Further experimental evidence of the compressibility of arteries

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Abstract

Further experimental evidence on the compressibility of arteries under normal physiological pressure range is provided using the experimental apparatus introduced in Yosibash et al., *JMBBM* **39**(2014):339-354. We enlarged the experimental database by including almost twice the number of experiments, we considered a different artery - the porcine common carotid that allowed longer and larger diameters.

In the physiological pressure range of 50 to 200 mmHg, a relative volume change of 5% was obtained, lower compared to the sapheneous and femoral arteries (2-6%). Most of the arteries had a relative volume change of 1.5%.

The relative volume change is found to be almost linearly proportional to the pressure, and inversely proportional to the dimensions of the experimented arteries (especially the artery length). The smaller the artery tested, the larger the relative volume change (such a phenomenon was also realized in Yosibash et al., *JMBBM* **39**(2014):339-354.).

We realized in recent past publications a flaw in the experimental protocol that results in an overestimation of the relative volume change (thus underestimating the bulk modulus). It is due to the consideration of experimental observations close to the zero pressure. Nonetheless, in view of the experimental evidence, the pre-assumption of incompressibility in

many phenomenological constitutive models of artery walls should be re-evaluated.

Keywords: Artery, compressibility, experimental observations

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1. Introduction

Common constitutive models of arteries, aimed at predicting their passive response, are based on hyperelasticity assumption, pre-assuming the incompressibility of the artery tissue under physiological conditions, see e.g. the review [6]. The motivation for this assumption is the high content of water in the artery wall, which is considered incompressible.

A definitive experimental-based answer on the level of compressibility in artery walls is therefore of biomechanical interest but not easily answered because of difficulties to measure accurately very small differences in volume under physiological pressure. In [8] an accurate and calibrated experimental system was described for the measurement of volume changes in arteries under physiological conditions. These experiments were performed ex-vivo on porcine arteries harvested the same day or a previous day, loaded by internal pressure that well represents physiological conditions. Ten saphenous and femoral arteries were considered (one carotid artery also) which demonstrated experimentally a considerable volume change: for the physiological normal pressure range ≈50–200 mmHg the relative volume change was ≈2–6%. Recently, further studies demonstrating a considerable volume change in arteries were reported. Norman and McGarry [7] report on experiments on 13 excised circular disks of diameter of 10 mm from the descending aorta of six sheep. At an average stress of about 60 mmHg an average relative volume change $9 \pm 3\%$ is reported. This high compressibility at that low stress is probably overestimated possibly due to the excision and inaccuracies of the measurements of a small overall volume of the specimen (estimated to about 80 mm^3), but more importantly because of consideration of the very high volume changes at very low applied stresses.

These recent experimental observations demonstrate the compressibility of arterial wall tissues in three different artery types in two animals. Here we extend the experimental database and further investigate more quantitatively the compressibility of the arterial wall tissue by using the calibrated experimental apparatus described in details in [8]. In addition to the sapheneous and femoral arteries in [8], we herein report on eighteen porcine common carotid arteries, thus enlarging both the type and number of specimens. The compressibility values observed in this study are compared to the ones in [8]. Furthermore, we investigate if pressurizing the arteries to 300 mmHg (above the physiological range as performed in [8]) may have an influence on the relative volume change after removing the internal pressure and reapply it. We also investigate artifacts in the experimental observations that may be contributed to the size of the specimens, and what is the influence of wall thickness measurement error on the error in relative volume change.

2. Methods

2.1. The experimental apparatus

The experimental apparatus used for measuring artery's volume change in a "physiological" condition (inflating the lumen by inserting liquid to simulate the blood pressure, allowing the artery to expand) is based on the ideas in [3] and thoroughly described in [8]. It is briefly described here: the *test chamber* (see Fig. 1) was a PMMA tube, with an internal diameter of 32 mm, sealed at both ends by rigid plastic caps. Through the center of each cap a hollow small diameter metallic tube was inserted, enclosing the main part of the pressurized volume V_p. One cap was perforated at two locations, that were connected to plastic tubes. These needles were used to fill the test chamber with water and to allow the exit of air bubbles trapped inside the test chamber.

The artery specimen was tied to the metal hollow tubes inserted from either side of the test chamber with a pressure sensor inserted via a catheter into the tied artery. Colored water V_{in} was inserted through the hollow tube inflating the artery. The volume of the extruded water V_{ext} from the PMMA tube due to the inflation of the outer surface of the artery was measured. The setup of the testing apparatus and all its components are shown in Fig. 2.

The experimental apparatus was thoroughly calibrated. The various calibration tests are documented in [8]

2.2. Experiment protocol

Porcine common carotid arteries were extracted from female pigs sacrificed for medical research not associated with the vascular system. Prior to excision, heparan sulfate was given to the sedated animal to prevent blood clots in the arteries. The excised specimens were kept in saline solution at 2-4 C$^\circ$ for at most 24 hours. The arteries were skeletonized (connective tissue removed around the arteries), cut to an appropriate length and attached to the metallic tubes by surgical thread. Colored water was then inserted into the lumen to remove trapped air and to check for leaks (by increasing the inner pressure to a value of $\approx$ 200 mmHg).

A preconditioning protocol was then followed by determining the amount of inlet water ($V_{in,200mmHg}$) that produced 200 mmHg pressure within the lumen, and repeatedly pumping it in and out until peak pressure in consecutive cycles remained constant. Following preconditioning, increments of a tenth of $V_{in,200mmHg}$ were inserted at a pumping rate of $400\frac{\mu L}{min}$. Immediately after each dose, the pressure and the water level in the measurement tube were recorded. Denoting water volume pumped into the artery by V_{in} and the volume extruded from the test chamber by V_{ext}, the tissue volume-change was $\Delta V = V_{in} - V_{ext}$ and relative volume-change was $\frac{\Delta V}{V_0}[\%]$ where V_0 is the initial volume of the examined specimen. Each experiment was repeated 3-4 times.

An experiment was performed to assess wether pressures above 300 mmHg (used in all arteries in [8] and several arteries in this study) may have induced damage to the tissue which affects relative volume-change results. On the third inflation repetition, three additional doses were pumped into the artery creating an internal pressure of up to 400 mmHg. Subsequently, two more inflation repetitions were performed and the relative volume-change calculated.

Following the experiment each artery was placed on a millimetric grid, and measured by photograph analysis. Edges of the artery outside the securing strings were trimmed, and the specimen was cut along its length. A micro slide was placed on the flattened artery and photographed. The flattened artery thickness was measured by a height indicator in three points along the micro slide and the average is considered the wall thickness. Note that the micro slide area was larger than the flattened arteries thus maximizing their contact area, and minimizing the asserted pressure by the height indicator. Volume was determined by area and wall thickness. To estimate the wall thickness measurement error, a micro slide and a height indicator as described above were used. Secondly, some specimens were excised into two equal rectangular pieces which were placed on top of each other the thickness was measured again.

We concluded that the error in wall thickness measurement is smaller than 10%. Specimens volume and wall thickness are proportional, therefore the error in specimens volume due to wall thickness error is below 10%. This will induce an error in $\Delta V/V_0$ of approximately 10%. For example, for an artery of 5% relative volume change and $V_0 = 100mm^3$, a 10% error in V_0 will result in a relative volume change of 4.54 to 5.54%.

Rubber specimens of two diameters and different lengths were used in [8] to identify the "system's overall bias from incompressibility". Since preliminary tests of rubber specimens in this study have shown similar results to [8] we use the data reported in that publication to adjust the volume change by the apparatus overall bias.

2.3. *Specimens*

Nineteen specimens from fourteen female porcine (*Sus scrofa domestica*, a crossbred of mainly large white X landrace) were used in our experiments. A summary is given in Table 1.

2.4. *Data analysis*

Pressure measurements were recorded from 0 mmHg but the data was analyzed starting at 50 mmHg, considered the lowest physiological limit (normal porcine blood pressure is $\approx 80/130$ mmHg [5, 2]). Because the common carotid arteries are large vessels, the normal blood pressure in them is $\approx 80/130$ mmHg, then the physiological pressures we consider varies between ≈ 50 mmHg and ≈ 250 mmHg representing an intense activity. Relative volume-change was calculated in relation to the volume at a pressure of ≈ 50 mmHg (the exact value varied between experiments).

The differences between the common carotid artery investigated here with the femoral and sapheneous arteries investigated in [8], from same species (porcine) and (almost) same experimental protocol and instrumentation, are visualized by box plots, see Fig.3. Red line indicates the median of the data set, the edges of the boxes show the 25th and 75th percentiles and the whiskers extend to the most extreme data points which were not considered outliers.

Data was statistically analyzed by SPSS version 23 (SPSS IBM, New york, USA). The influence of pressure on relative change in volume was analyzed by a multivariate analysis (linear regression) with logarithmic pressure and logarithmic relative change in volume, with a restricted maximum likelihood (REML) estimation. We considered artery length, circumference, initial volume and wall thickness as influencing parameters. The model accounted for animal as clusters. The clustered structure of the data had to be accounted for due to correlation assumed between observations belonging to one animal. Specification of a clustered structure in the regression model yielded an unbiased statistical estimation. The model used in this analysis was linear with robust standard errors. A 95% confidence interval level was set for all tests, with a p-value< 0.05 considered significant.

3. Results

The comparison between the arteries investigated in this study and these in [8] are presented in Table 2 and Figure 3. One notices that the average length, thickness and volume of the common carotid arteries in this study is larger than the femoral and sapheneous arteries in [8], whereas the average circumference is similar.

Relative volume-change before and after a high pressure of 300 mmHg was induced at the third repletion were not significantly different. Raw data which shows this specific experiment is provided in Table A.18.

The arteries' shapes at 0 pressure and under internal pressure are shown in Fig. 4. All long arteries exhibited "buckling" shapes when inflated. A similar behavior was observed in other experimental studies such as [1, 4].

3.1. Statistical analysis

Among all relations that were analysed statistically a multi-linear dependance was found between the logarithm of the relative volume change $ln\frac{\Delta V}{V_0}$ to the logarithm of the length, the logarithm of the shifted pressure and the logarithm of the width. Therefore, the following relation was determined:

$$\ln\frac{\Delta V}{V_0} = a - b\cdot\ln L + c\cdot\ln(P-50) - d\cdot\ln W \quad\Rightarrow\quad \frac{\Delta V}{V_0} = e^a\cdot\frac{(P-50)^c}{L^b\cdot W^d} \qquad (1)$$

where a, b, c, d are parameters estimated by the statistical analysis, see Table 3. We plot $\frac{\Delta V}{V_0}$ versus P according to (1) in Fig. 5. Since rubber is considered incompressible, we relate any relative volume-change detected in rubber experiments in [8] as experimental error, thus, we subtract the relative volume-change found for rubber from the relative volume-change found for arteries according to (2) and plot the corrected relative volume-change for arteries in Fig. 6. Parameters estimates for rubber were determined according to (1) and presented in Table 3.

$$\frac{\Delta V}{V_0} = e^{a_A} \cdot \frac{(P-50)^{c_A}}{L^{b_A} \cdot W^{d_A}} - e^{a_R} \cdot \frac{(P-50)^{c_R}}{L^{b_R} \cdot W^{d_R}} \tag{2}$$

4. Discussion

Experimental evidence on carotid arteries have shown that they cannot be considered incompressible under normal physiological pressures, which strengthens the argument already presented in [8] where the same phenomenon has been observed for the sapheneous and femoral arteries. This study enlarges the experimental database by including almost twice the number of experiments, addressing arteries that are longer on average and having a larger diameter, and using a different artery type - the common carotid. In the physiological pressure range of 50 to 200 mmHg, these arteries change their relative volume by about 5%, lower compared to the sapheneous and femoral arteries (2-6% [8]). Most of the arteries in the current study had a relative volume change of 1.5% in the pressure range of 50-200 mmHg.

The relationship between the relative volume change was found to be almost linearly proportional to the pressure, and inversely proportional to the dimensions of the experimented arteries (especially the artery length). The smaller the artery tested, the larger the relative volume change. This interesting phenomenon, also realized in [8] is not well explained yet, and further experiments are necessary with much longer arteries (this is a very challenging task since the longer the artery the more bifurcations it has). The ”boundary layer effects” (artery ends that are tied to the metallic tubes) may pollute the relative volume change observations and thus affect the bias of the experimental results.

Although an almost linear relationship was found between relative volume change and pressure, a simple linear correlation such as:

$$\frac{\Delta V}{V_0} = a + b \cdot L + c \cdot (P - 50) + d \cdot W \tag{3}$$

is not appropriate because $\frac{\partial(\Delta V \backslash V_0)}{\partial P}$ does not contain the specimen's dimensions (e.g L, W), resulting in a single identical slope in the graph of $\Delta V \backslash V_0$ versus P for all specimens. Of course that a more complex linear relationship, in which the pressure term is multiplied by a function of L and W may be considered in the future.

To illustrate the predicted relative volume change between the current study using (2) and the corresponding correlation from [8], we consider two typical arteries (length 22/15 mm, circumference 11.75/10.c mm, wall thickness 0.65/0.53 mm) and plot $\Delta V / V_0$ as a function of the physiological pressure in Figure 7.

Although there is a small difference between the two predictions, both predict very similar relative volume changes with a maximum difference of about 1% in relative difference at 200 mmHg.

The impact of compressibility on the biomechanical response of arteries is proportional to the ratio of bulk and shear modules, therefore, the bulk modulus determination was another motivation in this follow on research on artery compressibility. To allow an estimation of the bulk modulus, several photos of the artery at different internal pressures were taken to determine the change of outer diameter along the artery. However, due to the low transparency quality of the container, the photos were not of high enough quality to determine accurately these diameters. Furthermore, we don't have available at this time the necessary apparatus to identify the inner artery diameter. Future experiments with improved apparatus may allow to determine bulk modulus of arteries once convincing evidence is provided that there is a non-vanishing compressibility.

An artifact we realized in our study, common in recent past publications [7, 3], and may lead to misleading conclusions related to overestimation of the relative volume change (thus underestimating the bulk modulus), is due to the consideration of experimental observations close to the zero pressure. The aforementioned recent studies present the relative volume change from 0 pressure to a given pressure value, with a high relative volume changes in the order of 10% or more. Such a representation is misleading since very small pressure changes close to low pressures (which are unphysiological) cause a large change in relative volume. For example, in Figure 8 we show that all our tested arteries experience a very large relative volume change in the pre-physiological range, especially close to the 0-10 mmHg, but immediately thereafter the relative volume change is slowly changing as a function of the increase of internal pressure. *Thus, relative volume changes must be considered in the physiological pressure range, away from zero pressure, and relative to the lowest possible physiological pressure.*

Because of the effect of ”buckling” under pressure at axial stretch rations of 1, the change in diameter of the artery is not constant along the artery, thus the attempts to estimate the bulk modulus were unsuccessful. In [1, 4] same phenomenon of a decrease in axial force due to an increase in pressure is reported, causing compression forces in the load cell. Since in our experimental setup the artery is tied to the metallic tubes with an axial stretch ratio of 1, applying an internal pressure results in the ”buckling-like” shape.

Because of our use of the same experimental apparatus as in [8], same limitations listed there are extended to our study here.

The precise experimental apparatus together with extending the type and sizes of arteries addressed, we may further strengthen the conclusion that small relative volume change occurs in arteries under physiological pressure range. Therefore, any constitutive model for arteries must not a-priori assume an incompressible kinematic constrain.

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Conflict of interest None of the authors have any conflict of interest to declare that could bias the presented work.

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Table 1: Summary of the arteries (all female porcine).

Specimen	**Animal**	**L**	**W**	**T**	$\boldsymbol{V_0}$	**Weight**
#	#	*mm*	*mm*	*mm*	mm^3	*kg*
1 (exld)	1	14.5	3.8	0.52	89.8	60
2	2	38	12	0.83	378.5	41
3	3	34.5	12.8	0.63	279.3	73
4	4	44	12	0.46	242.9	60
5	5	36	11.5	0.9	372.6	50
6	6	32	11.8	0.5	188	45
7	7	28.5	13.5	0.77	296.3	50
8	8	35	15.5	0.88	477.4	90
9	8	23.5	13	0.66	201.6	90
10	9	28	13	0.79	267.5	70
11	10	32	12	0.75	288	74
12	10	19	13.5	0.75	192.4	74
13	11	21	13	0.91	248.4	77
14	11	30	12	0.95	342	77
15	11	15	13	0.9	175.5	77
16	12	30	15	0.82	381.3	82
17	13	34	11.5	0.7	273.7	45
18	14	12	12	0.6	86.4	30
19	14	15	12	0.65	117	30

Table 2: Averages of specimen's dimensions for the current study compared to [8] as well as average weight of the animals. N indicates the number of specimens used in the multi-linear regression analysis to estimate correlation's parameters.

	N	**Type**	**L**	**W**	**T**	**V_0**	**Weight**
	#		*mm*	*mm*	*mm*	mm^3	*Kg*
Current	18	Common Carotid	29.00	13.34	0.78	271.37	63.05
[8]	10	Saphenous, Femoral (1 Carotid)	15.63	10.51	0.53	89.34	70.70

Table 3: Parameter estimates from statistical analysis. A - for arteries, R - for rubber control specimens

	Estimate	**Std. Err**	**p<**
a_A	6.205	1.2423	0.000
b_A	1.087	0.1552	0.000
c_A	0.940	0.0321	0.000
d_A	2.602	0.6553	0.000
a_R	-9.075	1.2381	0.000
b_R	0.411	0.1161	0.000
c_R	0.953	0.0183	0.000
d_R	-2.125	0.2705	0.000

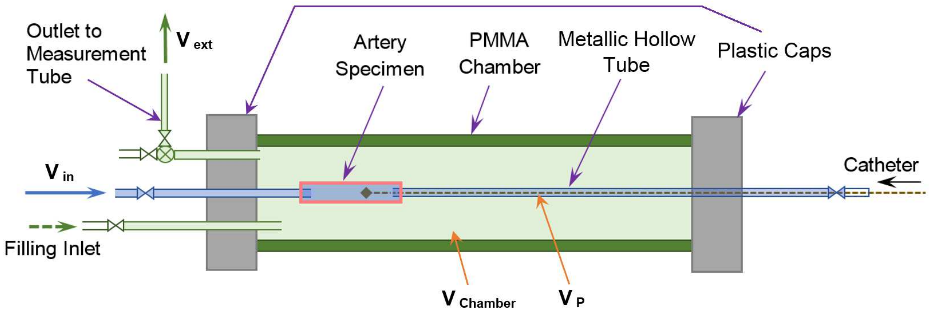

Figure 1: A schematic figure of the testing apparatus (from [8]).

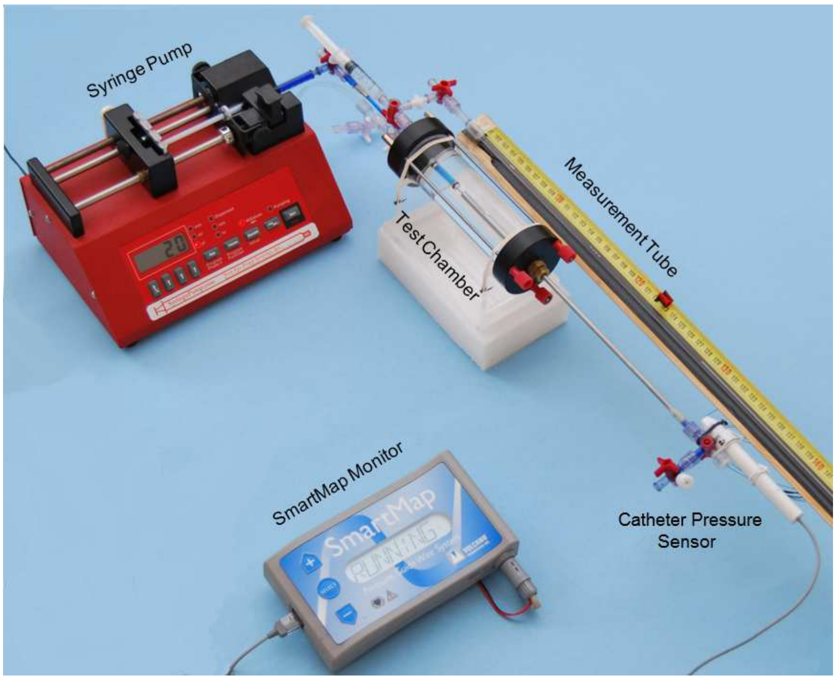

Figure 2: Photograph of the testing apparatus' components (from [8].)

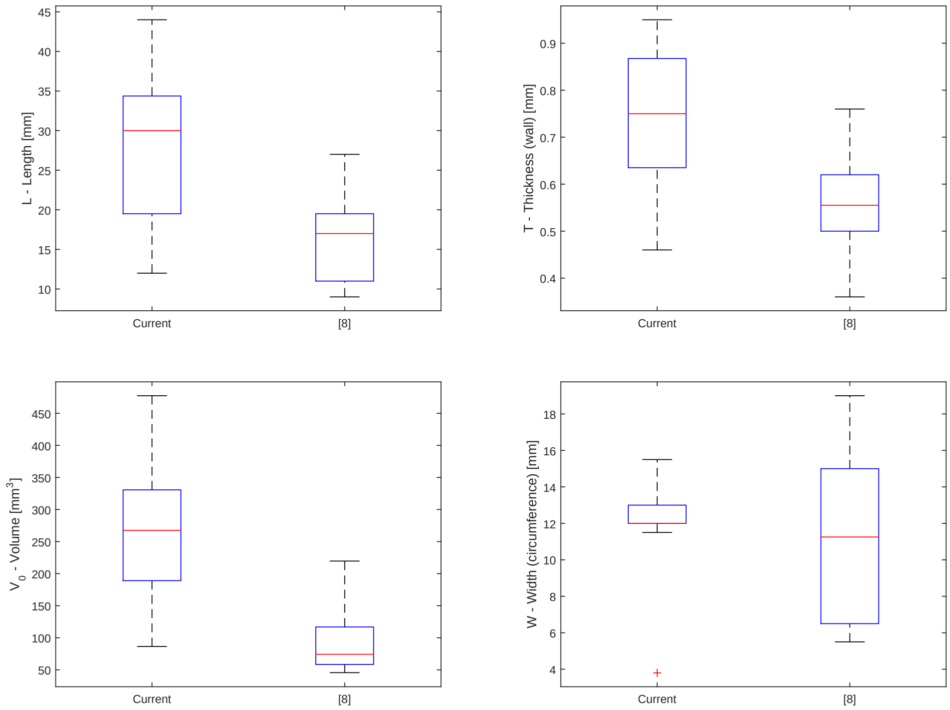

Figure 3: Arteries' dimensions statistics compared to [8]. Measurements were made on rectangular pieces after performing a longitudinal cut, therefore approximate specimens's average circumference are termed width.

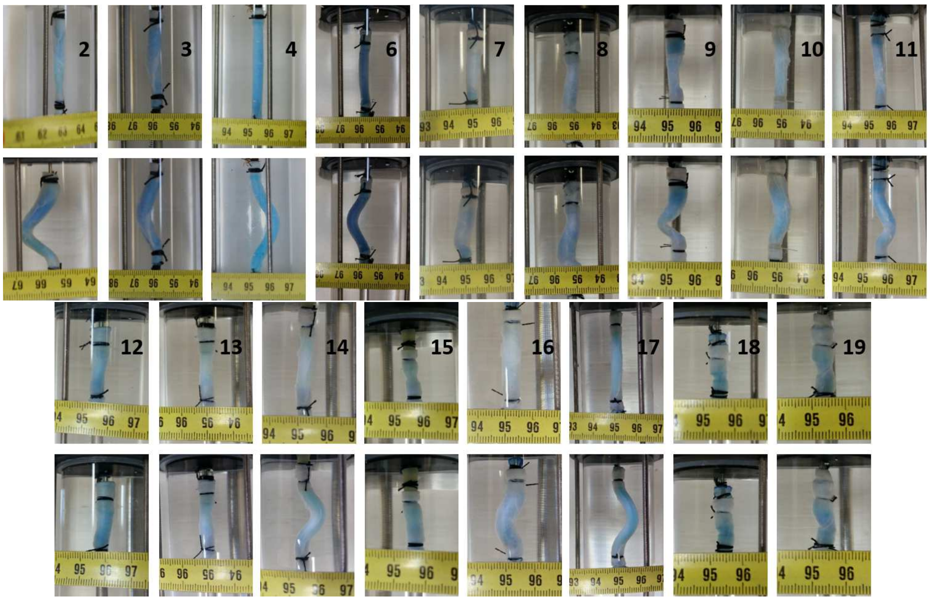

Figure 4: Photographs of all specimens included in statistical analysis (photo of specimen 5 is missing). In the first row of every group, the internal pressure is $0 mmHg$. In the second row of each group the internal pressure is $\approx 240 mmHg$. Axial stretch ratio is 1 in all experiments, therefore inflation resulted in a ”buckling-like” shape.

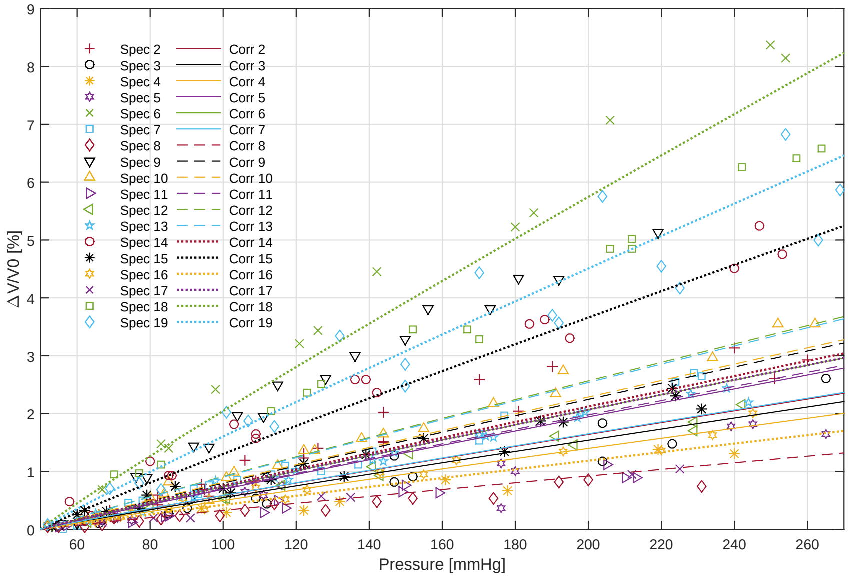

Figure 5: Relative volume-change for arteries. The correlation lines are evaluated by (1) with the parameters given in Table 3.

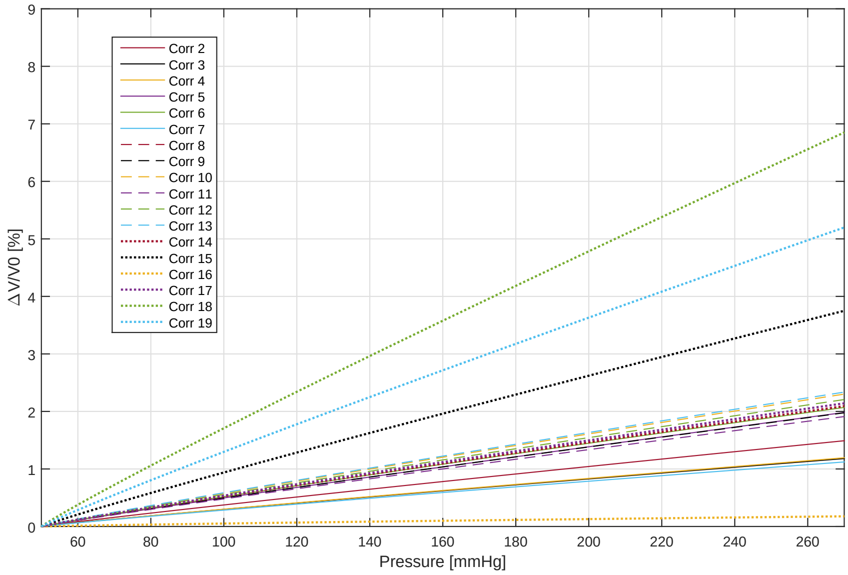

Figure 6: Corrected relative volume-change in arteries calculated by (2). One artery, Corr 8, has very small negative values and is not visible in this figure.

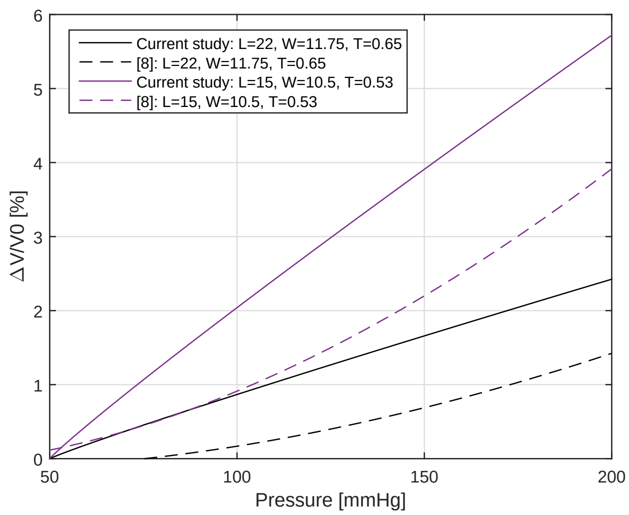

Figure 7: Predicted relative volume change as a function of the pressure in two typical arteries - comparison between the current study using (2) and the corresponding correlation from [8].

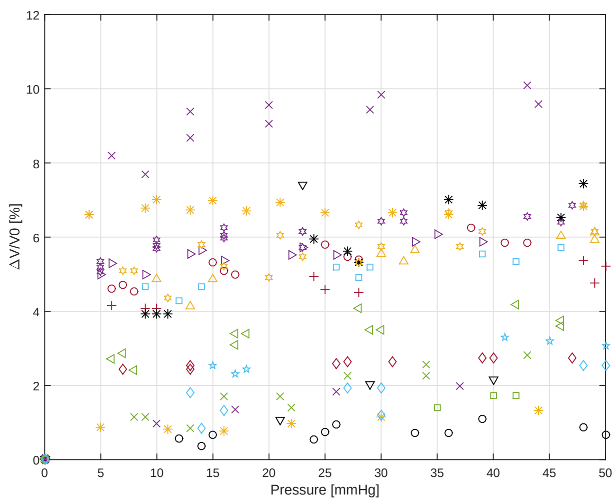

Figure 8: Raw data of relative volume-change in arteries between 0 and 50 mmHg.

Appendix A. Experimental Raw Data

Appendix A.1. Excluded artery

Table A.4: Spec 1

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	12	10.58	11	1.57
1	24	21.72	19	2.52
1	33	32.86	32	3.48
1	48	43.45	47	5.05
1	60	54.03	67	6.63
1	72	64.62	97	8.2
1	84	74.65	132	10.4
1	96	83.56	180	13.83
1	108	93.03	220	16.65
2	0	0	0	0
2	12	10.58	11	1.57
2	24	21.16	20	3.14
2	33	32.86	35	3.48
2	48	43.45	53	5.05
2	60	54.59	78	6.01
2	72	64.62	112	8.2
2	84	74.09	161	11.02
2	96	83	210	14.45
2	108	92.47	302	17.27
3	0	0	0	0
3	12	7.24	12	5.29
3	24	18.38	23	6.24
3	33	29.52	39	7.2
3	48	40.66	57	8.15
3	60	51.25	83	9.73
3	72	61.83	121	11.3
3	84	71.86	175	13.5
3	96	81.33	245	16.31
3	108	90.8	326	19.13

Appendix A.2. Raw data

Table A.5: Spec 2

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0.00	0	0.00
1	40	24.51	9	4.09
1	80	62.95	28	4.50
1	120	101.95	49	4.77
1	160	139.83	66	5.33
1	200	178.83	80	5.59
1	240	217.83	94	5.86
1	280	256.27	106	6.27
1	320	295.82	122	6.39
1	360	333.15	144	7.09
1	400	371.03	170	7.65
1	440	408.91	240	8.21
2	0	0	0	0.00
2	40	24.51	10	4.09
2	80	62.67	25	4.58
2	120	100.28	50	5.21
2	160	138.16	69	5.77
2	200	177.16	82	6.03
2	240	217.27	96	6.01
2	280	256.27	109	6.27
2	320	294.15	126	6.83
2	360	333.70	144	6.95
2	400	368.80	190	8.24
2	440	408.36	260	8.36
3	0	0.00	0	0.00
3	40	24.23	6	4.17
3	80	61.28	24	4.95
3	120	99.72	48	5.36
3	160	138.16	66	5.77
3	200	177.16	82	6.03
3	240	216.16	95	6.30
3	280	255.71	108	6.42
3	320	294.15	122	6.83
3	360	333.15	144	7.09
3	400	371.03	181	7.65
3	440	408.91	251	8.21

Table A.6: Spec 3

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0.00	0	0
1	30	28.13	15	0.67
1	60	57.38	26	0.94
1	90	86.91	39	1.11
1	120	116.43	52	1.28
1	150	145.96	67	1.45
1	180	174.93	91	1.81
1	210	203.90	112	2.18
1	240	232.87	147	2.55
1	270	261.28	204	3.12
1	300	289.14	265	3.89
2	0	0.00	0	0
2	30	28.97	14	0.37
2	60	57.94	25	0.74
2	90	88.02	36	0.71
2	120	118.11	50	0.68
2	150	147.08	70	1.05
2	180	176.60	90	1.22
2	210	206.13	109	1.39
2	240	235.10	152	1.76
2	270	263.51	223	2.32
2	300	292.48	290	2.69
3	0	0.00	0	0
3	30	28.41	12	0.57
3	60	58.50	24	0.54
3	90	88.02	33	0.71
3	120	117.55	48	0.88
3	150	147.08	66	1.05
3	180	176.60	85	1.22
3	210	206.13	112	1.39
3	240	235.10	147	1.76
3	270	264.07	204	2.12
3	300	292.48	291	2.69

Table A.7: Spec 4

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	40	23.96	4	6.61
1	80	62.95	10	7.02
1	120	103.06	15	6.97
1	160	143.18	21	6.93
1	200	183.84	31	6.65
1	240	223.40	48	6.84
1	280	262.40	75	7.25
1	320	302.51	101	7.20
1	360	342.06	132	7.39
1	400	381.62	178	7.57
1	440	420.06	240	8.21
2	0	0	0	0
2	40	23.96	4	6.61
2	80	63.51	9	6.79
2	120	103.62	13	6.74
2	160	143.73	18	6.70
2	200	183.84	25	6.65
2	240	223.96	36	6.61
2	280	263.23	56	6.90
2	320	303.06	83	6.97
2	360	342.62	109	7.16
2	400	382.17	143	7.34
2	440	421.17	193	7.75
3	0	0	0	0
3	40	37.88	5	0.87
3	80	77.99	11	0.83
3	120	118.11	16	0.78
3	160	157.66	22	0.96
3	200	197.21	30	1.15
3	240	236.77	44	1.33
3	280	276.32	68	1.51
3	320	315.88	94	1.70
3	360	355.99	122	1.65
3	400	394.71	161	2.18
3	440	433.43	219	2.71

Table A.8: Spec 5

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	40	20.06	5	5.35
1	80	57.94	10	5.92
1	120	96.66	16	6.26
1	160	138.72	23	5.71
1	200	175.21	32	6.65
1	240	214.48	47	6.85
1	280	253.76	71	7.04
1	320	291.64	116	7.61
1	360	333.15	176	7.21
1	400	367.69	245	8.67
2	0	0	0	0
2	40	20.61	5	5.20
2	80	58.50	10	5.77
2	120	97.49	16	6.04
2	160	137.05	23	6.16
2	200	176.04	32	6.43
2	240	215.60	43	6.55
2	280	254.60	75	6.82
2	320	293.04	111	7.24
2	360	331.48	180	7.66
2	400	369.08	265	8.30
3	0	0	0	0
3	40	21.17	5	5.05
3	80	58.77	10	5.70
3	120	97.77	16	5.97
3	160	137.05	23	6.16
3	200	176.04	30	6.43
3	240	216.16	46	6.40
3	280	254.87	71	6.74
3	320	293.31	106	7.16
3	360	331.48	176	7.66
3	400	369.08	239	8.30

Table A.9: Spec 6

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	25	22.84	8	1.15
1	50	46.80	21	1.70
1	75	70.19	34	2.56
1	100	94.15	53	3.11
1	125	116.43	85	4.56
1	150	137.60	126	6.59
1	175	158.77	185	8.63
1	200	178.27	250	11.56
2	0	0	0	0
2	25	22.84	9	1.15
2	50	46.80	16	1.70
2	75	70.75	27	2.26
2	100	94.71	43	2.82
2	125	117.55	67	3.96
2	150	139.28	98	5.70
2	175	160.45	142	7.74
2	200	180.50	206	10.37
3	0	0	0	0
3	25	23.40	13	0.85
3	50	47.35	22	1.41
3	75	70.75	34	2.26
3	100	94.15	54	3.11
3	125	116.43	83	4.56
3	150	138.16	121	6.30
3	175	159.33	180	8.33
3	200	178.83	254	11.26

Table A.10: Spec 7

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	35	21.17	14	4.67
1	70	54.60	29	5.20
1	105	88.02	46	5.73
1	140	122.56	60	5.89
1	175	156.27	78	6.32
1	210	190.53	96	6.57
1	245	224.51	117	6.92
1	280	258.77	141	7.16
1	315	291.92	177	7.79
1	350	324.79	229	8.51
2	0	0	0	0
2	35	22.28	12	4.29
2	70	55.43	28	4.92
2	105	89.14	42	5.35
2	140	123.40	61	5.60
2	175	157.38	74	5.95
2	210	191.92	93	6.10
2	245	225.91	113	6.45
2	280	260.45	137	6.60
2	315	293.87	170	7.13
2	350	326.18	224	8.04

Table A.11: Spec 8

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	40	28.41	7	2.43
1	80	67.41	31	2.64
1	120	106.96	47	2.73
1	160	146.52	62	2.82
1	200	186.07	77	2.92
1	240	225.63	99	3.01
1	280	265.18	128	3.10
1	320	304.18	174	3.31
1	360	343.18	231	3.52
2	0	0	0	0
2	40	27.86	13	2.54
2	80	67.41	27	2.64
2	120	106.96	40	2.73
2	160	145.96	55	2.94
2	200	185.24	70	3.09
2	240	225.07	88	3.13
2	280	264.07	114	3.34
2	320	303.62	152	3.43
2	360	342.06	200	3.76
3	0	0	0	0
3	40	28.41	13	2.43
3	80	67.69	26	2.58
3	120	106.96	39	2.73
3	160	146.24	52	2.88
3	200	186.07	67	2.92
3	240	225.63	83	3.01
3	280	264.90	106	3.16
3	320	304.18	142	3.31
3	360	342.62	192	3.64

Table A.12: Spec 9

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	20	15.88	29	2.04
1	40	33.43	60	3.26
1	60	51.81	79	4.06
1	80	70.75	96	4.59
1	100	89.69	111	5.11
1	120	108.36	128	5.77
1	140	127.02	150	6.44
1	160	145.96	173	6.96
1	180	164.90	192	7.49
1	200	183.29	219	8.29
2	0	0	0	0
2	20	25.07	23	7.40
2	40	43.18	54	8.34
2	60	61.28	72	9.28
2	80	80.22	85	9.81
2	100	99.16	103	10.33
2	120	118.66	117	10.58
2	140	136.49	134	11.66
2	160	155.43	156	12.18
2	180	174.09	180	12.85
2	200	192.48	205	13.65
3	0	0	0	0
3	20	17.83	21	1.08
3	40	35.65	40	2.16
3	60	54.60	56	2.68
3	80	72.98	76	3.48
3	100	91.92	92	4.01
3	120	110.86	104	4.53
3	140	129.81	115	5.06
3	160	148.75	136	5.58
3	180	167.13	156	6.38
3	200	186.07	181	6.91

Table A.13: Spec 10

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	40	27.02	15	4.85
1	80	65.74	32	5.33
1	120	104.18	49	5.92
1	160	143.18	65	6.29
1	200	181.62	85	6.87
1	240	221.17	103	7.04
1	280	260.17	125	7.42
1	320	299.16	155	7.79
1	360	336.49	193	8.79
1	400	374.37	252	9.58
2	0	0	0	0
2	40	28.97	13	4.12
2	80	64.90	33	5.64
2	120	103.62	49	6.12
2	160	142.62	66	6.50
2	200	181.62	84	6.87
2	240	220.89	101	7.14
2	280	259.61	122	7.62
2	320	298.89	144	7.89
2	360	337.05	191	8.58
2	400	373.82	262	9.79
3	0	0	0	0
3	40	27.02	10	4.85
3	80	65.18	30	5.54
3	120	103.90	46	6.02
3	160	142.90	62	6.39
3	200	182.17	80	6.67
3	240	222.28	96	6.62
3	280	260.72	115	7.21
3	320	299.44	138	7.69
3	360	337.88	174	8.27
3	400	375.77	234	9.06

Table A.14: Spec 11

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	30	15.60	9	5.00
1	60	44.57	16	5.36
1	90	74.09	26	5.52
1	120	103.06	39	5.88
1	150	132.59	57	6.04
1	180	162.12	85	6.21
1	210	191.36	115	6.47
1	240	220.61	150	6.73
1	270	249.58	205	7.09
2	0	0	0	0
2	30	14.76	6	5.29
2	60	43.73	14	5.65
2	90	73.54	23	5.72
2	120	102.51	35	6.07
2	150	132.03	55	6.24
2	180	161.56	84	6.40
2	210	191.09	117	6.57
2	240	220.33	159	6.83
2	270	249.58	213	7.09
3	0	0	0	0
3	30	15.60	5	5.00
3	60	44.01	13	5.55
3	90	74.09	22	5.52
3	120	103.06	33	5.88
3	150	133.15	52	5.85
3	180	162.67	75	6.02
3	210	192.20	111	6.18
3	240	221.17	149	6.54
3	270	250.42	210	6.80

Table A.15: Spec 12

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	8	3.34	8	2.42
1	16	9.47	18	3.39
1	24	16.16	28	4.08
1	32	23.96	42	4.18
1	40	31.20	63	4.58
1	48	38.72	80	4.82
1	56	45.96	116	5.22
1	64	52.92	151	5.76
1	72	60.17	229	6.15
2	0	0	0	0
2	8	2.51	7	2.86
2	16	9.47	17	3.39
2	24	17.27	30	3.50
2	32	24.79	46	3.75
2	40	32.03	69	4.14
2	48	39.55	101	4.39
2	56	46.80	143	4.78
2	64	53.76	196	5.32
2	72	61.00	229	5.72
3	0	0	0	0
3	8	2.79	6	2.71
3	16	10.03	17	3.10
3	24	17.27	29	3.50
3	32	25.07	46	3.60
3	40	32.31	69	4.00
3	48	39.83	100	4.25
3	56	46.80	141	4.78
3	64	53.76	191	5.32
3	72	60.72	242	5.86

Table A.16: Spec 13

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	8	1.95	18	2.44
1	16	8.36	50	3.08
1	24	15.32	78	3.49
1	32	22.01	98	4.02
1	40	29.53	120	4.22
1	48	36.77	147	4.52
1	56	44.01	170	4.83
1	64	51.25	197	5.13
1	72	58.22	228	5.55
2	0	0	0	0
2	8	2.23	17	2.32
2	16	8.08	45	3.19
2	24	15.32	63	3.49
2	32	22.28	92	3.91
2	40	29.53	118	4.22
2	48	36.77	144	4.52
2	56	43.73	174	4.94
2	64	50.70	198	5.36
2	72	58.22	244	5.55
3	0	0	0	0
3	8	1.67	15	2.55
3	16	7.80	41	3.30
3	24	14.48	65	3.83
3	32	21.73	88	4.14
3	40	28.97	114	4.44
3	48	36.21	141	4.75
3	56	43.18	172	5.16
3	64	50.14	199	5.58
3	72	57.10	238	6.00

Table A.17: Spec 14

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	30	14.21	6	4.62
1	60	42.62	16	5.08
1	90	71.59	28	5.38
1	120	100.00	43	5.85
1	150	127.86	64	6.48
1	180	155.43	86	7.18
1	210	183.01	109	7.89
1	240	209.75	139	8.85
1	270	236.49	184	9.80
1	300	263.23	240	10.75
2	0	0	0	0
2	30	14.48	8	4.54
2	60	42.90	17	5.00
2	90	71.31	27	5.47
2	120	100.00	41	5.85
2	150	128.13	62	6.39
2	180	155.43	85	7.18
2	210	183.29	109	7.81
2	240	210.58	142	8.60
2	270	237.33	195	9.55
2	300	262.40	253	11.00
3	0	0	0	0
3	30	13.93	7	4.70
3	60	41.78	15	5.33
3	90	70.19	25	5.79
3	120	98.61	38	6.26
3	150	127.02	58	6.72
3	180	154.60	80	7.43
3	210	182.45	103	8.06
3	240	209.75	136	8.85
3	270	236.21	188	9.88
3	300	260.72	247	11.48

Table A.18: Spec 15

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	8	1.11	9	3.92
1	16	6.69	28	5.31
1	24	12.53	46	6.53
1	32	19.22	62	7.28
1	40	26.74	79	7.56
1	48	34.54	100	7.67
1	56	41.78	122	8.10
1	64	49.03	155	8.53
1	72	56.55	193	8.81
1	80	63.51	223	9.40
2	0	0	0	0
2	8	1.11	11	3.92
2	16	6.13	27	5.62
2	24	11.98	39	6.85
2	32	19.22	53	7.28
2	40	26.74	68	7.56
2	48	33.98	87	7.99
2	56	41.78	113	8.10
2	64	49.03	139	8.53
2	72	55.99	187	9.12
2	80	63.23	224	9.55
3	0	0	0	0
3	8	1.11	10	3.92
3	16	5.57	24	5.94
3	24	11.70	36	7.01
3	32	18.94	48	7.44
3	40	26.18	60	7.87
3	48	33.98	77	7.99
3	56	41.50	102	8.26
3	64	49.03	133	8.53
3	72	56.27	177	8.96
3	80	62.95	231	9.71

Table A.19: Spec 16

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	35	18.38	11	4.36
1	70	51.25	20	4.92
1	105	84.68	28	5.33
1	140	118.11	37	5.74
1	175	151.53	49	6.15
1	210	185.52	65	6.42
1	245	219.78	85	6.61
1	280	253.48	109	6.95
1	315	287.47	143	7.22
1	350	321.17	193	7.56
1	385	353.76	245	8.19
2	0	0	0	0
2	35	15.60	8	5.09
2	70	50.14	16	5.21
2	105	84.12	23	5.48
2	140	118.11	30	5.74
2	175	151.53	39	6.15
2	210	185.52	52	6.42
2	245	220.06	72	6.54
2	280	254.60	95	6.66
2	315	288.30	123	7.00
2	350	321.45	164	7.49
2	385	354.87	234	7.90
3	0	0	0	0
3	35	15.60	7	5.09
3	70	47.91	14	5.79
3	105	81.89	21	6.06
3	140	115.88	28	6.33
3	175	149.58	36	6.67
3	210	183.84	48	6.86
3	245	217.83	65	7.13
3	280	251.25	89	7.54
3	315	286.35	117	7.51
3	350	319.78	155	7.93
3	385	353.20	220	8.34
3	420	385.52	291	9.04
3	455	418.94	394	9.46

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
4	0	0	0	0
4	35	16.99	7	4.72
4	70	48.47	14	5.65
4	105	83.57	20	5.62
4	140	117.55	26	5.89
4	175	151.53	33	6.15
4	210	186.07	42	6.28
4	245	220.06	56	6.54
4	280	254.04	76	6.81
4	315	288.02	99	7.08
4	350	322.01	128	7.34
4	385	355.99	170	7.61
4	420	389.14	243	8.09
4	455	421.17	350	8.87
5	0	0	0	0
5	35	18.94	5	4.21
5	70	51.25	12	4.92
5	105	85.79	18	5.04
5	140	119.78	23	5.3
5	175	153.76	30	5.57
5	210	188.3	38	5.69
5	245	222.28	49	5.96
5	280	256.82	66	6.08
5	315	290.81	87	6.34
5	350	324.79	113	6.61
5	385	358.77	148	6.88
5	420	392.2	209	7.29
5	455	424.51	304	8

Table A.20: Spec 17

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	35	32.31	10	0.98
1	70	66.30	17	1.35
1	105	100.00	26	1.83
1	140	134.54	37	1.99
1	175	168.80	56	2.26
1	210	203.06	78	2.53
1	245	237.60	100	2.70
1	280	271.87	162	2.97
1	315	304.74	257	3.75
2	0	0	0	0
2	35	13.93	9	7.70
2	70	46.24	13	8.68
2	105	80.22	20	9.05
2	140	114.21	29	9.42
2	175	148.75	44	9.59
2	210	183.29	67	9.76
2	245	218.11	91	9.83
2	280	252.09	135	10.20
2	315	285.79	225	10.67
3	0	0	0	0
3	35	12.53	6	8.21
3	70	44.29	13	9.39
3	105	78.83	20	9.56
3	140	113.09	30	9.83
3	175	147.35	43	10.10
3	210	181.62	66	10.37
3	245	216.43	81	10.44
3	280	250.42	127	10.81
3	315	284.40	212	11.18

Table A.21: Spec 18

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	4	2.79	35	1.41
1	8	5.29	70	3.13
1	12	8.36	113	4.22
1	16	11.14	152	5.62
1	20	13.93	206	7.03
1	24	16.71	242	8.43
2	0	0	0	0
2	4	2.51	42	1.73
2	8	5.29	83	3.13
2	12	8.08	127	4.54
2	16	11.42	170	5.30
2	20	13.93	212	7.03
2	24	16.71	257	8.43
3	0	0	0	0
3	4	2.51	40	1.73
3	8	5.29	77	3.13
3	12	8.08	123	4.54
3	16	11.14	167	5.62
3	20	13.93	212	7.03
3	24	16.43	264	8.76

Table A.22: Spec 19

Cycle	V in	V ext	p in	$\frac{\Delta V}{V_0}$
#	μL	μL	$mmHg$	%
1	0	0	0	0
1	6	3.90	13	1.80
1	12	9.75	27	1.92
1	18	15.04	48	2.53
1	24	19.50	69	3.85
1	30	23.96	101	5.17
1	36	28.41	132	6.49
1	42	33.15	170	7.57
1	48	37.60	204	8.89
1	54	42.34	254	9.97
2	0	0	0	0
2	6	5.01	14	0.84
2	12	10.58	30	1.21
2	18	15.88	52	1.81
2	24	21.17	83	2.42
2	30	25.91	114	3.50
2	36	30.64	150	4.58
2	42	35.65	190	5.42
2	48	40.67	220	6.27
2	54	45.13	269	7.59
3	0	0	0	0
3	6	4.46	16	1.32
3	12	9.75	30	1.92
3	18	15.04	50	2.53
3	24	19.50	77	3.85
3	30	24.23	107	4.93
3	36	29.53	150	5.53
3	42	34.26	192	6.61
3	48	39.55	225	7.22
3	54	44.57	263	8.06