Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *85* (3): 329-338, jul./sep. 1990

STUDY ON THERMOSTABILIZERS FOR TRIVALENT ORAL POLIOMYELITIS VACCINE

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Different formulations of Trivalent Oral Poliomyelitis Vaccine were tested, in order to obtain better thermostability, reduce corrosion of machinery and improve production costs.

Magnesium chloride, sucrose, arginine and 199-Hank's medium were used in the formulations. The most appropriate formulation was a mixture of MgCl2 and arginine, which was highly thermostable, and had low production costs.

The low corrosive formulation was rejected, due to low thermostability on storage.

Key words: poliovaccine – thermostabilizers

In 1984, the Poliomyelitis Laboratory, with the technical cooperation of the Japan Poliomyelitis Research Institute (JPRI) of Tokyo, started work on the technological aspects of formulation, in Brazil, of a Trivalent Oral Poliomyelitis Vaccine (TOPV).

In 1984, this Laboratory formulated the first batches of TOPV, using sucrose as thermostabilizer, with the JPRI technology.

Due to the importation of Poliovirus concentrate standardized and pre-stabilized with MgCl2, our vaccine was reformulated using MgCl2 as thermostabilizer.

After producing TOPV for one year, it was found that the corrosion, caused by MgCl2, would drastically reduce the expected service time of machinery and equipment. There was also some concern about production costs and dependence on imported ingredients.

Based on these two points, our Laboratory decided to search for a less agressive low cost formulation. This formulation should also improve (or at least maintain) the thermostability presently achieved by the current formulation for refrigeration and environment temperatures of Brazil, specially for Poliovirus types I and III, prevalent in confirmed cases of Brazilian poliomyelitis (Poliomielite, 1989; WIN, 1989).

The formulations tested involved raw materials available in the Brazilian market, at costs compatible with the final costs of the vaccine and guaranteeing the quality of the vaccine.

Considering the information of Wallis & Melnick (1961), Melnick (1963), Imam (1976), Magrath (1976), Mirchamsy et al. (1978), and our own experience, formulations based on MgCl2, sucrose, arginine and 199-Hank's medium were investigated.

The temperatures selected for the test were 20 °C, 26 °C and 37 °C, corresponding to the expected environmental temperatures in different regions of the country.

Titration was carried out for the trivalent vaccine and for each type of virus, to detect individual strain stability with a given termostabilizer (Magrath, 1976; Mauler & Gruschkau, 1978; Peetermans & Colinet, 1980).

MATERIALS AND METHODS

Vaccine – Three different formulations were prepared, using the same Poliovirus concentrates (I, II and III), as:

Received 13 March 1990.
Accepted 21 May 1990.

1) TOPV – SK – RIT* pH6.4
2) Sucrose 35%, Arginine 0.05M in H2O pH 6.9
3) MgCl2 1M, Arginine 0.05M in H2O pH 6.6
4) MgCl2 1M in 199-Hank's medium pH 6.6

3 ml vials were filled with 2.2 ml of the different preparations, corresponding to 20 human doses.

The preparations were subjected to different temperatures for varying times and subsequently kept at −20 °C until titration.

Virus titration – Samples in each series were tested toghether.

The technique used was that of the JPRI, adapted to meet WHO Minimum Requirements.

Cells used were GMK-2 (Green Monkey Kidney Cell), cultivated in 199-Earle's medium with 10% fetal calf serum (FCS) plus 0.11% NaHCO3.

Cell suspension was prepared immediately before titration, using 199-Earle's medium plus 2% FCS and 0.225% NaHCO3. This suspension contained 250,000 cells/ml and was distributed into microplates of 96 wells (0.1 ml/well).

Test samples and the reference vaccine were diluted with 199 Earle's medium in intervals of 0.5 log10. To find the monovalent titers, the undesired types of virus were neutralized using appropriate anti-sera as necessary. Fifty microliters of each dilution were inoculated in each of eight wells of the microplate.

Microplates were incubated at 36 °C in CO2 incubator and results finally read by the observation of CPE (citopathogenic effect) on the seventh day, to determine the virus titer.

Calculations were done by computer, using the Reed-Muench method.

* This vaccine was used as reference standard, for thermostability as it has been used in throughout the country for vaccination activities in the last 10 years.

RESULTS

Titers given in the tables are results of geometric mean of four titrations, analyzed by linear regression (Greiff & Rightsel, 1964; Peetermans & Colinet, 1980; Allison et al., 1981), to compare the stability of each trivalent titer and individual virus type titers in each formulation (Tables I, III, V, VII).

Considering the slope (k) obtained at the linear regression analysis, the data obtained on trivalent, and types I, II and III titers were subjected to the Arrhenius calculation, in order to estimate the half-life of the trivalent vaccines and of each virus type at 4 °C, (Tables II, IV, VI, VIII for trivalent, types I, II and III respectively).

Figure 1 shows plots of the data obtained for each formulation tested, including the trivalent and monovalent titers, and it can be observed that the standard reference preparation is significantly more stable, followed by formulation 3 for the trivalent titer. Regarding monovalent titers, results for type I are similar for formulations 3 and 4, followed by formulation 2; for type II the three formulations follow different degradation patterns, by order, 4, 2 and 3; for type III, also small differences give the order 2, 3 and 4.

Figure 2 compares more clearly the thermostability pattern of the individual virus according to each formulation. We can observe that the most homogeneous degradation curves for the three types of virus is offered by formulation 3 (MgCl2 + ARG).

Figure 3 compares directly the profiles of formulation 3 (the one that showed the most coherent degradation curve for all virus types), and formulation 4 (the one being used for production). For the trivalent titer, formulation 3 shows better performance. We also can observe that type I shows similar loss of titer with both formulations, while type III has a slight improvement in its stability with formulation 3. Type II is more stable in formulation 4.

TABLE I

Assay results and linear regression calculations of various formulations of Oral Poliomyelites Vaccine Trivalent

Vaccine	Exposure temp. °C	TCID50/dose Exposure time (days) 0	1	2	4	7	14	21	28	35	42	Correlat. (r)	Interc. time zero	Slope (k)
	37		5.93	6.29	5.27	5.01	3.80	2.55	–	–	–	0.986 c	6.18	0.17181
RIT	26	6.00		–	5.98	5.91	5.77	5.56	5.23	5.17	–	0.984 c	6.07	0.02636
	20		–	–	6.14	5.99	5.93	5.83	5.74	5.81	5.55	0.918 b	6.08	0.01097
	37		6.05	5.85	5.46	4.83	3.52	2.17	–	–	–	0.999 c	6.21	0.19239
SUC-ARG	26	6.16	–	–	6.06	6.02	5.58	5.43	5.21	4.91	–	0.992 c	6.19	0.03629
	20		–	–	6.25	6.12	5.94	5.88	5.74	5.51	5.29	0.979 c	6.26	0.02143
	37		5.95	5.72	5.17	4.68	3.15	2.05	–	–	–	0.998 c	6.08	0.19810
MgCl2-ARG	26	6.15	–	–	6.02	6.06	5.91	5.33	5.10	4.94	–	0.974 c	6.23	0.03784
	20		–	–	6.15	6.26	5.99	5.92	5.70	5.63	5.64	0.950 c	6.22	0.01557
	37		5.75	5.60	5.36	4.64	3.38	2.26	–	–	–	0.999 c	5.95	0.17799
MgCl2-199	26	5.93	–	–	5.95	5.87	5.50	5.22	5.25	4.59	–	0.962 c	6.05	0.03688
	20		–	–	6.01	5.90	6.04	5.83	5.65	5.51	5.22	0.906 b	6.08	0.01682

b) $P < 0.01$
c) $P < 0.001$

TABLE II

Arrhenius' plot calculations for various formulations of Poliomyelitis Oral Poliovaccine Trivalent

Vaccine	Exposure temp. °C	1/°Ax10E6	Arith. (k)	Derived/Corrected values Correlation (r)	Arrhenius slope	Arith. (K)	Half life (days)
	37	3226	0.17181			0.16618	1.8
RIT	26	3344	0.02636	0.998	0.00644	0.02885	10.4
	20	3413	0.01097	($P < 0.05$)		0.01036	29
	04	3610	–			0.00056	536
	37	3226	0.19239			0.17973	1.7
SUC-ARG	26	3344	0.03629	0.990	0.00521	0.04364	6.9
	20	3413	0.02143	($P < 0.1$)		0.01907	15.7
	04	3610	–			0.00180	167
	37	3226	0.19810			0.19571	1.5
MgCl2-ARG	26	3344	0.03784	0.999	0.00593	0.03910	7.7
	20	3413	0.01557	($P < 0.05$)		0.01525	19.7
	04	3610	–			0.00104	288
	37	3226	0.17799			0.17437	1.7
MgCl2-199	26	3344	0.03688	0.999	0.00551	0.03899	7.7
	20	3413	0.01682	($P < 0.05$)		0.01624	18.5
	04	3610	–			0.00133	226

TABLE III

Assay results and linear regression calculations for Type I virus contained in various formulations of Oral Poliomyelitis Trivalent Vaccine

Vaccine	Exposure temp. °C	TCID50/dose Exposure time (days) 0	1	2	4	7	14	21	28	35	42	Correlat. (r)	Interc. time zero	Slope (k)
	37		5.79	5.74	5.20	4.94	3.64	2.69	–	–	–	0.997 c	5.98	0.15984
RIT	26	6.04	–	–	5.98	5.71	5.60	5.40	5.32	5.05	–	0.987 c	6.01	0.02707
	20		–	–	6.00	5.88	5.95	5.74	5.78	5.80	5.61	0.900 b	6.01	0.00855
	37		5.72	5.58	5.21	4.65	3.35	2.04	–	–	–	0.999 c	5.96	0.18656
SUC-ARG	26	6.01	–	–	5.82	5.74	5.52	5.31	4.95	4.77	–	0.997 c	6.00	0.03530
	20		–	–	6.10	5.85	5.99	5.66	5.50	5.47	5.22	0.952 c	6.09	0.01952
	37		5.70	5.62	5.16	4.43	2.88	1.94	–	–	–	0.995 b	5.93	0.19945
MgCl2-ARG	26	6.02	–	–	5.87	5.83	5.42	5.28	5.08	4.77	–	0.993 c	6.02	0.03520
	20		–	–	5.94	5.94	5.91	5.72	5.70	5.44	5.22	0.962 c	6.07	0.01768
	37		5.73	5.46	5.36	4.65	3.36	2.29	–	–	–	0.997 c	5.95	0.17805
MgCl2-199	26	6.09	–	–	6.03	5.79	5.53	5.14	4.98	4.68	–	0.994 c	6.11	0.04163
	20		–	–	6.07	6.03	5.98	5.71	5.62	5.57	5.51	0.975 b	6.12	0.01560

b) $P < 0.01$
c) $P < 0.001$

TABLE IV

Arrhenius' plot calculations for Type I virus contained in various formulations of Oral Poliomyelitis Trivalent Vaccine

Vaccine	Exposure temp. °C	Exposure temp. 1/°Ax10E6	Arith. (k)	Derived/Corrected values Correlation (r)	Derived/Corrected values Arrhenius slope	Arith. (K)	Half life (days)
	37	3226	0.15984			0.16263	1.8
RIT	26	3344	0.02707	0.999	0.00677	0.02583	12
	20	3413	0.00855	($P < 0.05$)		0.00881	34
	04	3610	–			0.00041	732
	37	3226	0.18656			0.17608	1.7
SUC-ARG	26	3344	0.03530	0.993	0.00534	0.04129	7.3
	20	3413	0.01952	($P < 0.1$)		0.01768	17
	04	3610	–			0.00157	191
	37	3226	0.19945			0.18983	1.6
MgCl2-ARG	26	3344	0.03520	0.996	0.00571	0.04024	7.4
	20	3413	0.01768	($P = 0.05$)		0.01625	18.5
	04	3610	–			0.00122	246
	37	3226	0.17805			0.18165	1.6
MgCl2-199	26	3344	0.04163	0.999	0.00562	0.03943	7.6
	20	3413	0.01560	($P < 0.05$)		0.01614	18.6
	04	3610	–			0.00126	238

TABLE V

Assay results and linear regression calculations for Type II virus contained in various formulations of Oral Poliomyelitis Trivalent Vaccine

Vaccine	Exposure temp. °C	TCID50/dose Exposure time (days) 0	1	2	4	7	14	21	28	35	42	Correlat. (r)	Interc. time zero	Slope (k)
	37		4.64	4.30	3.98	3.62	2.63	1.79	–	–	–	0.996 c	4.66	0.14066
RIT	26	4.77	–	–	4.64	4.37	4.25	4.00	3.95	3.85	–	0.962 c	4.67	0.02614
	20		–	–	4.63	4.80	4.65	4.70	4.54	4.52	4.38	0.873 b	4.77	0.00799
	37		4.83	4.65	4.29	3.76	2.50	1.25	–	–	–	0.999 c	5.03	0.18058
SUC-ARG	26	5.09	–	–	4.90	4.79	4.69	4.16	4.14	3.78	–	0.983 c	5.07	0.03641
	20			–	4.93	4.79	4.93	4.70	4.59	4.42	4.40	0.952 c	5.03	0.01565
	37		4.76	4.64	4.26	3.77	2.20	1.66	–	–	–	0.989 c	4.94	0.16808
MgCl2-ARG	26	5.04	–	–	4.91	4.89	4.57	4.21	3.89	3.88	–	0.984 c	5.06	0.03729
	20		-	–	5.03	5.14	5.02	4.87	4.69	4.49	4.39	0.953 c	5.16	0.01748
	37		4.66	4.56	4.20	3.84	2.57	1.76	–	–	–	0.998 c	4.83	0.15041
MgCl2-199	26	4.86	-	–	4.81	4.80	4.54	4.18	4.05	3.82	–	0.989 c	4.93	0.03193
	20		–	–	4.83	4.77	4.88	4.87	4.58	4.64	4.39	0.828 a	4.91	0.00956

a) $P < 0.05$
b) $P < 0.01$
c) $P < 0.001$

TABLE VI

Arrhenius' plot calculations for Type II virus contained in various formulations of Oral Poliomyelitis Trivalent Vaccine

Vaccine	Exposure temp. °C	Exposure temp. 1/°Ax10E6	Arith. (k)	Correlation (r)	Derived/Corrected values Arrhenius slope	Arith. (K)	Half life (days)
	37	3226	0.14066			0.14502	2
RIT	26	3344	0.02614	0.999	0.00661	0.02406	12.5
	20	3413	0.00799	($P < 0.05$)		0.00842	35.6
	04	3610	–			0.00042	714
	37	3226	0.18058			0.17808	1.7
SUC-ARG	26	3344	0.03641	0.999	0.00570	0.03781	7.9
	20	3413	0.01565	($P < 0.05$)		0.01528	19.6
	04	3610	–			0.00115	261
	37	3226	0.16808			0.16498	1.8
MgCl2-ARG	26	3344	0.03729	0.999	0.00529	0.03922	7.6
	20	3413	0.01748	($P < 0.05$)		0.01693	17.7
	04	3610	–			0.00154	195
	37	3226	0.15041			0.15741	1.9
MgCl2-199	26	3344	0.03193	0.997	0.00632	0.02823	10.6
	20	3413	0.00956	($P < 0.05$)		0.01033	29
	04	3610	–			0.00059	508

TABLE VII

Assay results and linear regression calculations for Type III virus contained in various formulations of Oral Poliomyelitis Trivalent Vaccine

Vaccine	Exposure temp. °C	TCID50/dose Exposure time (days) 0	1	2	4	7	14	21	28	35	42	Correlat. (r)	Interc. time zero	Slope (k)
	37		5.51	5.46	4.94	4.69	3.34	2.34	–	–		0.997 c	5.67	0.15961
RIT	26	5.58	–	–	5.45	5.42	5.26	5.05	4.84	4.72		0.997 c	5.58	0.02506
	20		–	–	5.72	5.37	5.66	5.44	5.30	5.26	5.14	0.832 a	5.64	0.01118
	37		5.58	5.38	4.97	4.37	2.95	1.54	–	–	–	0.999 c	5.78	0.20222
SUC-ARG	26	5.79	–	–	5.68	5.27	5.52	5.02	4.79	4.61	–	0.948 b	5.75	0.03293
	20		–	–	5.74	5.72	5.67	5.36	5.52	5.17	5.11	0.946 c	5.82	0.01658
	37		5.63	5.28	4.77	4.43	2.72	1.66	–	–	–	0.996 c	5.75	0.20156
MgCl2-ARG	26	5.88	–	–	5.77	5.43	5.22	4.95	4.74	4.55		0.986 c	5.82	0.03836
	20		–	–	5.61	5.92	5.68	5.47	5.44	5.30	5.12	0.928 c	5.87	0.01680
	37		5.12	4.55	4.72	4.12	2.82	1.93	–	–	–	0.990 c	5.19	0.15926
MgCl2-199	26	5.30	–	–	5.14	4.99	4.72	4.54	4.43	4.06	–	0.991 c	5.26	0.03316
	20		–	–	5.26	5.25	5.25	5.03	5.00	4.79	4.51	0.950 c	5.38	0.01746

a) $P < 0.05$
b) $P < 0.01$
c) $P < 0.001$

TABLE VIII

Arrhenius' plot calculations for Type III virus contained in various formulations of Oral Poliomyelitis Trivalent Vaccine

Vaccine	Exposure temp. °C	Exposure temp. 1/°Ax10E6	Arith. (k)	Correlation (r)	Derived/Corrected values Arrhenius slope	Arith. (K)	Half life (days)
	37	3226	0.15961			0.15308	2
RIT	26	3344	0.02506	0.997	0.00624	0.02807	11
	20	3413	0.01118	($P < 0.05$)		0.01041	28.8
	04	3610	–			0.00061	492
	37	3226	0.20222			0.19103	1.6
SUC-ARG	26	3344	0.03293	0.994	0.00690	0.03842	7.8
	20	3413	0.01658	($P < 0.1$)		0.01504	20
	04	3610	–			0.00103	291
	37	3226	0.20156			0.19719	1.5
MgCl2-ARG	26	3344	0.03836	0.999	0.00581	0.04071	7.4
	20	3413	0.01680	($P < 0.05$)		0.01618	18.5
	04	3610	–			0.00116	259
	37	3226	0.15926			0.15272	2
MgCl2-199	26	3344	0.03316	0.996	0.00520	0.03715	8.1
	20	3413	0.01746	($P = 0.05$)		0.01625	18.5
	04	3610	–			0.00153	196

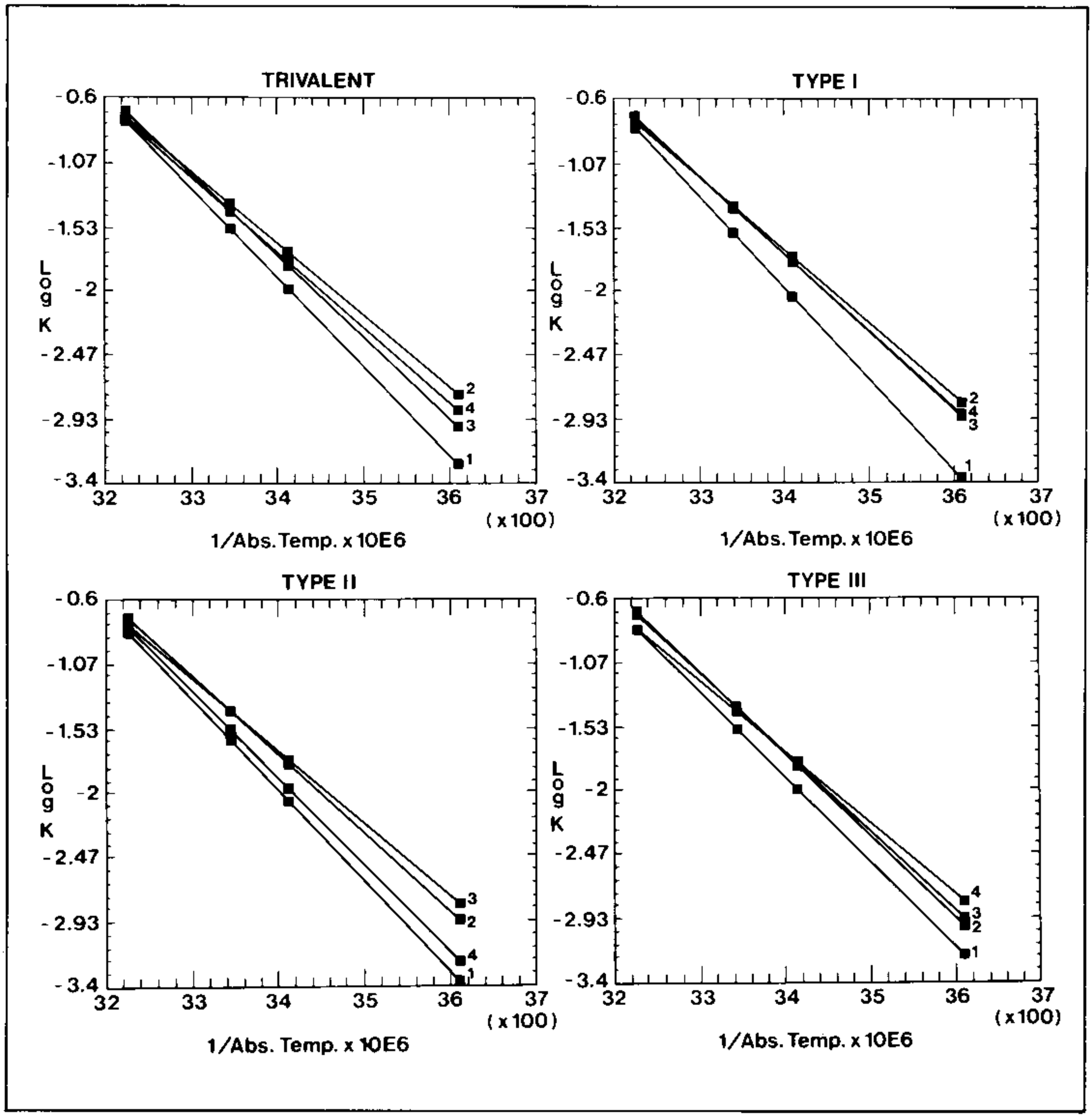

Fig. 1: Arrhenius' plot on various formulations of Oral Poliomyelitis Trivalent Vaccine.
Code of lines: 1-RIT, 2-SUC+ARG, 3-MgCl2+ARG, 4-MgCl2+199

DISCUSSION

The search for a high thermostable TOPV formulation with reduced cost and non-corrosive activity for the equipment and machinery involved in the production process resulted the option for formulation 2 (SUC + ARG) being discarded. Even though this formulation had the lowest cost and non-corrosive characteristics. The formulation gives reduced thermostabilization of trivalent titers at low temperatures (Peetermans & Colinet, 1980), resulting in a shorter life-span on conservation, an absolutely undesirable aspect for TOPV formulation (Fig. 1).

The three types of virus demonstrated very similar thermostability pattern with each formulation. An exception being formulation 4, where types I and III diverge from type II in thermostability. Formulation 3 shows the most homogeneous degradation rate for the three types of virus (Fig. 2). This same formulation showed high standards in thermostabilization of trivalent, type I and type III titers, the ones prevalent in Brazil (Fig. 3). For type II the stability is less pronounced than in formulation 4.

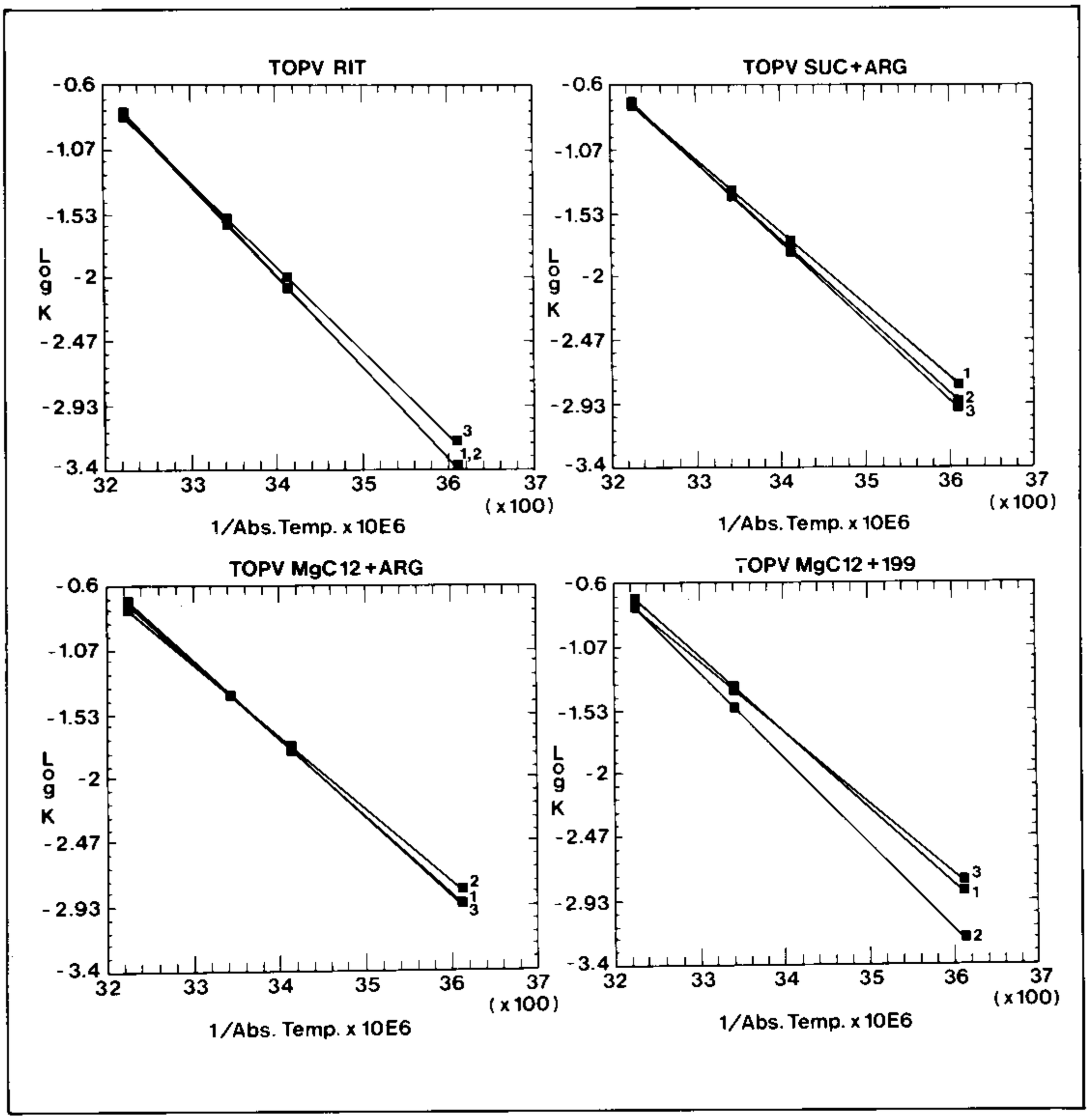

Fig. 2: Arrhenius' plot on Poliovirus Type I, II and III contained in various formulations of oral Poliomyelitis Trivalent Vaccine.
Code of lines: 1-Type I, 2-Type II, 3-Type III

Considering the recent WHO recommendations for virus content per dose (I-10E6, II-10E5 and III-10E5.5 ± 0.5 log10), plus the Minimum Requirements for TOPV thermostability the results obtained with this accelerated thermostability study for formulation 3 (MgCl2 + ARG), surpass the required standards recommended by WHO. To reach the limit loss of trivalent titer accepted by the Minimum Requirements, formulation 3 has to be subjected to 37 °C for 2.6 days, or to 26 °C for 12.8 days, or to 20 °C for 32.8 days, or to 4 °C for 480 days (WHO, 1989).

Formulation 3, besides fulfilling all quality characteristics for the TOPV, is less expensive as its thermostabilizer is composed of more economic ingredients, and can be processed in a more economic manner. The other objective, finding a formulation that is less corrosive to our process equipment and machinery, still remain to be reached.

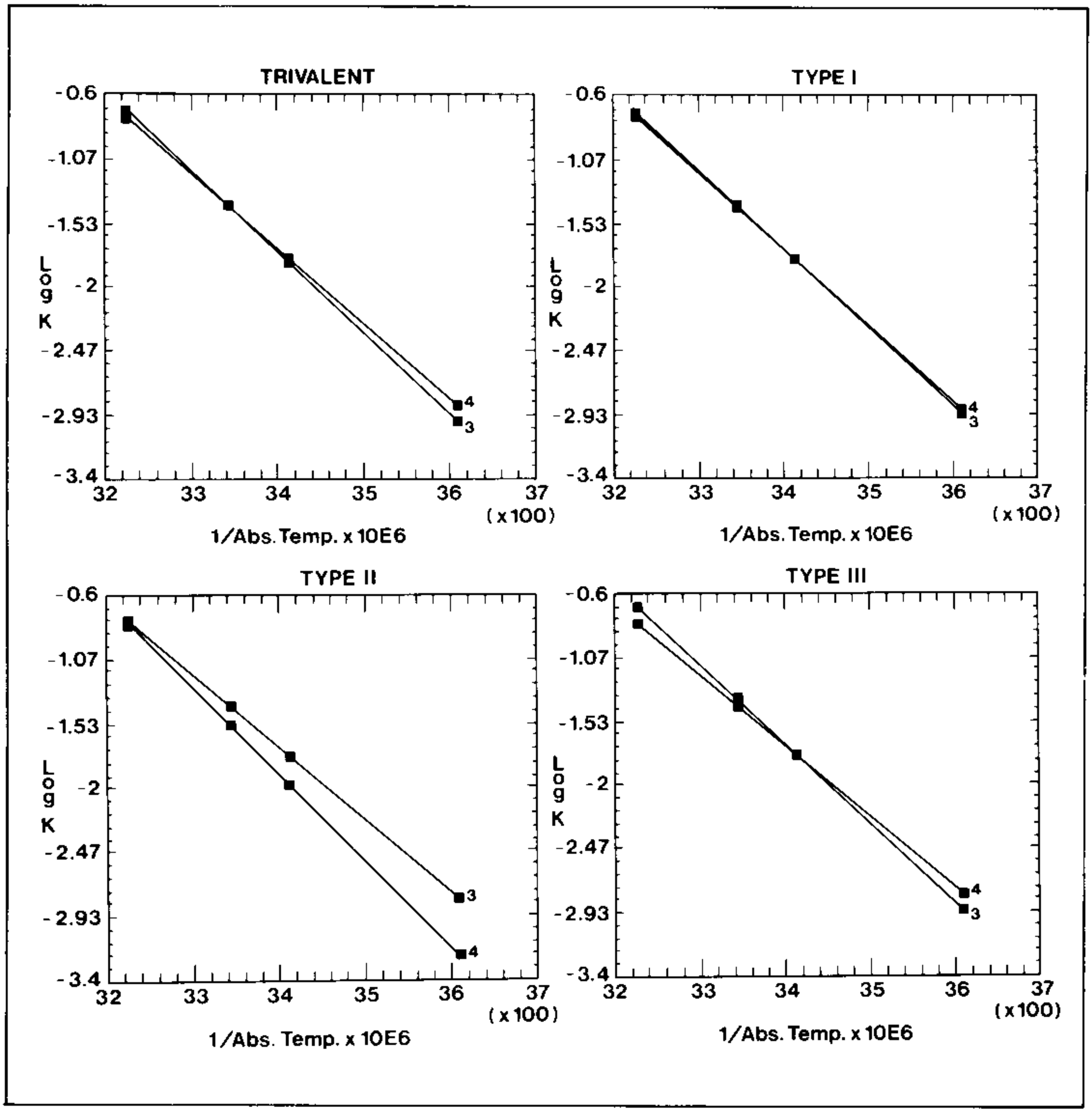

Fig. 3: Arrhenius' plot on formulations of Oral Poliomyelitis Trivalent Vaccine with MgCl2+ARG and MgCl2+199.
Code of lines: 3-MgCl2+ARG, 4-MgCl2+199

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