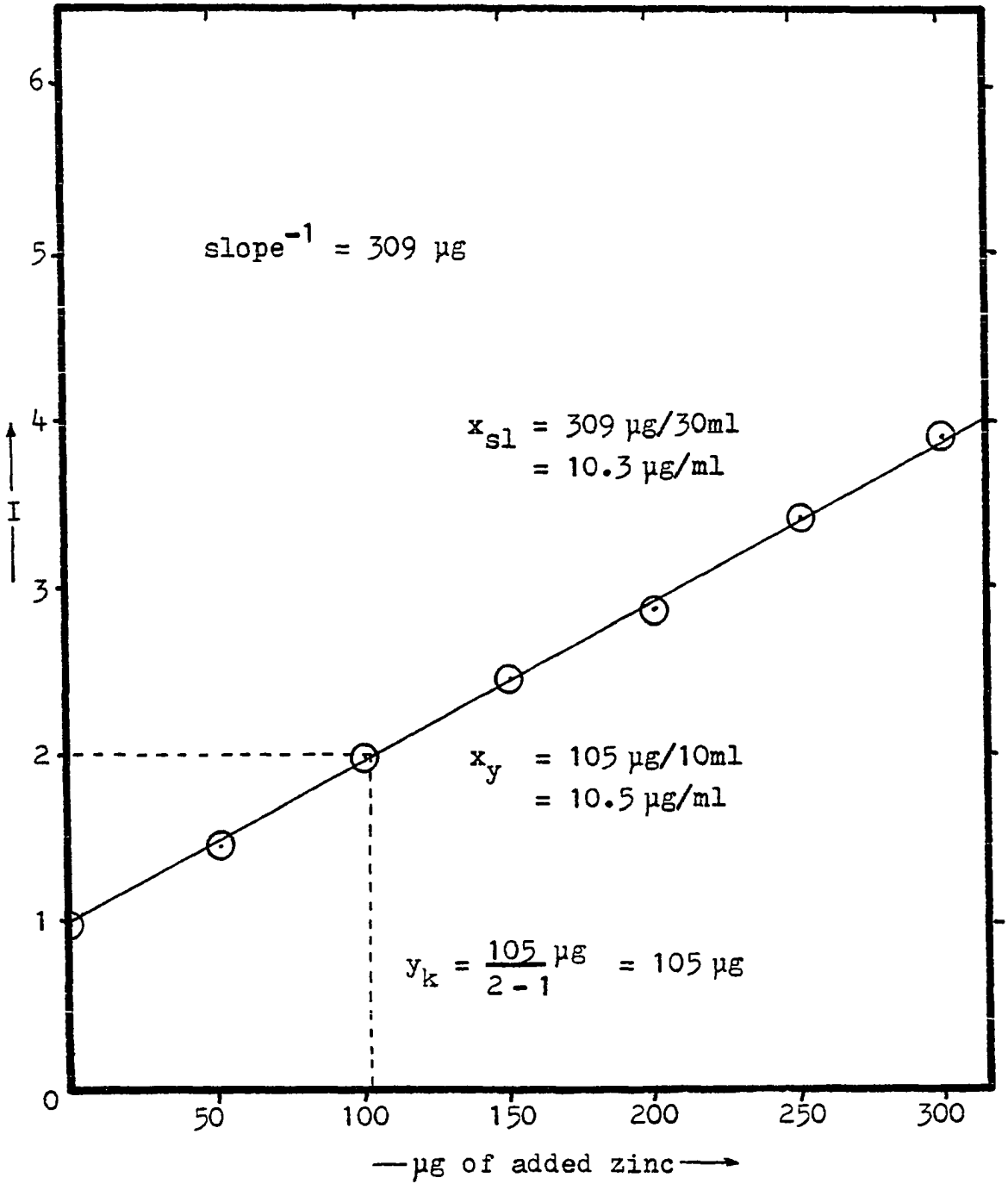

Figure H.2. Extraction Involving 61 µg/ml Pb and Ni

Table H.3

Extraction Involving 56 µg/ml Cd, Co , Cu and Ni

K = 2	Kx > SR	SR = 1.41 µmole
x_{kn}	9.94µg/ml = 1.52µmole/10ml	pH = 7.20
y	10.0µg/ml = 1.53µmole/10ml	pH = 6.90
INTERCEPT = 1.00	x_y = 10.3µg/ml	x_{sl} = 10.3µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.04	0	0	2782	-
10	1.52	0	0	2802	0.99
10	1.52	5	0.77	1883	1.48
10	1.52	10	1.53	1391	2.00
10	1.52	15	2.30	1124	2.48
10	1.52	20	3.06	969	2.87
10	1.52	25	3.82	802	3.47

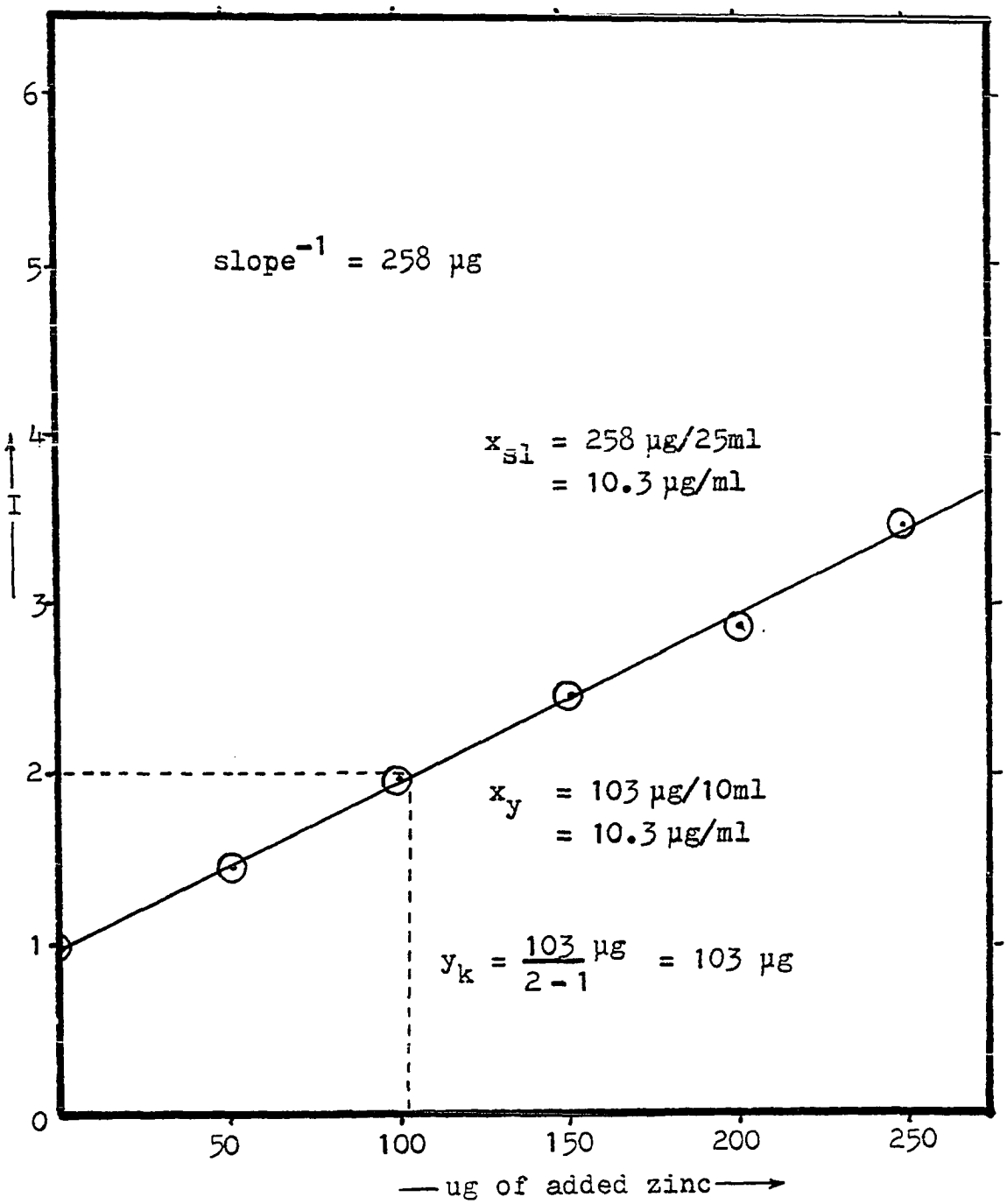

Figure H.3. Extraction Involving 56 µg/ml Cd, Co, Cu and Ni

Table H.4

Extraction Involving 185 µg/ml Cd

K = 2	Kx > SR	SR = 1.45 µmole
x_{kn}	9.93µg/ml = 1.52µmole/10ml	pH = 7.60
y	10.0µg/ml = 1.53µmole/10ml	pH = 7.60
INTERCEPT = 1.00	x_y = 10.3µg/ml	x_{sl} = 10.3µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.04	0	0	5198	-
10	1.52	0	0	5244	0.99
10	1.52	5	0.77	3420	1.52
10	1.52	10	1.53	2646	1.96
10	1.52	15	2.30	2142	2.43
10	1.52	20	3.06	1804	2.88
10	1.52	25	3.82	1497	3.47

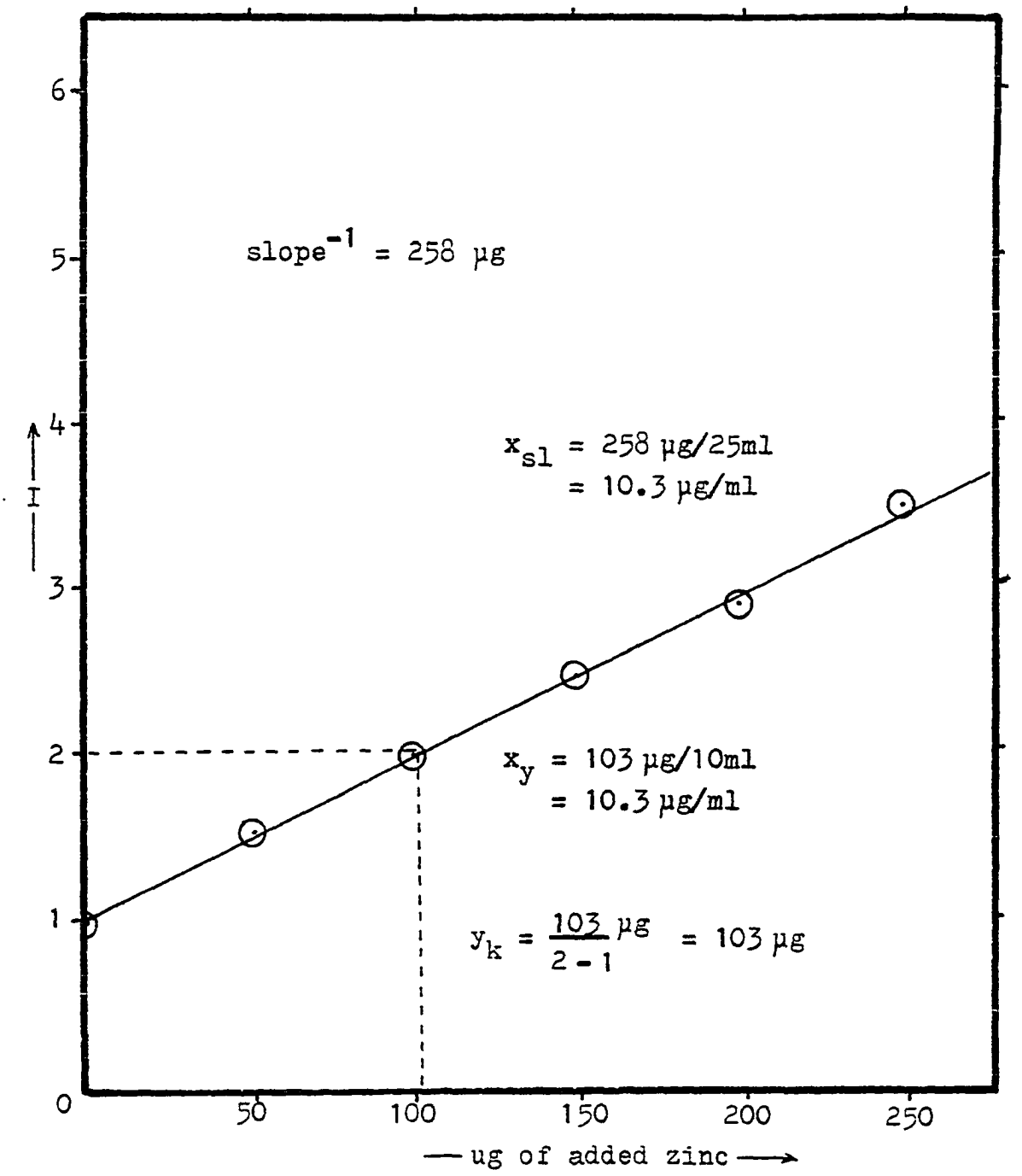

Figure H.4. Extraction Involving 185 µg/ml Cd

Table H.5

Extraction Involving 1536 µg/ml Co

K = 2	Kx > SR	SR = 1.45 µmole
x_{kn}	9.92µg/ml = 1.52µmole/10ml	pH = 7.60
y	10.0µg/ml = 1.53µmole/10ml	pH = 7.40
INTERCEPT = 0.96	x_y = 11.4µg/ml	x_{sl} = 11.0µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.04	0	0	5937	-
10	1.52	0	0	5946	1.00
10	1.52	5	0.77	4298	1.38
10	1.52	10	1.53	3184	1.86
10	1.52	15	2.30	2600	2.28
10	1.52	20	3.06	2096	2.83
10	1.52	25	3.82	1842	3.22

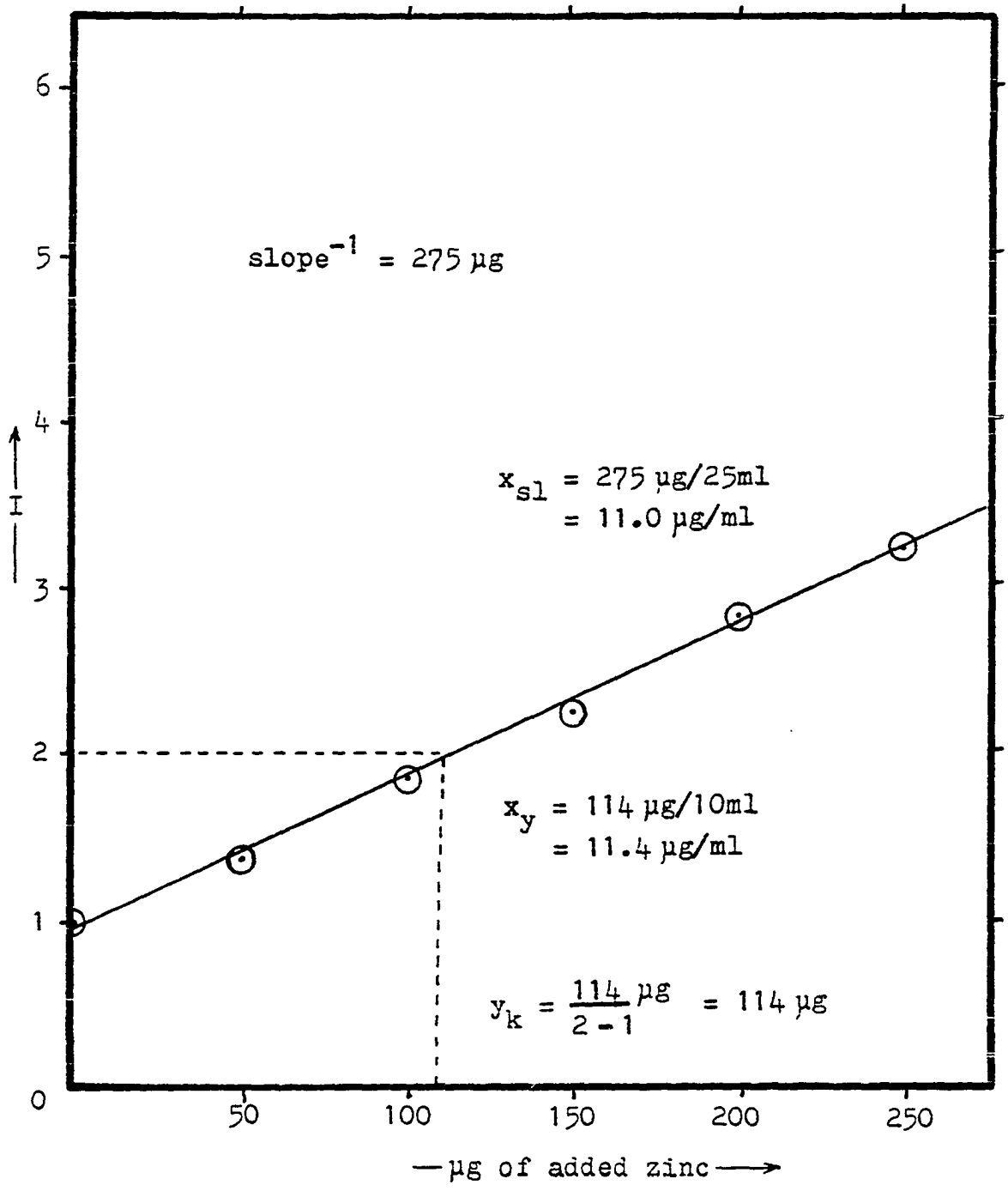

Figure H.5. Extraction Involving 1536 µg/ml Co

Table H.6

Extraction Involving 435 µg/ml Ni

K = 2	Kx > SR	SR = 1.41 µmole
x_{kn}	9.86µg/ml = 1.51µmole/10ml	pH = 7.45
y	10.0µg/ml = 1.53µmole/10ml	pH = 7.85
INTERCEPT = 0.93	x_y = 10.3µg/ml	x_{sl} = 9.63µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.03	0	0	6362	-
10	1.51	0	0	6402	1.01
10	1.51	5	0.77	4506	1.41
10	1.51	10	1.53	3300	1.93
10	1.51	15	2.30	2576	2.47
10	1.51	20	3.06	2132	2.98
10	1.51	25	3.82	1771	3.59

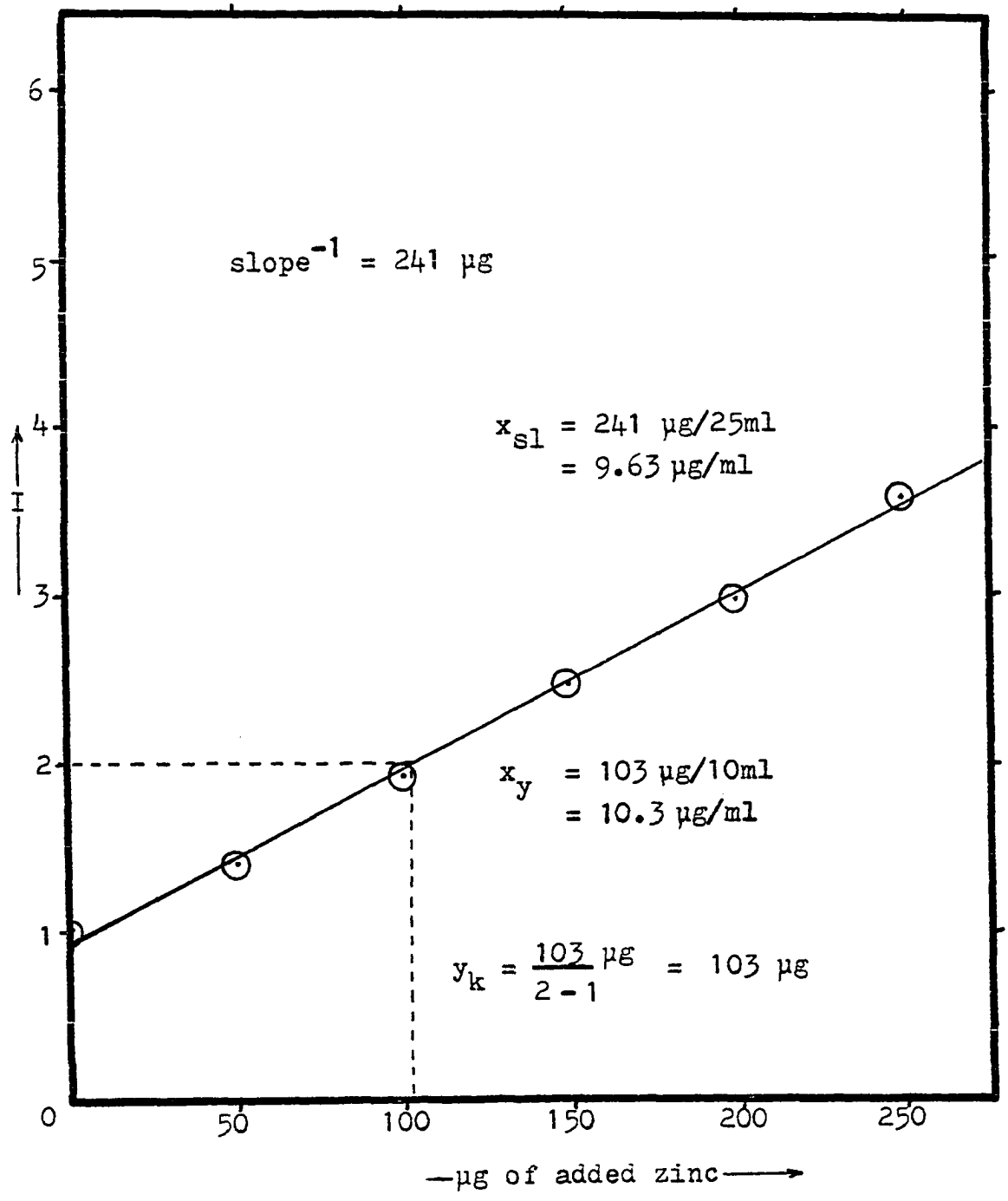

Figure H.6. Extraction Involving 435 µg/ml Ni

Table H.7

Extraction Involving over 2100 µg/ml Cd, Co and Ni

K = 2	Kx > SR	SR = 1.45 µmole
x_{kn}	9.81µg/ml = 1.50µmole/10ml	pH = 7.60
y	10.0µg/ml = 1.53µmole/10ml	pH = 7.55
INTERCEPT = 0.99	x_y = 10.1µg/ml	x_{sl} = 10.0µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.00	0	0	7190	-
10	1.50	0	0	7011	0.98
10	1.50	4	0.61	5160	1.39
10	1.50	8	1.22	4003	1.80
10	1.50	12	1.83	3294	2.18
10	1.50	16	2.45	2750	2.62
10	1.50	20	3.06	2430	2.96

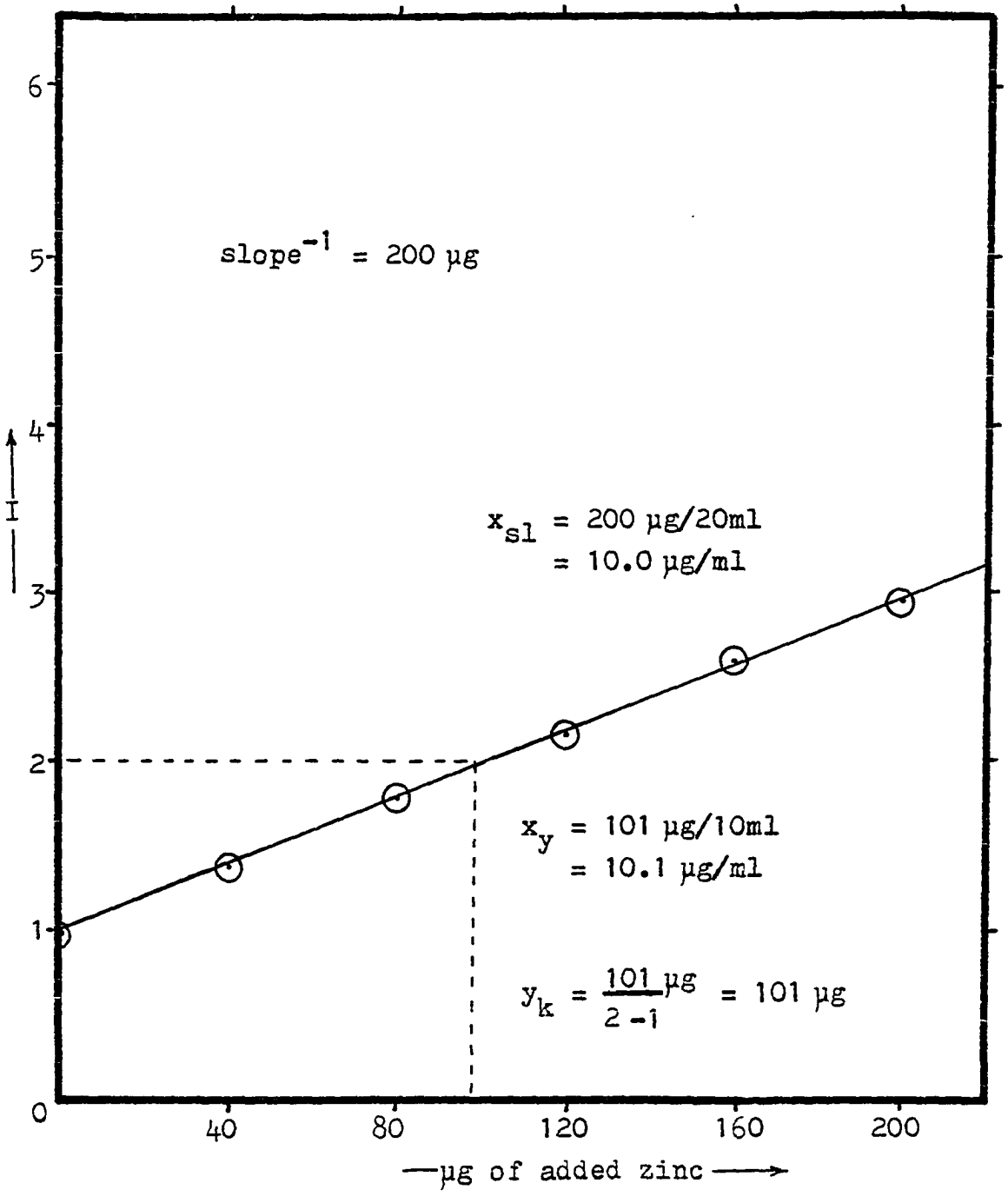

Figure H.7. Extraction Involving over 2100 µg/ml Cd, Co and Ni

APPENDIX I

STATE HEALTH DEPARTMENT

COMPARISON STUDY

Table I.1

Comparison Study versus State Health Department - Run 1A

K = 2	Kx > SR	SR = 0.55 µmole
x_{kn}	2.9µg/ml = 0.44µmole/10ml	pH = 7.95
y	13 µg/ml = 2.0 µmole/10ml	pH = 7.50
INTERCEPT = 1.05	x_y = 2.6µg/ml	x_{sl} = 2.7µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.89	0	0	41715	-
10	0.44	0	0	40026	1.04
10	0.44	2	0.4	20863	2.00
10	0.44	4	0.8	13681	3.05
10	0.44	6	1.2	10646	3.92
10	0.44	8	1.6	8489	4.91
10	0.44	10	2.0	7055	5.91

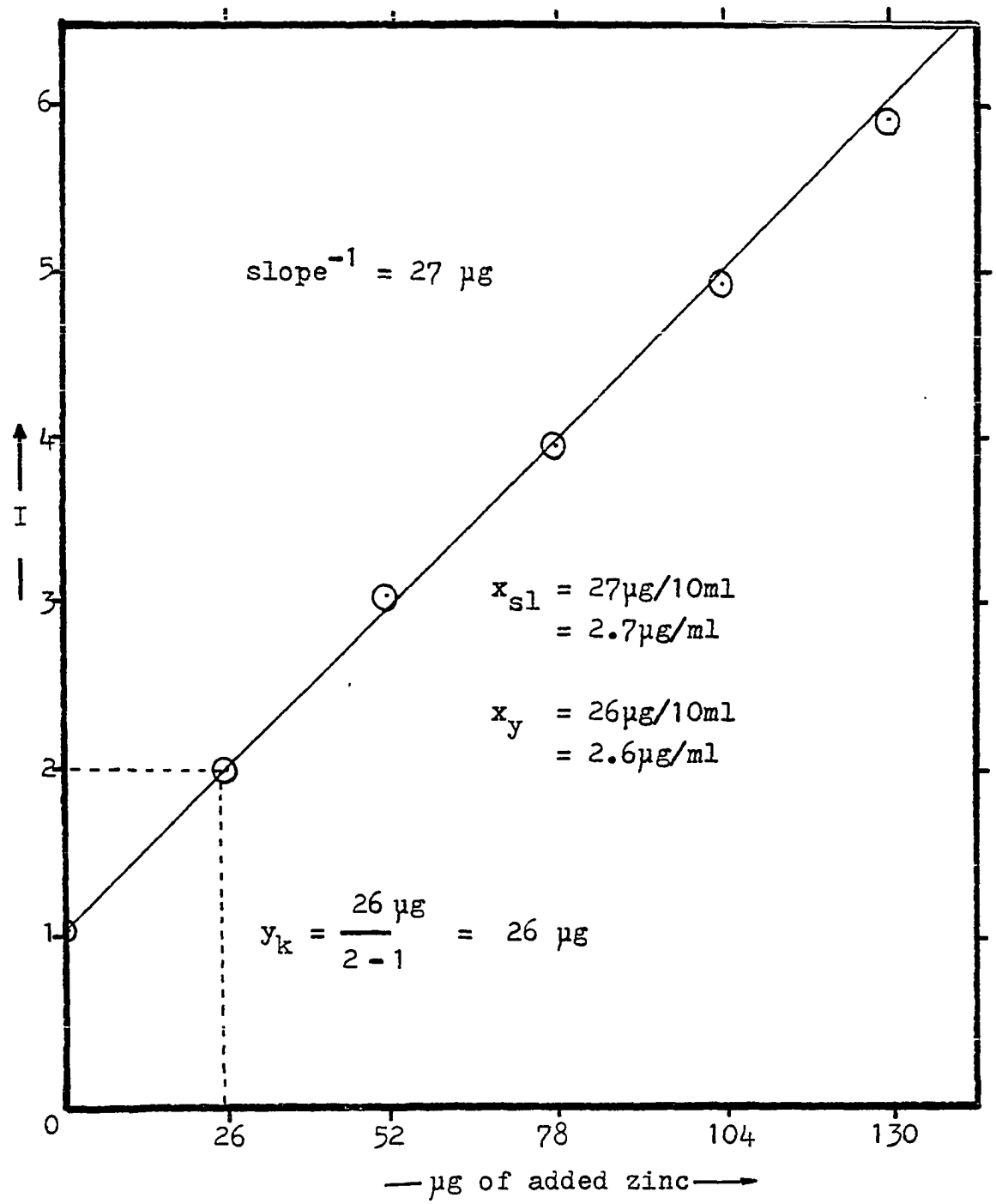

Figure I.1. Comparison Study versus State Health Department- Run 1A

Table I.2

Comparison Study versus State Health Department- Run 2A

K = 2	Kx > SR	SR = 0.55 µmole
x_{kn}	2.9µg/ml = 0.44µmole/10ml	pH = 7.95
y	13 µg/ml = 2.0 µmole/10ml	pH = 7.50
INTERCEPT = 1.09	x_y = 2.4µg/ml	x_{sl} = 2.7µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.89	0	0	43053	-
10	0.44	0	0	42626	1.01
10	0.44	2	0.4	20293	2.11
10	0.44	4	0.8	13520	3.18
10	0.44	6	1.2	10860	3.98
10	0.44	8	1.6	8399	5.13
10	0.44	10	2.0	7239	5.95

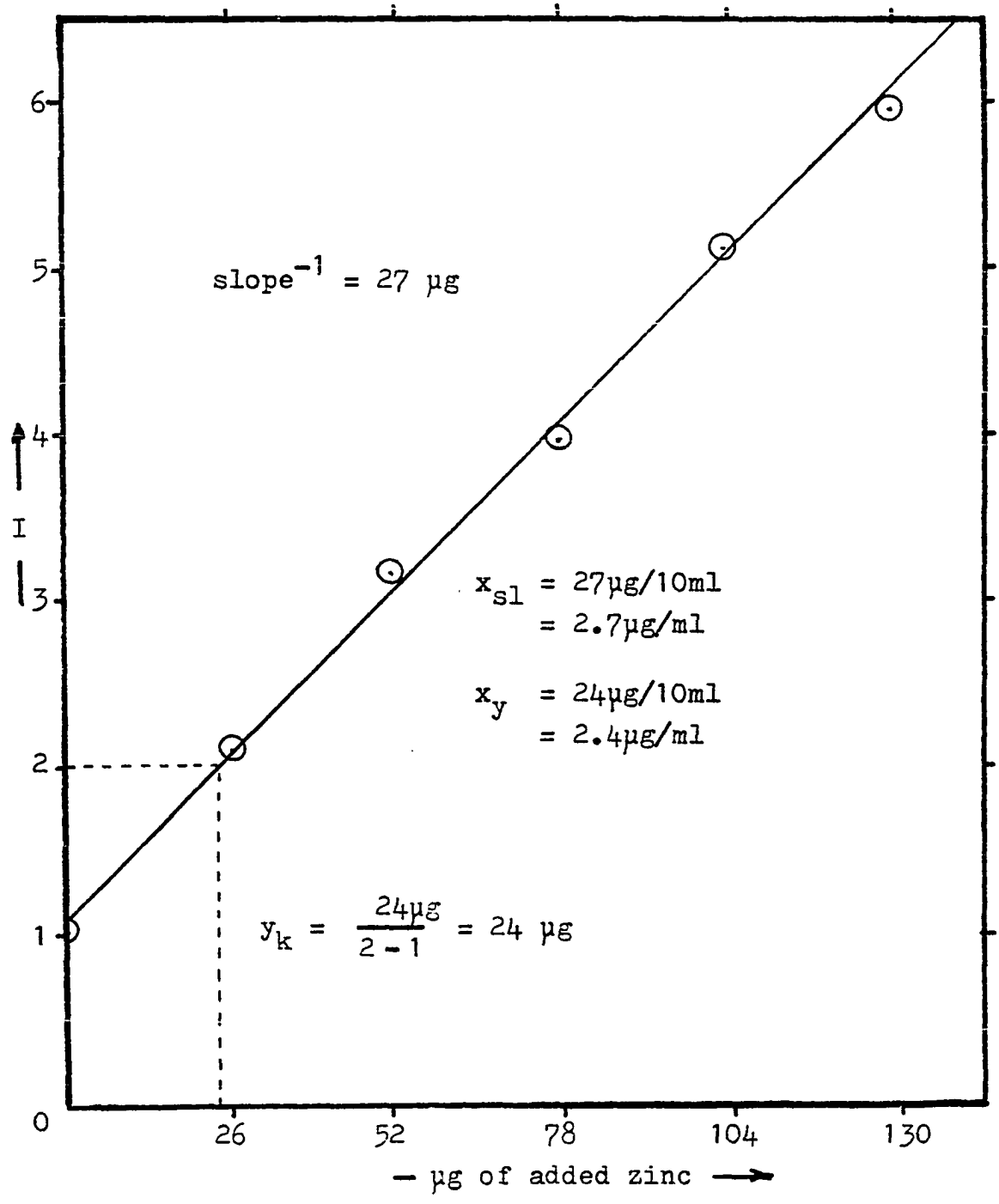

Figure I.2. Comparison Study versus State Health Department- Run 2A

Table I.3

Comparison Study versus State Health Department- Run 3A

K = 2	Kx > SR	SR = 0.55 µmole
x_{kn}	2.9µg/ml = 0.44µmole/10ml	pH = 7.95
y	13 µg/ml = 2.0 µmole/10ml	pH = 7.70
INTERCEPT = 1.10	x_y = 2.3µg/ml	x_{sl} = 2.6µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.89	0	0	42293	-
10	0.44	0	0	39064	1.08
10	0.44	2	0.4	20458	2.07
10	0.44	4	0.8	13299	3.18
10	0.44	6	1.2	10061	4.20
10	0.44	8	1.6	8191	5.16
10	0.44	10	2.0	6907	6.12

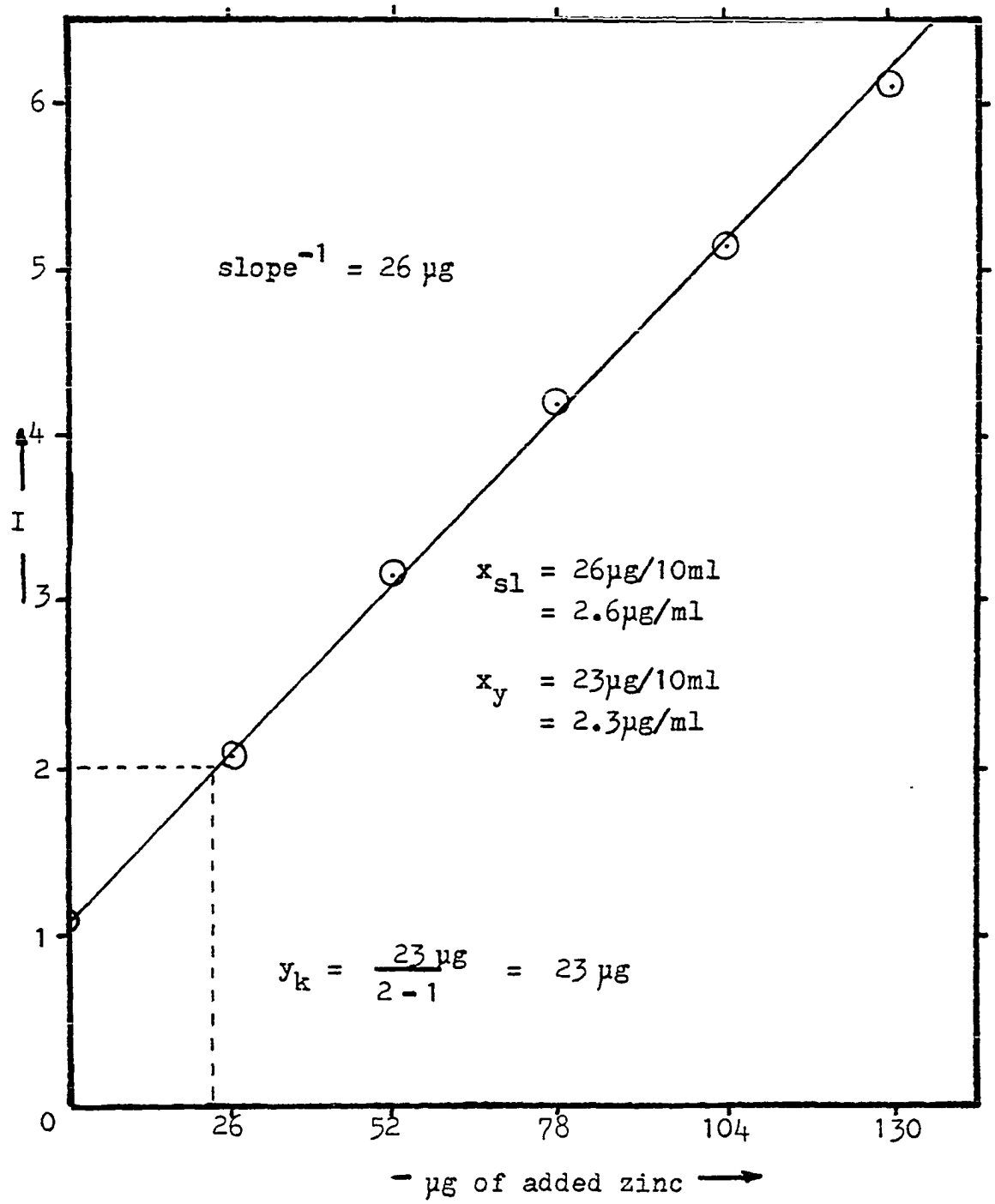

Figure I.3. Comparison Study versus State Health Department- Run 3A

Table I.4

Comparison Study versus State Health Department- Run 1B

K = 2		Kx > SR		SR = 0.15 µmole	
x_{kn}	1.2µg/ml = 0.37µmole/10ml			pH = 7.95	
y	4.0µg/ml = 0.61µmole/10ml			pH = 8.30	
INTERCEPT = 1.14		x_y = 1.1µg/ml		x_{sl} = 1.3µg/ml	
x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.37	0	0	12588	-
10	0.18	0	0	10678	1.18
10	0.18	2	0.12	6984	1.80
10	0.18	4	0.24	5361	2.35
10	0.18	6	0.36	4242	2.97
10	0.18	8	0.49	3664	3.44
10	0.18	10	0.61	2860	4.40

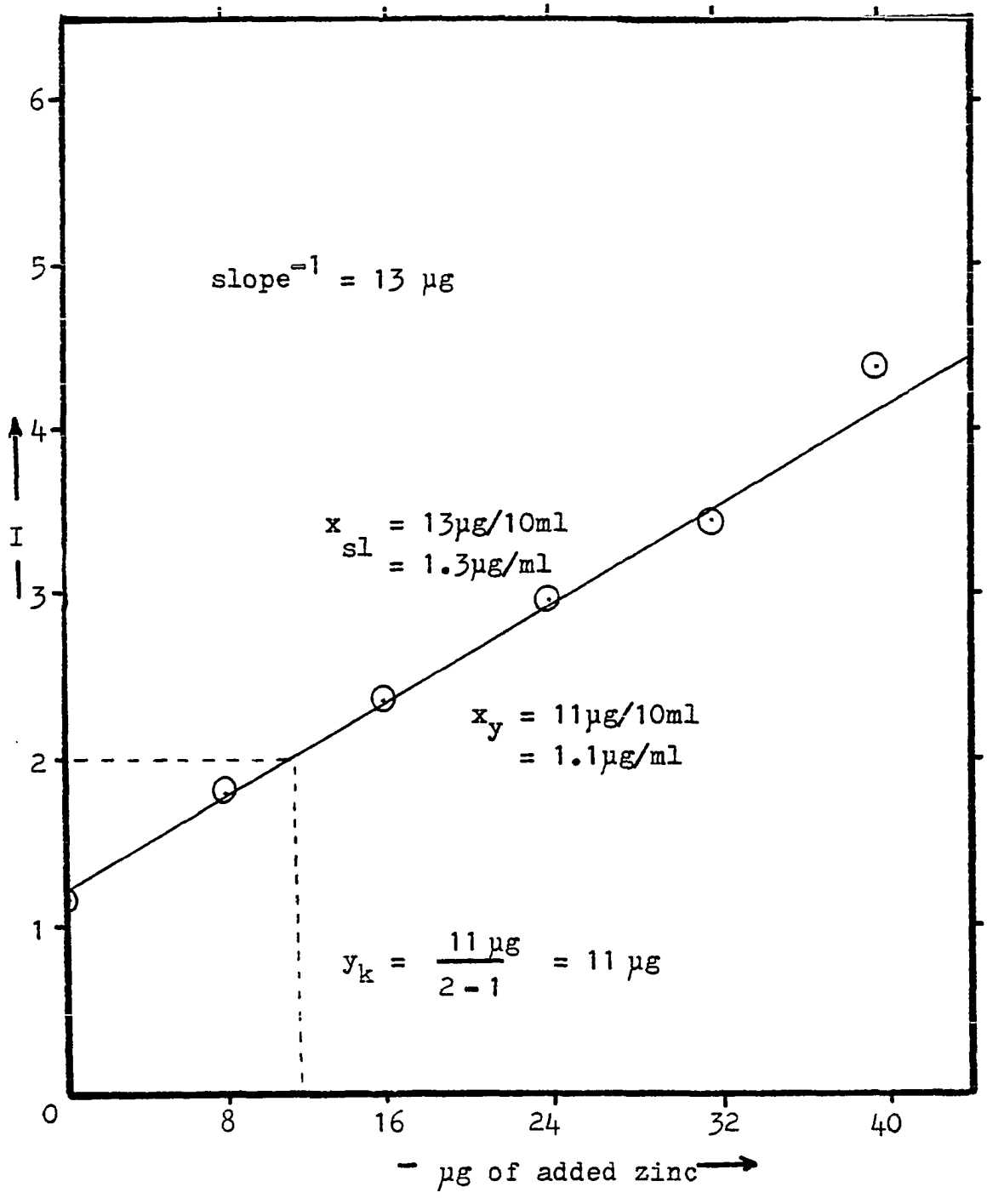

Figure I.4. Comparison Study versus State Health Department- Run 1B

Table I.5

Comparison Study versus State Health Department- Run 2B

K = 2	Kx > SR	SR = 0.15 μmole
x_{kn}	1.2μg/ml = 0.37μmole/10ml	pH = 7.95
y	4.0μg/ml = 0.61μmole/10ml	pH = 8.30
INTERCEPT = 1.00	x_y = 1.0μg/ml	x_{sl} = 1.0μg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	μmole	ml	μmole		
20	0.37	0	0	13862	-
10	0.18	0	0	13412	1.03
10	0.18	2	0.12	7839	1.77
10	0.18	4	0.24	5443	2.55
10	0.18	6	0.36	4219	3.29
10	0.18	8	0.49	3281	4.23
10	0.18	10	0.61	2834	4.89

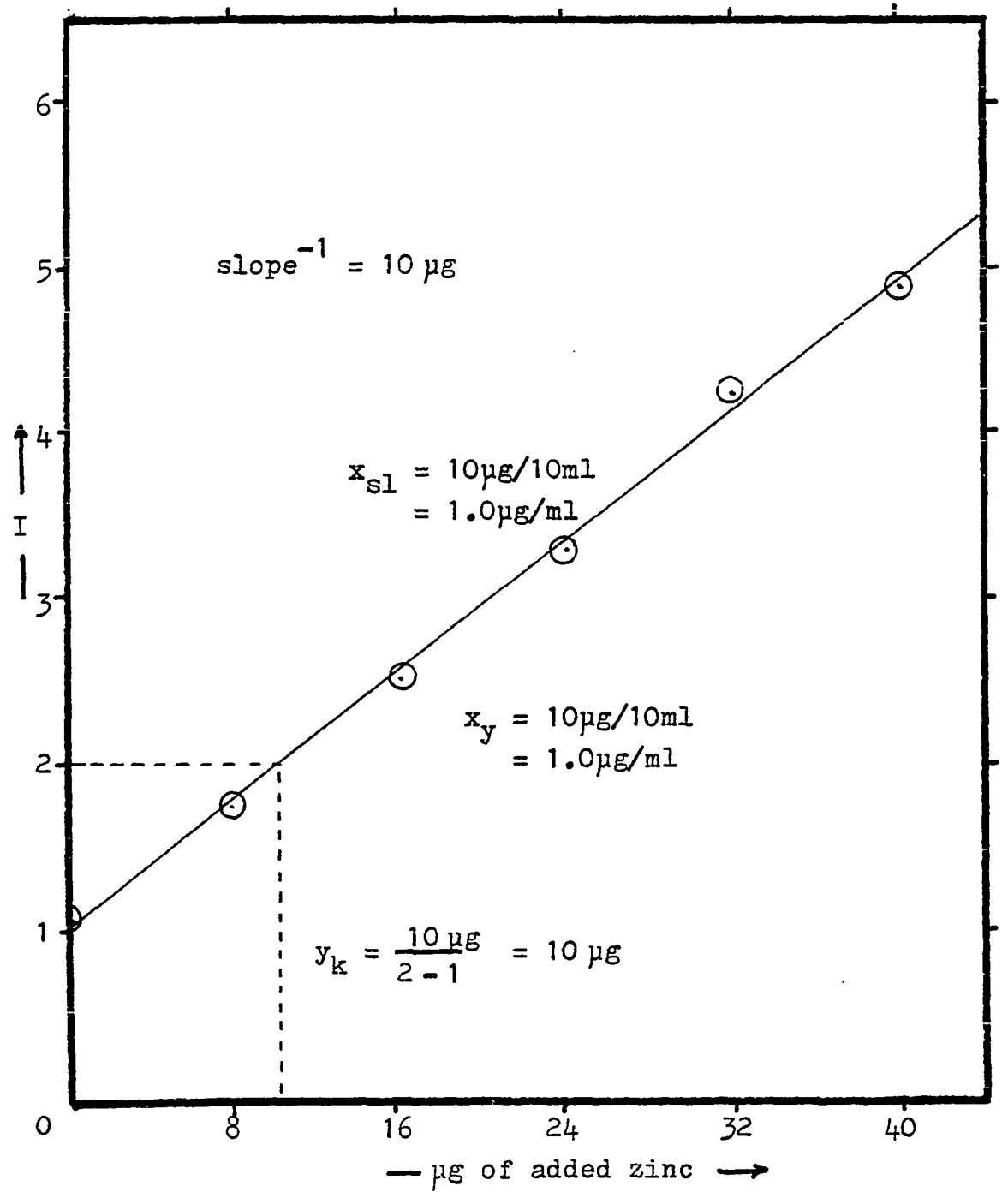

Figure H.5. Comparison Study versus State Health Department- Run 2B

Table I.6

Comparison Study versus State Health Department- Run 3B

K = 2	Kx > SR	SR = 0.15 µmole
x_{kn}	1.2µg/ml = 0.37µmole/10ml	pH = 7.95
y	4.0µg/ml = 0.61µmole/10ml	pH = 8.30
INTERCEPT = 1.13	x_y = 1.1µg/ml	x_{sl} = 1.3µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.37	0	0	19513	-
10	0.18	0	0	18406	1.06
10	0.18	2	0.12	11333	1.72
10	0.18	4	0.24	8011	2.44
10	0.18	6	0.36	6414	3.04
10	0.18	8	0.49	5461	3.57
10	0.18	10	0.61	4785	4.08

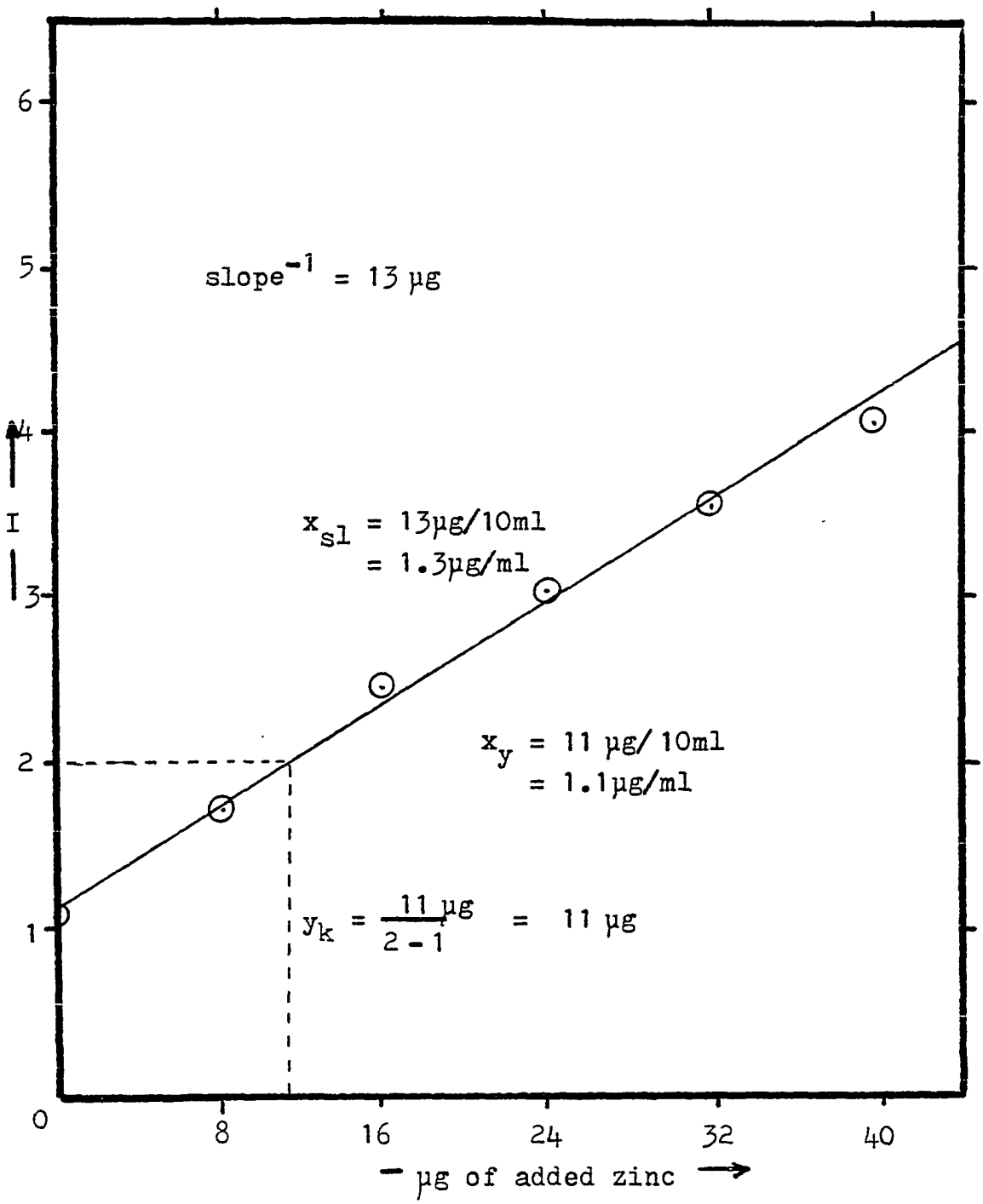

Figure I.6. Comparison Study versus State Health Department= Run 3B

Table I.7

Comparison Study versus State Health Department- Run 1C

K = 2	Kx > SR	SR = 0.06 µmole
x_{kn}	0.19µg/ml = 0.03µmole/10ml	pH = 7.50
y	0.76µg/ml = 0.12µmole/10ml	pH = 7.70
INTERCEPT = 1.07	x_y = 0.29µg/ml	x_{sl} = 0.32µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.06	0	0	14542	-
10	0.03	0	0	12835	1.13
10	0.03	2	0.02	9683	1.50
10	0.03	4	0.05	7054	2.06
10	0.03	6	0.07	5900	2.46
10	0.03	8	0.09	5040	2.88
10	0.03	10	0.12	4048	3.59

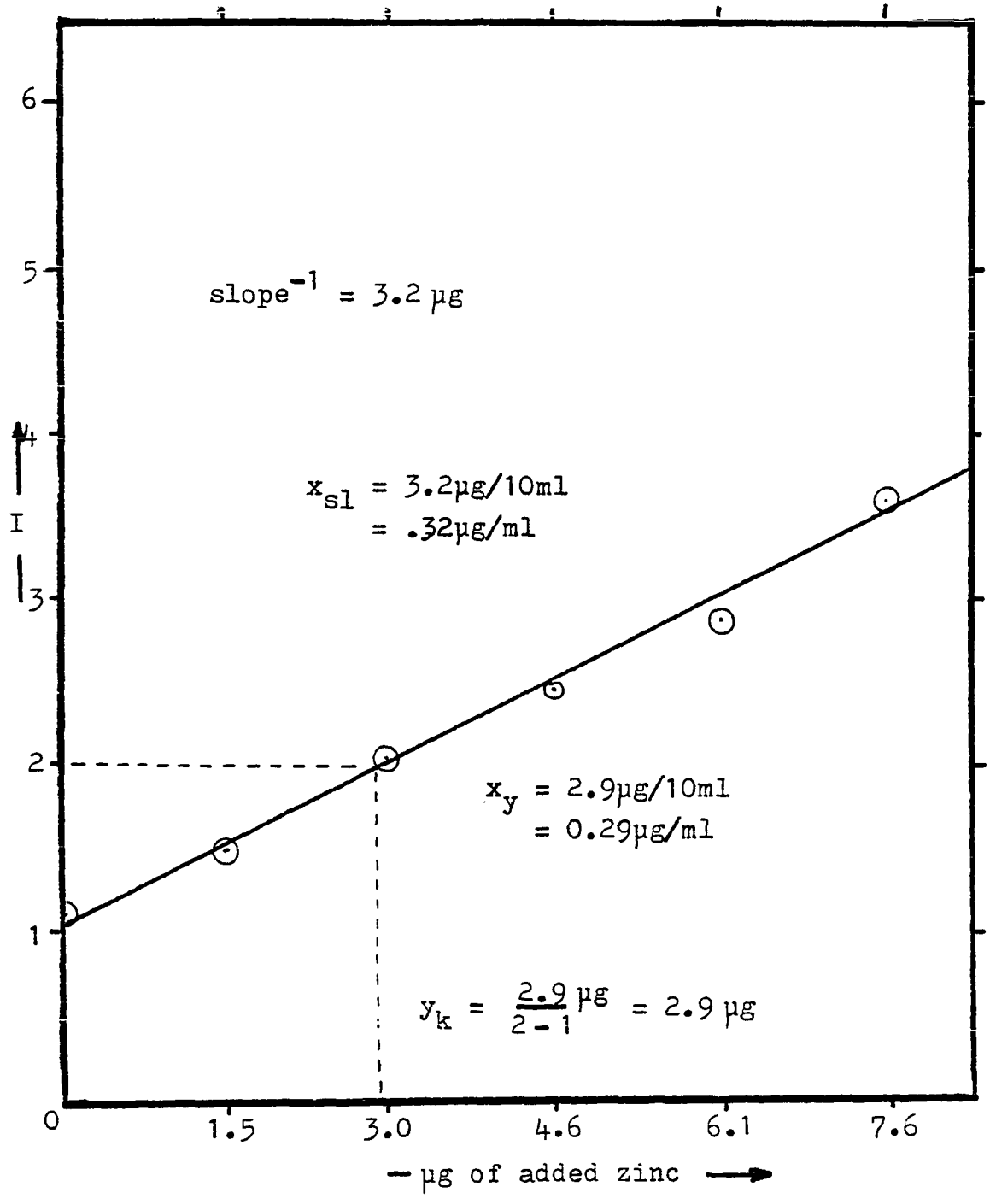

Figure I.7. Comparison Study versus State Health Department- Run 1C

Table I.8

Comparison Study versus State Health Department- Run 2C

K = 2	Kx > SR	SR = 0.06 µmole
x_{kn}	0.19µg/ml = 0.03µmole/10ml	pH = 7.50
y	0.76µg/ml = 0.12µmole/10ml	pH = 7.70
INTERCEPT = 1.04	x_y = 0.28µg/ml	x_{sl} = 0.29µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.06	0	0	20121	-
10	0.03	0	0	18273	1.10
10	0.03	2	0.02	12742	1.58
10	0.03	4	0.05	9696	2.08
10	0.03	6	0.07	8046	2.50
10	0.03	8	0.09	6448	3.12
10	0.03	10	0.12	5312	3.79

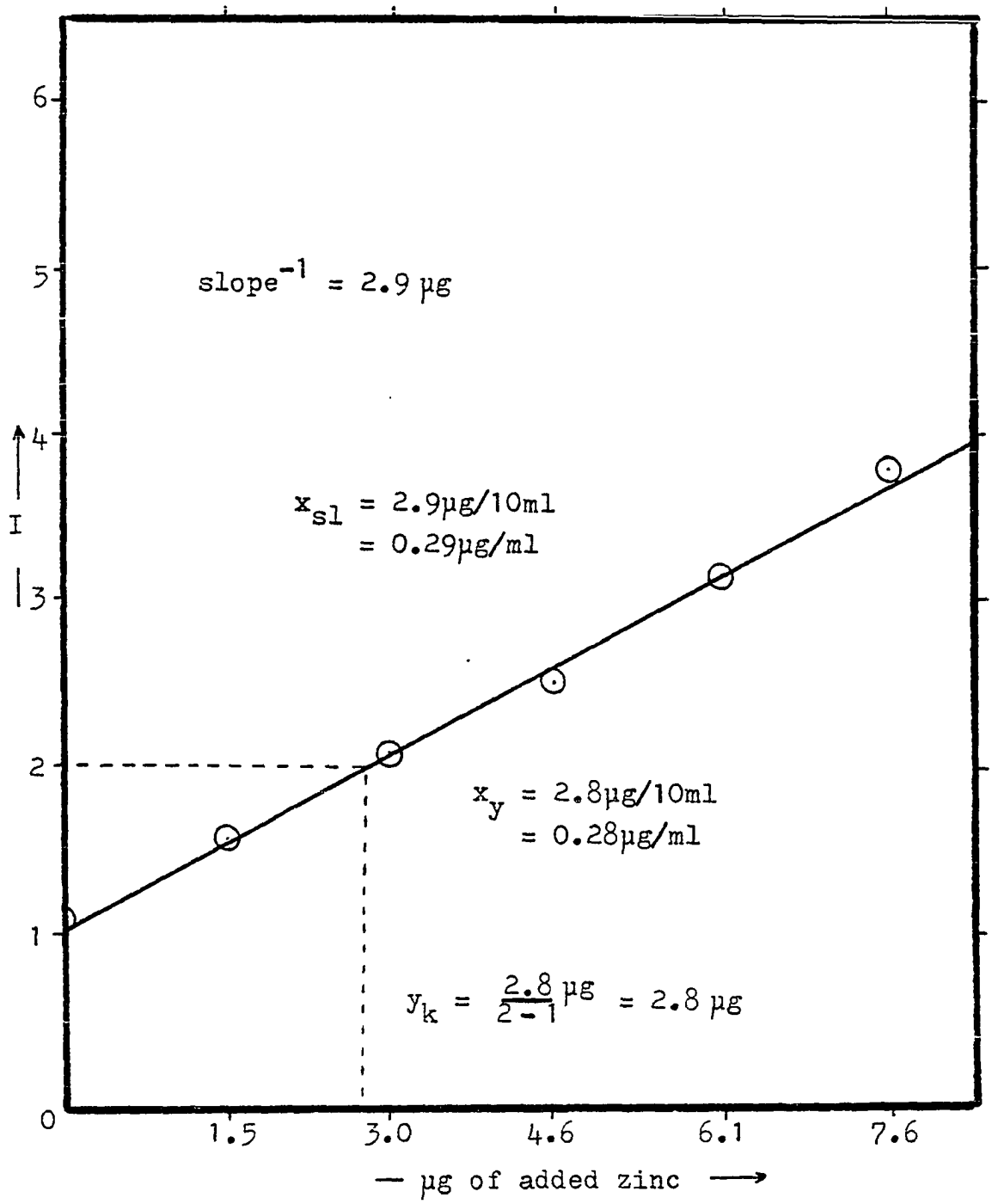

Figure I.8. Comparison Study versus State Health Department- Run 2C

Table I.9

Comparison Study versus State Health Department- Run 3C

K = 2	Kx > SR	SR = 0.06 µmole
x_{kn}	0.19µg/ml = 0.03µmole/10ml	pH = 7.50
y	0.76µg/ml = 0.12µmole/10ml	pH = 7.70
INTERCEPT = 1.23	x_y = 0.26µg/ml	x_{sl} = 0.34µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.06	0	0	17077	-
10	0.03	0	0	14096	1.21
10	0.03	2	0.02	10267	1.66
10	0.03	4	0.05	7863	2.17
10	0.03	6	0.07	6672	2.56
10	0.03	8	0.09	5621	3.04
10	0.03	10	0.12	4360	3.92

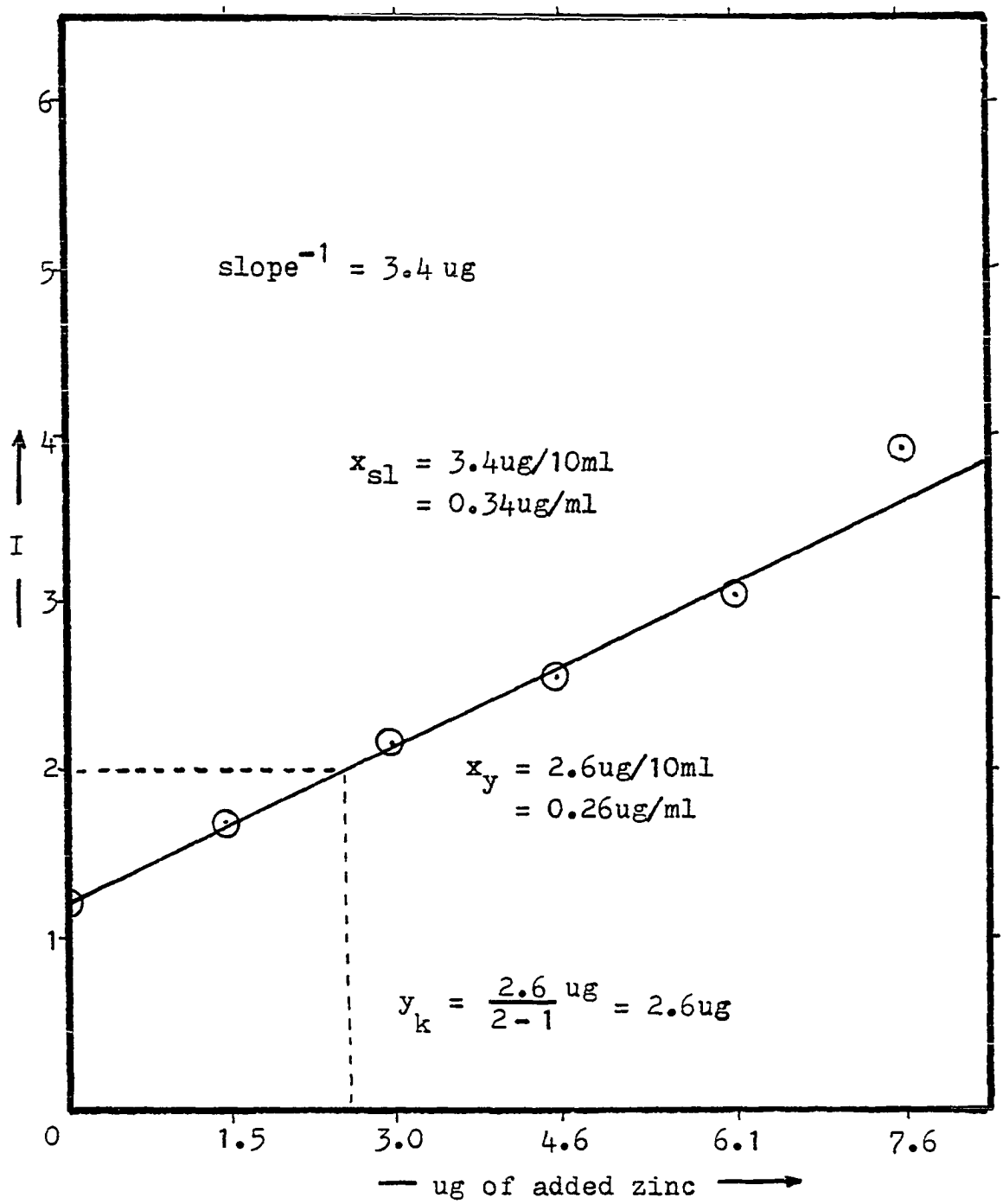

Figure I.9. Comparison Study versus State Health Department- Run 3C

APPENDIX J

EFFECTS OF VARYING AMOUNTS OF DITHIZONE

Table J.1

Effect of Increased Amounts of Dithizone- Run 1

K = 2	Kx > SR	SR = 2.55 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 1.00	x_y = 11.0µg/ml	x_{sl} = 10.9µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	4174	-
10	1.53	0	0	4078	1.02
10	1.53	2	1.53	2151	1.94
10	1.53	4	3.06	1502	2.78
10	1.53	6	4.59	1125	3.71
10	1.53	8	6.12	898	4.65
10	1.53	10	7.65	744	5.61

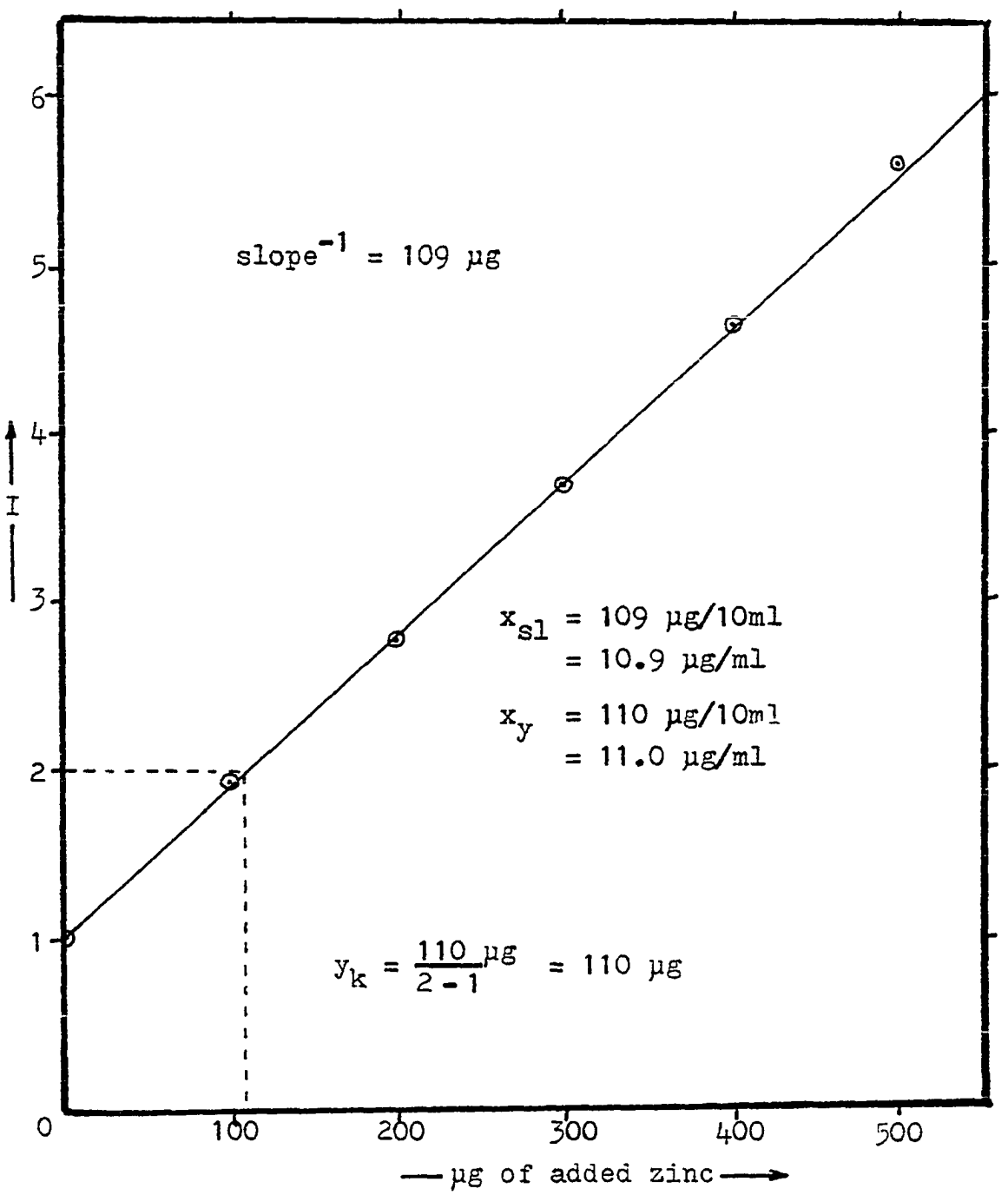

Figure J.1. Effect of Increased Amounts of Dithizone-Run 1

Table J.2

Effect of Increased Amounts of Dithizone- Run 2

K = 2	Kx > SR	SR = 3.31 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 1.08	x_y = 11.0µg/ml	x_{sl} = 11.9µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	5036	-
10	1.53	0	0	2702	1.86
10	1.53	2	1.53	2396	2.10
10	1.53	4	3.06	1800	2.80
10	1.53	6	4.59	1368	3.68
10	1.53	8	6.12	1215	4.14
10	1.53	10	7.65	925	5.44

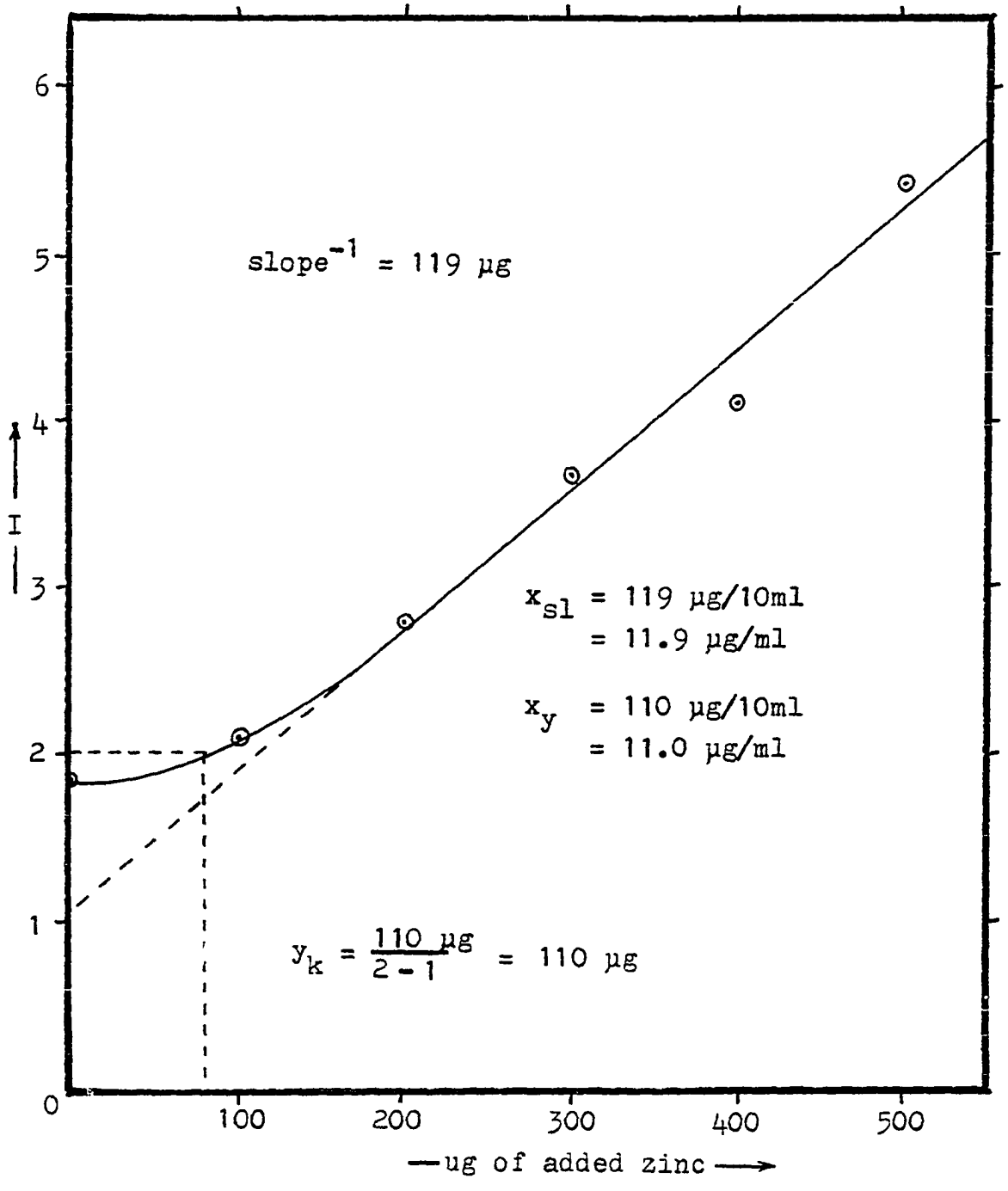

Figure J.2. Effect of Increased Amounts of Dithizone-Run 2

Table J.3

Effect of Increased Amounts of Dithizone- Run 3

K = 2	Kx > SR	SR = 3.82 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 1.55	x_y = 6.34µg/ml	x_{sl} = 14.3µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	3925	-
10	1.53	0	0	1624	2.42
10	1.53	2	1.53	1875	2.09
10	1.53	4	3.06	1381	2.84
10	1.53	6	4.59	1043	3.76
10	1.53	8	6.12	880	4.46
10	1.53	10	7.65	796	4.93

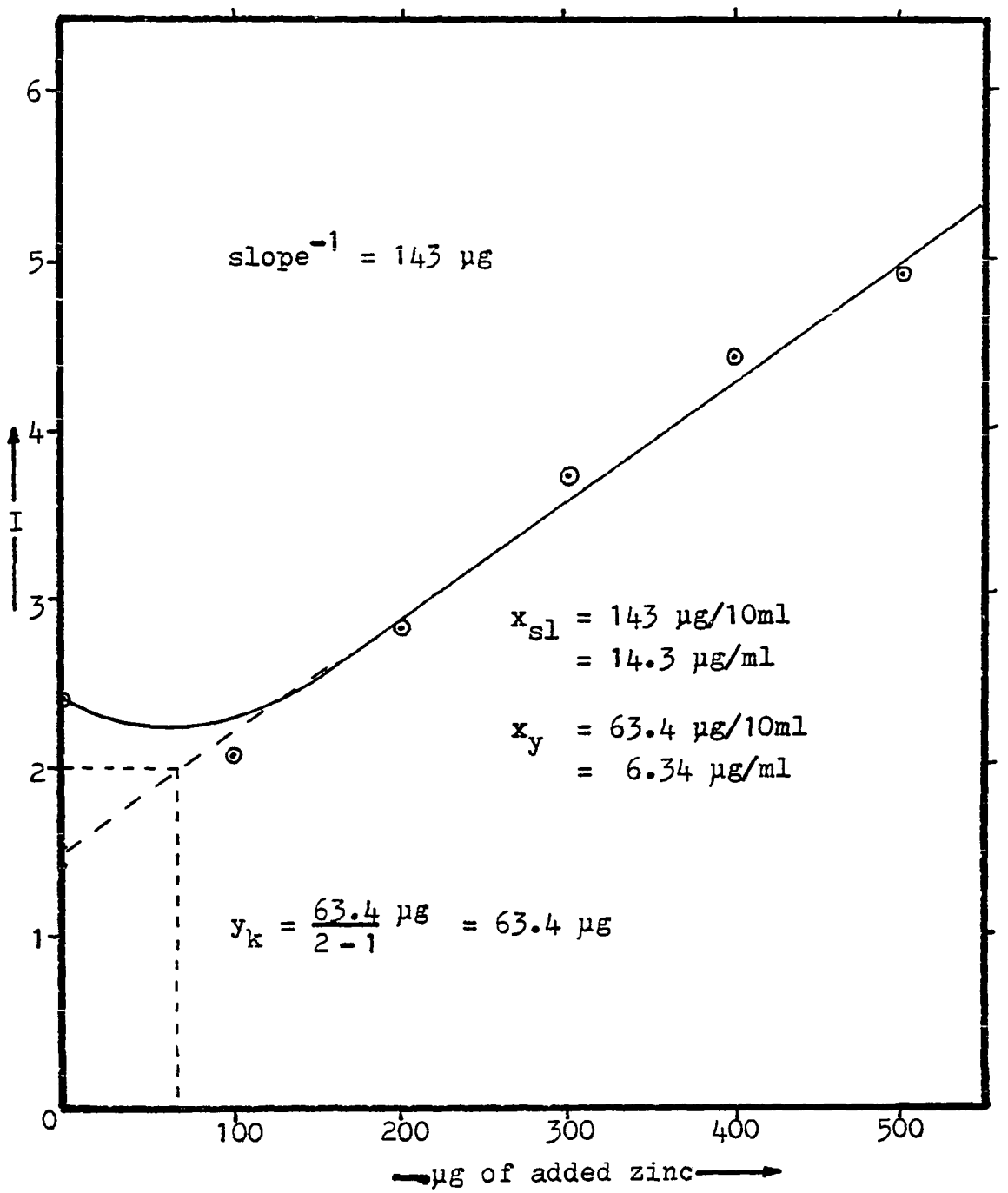

Figure J.3. Effect of Increased Amounts of Dithizone-Run 3

Table J.4

Effect of Increased Amounts of Dithizone- Run 4

K = 2	Kx > SR	SR = 5.10 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 1.35	x_y = 17.9µg/ml	x_{sl} = 17.6µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	4706	-
10	1.53	0	0	1266	3.72
10	1.53	2	1.53	2767	1.70
10	1.53	4	3.06	2236	2.10
10	1.53	6	4.59	1720	2.74
10	1.53	8	6.12	1475	3.19
10	1.53	10	7.65	1227	3.84

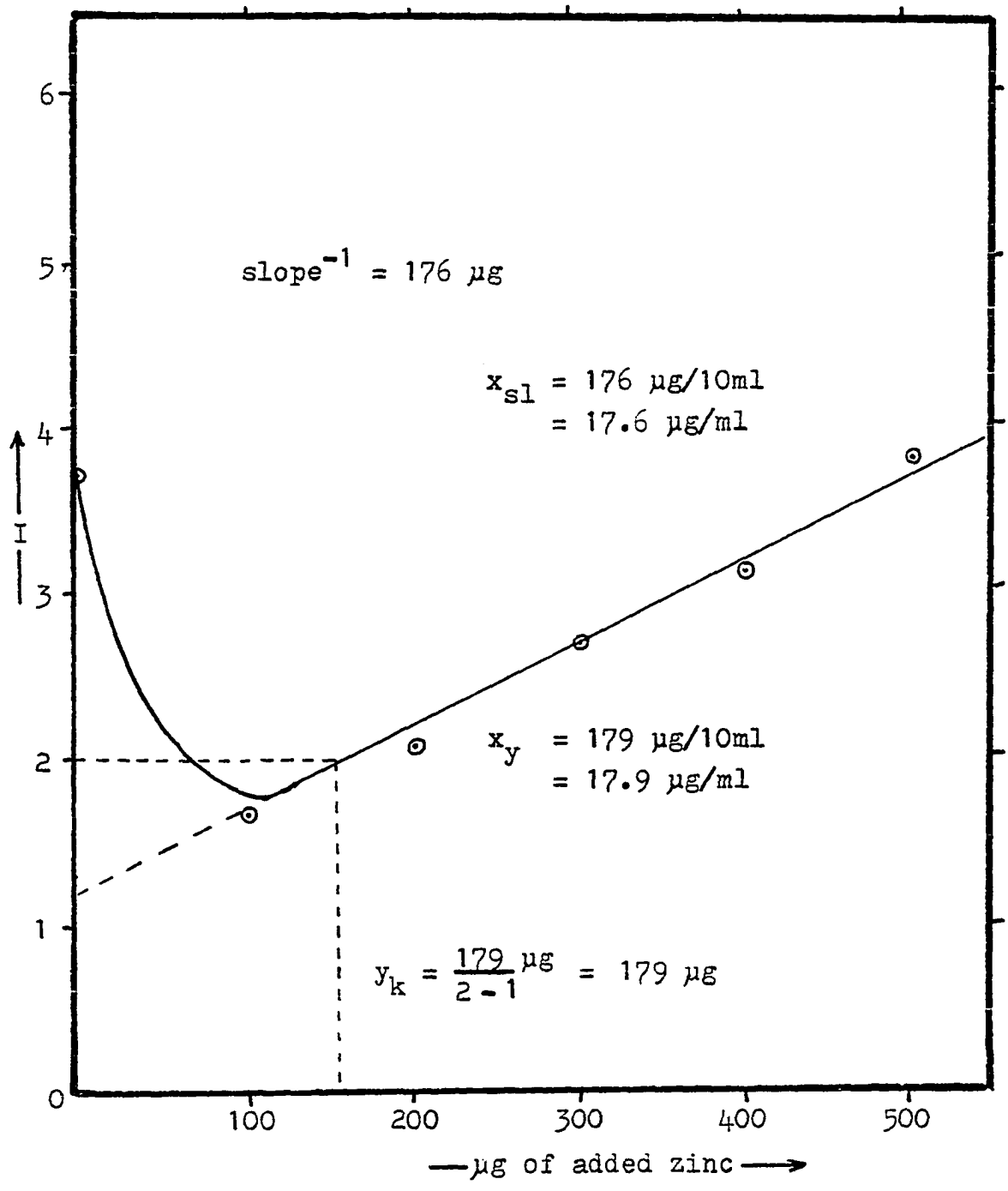

Figure J.4. Effect of Increased Amounts of Dithizone-Run 4

Table J.5

Effect of Increased Amounts of Dithizone- Run 5

K = 2	SR > Kx	SR = 7.65 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 2.06	x_y > 25µg/ml	x_{sl} = 109µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	1597	-
10	1.53	0	0	435	3.67
10	1.53	2	1.53	732	2.18
10	1.53	4	3.06	701	2.28
10	1.53	6	4.59	744	2.14
10	1.53	8	6.12	656	2.43
10	1.53	10	7.65	630	2.53

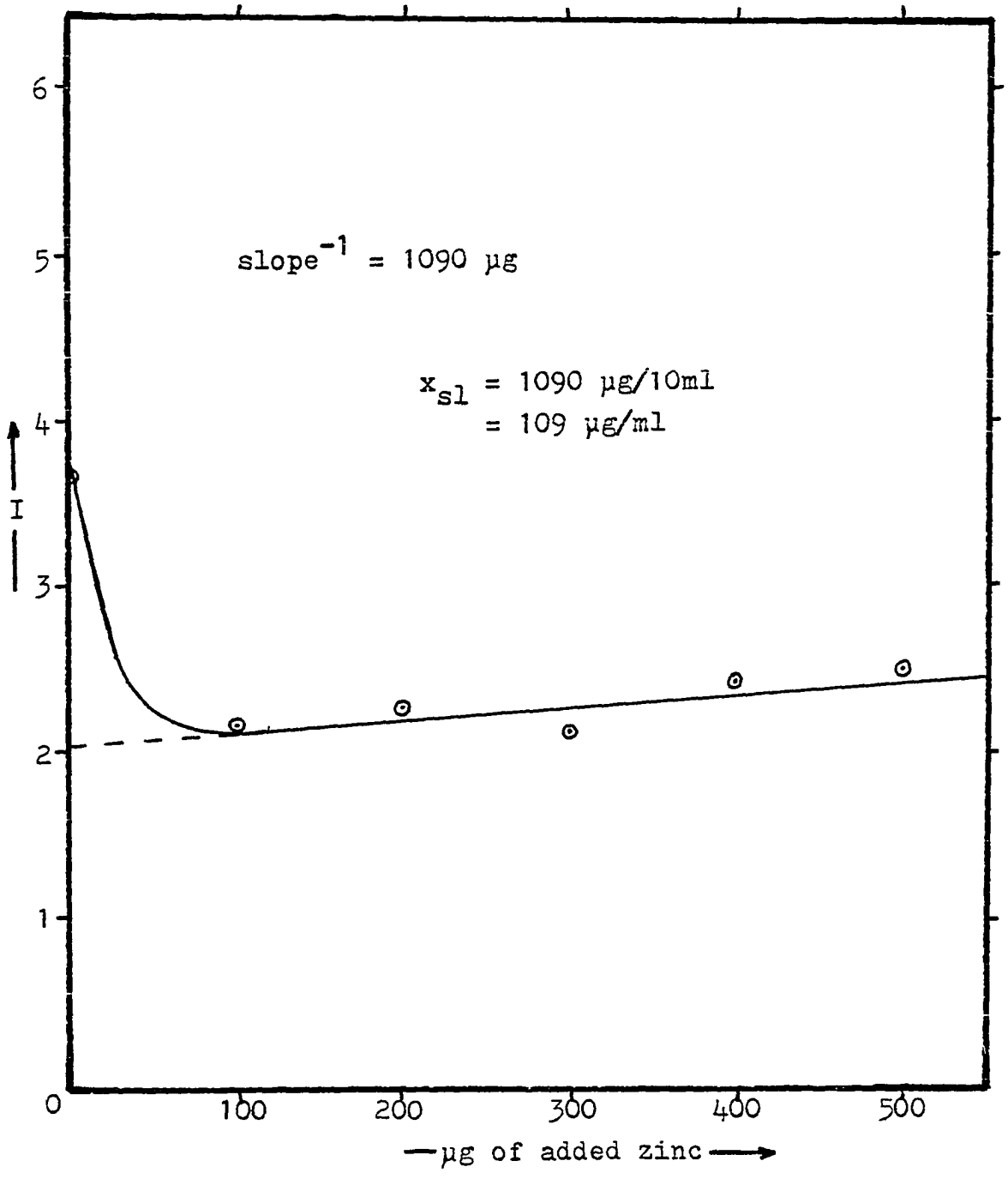

Figure J.5. Effect of Increased Amounts of Dithizone-Run 5

Table J.6

Effect of Increased Amounts of Dithizone- Run 6

K = 2	Kx > SR	SR = 3.82 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 0.95	x_y = 10.7µg/ml	x_{sl} = 10.2µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	5728	-
10	1.53	0	0	4129	1.39
10	1.53	2	1.53	3010	1.90
10	1.53	4	3.06	1984	2.89
10	1.53	6	4.59	1454	3.94
10	1.53	8	6.12	1166	4.91
10	1.53	10	7.65	991	5.78

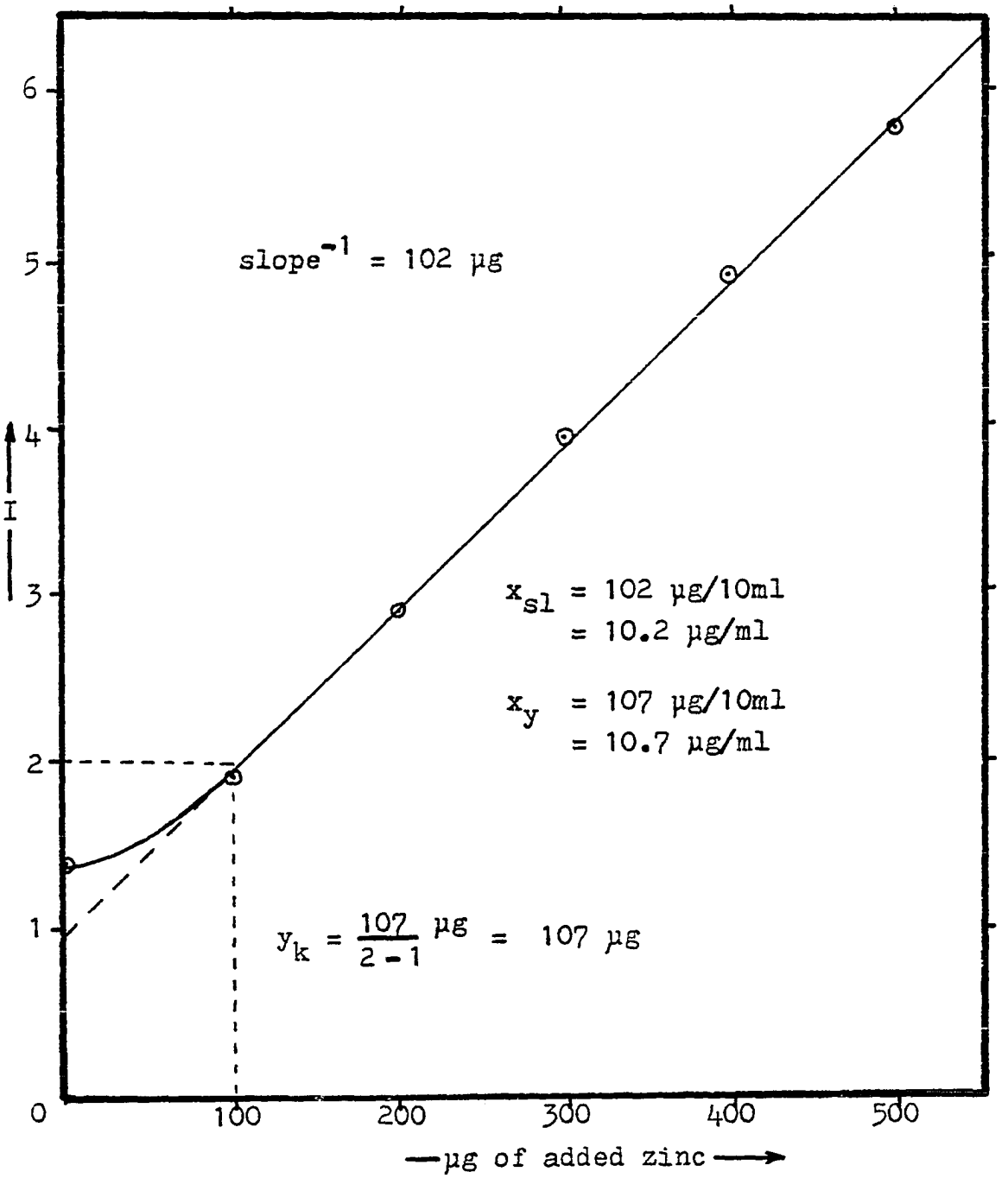

Figure J.6. Effect of Increased Amounts of Dithizone-Run 6

Table J.7

Effect of Increased Amounts of Dithizone- Run 7

K = 2	Kx > SR	SR = 5.10 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 1.02	x_y = 10.5µg/ml	x_{sl} = 10.6µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	8701	-
10	1.53	0	0	4477	1.94
10	1.53	2	1.53	4454	1.95
10	1.53	4	3.06	3025	2.88
10	1.53	6	4.59	2268	3.84
10	1.53	8	6.12	1802	4.83
10	1.53	10	7.65	1534	5.67

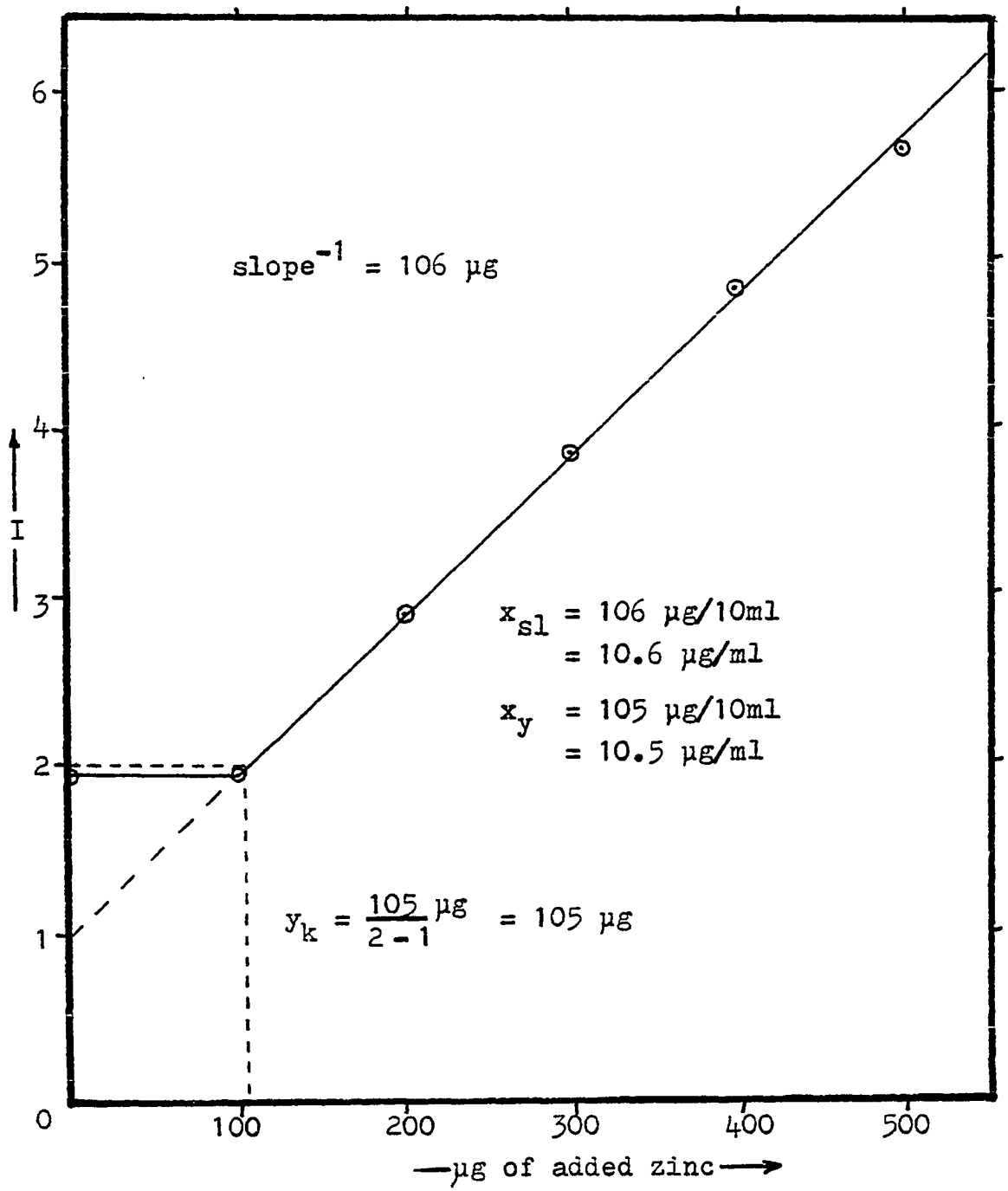

Figure J.7. Effect of Increased Amounts of Dithizone-Run 7

Table J.8

Effect of Increased Amounts of Dithizone- Run 8

K = 2	SR > Kx	SR = 7.65 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT = 0.99	x_y = 13.5µg/ml	x_{sl} = 13.3µg/ml

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	7079	-
10	1.53	0	0	2979	2.38
10	1.53	2	1.53	3380	2.09
10	1.53	4	3.06	2840	2.49
10	1.53	6	4.59	2181	3.25
10	1.53	8	6.12	1784	3.97
10	1.53	10	7.65	1491	4.75

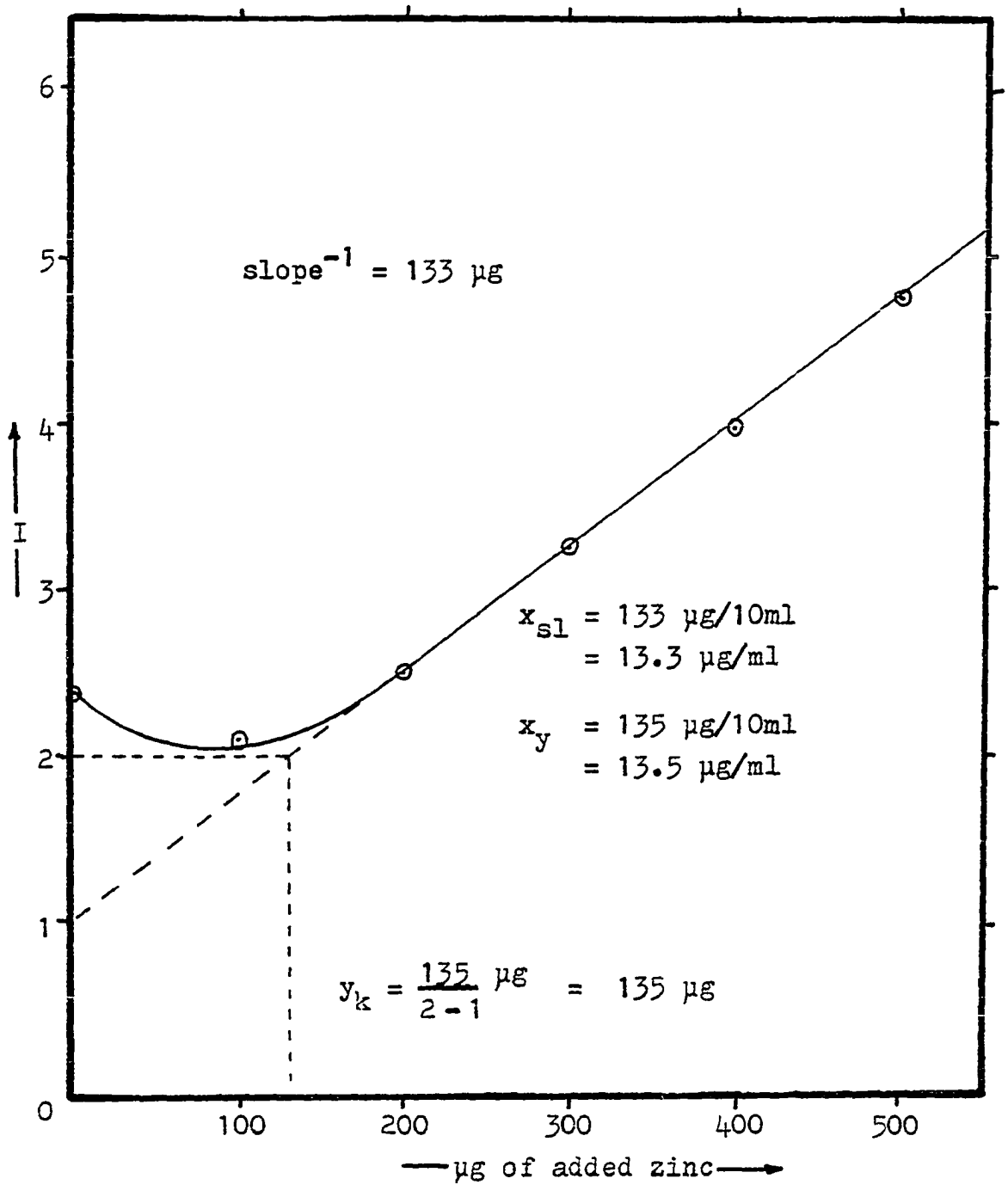

Figure J.8. Effect of Increased Amounts of Dithizone-Run 8

Table J.9

Effect of Increased Amounts of Dithizone- Run 9

K = 2	SR > Kx	SR = 15.3 µmole
x_{kn}	10.0µg/ml = 1.53µmole/10ml	pH = 8.6
y	50.0µg/ml = 7.65µmole/10ml	pH = 8.6
INTERCEPT =	x_y > 50µg/ml	x_{sl} =

x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	3.06	0	0	8116	-
10	1.53	0	0	3026	2.68
10	1.53	2	1.53	3984	2.04
10	1.53	4	3.06	4771	1.70
10	1.53	6	4.59	5047	1.61
10	1.53	8	6.12	4528	1.79
10	1.53	10	7.65	4284	1.89

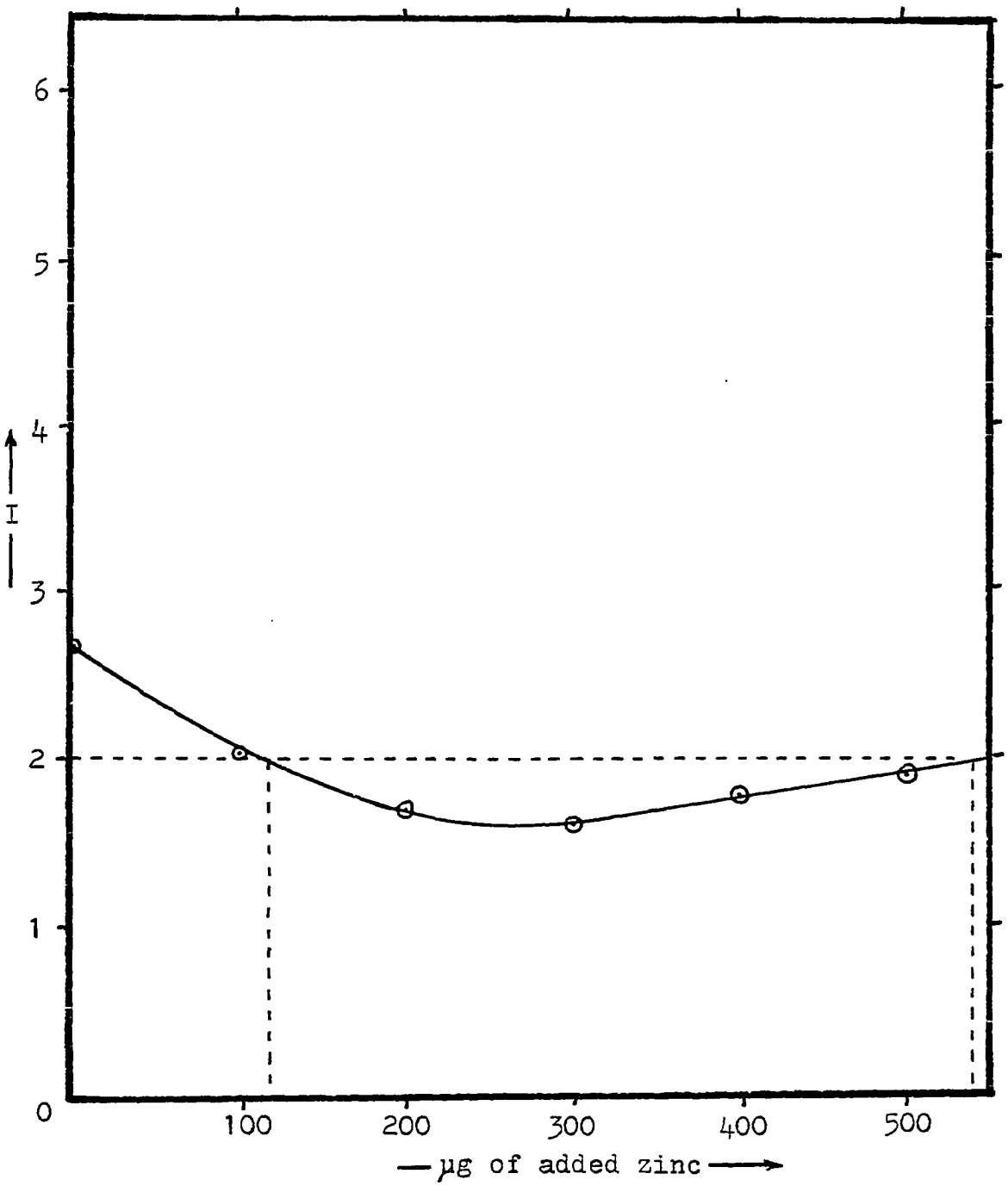

Figure J.9. Effect of Increased Amounts of Dithizone-Run 9

Table J.10

Effect of Increased Amounts of Dithizone- Run 10

K = 2		SR > Kx		SR = 5.07 µmole	
x_{kn}	2.0µg/ml = 0.31µmole/10ml			pH = 8.6	
y	10.0µg/ml = 0.77µmole/10ml			pH = 8.6	
INTERCEPT = 2.48		x_y = 3.35µg/ml		x_{sl} = -6.14µg/ml	
x		y		CORRECTED COUNT PER MINUTE	I
ml	µmole	ml	µmole		
20	0.61	0	0	5336	-
10	0.31	0	0	2026	2.63
10	0.31	2	0.31	2654	2.01
10	0.31	4	0.61	2851	1.87
10	0.31	6	0.92	3251	1.68
10	0.31	8	1.22	3870	1.38
10	0.31	10	1.53	5179	1.03

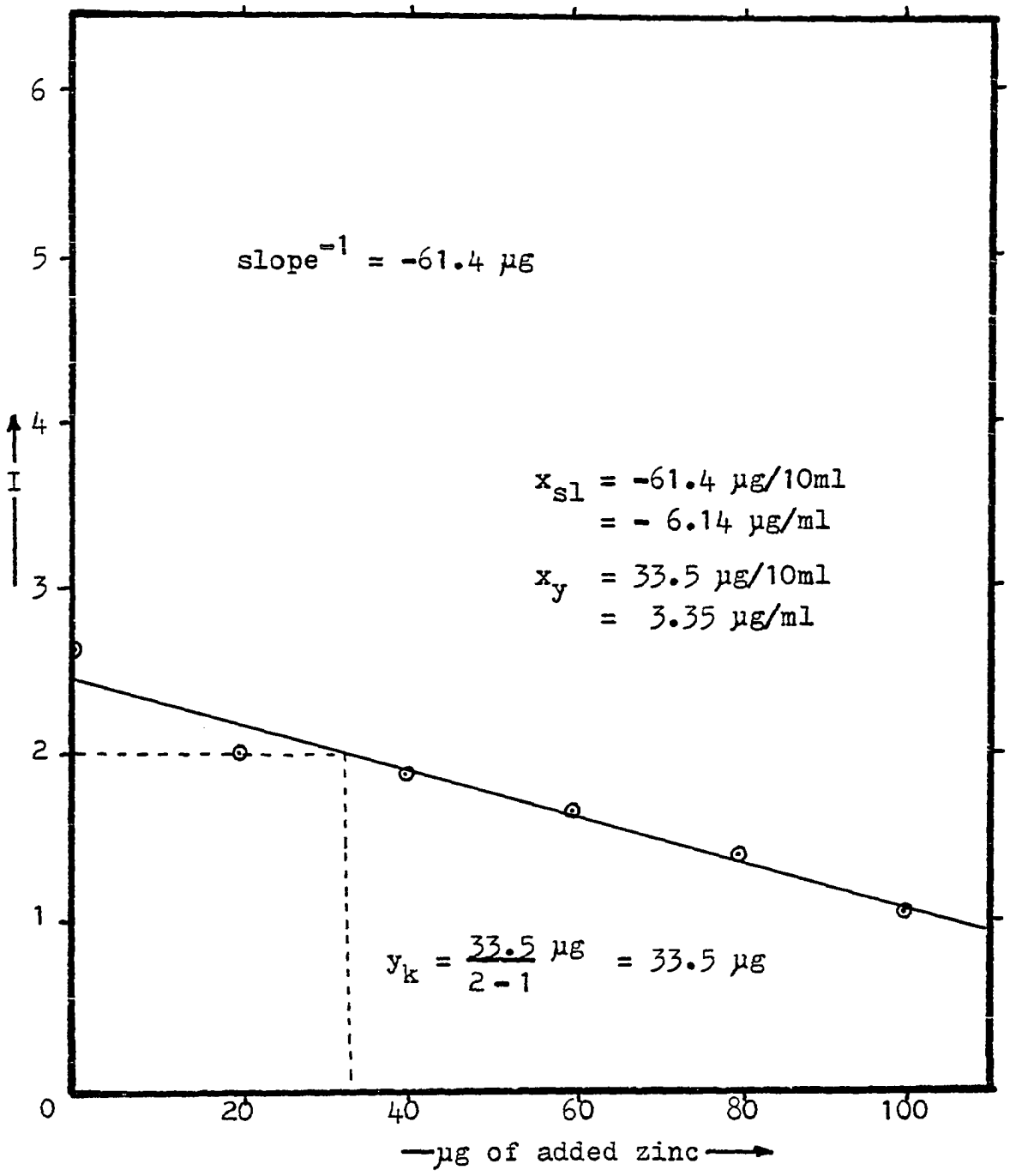

Figure J.10. Effect of Increased Amounts of Dithizone-Run 10