

An *in vitro* study on the compatibility and concentrations of combinations of vancomycin, amikacin, and dexamethasone in human vitreous

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LABORATORY STUDY

Abstract

Purpose To investigate the precipitation process of a mixture of vancomycin, amikacin, and dexamethasone by equilibrium dialysis and its subsequent effect on the levels of available-free antibiotics and steroid.

Methods Concentrations of amikacin, vancomycin, and dexamethasone in an equilibrium dialysis chamber were measured during the equilibrium process by high-performance liquid chromatography and fluorescence polarisation immunoassay. Vitreous were used as the medium of dialysis, with the three medications prepared in normal saline (NS) and balanced salt solution plus (BSS Plus) separately.

Results Amikacin showed no measurable loss in NS or BSS Plus, either alone or when mixed with vancomycin or dexamethasone. Vancomycin showed minimal loss in BSS Plus, either alone or when mixed with amikacin or dexamethasone. Dexamethasone showed a median loss of 16 and 15% when incubated alone in NS and BSS Plus, respectively, at 48 h. When mixed with vancomycin or amikacin in BSS Plus, it showed a median loss of 13 and 12%, respectively, at 48 h. There was no statistically significant difference in the loss of dexamethasone under various conditions. In equilibrium dialysis in vitreous, amikacin, vancomycin, and dexamethasone reached equilibrium within 24 h and with no loss up to 192 h. There was no difference observed when the medications were prepared in NS or BSS Plus.

Conclusions Both amikacin and vancomycin did not show precipitation or decrease in concentration in NS or BSS Plus.

Dexamethasone showed relatively small percentage loss. As a result, treatment of endophthalmitis with vancomycin and amikacin combination is preferred.

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Introduction

Infective endophthalmitis is a serious condition with a potential to cause blindness.¹ Owing to its urgent nature, empirical combination of antibiotics with coverage against both Gram-positive and Gram-negative organisms are commonly employed.^{2,3} Frequently used regimens include combination of vancomycin and aminoglycosides, or vancomycin with ceftazidime.^{4,5} In addition, to attenuate the inflammatory response of endophthalmitis, which may be responsible for the resultant visual loss, some authorities also advocate the use of intravitreal steroid, such as dexamethasone.^{6–8}

Although such empirical antibiotics are commonly used, their physical–chemical incompatibility was also known for many years.⁹ We had previously explored the precipitate formation of vancomycin and ceftazidime,¹⁰ vancomycin and ciprofloxacin.¹¹

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We had showed that both ceftazidime and ciprofloxacin precipitated in vitreous, with resultant reduced-free antibiotic concentration. In particular, ceftazidime concentration can fall to below the MIC₉₀ of the drug against many commonly encountered Gram-negative organisms.

In this study, we aimed to explore the other commonly used regimen, vancomycin and amikacin, with respect to its precipitation process. In addition, we also explored the precipitation process of dexamethasone when added with vancomycin and amikacin. We investigated the process by the use of checkerboard titration and equilibrium dialysis in vitreous medium.

Materials and methods

All experiments were conducted in duplicate, and the mean values taken as results. Vitreous were obtained from cadaveric donor eyes during post-mortem examination. The vitreous obtained were immediately stored at -70°C and used within 3 months. The standard intravitreal dosage of vancomycin (Abbott, Chicago, IL, USA), amikacin (Bristol-Myers Squibb, Puerto Rico, USA) and dexamethasone sodium phosphate (David Bull Laboratories, UK) for the treatment of infective endophthalmitis was 1.0, 0.4, and 0.4 mg, respectively. The medication was prepared in 0.1 ml of 0.9% normal saline (NS) (Otsuka, Guandong, China) or balanced salt solution plus (BSS Plus, Alcon, Fort Worth, TX, USA). The vitreous volume of an adult emmetropic human eye is about 4 ml, giving respective empirical concentrations of 0.25, 0.1, and 0.1 mg/ml for vancomycin, amikacin, and dexamethasone sodium phosphate (dexamethasone) when injected into the vitreous. The actual concentration would be different, depending on the extent of precipitation and the actual vitreous volume.

Assay of antibiotics

Vancomycin and amikacin were assayed by a fluorescence polarisation immunoassay (TDx, Abbott laboratories, Diagnostic division, Abbott Park, IL, USA). Dexamethasone sodium phosphate was assayed by high-performance liquid chromatography (HPLC) as described by Kwak *et al*¹² with modification of using 55:45 mixture of methanol:water as a solvent. Its result was expressed in terms of the salt measured.

Study 1: visual test

Standard mixture solutions of 1.0 mg vancomycin, 0.4 mg amikacin, and 0.4 mg dexamethasone in 0.1 mg of 0.9% NS or BSS Plus were mixed separately with 4.0 ml of NS, BSS Plus, and vitreous for incubation at 37°C.

Study 2: checkerboard analysis

In the checkerboard titration study, one of the drug combinations was diluted along the rows of the microtitre plate, the other drug was diluted along the columns of the plate. Therefore, each well in the plate contains mixture of drugs at various concentrations.¹³ Using this technique, vancomycin, amikacin, and dexamethasone combinations were prepared in NS or BSS Plus and were incubated at 37°C in microtitre plates (Table 1) with cover by paraffin foil. Aliquots were taken at 24, 48, and 72 h for assay by HPLC (dexamethasone) and TDx (vancomycin and amikacin) to determine the concentration and amount of free drugs.

Study 3: equilibrium dialysis in vitreous

In equilibrium dialysis study, a chamber separated into two halves by a semipermeable membrane. The drugs to be assessed are injected into one side of the chamber (chamber A), it will then diffuse into the other chamber (chamber B). The time to reach equilibrium and any loss can then be assessed by measuring the drug concentrations in chamber B.¹⁴ Equilibrium dialysis studies were performed in an equilibrium dialyser (Spectrum Medical Industries, Los Angeles, CA, USA) using Teflon cells with a half cell working volume of 1.0 ml. Vancomycin (250 µg), amikacin (100 µg), and dexamethasone (100 µg) were prepared in NS and added into half cell chamber A, which was separated from half cell chamber B by a cellulose semipermeable Spectrapor dialysis membrane with a molecular weight cutoff at

Table 1 Initial concentrations (mg/l) of (a) amikacin/vancomycin (b) dexamethasone/vancomycin, (c) dexamethasone/amikacin prepared in microtitre plate

(a)				
0/0	0/250	0/125	0/62.5	0/31.25
100/0	100/250	100/125	100/62.5	100/31.25
50/0	50/250	50/125	50/62.5	50/31.25
25/0	25/250	25/125	25/62.5	25/31.25
12.5/0	12.5/250	12.5/125	12.5/62.5	12.5/31.25
(b)				
0/0	0/250	0/125	0/62.5	0/31.25
100/0	100/250	100/125	100/62.5	100/31.25
50/0	50/250	50/125	50/62.5	50/31.25
25/0	25/250	25/125	25/62.5	25/31.25
12.5/0	12.5/250	12.5/125	12.5/62.5	12.5/31.25
(c)				
0/0	0/100	0/50	0/25	0/12.5
100/0	100/100	100/50	100/25	100/12.5
50/0	50/100	50/50	50/25	50/12.5
25/0	25/100	25/50	25/25	25/12.5
12.5/0	12.5/100	12.5/50	12.5/25	12.5/12.5

6000–8000 D (Spectrum Medical Industries). Both chambers were filled with 1.0 ml of vitreous. The whole system was incubated at 37°C Aliquots were taken from half cell chamber B at appropriate time intervals up to 192 h for drug assay. The experiment was then repeated with the drugs prepared in BSS Plus.

Results

Study 1: visual test

Precipitate was not visually detected in NS, BSS Plus, and vitreous after incubation for 1 day at 37°C.

Study 2: checkerboard analysis in microtitre plates

Amikacin alone showed no measurable loss in NS or BSS Plus up to 72 h of incubation (first rows, Tables 2g–l). Vancomycin alone in NS showed no measurable loss up to 72 h of incubation (first rows, Tables 2a–c). While in BSS Plus, vancomycin alone showed minimal loss (median 0%, first rows, Tables 2d–f).

Dexamethasone alone in NS showed measurable decrease of 0–12% (median 5%) after 24 h of incubation.

Table 2 (a) Percentage loss of dexamethasone/vancomycin after (a) 24 h in NS (b) 48 h in NS (c) 72 h in NS (d) 24 h in BSS Plus (e) 48 h in BSS Plus (f) 72 h in BSS Plus. Percentage loss of dexamethasone/amikacin after (g) 24 h in NS (h) 48 h in NS (i) 72 h in NS (j) 24 h in BSS Plus (k) 48 h in BSS Plus (l) 72 h in BSS Plus

(a)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
3/0 ^a	7/0 ^a	5/0 ^a	5/0 ^a	5/0 ^a
9/0 ^a	9/0 ^a	11/0 ^a	11/0 ^a	12/0 ^a
2/0 ^a	3/0 ^a	6/0 ^a	4/0 ^a	7/0 ^a
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
(b)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
11/0 ^a	10/0 ^a	12/0 ^a	11/0 ^a	8/0 ^a
12/0 ^a	13/0 ^a	13/0 ^a	10/0 ^a	12/0 ^a
25/0 ^a	24/0 ^a	25/0 ^a	25/0 ^a	24/0 ^a
20/0 ^a	35/0 ^a	37/0 ^a	32/0 ^a	34/0 ^a
(c)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
9/0 ^a	9/0 ^a	4/0 ^a	8/0 ^a	4/0 ^a
4/0 ^a	7/0 ^a	6/0 ^a	9/0 ^a	7/0 ^a
26/0 ^a	22/0 ^a	21/0 ^a	23/0 ^a	23/0 ^a
16/0 ^a	12/0 ^a	8/0 ^a	0 ^a /0 ^a	10/0 ^a
(d)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
8/0 ^a	5/0 ^a	5/0 ^a	16/0 ^a	12/0 ^a
8/0 ^a	8/0 ^a	10/0 ^a	9/0 ^a	10/0 ^a
16/0 ^a	13/0 ^a	13/0 ^a	13/0 ^a	14/0 ^a
18/0 ^a	27/0 ^a	27/0 ^a	30/0 ^a	26/0 ^a

Table 2 (Continued)

(e)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
24/0 ^a	22/0 ^a	22/0 ^a	24/0 ^a	22/0 ^a
11/0 ^a	10/0 ^a	10/0 ^a	13/0 ^a	12/0 ^a
14/0 ^a	14/0 ^a	13/0 ^a	12/0 ^a	12/0 ^a
29/0 ^a	32/0 ^a	33/0 ^a	30/0 ^a	32/0 ^a
(f)				
0 ^a /0 ^a	0 ^a /5	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
20/0 ^a	16/0 ^a	17/0 ^a	21/2	17/0 ^a
10/0 ^a	10/4	11/0 ^a	8/0 ^a	4/0 ^a
9/0 ^a	10/0 ^a	6/0 ^a	10/3	11/0 ^a
0 ^a /0 ^a	0 ^a /0 ^a	3/0 ^a	1/0	3/0 ^a
(g)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
12/0 ^a	8/0 ^a	8/0 ^a	10/0 ^a	10/0 ^a
7/0 ^a	8/0 ^a	13/0 ^a	9/0 ^a	10/0 ^a
8/0 ^a	10/0 ^a	6/0 ^a	10/0 ^a	9/0 ^a
0 ^a /0 ^a	1/0 ^a	3/0 ^a	6/0 ^a	4/0 ^a
(h)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
5/0 ^a	7/0 ^a	5/0 ^a	6/0 ^a	6/0 ^a
6/0 ^a	3/0 ^a	7/0 ^a	7/0 ^a	2/0 ^a
22/0 ^a	24/0 ^a	24/0 ^a	21/0 ^a	21/0 ^a
21/0 ^a	28/0 ^a	20/0 ^a	23/0 ^a	22/0 ^a
(i)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
8/0 ^a	5/0 ^a	7/0 ^a	9/0 ^a	8/0 ^a
13/0 ^a	13/0 ^a	8/0 ^a	10/0 ^a	10/0 ^a
22/0 ^a	24/0 ^a	25/0 ^a	23/0 ^a	20/0 ^a
18/0 ^a	18/0 ^a	18/0 ^a	18/0 ^a	19/0 ^a
(j)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
16/0 ^a	18/0 ^a	18/0 ^a	16/0 ^a	17/0 ^a
4/0 ^a	9/0 ^a	8/0 ^a	6/0 ^a	4/0 ^a
14/0 ^a	14/0 ^a	12/0 ^a	14/0 ^a	8/0 ^a
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	2/0 ^a	14/0 ^a
(k)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
17/0 ^a	17/0 ^a	12/0 ^a	12/0 ^a	14/0 ^a
8/0 ^a	6/0 ^a	8/0 ^a	6/0 ^a	8/0 ^a
11/0 ^a	12/0 ^a	10/0 ^a	5/0 ^a	10/0 ^a
22/0 ^a	17/0 ^a	18/0 ^a	17/0 ^a	20/0 ^a
(l)				
0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a	0 ^a /0 ^a
18/0 ^a	23/0 ^a	21/0 ^a	23/0 ^a	25/0 ^a
10/0 ^a	10/0 ^a	12/0 ^a	11/0 ^a	13/0 ^a
14/0 ^a	12/0 ^a	13/0 ^a	6/0 ^a	13/0 ^a
0 ^a /0 ^a	14/0 ^a	0 ^a /0 ^a	8/0 ^a	8/0 ^a

^aNo measurable loss.

After 48 h, its concentration decreased by 5–25%, (median 16%). At 72 h, the loss was 4–26%, median 14% (first columns, Tables 2a–c, g–l). While in BSS Plus, it

showed a decrease of 0–18% (median 11%) at 24 h of incubation. The decrease was 8–29% (median 15%) at 48 h. At 72 h, the decrease was 0–20%, median 10% (first columns, Tables 2d–f, j–l). There was no statistically significant difference in dexamethasone concentration when incubated alone in NS or BSS Plus (Student's *t* test, $P > 0.05$).

In mixture of amikacin and vancomycin, there was no measurable loss of amikacin in NS or BSS Plus up to 72 h of incubation. For vancomycin, it showed no loss in this mixture for up to 72 h of incubation in NS. In BSS Plus, it showed minimal loss with median 0% (results not shown). For mixture of dexamethasone and vancomycin, vancomycin again showed no measurable loss in NS up to 72 h. It showed a loss of 0–4% (median 0%) in BSS Plus. Dexamethasone showed loss in NS of 0–37% (median 9%). Its loss was 0–33%, median 13% in BSS Plus (Tables 2a–f). For mixture of dexamethasone and amikacin, amikacin showed no measurable loss in NS or BSS Plus up to 72 h. Dexamethasone loss was 1–28%, median 10% in NS. While in BSS Plus, dexamethasone loss was 0–25%, median 12% (Tables 2g–l). There was no statistically significant difference in the loss of dexamethasone when it was incubated with amikacin or

vancomycin, or when incubated in NS or BSS Plus (Student's *t* test, $P > 0.05$).

Study 3: equilibrium dialysis in vitreous

The drugs were prepared in NS and injected into dialysis chamber A which was filled with vitreous. The vancomycin concentration in chamber B rose to about 44% of its original concentration in chamber A after about 24 h and then plateau off. Its concentration was able to maintain to about 40% of its original concentration at the end of the experiment at 192 h. For Amikacin, it rose to about 50% at 5 h, and then plateau off. For dexamethasone, it rose to about 45% at 12 h, it then fluctuated slightly but maintaining at about 50% till the end of the experiment. This experiment was repeated with the drugs prepared in BSS Plus and it showed similar results (Figures 1 and 2).

Discussion

The use of combination intravitreal antibiotics is widely employed in the treatment of endophthalmitis. It is also known to ophthalmologist that precipitation can occur

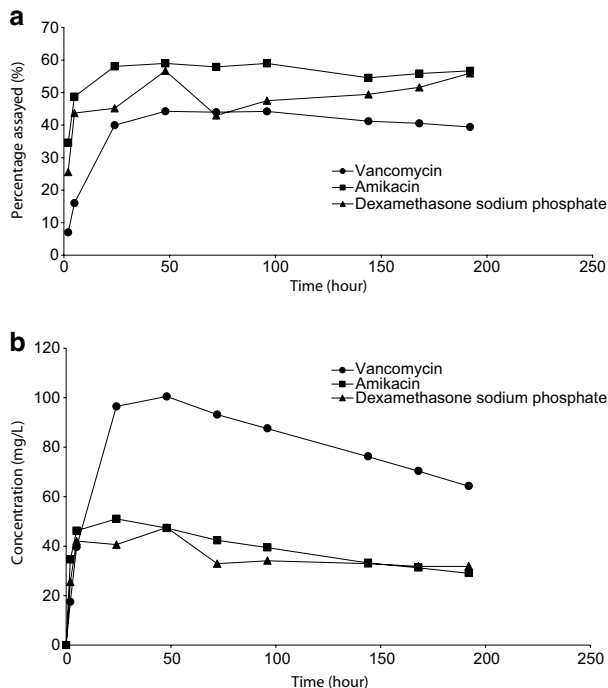


Figure 1 (a) Equilibrium dialysis in vitreous medium with vancomycin, amikacin, and dexamethasone sodium phosphate initially prepared in normal saline. Percentage change in concentration in vitreous of chamber B. (b) Equilibrium dialysis in vitreous medium with vancomycin, amikacin, and dexamethasone sodium phosphate initially prepared in normal saline. Actual change in concentration in vitreous of chamber B.

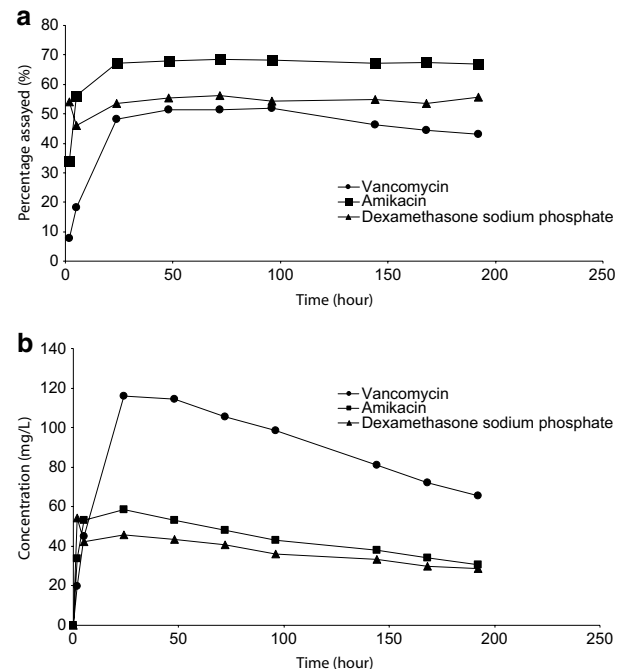


Figure 2 (a) Equilibrium dialysis in vitreous medium with vancomycin, amikacin, and dexamethasone sodium phosphate initially prepared in BSS Plus. Percentage change in concentration in vitreous of chamber B. (b) Equilibrium dialysis in vitreous medium with vancomycin, amikacin, and dexamethasone sodium phosphate initially prepared in BSS Plus. Actual change in concentration in vitreous of chamber B.

with the mixing of vancomycin and ceftazidime.¹⁵ We had previously explored this process by the use of checkerboard analysis and equilibrium dialysis, and showed that both ceftazidime and ciprofloxacin precipitated to various extent in vitreous, and the precipitation can be affected by pH, temperature and choice of preparation medium (NS or BSS Plus).

In this study, we explored the possible precipitation process of another commonly used antibiotic regimen in the treatment of endophthalmitis: vancomycin and amikacin, as well as the anti-inflammatory agent dexamethasone. Fluorescence polarisation immunoassay (TDx) is used for the measurement for vancomycin and amikacin. Its precision and accuracy had been previously validated and employed in other ophthalmic study.^{16–18} Dexamethasone assay was performed by HPLC as validated by Kwak *et al.*¹² The accuracy of the TDx and HPLC was ascertained by preliminary studies using known concentrations of vancomycin, amikacin, dexamethasone, as well as internal quality controls (data not shown).

In the checkerboard study, vancomycin had showed no loss in NS, either alone or in combination with amikacin or dexamethasone. It showed minimal loss when incubated in BSS Plus, also either alone or when in combination with amikacin or dexamethasone. This finding is consistent with our previous experiment, which also showed that vancomycin showed minimal loss when combined with ceftazidime or ciprofloxacin. This result suggests that vancomycin appears to be a stable chemical, and is capable of maintaining its free concentration despite the variety of media and combination of drugs being used.

Previous investigators had explored the effects on elimination of intravitreal vancomycin by intravitreal dexamethasone. However, animal experimental models with *Streptococcus pneumoniae* and *Staphylococcus epidermidis* had yielded conflicting results.^{19,20} As our *in vitro* study suggests that loss of vancomycin was minimal, either alone or in the presence of other medications or media, other *in vivo* variations, such as organism characteristics, host inflammatory response and breakdown of blood-ocular barrier, may account for the opposite results obtained by previous *in vivo* studies. Smith *et al*¹⁹ had suggested that the elimination of vancomycin through the canal of Schlemm may be facilitated by the presence of steroids, resulting in lower intraocular levels. However, as *S. epidermidis* is an organism of low virulence, as opposed to the *S. pneumoniae* used by Park *et al*, the degree of inflammation and the resultant effect of steroids may well be different. As suggested by Park *et al*, the beneficial effect of steroids in reducing blood-ocular barrier breakdown may have greater impact than the enhancement of elimination of

antibiotic. To further address the effect of steroids, further *in vivo* studies are warranted.

Amikacin also showed no measurable loss in the checkerboard study, whether it was in NS or BSS Plus, alone or in combination with vancomycin or dexamethasone. This appears extraordinary as other anti-Gram-negatives antibiotics previously tested, such as ceftazidime and ciprofloxacin, showed precipitation to various extent. This is of further interest as BSS Plus, with its advantages of containing appropriate bicarbonate, pH, ions, and glutathione, is preferred over NS for intravitreal irrigation for its ability to maintain retinal electrical activity and endothelial cell adenosine triphosphate. BSS Plus, however, increases the precipitation of ceftazidime.⁹ Therefore, amikacin would appear to be the most stable antibiotic tested so far, with its free drug concentration maintained adequately and not affected by the medium of preparation. This would also suggest that amikacin is preferred over ceftazidime or ciprofloxacin for its lack of precipitation and resultant loss of free drug.

Dexamethasone showed measurable loss when alone or in combination, in NS or BSS Plus. The precipitation process and the loss in free drug appeared independent of the medium used, and was not affected mixing with amikacin or vancomycin, as shown by the insignificant Student's *t* test results. The medians of decrease in dexamethasone concentration ranged from 5 to 16% in this checkerboard study. It had been suggested that rabbit fibroblast proliferation can be inhibited by dexamethasone at 200 µg/ml.²¹ However, whether this loss in the current study can contribute to clinical failure would require further studies.

In the equilibrium dialysis study, vancomycin, amikacin, and dexamethasone all distributed readily and evenly into the two dialysis chamber. Amikacin was the fastest to reach equilibrium by 5 h. Vancomycin, as in previous study, reached equilibrium at about 24 h. Dexamethasone reached equilibrium at about 12 h. All three drugs maintain their concentrations till the end of the experiment. There was no difference observed when the drugs were prepared in NS or BSS Plus. The results of this suggest that the combination regimen of vancomycin and amikacin is currently the most stable drugs when tested in our experiments. Adequate free drug concentration can be maintained, and was not affected by the choice of preparation medium of either NS or BSS Plus. Importantly, the antibiotics level should be higher than the MIC₉₀ of commonly encountered pathogens.¹⁰ Dexamethasone, although its use is controversial,^{22,23} also exhibited favourable pharmacokinetic profiles with small percentage loss only. Nonetheless, this loss should be taken into account when designing the intravitreal dosage for this medication.

With the limited number of antibiotics that can be used intravitreally, and the significant antibiotic loss with precipitation, the vancomycin–amikacin regimen would be the preferred choice in the treatment of infective endophthalmitis.

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