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Tympanic membrane pressure buffering function at quasi-static and low-frequency pressure variations

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Abstract

Deformation of the tympanic membrane is known to contribute to the pressure regulation processes in the middle ear cleft. In this paper we investigated pressure variations in the rabbit middle ear in response to sinusoidal varying pressures applied to the ear canal, with frequencies ranging from 0.5 Hz to 50 Hz and pressure amplitudes ranging between 0.25 kPa and 1 kPa. The transtympanic pressure difference was found to be smallest in the quasi-static range, and quickly increased as a function of frequency. The response curves showed asymmetry, with larger transtympanic pressures when positive pressures were applied in the ear canal. Normalized transtympanic pressure amplitudes remained fairly constant as a function of input pressure, with values in the range of 60% to 70% relative to the applied pressure. The total harmonic distortion of the middle ear pressure signal was calculated and was found to be very small ($\leq 2\%$) for low-pressure amplitudes and low frequencies. For pressure amplitudes in the order of 0.25 kPa to 0.5 kPa, it increased to about 10% at 50 Hz. When a 1 kPa pressure amplitude was applied, variation between animals became large and distortion values up to 30% at 50 Hz were observed. The results showed that pressure buffering due to tympanic membrane displacement was most effective for compensating small transtympanic pressure loads at low frequencies.

32 Keywords: middle ear pressure, ear canal pressure, pressure regulation, pressure buffering.

33
34 Abbreviations: EC, Ear canal; ECP, Ear canal pressure; ET, Eustachian tube; ME, Middle ear;
35 MEP, Middle ear pressure; THD, Total harmonic distortion; TM, Tympanic membrane.

36
37

38 **1. Introduction**

39

40 The ear is subject to pressure variations over a large frequency range. In the auditory range (20 Hz
41 – 20 kHz), the pain threshold of 120 dB SPL corresponds to a pressure amplitude of 20 Pa. In the
42 very low frequency range (<20 Hz), however, pressure variations occur with amplitudes which can
43 be several orders of magnitude larger: during an airplane liftoff or descent, or a dive under water,
44 pressure variations of several kPa are commonly encountered, and even a simple elevator trip of a
45 few floors leads to a pressure variation of several hundreds of Pascals. The gas exchange processes
46 between the blood perfusion in the middle ear (ME) mucosa and the gases in the ME cleft also lead
47 to a slow buildup of pressure differences between the ME and the environment (Loring and Butler,
48 1987).

49

50 Middle ear pressure (MEP) is regulated by a combination of Eustachian tube (ET) action, gas
51 exchange processes, and deformation of the tympanic membrane (TM). As the TM is flexible, it is
52 deformed by pressure gradients between the ME and the environment, thus changing the volume of
53 the gases enclosed in the ME, and buffering part of the pressure change. The TM is therefore an
54 important factor in ME pressure regulation, but at the same time a deficient regulation of pressure
55 loads can lead to TM pathologies. Sustained ME under-pressure is a common clinical condition
56 which can result in remodeling of the TM with atrophy, retraction pockets, atelectasis, and
57 cholesteatoma including ossicular destruction (Tos et al., 1984; Ars et al., 1989; Sadé and Ar,
58 1997).

59

60 Quasi-static pressure changes in the ME can be measured indirectly with tympanometry in clinical
61 circumstances (Thomsen, 1960) or directly using various other methods. Direct measurements can
62 be done through a perforation in the mastoid (Flisberg et al., 1963; Hergils et al., 1990) or the TM

63 (Buckingham and Ferrer, 1973; Sadé et al., 1976), as well as insertion of a pressure transducer
64 through the ET (Takahashi et al., 1987). Numerous studies have investigated the influence of such
65 pressures on the deformation of the TM (e.g. Dirckx and Decraemer, 1991; von Unge et al., 1993;
66 Vorwerk et al., 1999; Lee and Rosowski, 2001) and the displacement of the ME structures (e.g.
67 Hüttenbrink, 1988; Rosowski et al., 1999; Salih et al., 2016) using various techniques. Recently,
68 the buffering function of the TM in humans was investigated with measurements of MEP change
69 on test persons that were subjected to external pressure variations due to elevator trips (Padurariu
70 et al., 2016). In such experiments it is, however, not possible to systematically investigate the
71 dependence of the pressure buffering as a function of frequency and amplitude.

72
73 In the current work we used an animal model to measure pressure variation in the ME caused by
74 pressure variation in the ear canal (EC). Measurements were taken over a wide range of amplitudes
75 and frequencies, so the gap is bridged between the quasi-static pressure regime and the (very) low
76 auditory frequencies. As the study focuses on the purely mechanical effect of the TM,
77 measurements are done ex-vivo, so that the ET action or gas exchange effects are avoided.

78

79 **2. Materials and methods**

80

81 *2.1. Sample preparation*

82

83 Rabbits used in this study were sacrificed using intravenous injection of sodium pentobarbital 60
84 mg/kg (Dolethal, Ethical Agents Ltd, Auckland, New Zealand). The injection was performed in the
85 vein of the pinna after local surface anesthesia with lidocaine spray (Xylocaine, AstraZeneca,
86 Ussel, Belgium). All preparations were conducted according to the rules set by the Belgian
87 legislation and the local ethical committee of the University of Antwerp, and were in accordance
88 with the Guiding Principles for Research Involving Animals and Human Beings as adopted by the
89 American Physiological Society. The temporal bone was dissected from the skull. The EC was
90 connected with instant glue (Loctite 401, Loctite, Düsseldorf, Germany) to a 2 cm long plastic
91 tube, through which pressure was applied. At the medial side of the bullae, a hole of 2 mm was
92 drilled using a dental bur. Through the hole, a 2 cm long metal tube was glued with dental glue
93 (OptiBond Solo Plus, Kerr, Orange, CA, USA) to measure the MEP as a function of ear canal

94 pressure (ECP). A miniature pressure sensor (Endevco 8507C-1, Meggit Sensing Systems,
95 Basingstoke, UK) for measuring MEP was connected to the metal tube using a 3-way valve so that
96 the ME could be vented before starting measurements. Specimens were kept humid during the
97 preparation and measurement by using an ultrasonic humidifier (BU-1300, Bonaire, Salisbury,
98 Australia).

99

100 *2.2. Pressure generation*

101

102 A custom-built pressure generator was used to apply sinusoidal pressure changes to the EC. As
103 shown in Figure 1, the pressure setup consists of an electromagnetic actuator (Vibration Generator
104 (2185.00), Frederiksen, Endeavour Hills, Australia) that is attached to an adaptable volume
105 connected to a tube. When the actuator moves, the volume and hence the pressure of the enclosed
106 gas change, since the amount of gas remains constant. With a pressure sensor (PDCR 10/L, Druck,
107 Inc., New Fairfield, CT, USA) coupled to the tube, the pressure values were measured and used in
108 a custom-built feedback system (Aernouts and Dirckx, 2011). This way the actual pressure follows
109 the desired values with an accuracy of better than 2% over the entire frequency and pressure range.
110 The feedback control unit (FU) was connected to a function generator (TDS 210, Tektronix,
111 Beaverton, OR, USA), and was used to generate sinusoidal pressure changes with frequencies
112 varying from 0.1 to 100 Hz within the range of -2 to +2 kPa. The calibration of the pressure
113 generation system and the MEP pressure sensor were checked extensively, and proved to be
114 perfectly stable within the measuring resolution over long periods of time (months).

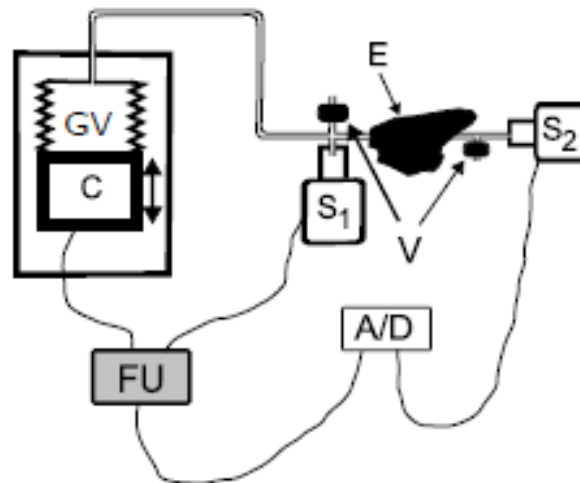
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116 *2.3. Measurement protocol*

117

118 With an A/D port (NI DAQPad-6015, National Instruments, Austin, TX, USA) connected to a PC,
119 pressure generation and measurement was controlled from a custom written software in Matlab
120 (Mathworks, Natick, MA, USA). The setup allowed to apply pressures with a precision of 10 Pa.
121 Measurements were conducted in fresh specimens, within less than 20 minutes after sacrificing the
122 animal. Four periods were recorded after completing two initial pressure cycles, so that the
123 specimen was preconditioned to reduce viscoelastic effects. Pressures at both the EC and ME were
124 measured simultaneously, so that the time-dependent response of the TM could be obtained. ECPs

125 with amplitudes of 0.25, 0.5 and 1 kPa and frequencies of 0.5, 1, 2, 5, 10, 20, 30, 40 and 50 Hz
 126 were applied. The specimens were ventilated before each cycle of pressure measurements. In this
 127 way the measurement always started at zero pressure. This measurement protocol allowed us to
 128 minimize static pressure gradient build-up, which can occur due to changes in environmental
 129 conditions (e.g. changing barometric pressure due to weather conditions, draft due to room
 130 ventilation systems etc.). Results using low-pressure amplitudes were recorded first to avoid
 131 possible effects of inelastic deformation caused by the higher pressure values. After the
 132 measurements, a static under-pressure and over-pressure of 2 kPa was applied to the ear to check
 133 for ET opening action, but no leakage was observed.
 134



135
 136 Figure 1: Schematic drawing of the experimental setup; (GV): compressible gas volume, (C):
 137 electromagnetic actuator, (S1): pressure sensor to measure the actual pressure generated by the
 138 system which is applied to the EC, (FU): feedback control unit, (S2): pressure sensor to
 139 measure the MEP, (V): two valves used to ventilate between measurements, (E): specimen,
 140 which is glued via two tubes to the pressure sensors and (A/D): A/D port that sends/receives the
 141 signals to/from a PC.

142 When the actuator (C) moves, the pressure in the gas volume (GV) changes as the total gas
 143 content remains fixed. With the feedback control unit (FU) a desired pressure value is obtained.
 144

145 2.4. Total harmonic distortion

146

147 It is well known that the TM and ME show nonlinear behavior in the quasi-static regime at large
148 pressure amplitudes (Hüttenbrink, 1988; Dirckx and Decraemer, 1991, 1992; Aerts and Dirckx,
149 2010). Consequently, a nonlinear pressure response as a function of ECP is to be expected. To
150 quantify the level of such a nonlinearity, the total harmonic distortion (THD) was calculated,
151 which is a popular method to specify nonlinearity in acoustic signals (Aerts and Dirckx, 2007).
152 This is achieved by considering the Fourier transform of the pressure signal at the excitation
153 frequency and the corresponding higher harmonics. To calculate the THD, the amplitudes of the
154 contributions of the higher harmonics are first squared and summed. Then, the square root of this
155 component is divided by the amplitude of the contribution of the excitation frequency.

156

157 **3. Results**

158

159 Figure 2 shows an example of the measured MEP and ECP signals obtained at 0.5 and 50 Hz
160 (amplitude of 1 kPa). The figure shows that the MEP follows the waveform of the ECP at lower
161 frequencies, while the deviation between the two waveforms increases at higher frequencies. The
162 figure also shows that both ECP and MEP curves have a small offset of a few Pa. For the higher
163 pressure amplitudes like the one in the figure, this offset and its variation over time negligible, but
164 for the lowest amplitudes (0.1 kPa) it causes a slight drift of the curve over time. Hence, only a
165 single period will be used to do calculations. The results obtained in the several periods are very
166 repetitive indicating that stable preconditioning has been reached.

167

168 In the time graphs shown in Figure 2 it is difficult to see the relationship between applied pressure
169 (ECP) and resulting pressure (MEP). Therefore, all results will be shown in a different
170 representation. For each measurement MEP is plotted as a function of ECP for one period of the
171 recorded time signals. For the 6 specimens, MEPs as a function of ECPs for amplitudes of 0.25,
172 0.5 and 1 kPa are presented in Figures 3, 4 and 5 respectively. All specimens exhibited similar
173 behavior for each amplitude and frequency, apart from specimen #R2, which shows markedly
174 larger hysteresis than the other samples. For 0.25 kPa amplitude, all curves are nearly straight lines
175 with a slope in the order of 0.3. Most curves go through (0, 0), although for some measurements
176 there is a small offset. For one ear the offset is a bit larger (0.05 kPa). At 50 Hz all curves start to
177 show a little hysteresis. The curves recorded for the higher pressure amplitudes (0.5 kPa and 1

178 kPa) show clearly larger hysteresis, which also increases with frequency. The hysteresis
179 contributes to distortion of the curve which we analyze further below. The overall slope of the
180 curves is larger for the 0.5 kPa measurements, with a value of about 0.4. For the 1 kPa
181 measurements the curves deviate clearly from a straight line and start to show the typical S-shape
182 found in other studies using pressure gradients in the range of several kPa.

183
184 To analyze the pressure buffering effect as a function of frequency, the maximal transtympanic
185 pressure gradient (ECP - MEP) as a function of frequency is presented in Figure 6. The figure
186 shows that the transtympanic pressure gradient rapidly increases when frequency increases from
187 0.5 Hz to a few Hz. Beyond a few Hz the gradient increases slowly as a function of frequency. The
188 ratios of transtympanic pressure over ECP are presented as a function of ECP in Figure 7. This
189 normalized value is fairly constant for all frequencies. Also as a function of ECP the value is
190 nearly constant between amplitudes 0.5 kPa and 1 kPa. At the lowest ECP amplitude (0.25 kPa)
191 the value is slightly higher.

192
193 As shown in Figure 2, the ECP has an almost perfect sinusoidal shape, and thus it has a very small
194 THD value (less than 2%). In order to take into account the small nonlinearity of the input signal,
195 we subtracted the THD of the ECP from the THD of the MEP. The results are shown in Figure 8
196 for input pressure amplitudes of 0.25, 0.5 and 1 kPa. For all ears and all pressures one sees that
197 THD increases as a function of frequency. For all pressure amplitudes the THD of the ME
198 response curve increases as a function of frequency, but the slope of the increase diminishes with
199 increasing frequency. For all pressure values the THD drops sharply at the lowest frequency (0.5
200 Hz). For ECP values 0.25 kPa and 0.5 kPa the variability between the ears is much smaller than for
201 ECP = 1 kPa where two ears show a THD which is three times larger than in the other ears.

202

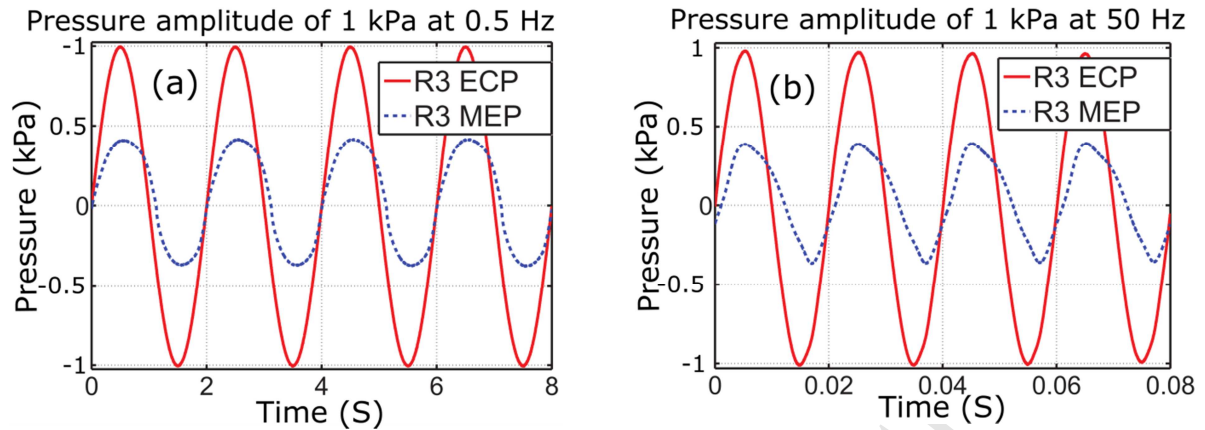


Figure 2: R3 ECP and MEP as a function of time at frequencies of: (a) 0.5 Hz and (b) 50 Hz.

Figure 2 shows that the MEP curve is not sinusoidal, meaning that the TM displacement response as a function of pressure is not linear. In section 4.3 and 4.4 these aspects will be analyzed in more detail.

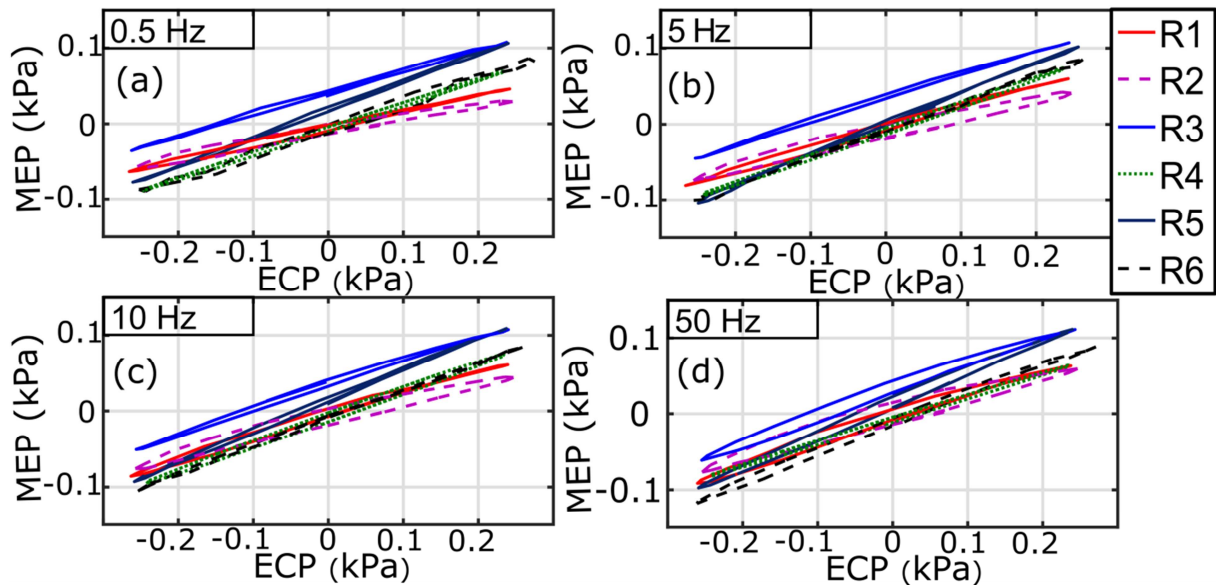
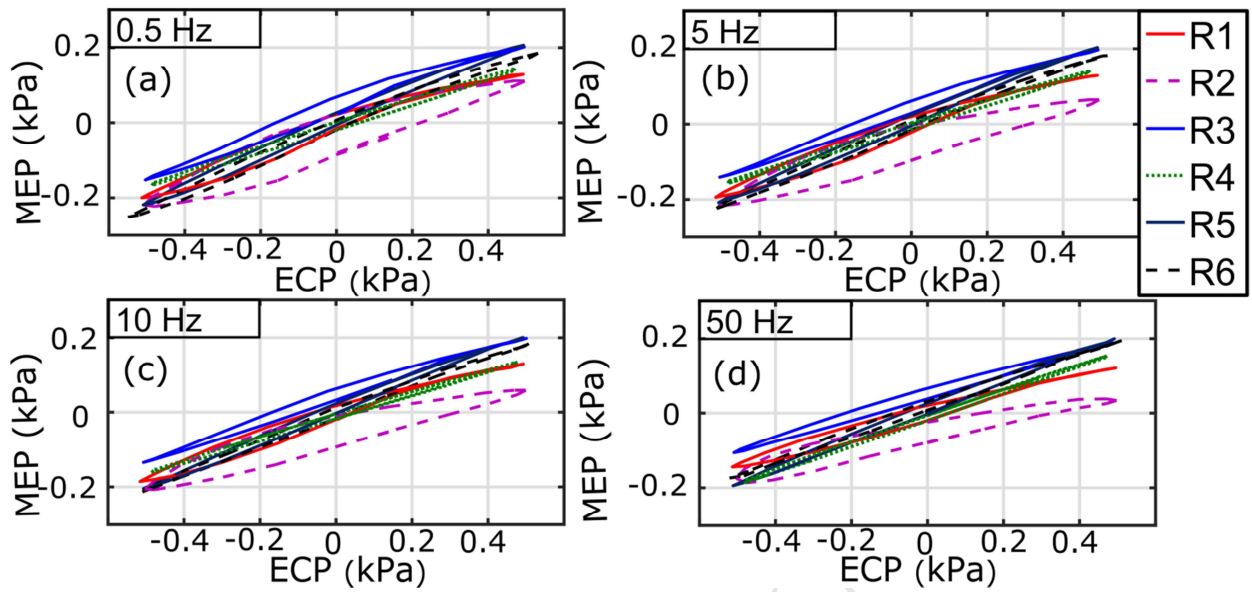


Figure 3: MEP as a function of ECP for an ECP amplitude of 0.25 kPa and frequencies of: (a) 0.5 Hz, (b) 5 Hz, (c) 10 Hz and (d) 50 Hz.



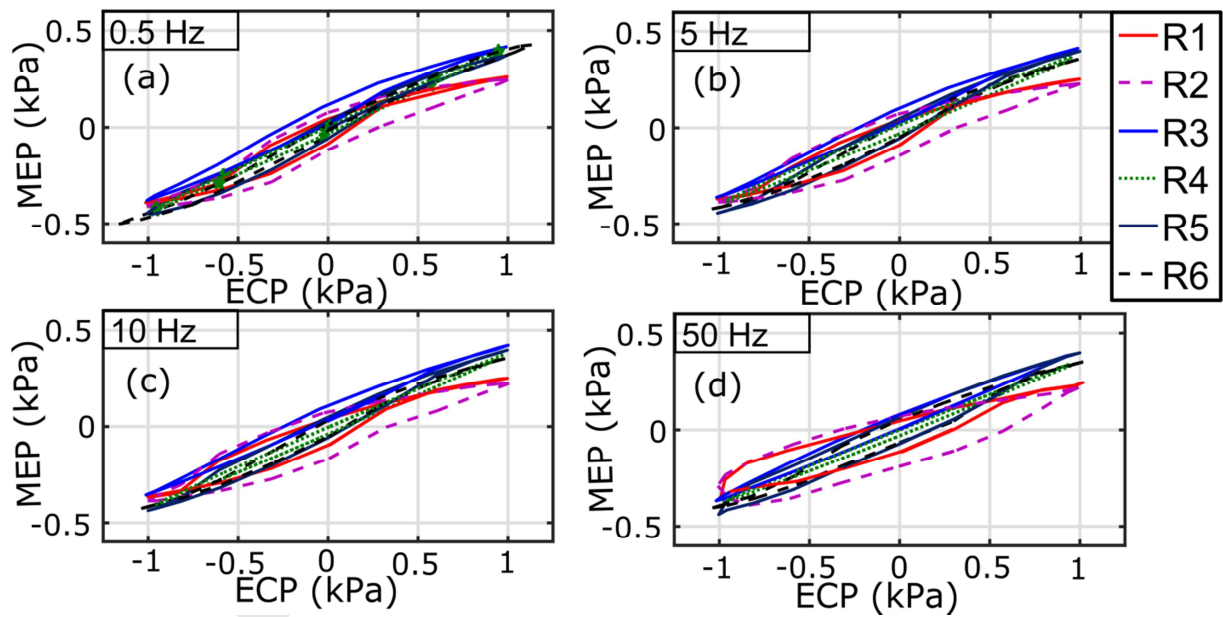
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Figure 4: MEP as a function of ECP for an ECP amplitude of 0.5 kPa and frequencies of: (a) 0.5 Hz, (b) 5 Hz, (c) 10 Hz and (d) 50 Hz.



217

218

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Figure 5: MEP as a function of ECP for an ECP amplitude of 1 kPa and frequencies of: (a) 0.5 Hz, (b) 5 Hz, (c) 10 Hz and (d) 50 Hz.

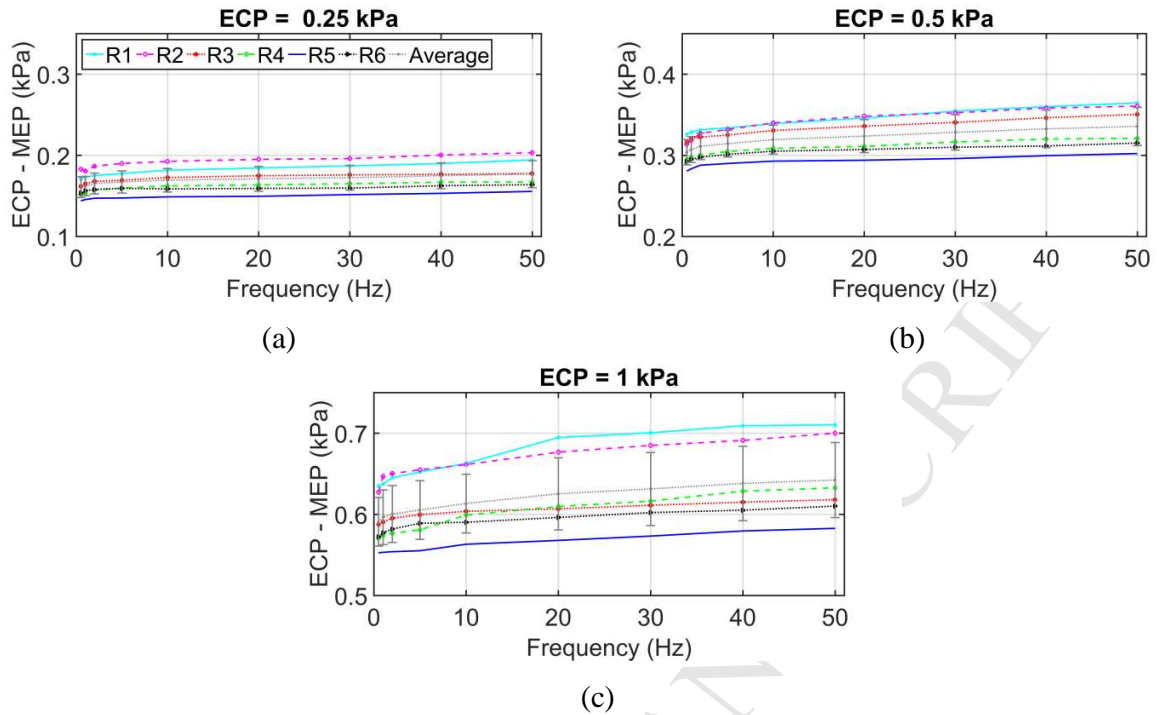


Figure 6: Transtympanic pressure as a function of frequency for ECP amplitudes of: (a) 0.25 kPa, (b) 0.5 kPa and (c) 1 kPa.

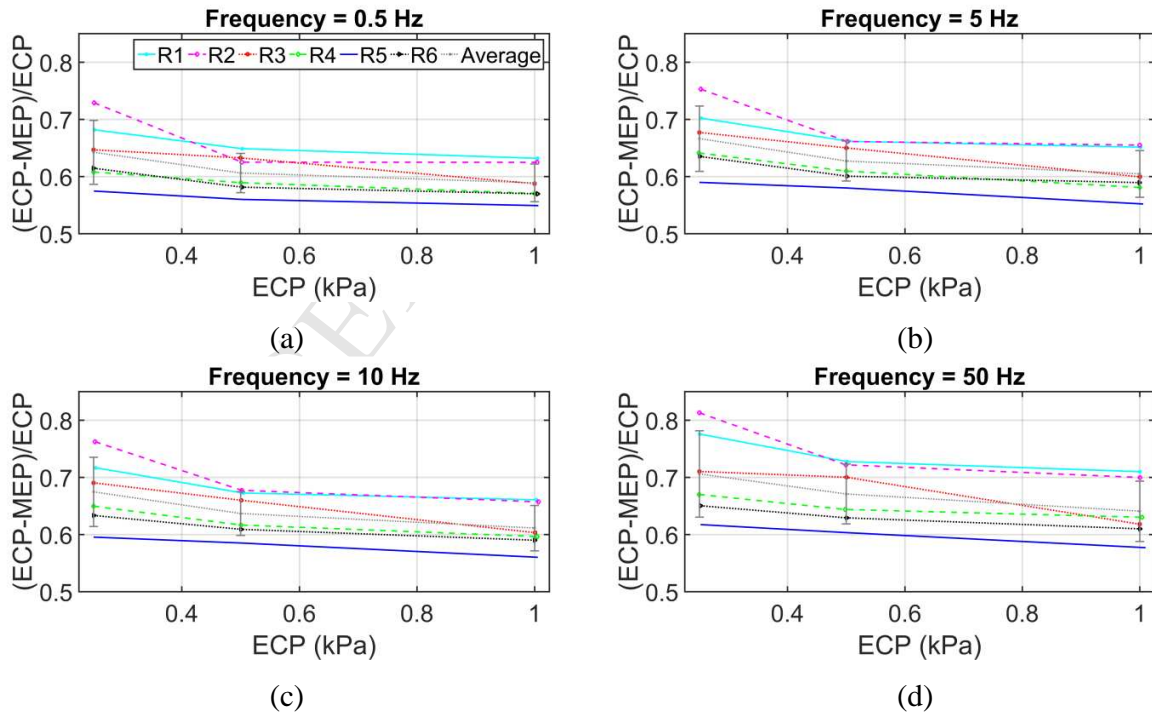
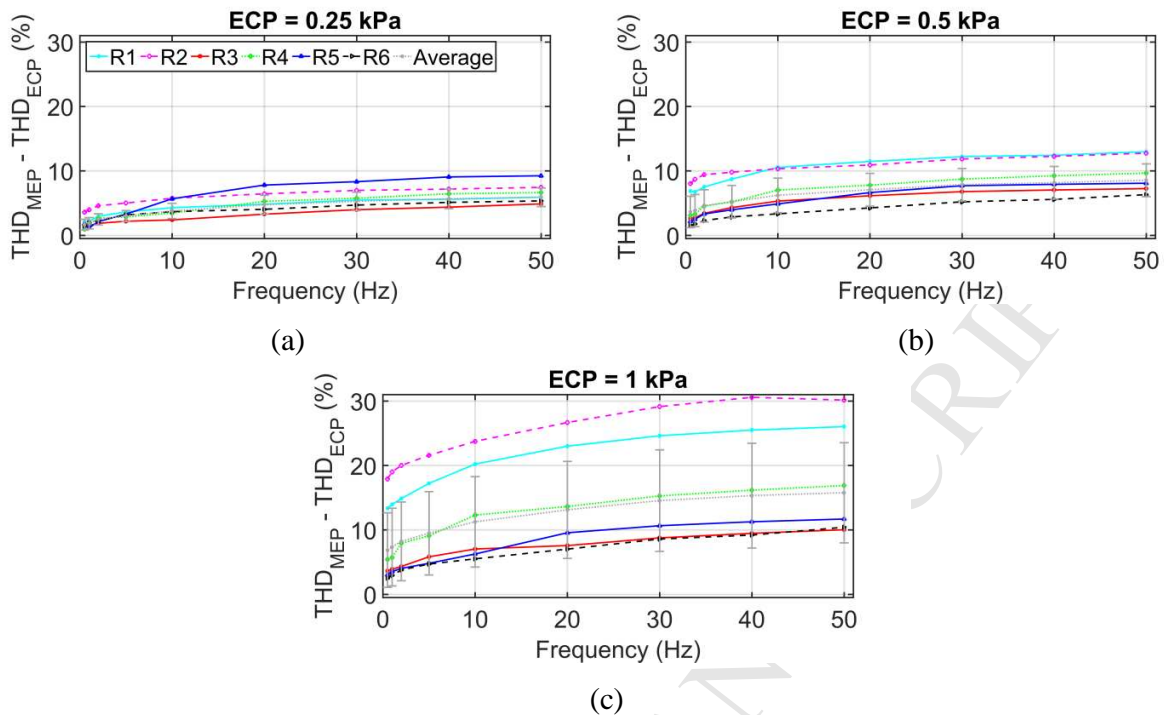


Figure 7: Normalized transtympanic pressure as a function of the input pressure (ECP) at frequencies of: (a) 0.5 Hz, (b) 5 Hz, (c) 10 Hz and (d) 50 Hz.



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237 4. Discussion

238

239 4.1. Choice of animal model

240

241 In hearing research, several animal models have been adopted, each with specific advantages and
 242 disadvantages regarding morphology, ME size and animal availability. For the current work we
 243 preferred rabbit over the more commonly used gerbil because the gerbil ME volume is even
 244 smaller than the rabbit ME volume, and the very thin-walled bulla makes it difficult to connect
 245 tubes going to the pressure transducer. Moreover, the dead volume added by the tubes and the
 246 transducer become relatively large as compared to the volume of the system under study.
 247 Chinchilla has a ME volume comparable to rabbit and has been used in several animal studies (e.g.
 248 Ruggero, 1990; Ruggero et al., 1997; Recio-Spinoso et al., 1998), but in Europe the animal is not
 249 available for experimental use. A small ME volume makes accurate measurement of pressure
 250 changes more challenging due to the inevitable dead volume added by the pressure sensor. In
 251 rabbit the total ME gas volume is about 300 μ l (Dirckx et al., 2008), while the dead volume of the

252 miniature *Endevco* pressure transducer is equal to 0.8 μl . In earlier work on ossicles movement
253 induced by low-frequency pressure variation, the rabbit was also used as animal model (cf. Salih et
254 al., 2016).

255
256 The human ME cavity is coupled to the mastoid, which adds a large volume of gas to the system,
257 while the rabbit ME (just like gerbil and chinchilla) is enclosed in a bulla. The surface area of the
258 rabbit TM is about 34 mm^2 (Salih et al., 2012), and the ME gas volume is of the order of 300 μl
259 (Dirckx et al., 2008), so the ratio is 113 m^{-1} . Recent detailed measurements of human TM surface
260 area reported an average value of $65.6 \pm 5.6 \text{ mm}^2$ (De Greef et al., 2015). Other reports gave values
261 of 68.3 mm^2 (Nummela, 1995; Hemilä et al., 1995), 60 mm^2 (Rosowski, 1994) and 57-64 mm^2
262 (Kirikae, 1960). Recently the average volume of the ME cleft was reported to be 9.7 ml (Padurariu
263 et al., 2016). Other authors report a ME cleft volume of 15 ml (Csakanyi et al., 2011) and mastoid
264 volumes of 10.4 ml (Cros et al., 2016), 9 ml (Park et al., 2000) and 6 ml (Cinamon and Sadé,
265 2003). Using the most recent data (De Greef et al., 2015; Padurariu et al., 2016), the ratio of TM
266 surface area over ME cleft volume is 6.7 m^{-1} , which is a factor of 17 smaller than the ratio in
267 rabbit. For the gerbil, the TM surface area is 19.9 mm^2 (Buytaert et al., 2011) and the ME volume
268 is 0.233 ml (Buytaert et al., 2011), yielding a surface to volume ratio of 85.4 m^{-1} , which is closer to
269 the value found in rabbit. For the chinchilla, the TM surface area is 60.44 mm^2 (Vrettakos et al.,
270 1988) and the ME volume is 1.52 ml (Vrettakos et al., 1988), giving a surface to volume ratio of
271 39.8 m^{-1} . These estimations suggest that quantitative values of e.g. buffering capacity may differ
272 strongly between rabbit and human. Despite these differences, the basic elements of the human
273 ME are also found in other terrestrial mammals, such as the rabbit, containing a specialized TM for
274 the reception of sound, an ossicular chain composed of three bony ossicles coupled and supported
275 by several ligaments, an air-filled ME cavity, an ET to maintain aeration of the cavity, and ME
276 muscles that tense the TM and ossicular ligaments causing alterations in sound transmission
277 (Rosowski, 1994). Therefore one could expect that the qualitative results of the current study,
278 namely better buffering at very low frequencies and at low pressure values, and harmonic
279 distortion of TM response at high pressure levels, also hold for human ears.

280

281 *4.2. Measurement setup*

282

283 The pressure generator coupled to a feedback system allowed us to apply a desired pressure value
284 to the EC with a resolution of 10 Pa. In this way the measurement accuracy ranged between 2%,
285 for 0.25 kPa, and 0.5% for 1 kPa. Moreover, the two sensitive pressure sensors allowed us to
286 measure the pressure at both the EC and ME simultaneously, which was an important factor to
287 quantify the TM pressure response.

288
289 The pressures were measured using calibrated pressure transducers. These transducers have a
290 linearity of better than 1%, and their calibration was checked using a high precision pressure
291 calibrator (Fluke 700PD2, Fluke, Everett, WA, USA). During the experiments no further control
292 measurements were needed as the calibration of the transducers and their amplification electronics
293 remain stable over many months. As we are measuring extremely low pressure values, trivial
294 environmental changes such as opening a door, changing weather conditions, a draft or wind can
295 lead to small offsets in the base pressure value. To exclude this effect, the ears were ventilated
296 between each recording. As shown in Figure 2, the average pressure value then remains stable over
297 the measurement period, and recordings can be made in a stable repeatable way.

298
299 Experiments in this study were performed *ex-vivo* because effect of gas exchange processes and
300 ET opening needed to be avoided. The system under study is governed purely by the passive
301 mechanical behavior of the TM and ossicles. However, in the living animal, one may expect that
302 voluntary or involuntary openings of the ET will occur, partly or completely equilibrating the
303 transtympanic pressure gradient. In the current work, we wanted to focus on the pressure buffering
304 exerted by the TM, thus the ET action needed to be excluded. Nonetheless, no ET leakages were
305 detected when applying static pressures of -2 and +2 kPa to the prepared specimens. From the
306 clinical point of view, the situation with excluded ET action is relevant to pathologies with
307 impaired or blocked ET function. As the pressure buffering by TM deformation is a passive
308 mechanical action, one may expect that it behaves the same for the living and the dead animal.

309
310 Specimens were preconditioned before the start of the measurements. Preconditioning is a
311 phenomenon in which tissue behavior changes due to repetitive loading-unloading experiments. In
312 hearing research, several studies have demonstrated this phenomenon (e.g. Gaihede, 1996;
313 Aernouts and Dirckx, 2012). TM preconditioning was reported to be relevant and necessary to

314 reduce artefacts, leading to more stable results of TM deformation in response to pressure loads,
315 and application of 3 preconditioning cycles has been shown to be adequate (Gaihede, 1996). In line
316 with this, Figure 2 shows that stable results are indeed obtained over subsequent pressure periods.

317

318 *4.3. Transtympanic pressure difference*

319

320 Figure 2 showed ECP and MEP as a function of time to give an example of the directly recorded
321 signals. To gain understanding of the pressure buffering effect it is more instructive to plot
322 resulting pressure (MEP) as a function of applied pressure (ECP), this representation was chosen in
323 Figures 3-5. In these figures, MEP is plotted as a function of ECP at frequencies ranging from 0.5
324 Hz to 50 Hz and for pressure amplitudes ranging from 0.25 to 1 kPa. Figure 3 showed a
325 methodological problem associated with the smallest pressure range: even though we ventilated
326 the ear immediately before the measurement, sometimes a small static pressure was present: for
327 most ears the curves were nicely centered around zero, but for a few measurements pressure
328 offsets up to about 0.05 kPa existed. These very small pressure offsets can be caused by changes in
329 environmental pressure or even by bending of a connection tube while closing the ventilation
330 valve. At all frequencies and pressure ranges, the relationship between ECP and MEP showed
331 some hysteresis. Sample #R2 is a bit different from the other ears, with significantly larger
332 hysteresis and smaller MEP values, especially for positive pressures. This behavior might be due
333 to TM pathology such as otitis media with effusion or myringosclerosis, which have been shown to
334 increase ME viscoelasticity (Gaihede et al., 2005) and hysteresis (Gaihede et al., 1997),
335 respectively. However, the MEs were checked to be air-filled, so the sample may simply represent
336 an outlier among healthy rabbits.

337

338 In general, one can see that MEP values were about half of the ECP values, demonstrating a clear
339 pressure buffering capacity of the TM. For positive ECPs, smaller absolute values of MEP were
340 observed than for negative ECPs, so transtympanic pressures were the largest for positive ECPs.
341 This effect may be a consequence of the conical shape of the TM and the presence of the ossicles,
342 making movements towards the ME more difficult than inflation movements towards the EC: if
343 the TM moves less, pressure compensation due to volume change is less, resulting in larger

344 transtympanic pressure. This agrees with the asymmetry associated with the “pumping direction”
345 in tympanometry as observed by Therkildsen and Gaihede (2005).

346
347 From Figure 6 one sees that the transtympanic pressure difference increases slightly as a function
348 of frequency, but it decreases rather sharply when the frequency decreases below 5 Hz. Under
349 normal physiologic conditions, very large pressure fluctuations mainly occur at these very low
350 frequencies, induced by processes such as gas exchange in the ME, changes in meteorological
351 conditions or altitude changes. At these very low frequencies, transtympanic pressure loads are the
352 smallest so pressure buffering due to TM displacement works at its best.

353
354 Figure 7 showed the normalized transtympanic pressure difference as a function of pressure
355 applied to the EC. Transtympanic pressure of course increases with ECP, so we plotted the
356 normalized value to better see the relative regulation effect of the TM. The normalized
357 transtympanic pressure remained fairly constant at values between 60% and 70% as a function of
358 ECP, except for the very small ECP value of 0.25 kPa, where the value increased a bit. This means
359 that at lower pressures less ECP was transferred to the TM and ME than at higher pressures.

360
361 *4.4. Nonlinearity of tympanic membrane response*

362
363 In Figure 8 the THD as a function of frequency was presented for the different applied pressure
364 amplitudes. At all pressure values one sees that THD increases as a function of frequency.
365 Especially at frequencies below 5 Hz, THD decreases strongly. The increase of THD with
366 frequency can possibly be attributed to the combined effect of inertia and viscoelasticity of the
367 TM. At 0.25 kPa, THD remains smaller than 10% for all ears, and all ears behave fairly the same.
368 However, at 1 kPa very strong differences exist between ears, with two ears remaining in the 5-
369 10% region over the measured frequency range, while three other ears showed THD values up to
370 18%, 25% and even 30%.

371
372 The asymmetry and nonlinearity in the response of TM displacement as a function of pressure can
373 have several sources. The asymmetrical shape of the TM may play an important role in the
374 asymmetry of the response: at ME positive pressures the TM only needs to bend when it is pushed

375 in the lateral direction, while at ME under-pressures it needs to stretch. One can expect that the
376 presence of the tangentially oriented collagen fibers strongly prevent this stretching, making
377 inward motion more difficult than outward motion. To fully understand this process, detailed
378 modelling of the membrane will be needed. The observed nonlinearity may also be due to the
379 viscoelastic properties of the TM as well as to the viscoelastic properties of the ligaments of the
380 ossicular chain. Again, modelling including soft tissue properties will be needed to fully
381 understand the mechanisms underlying the current measurement results.

382

383 *4.5. Clinical relevance*

384

385 As explained above, care needs to be taken when interpreting the current data in clinical context as
386 significant differences exist between the human and rabbit ear. Nevertheless, some of the main
387 mechanical findings can be expected to be general for mammal ears, despite the fact that absolute
388 values such as buffer capacity will be very different between species.

389

390 The current results show that pressure buffering strongly increases for the lowest frequencies. As
391 discussed in the introduction, long standing ME under-pressure is associated with remodeling of
392 the TM, retraction pockets and its sequelae. The higher pressure buffering capacity of the TM at
393 (ultra) low frequency pressure variations can be a protective mechanism to prevent development of
394 retraction pockets under normal conditions. In normal conditions the average MEP will be close to
395 ambient pressure but low frequency pressure variations will constantly occur as they are common
396 to daily life. In common meteorological conditions, it has been shown that in the course of a few
397 hours ambient fluctuations occur with amplitudes in the order of tens of Pascals (Didyk et al.,
398 2008). A simple trip in an elevator causes pressure fluctuations of 0.4 kPa over a time span of
399 several seconds (Padurariu et al., 2016). During our experiments we encountered pressure
400 fluctuations of 0.1 kPa when closing a door in a ventilated room. In an ear with pathologic pressure
401 regulation (e.g. blocked ET or dysfunctional gas exchange), the TM is constantly in its deformed
402 state and normal pressure buffering ceases to function.

403

404 **5. Conclusion**

405

406 We developed a setup which made it possible to measure MEP as a function of small and slow
407 sinusoidal pressure variations applied to the EC. The MEP was measured as a function of
408 sinusoidal varying ECP with pressure amplitudes ranging between 0.25 kPa and 1 kPa, and for
409 frequencies varying from 0.5 Hz to 50 Hz. It has been found that the transtympanic pressure load is
410 the lowest in the quasi-static range, and quickly increases when reaching the range of audible
411 frequencies. The THD in the resulting MEP was very small for low frequencies and pressure
412 amplitudes, which means that the overall TM motion followed the applied pressure well. The THD
413 increased for both higher pressure values as well as for higher frequencies.

414

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416

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419 measurement setup and preparing the specimens.

420

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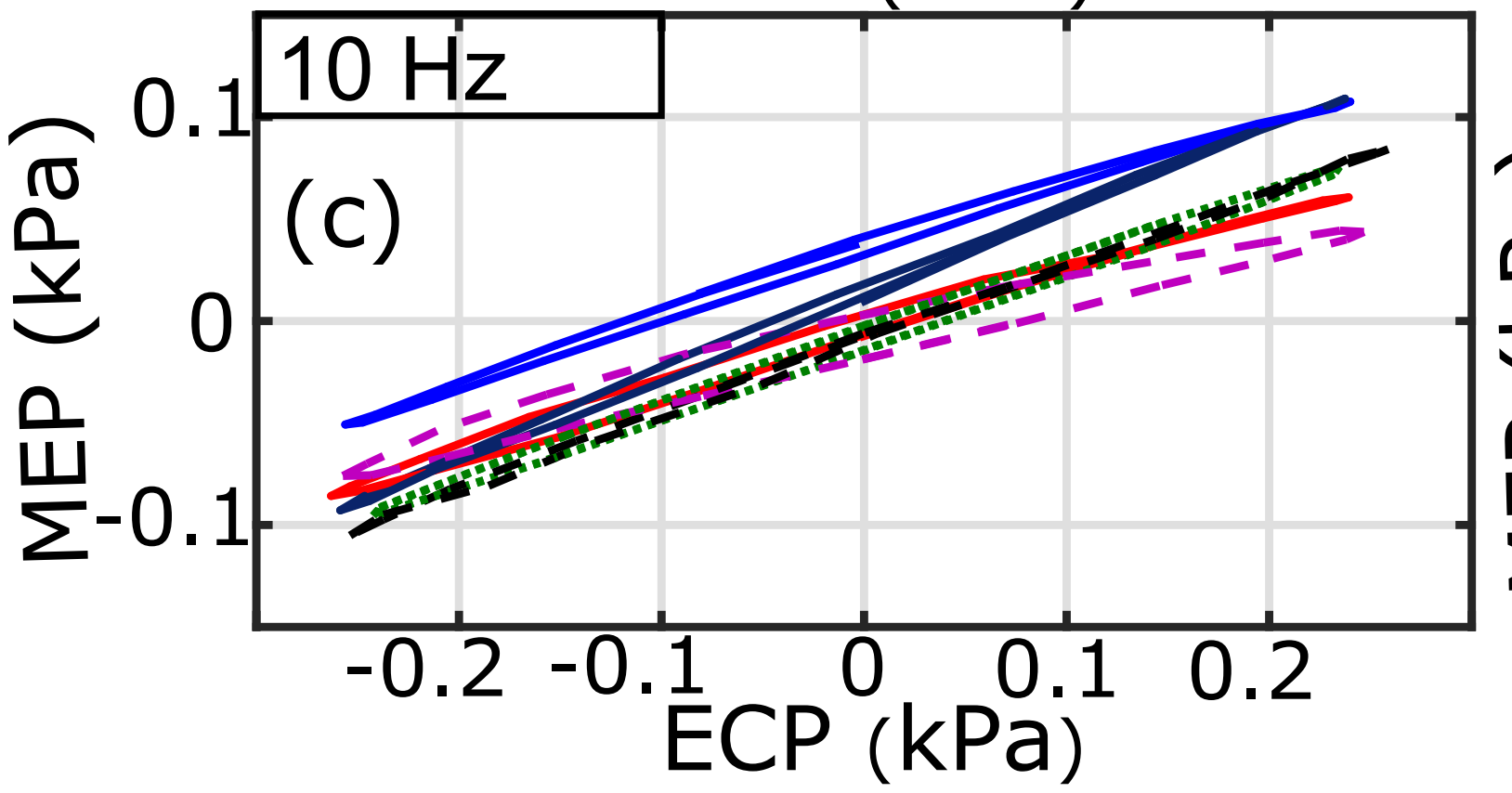
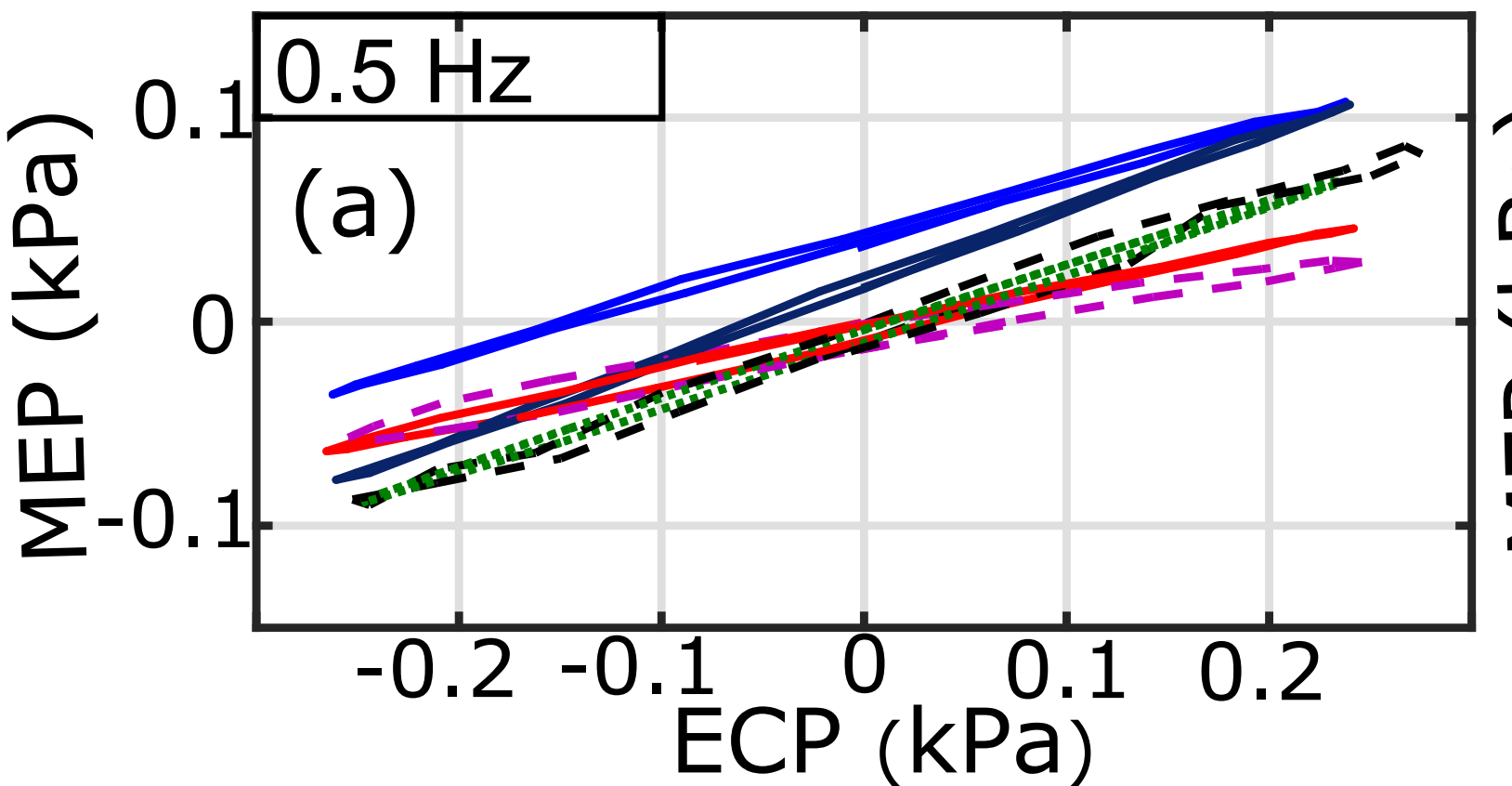
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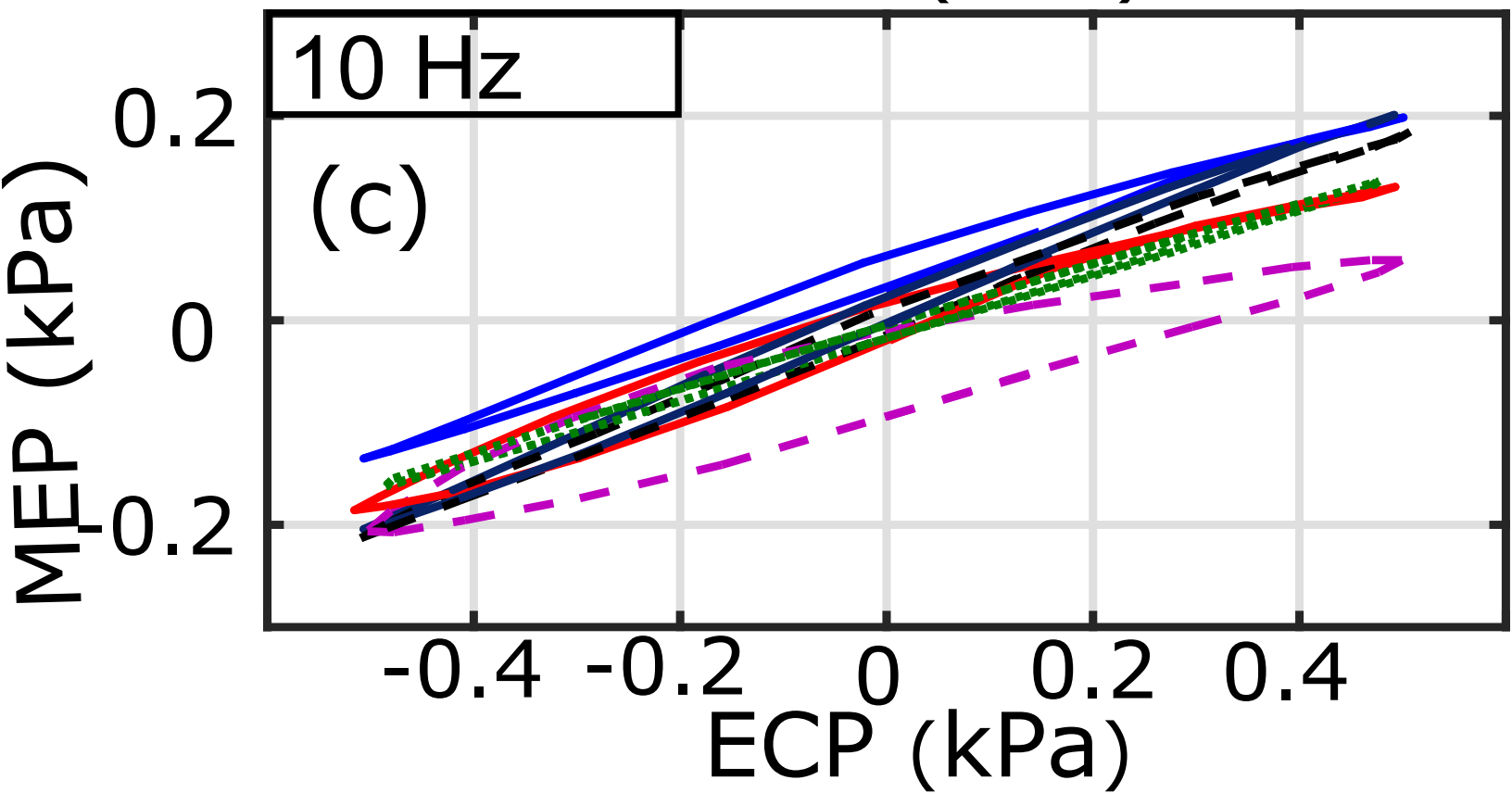
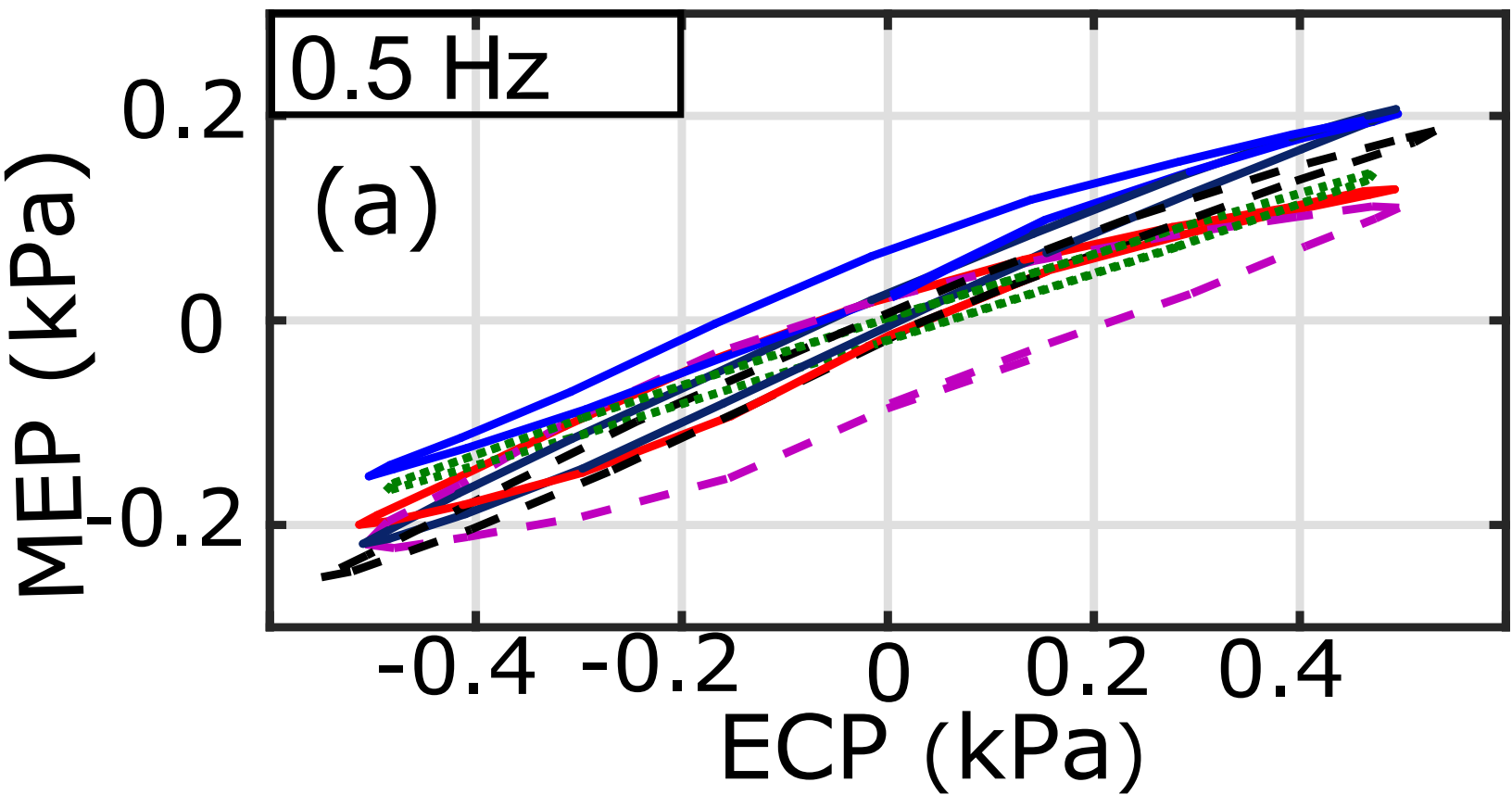
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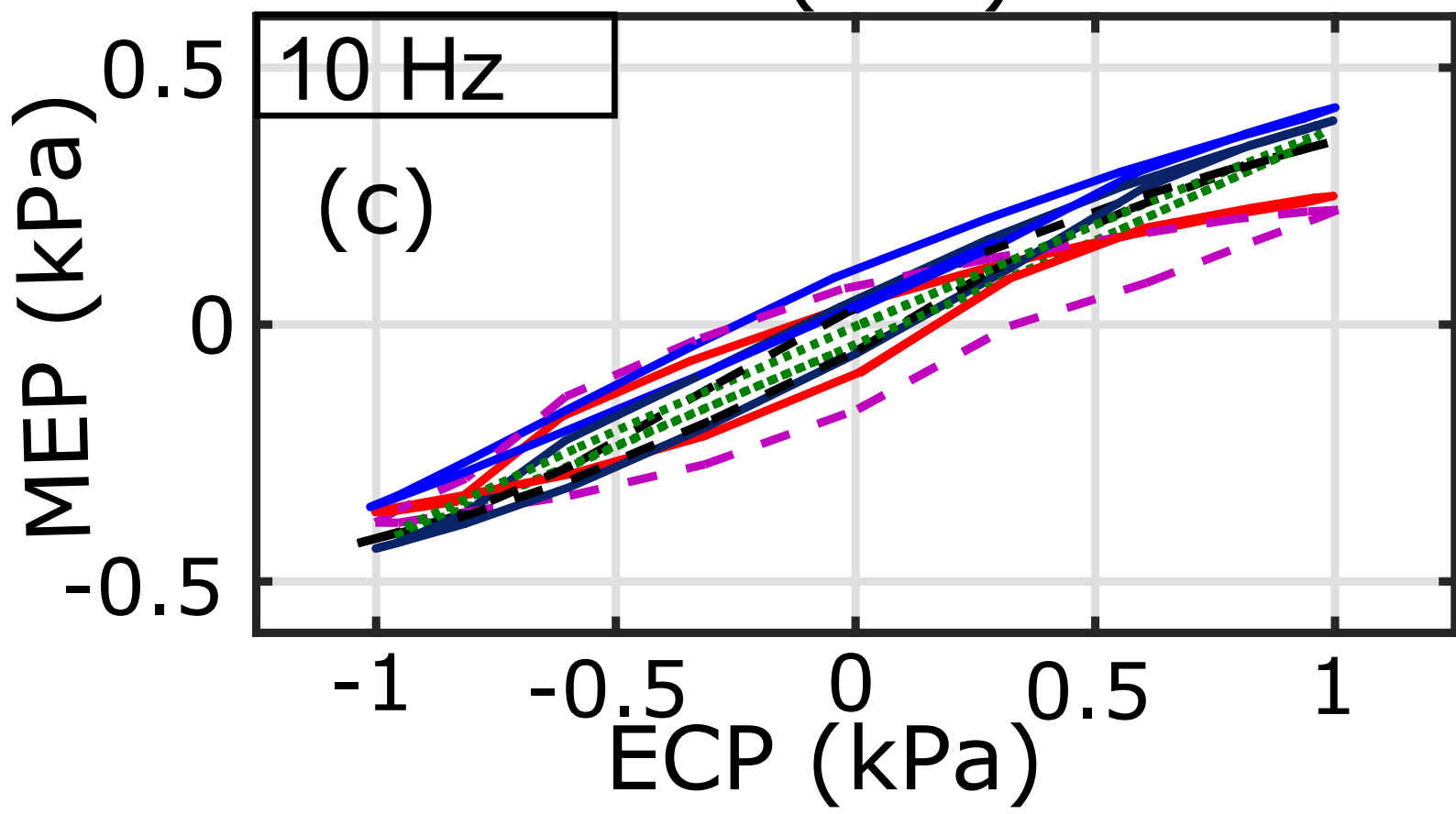
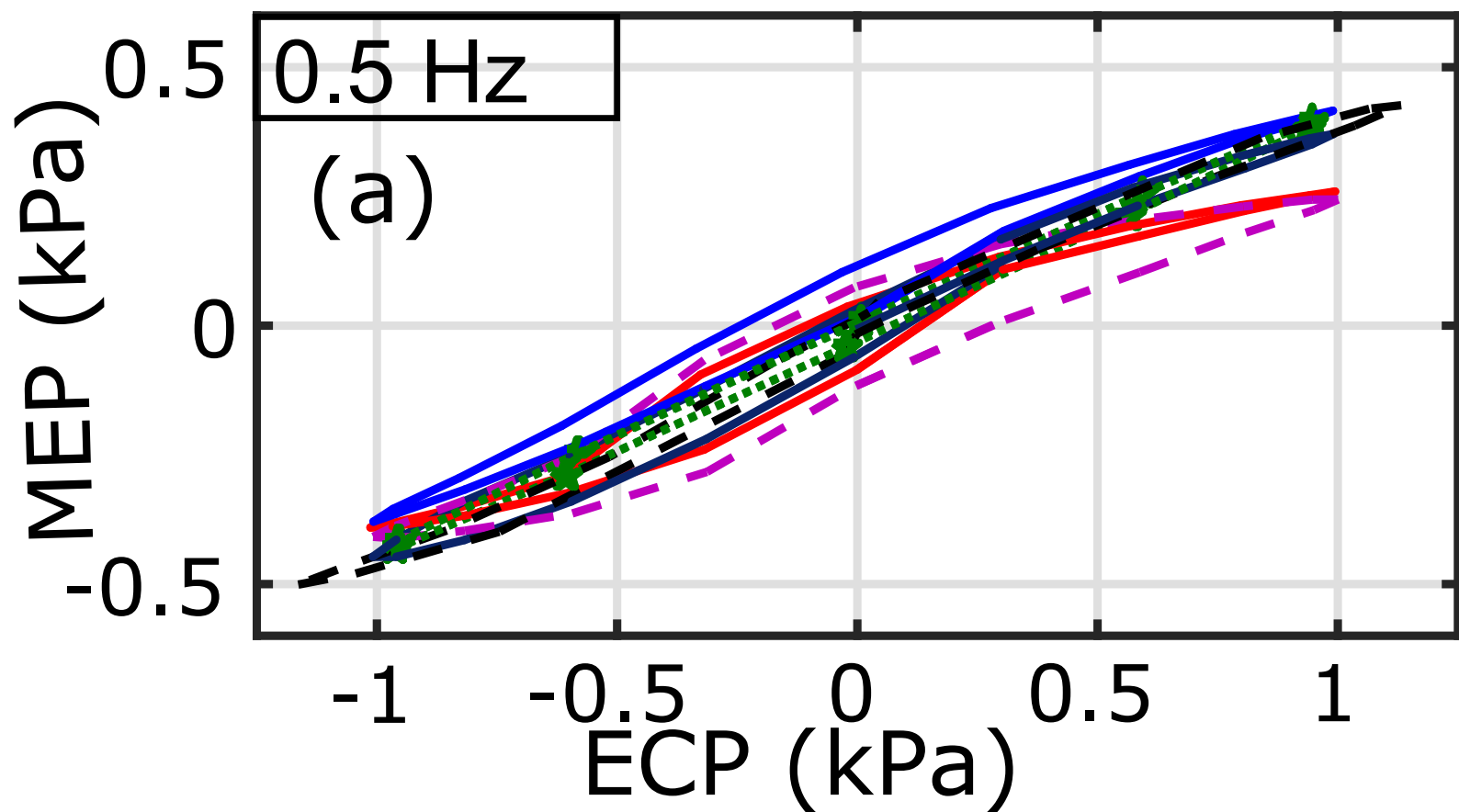
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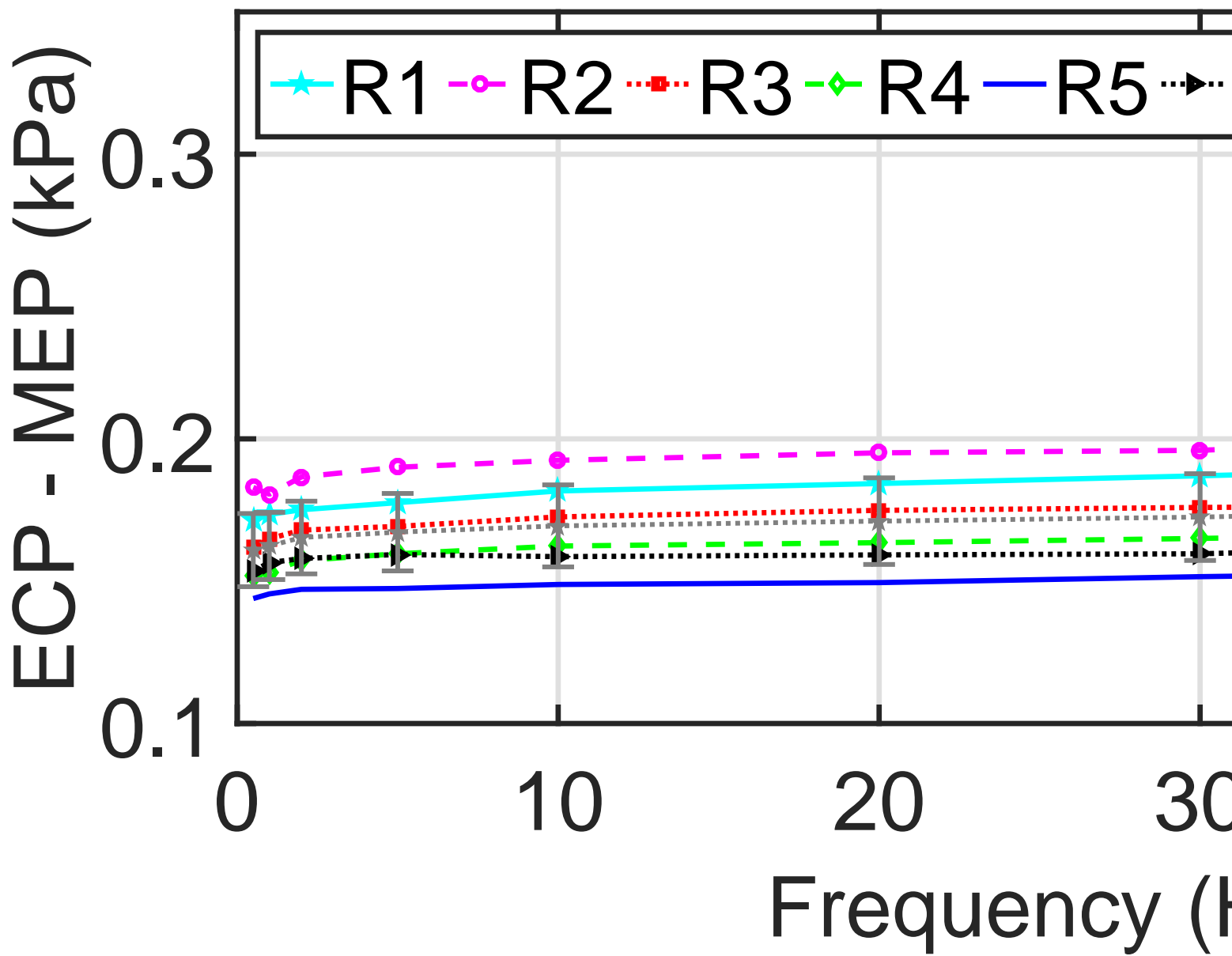
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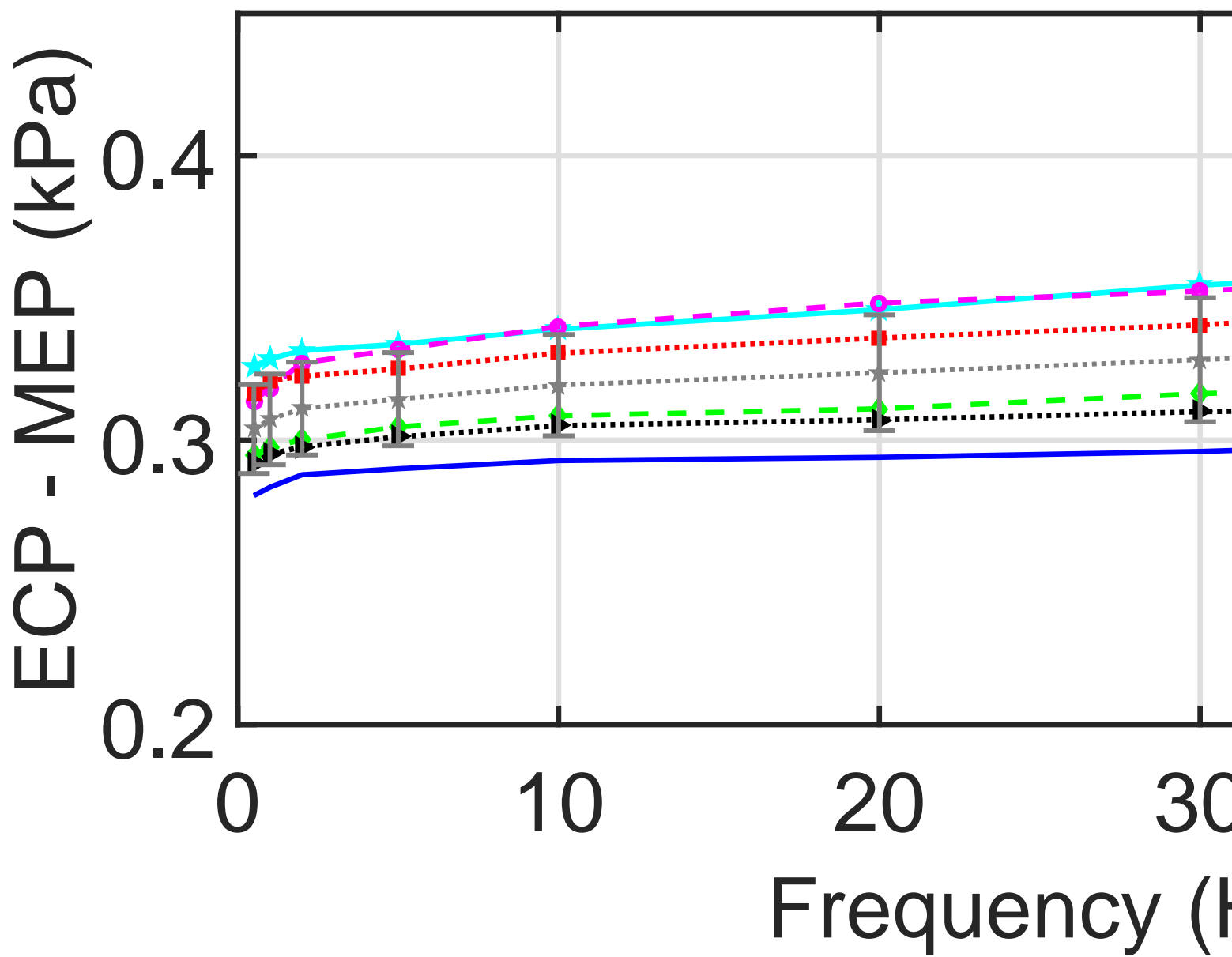


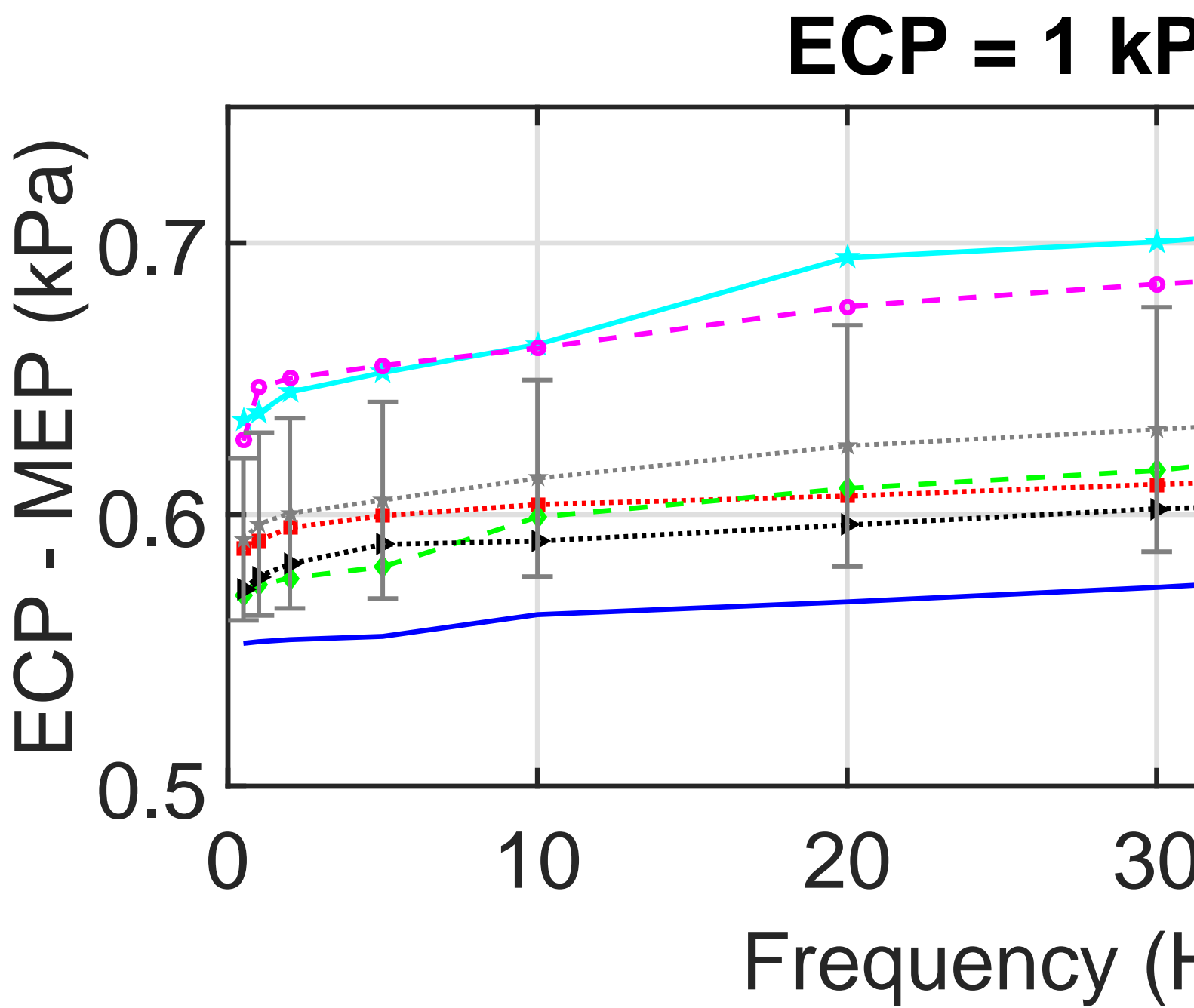


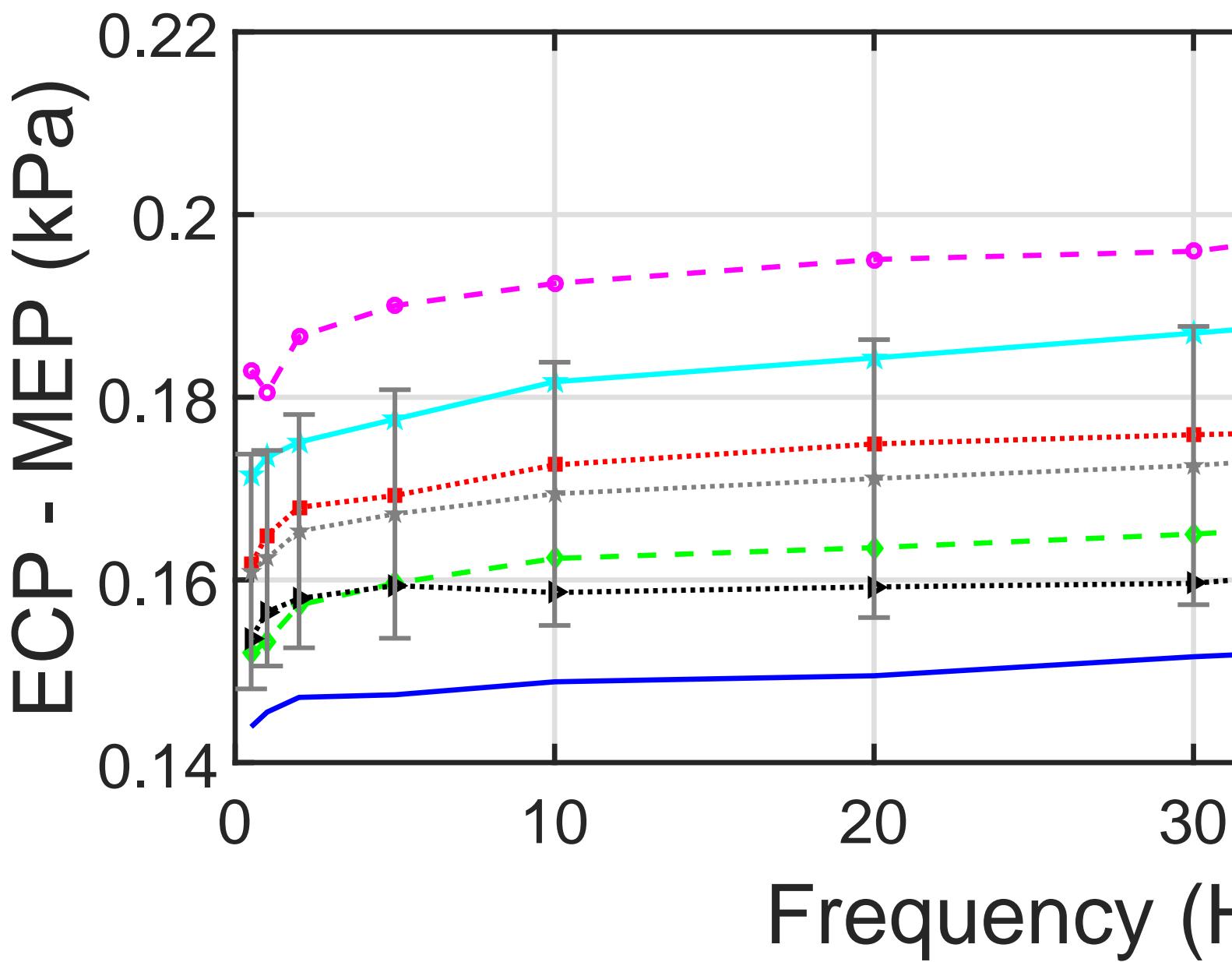


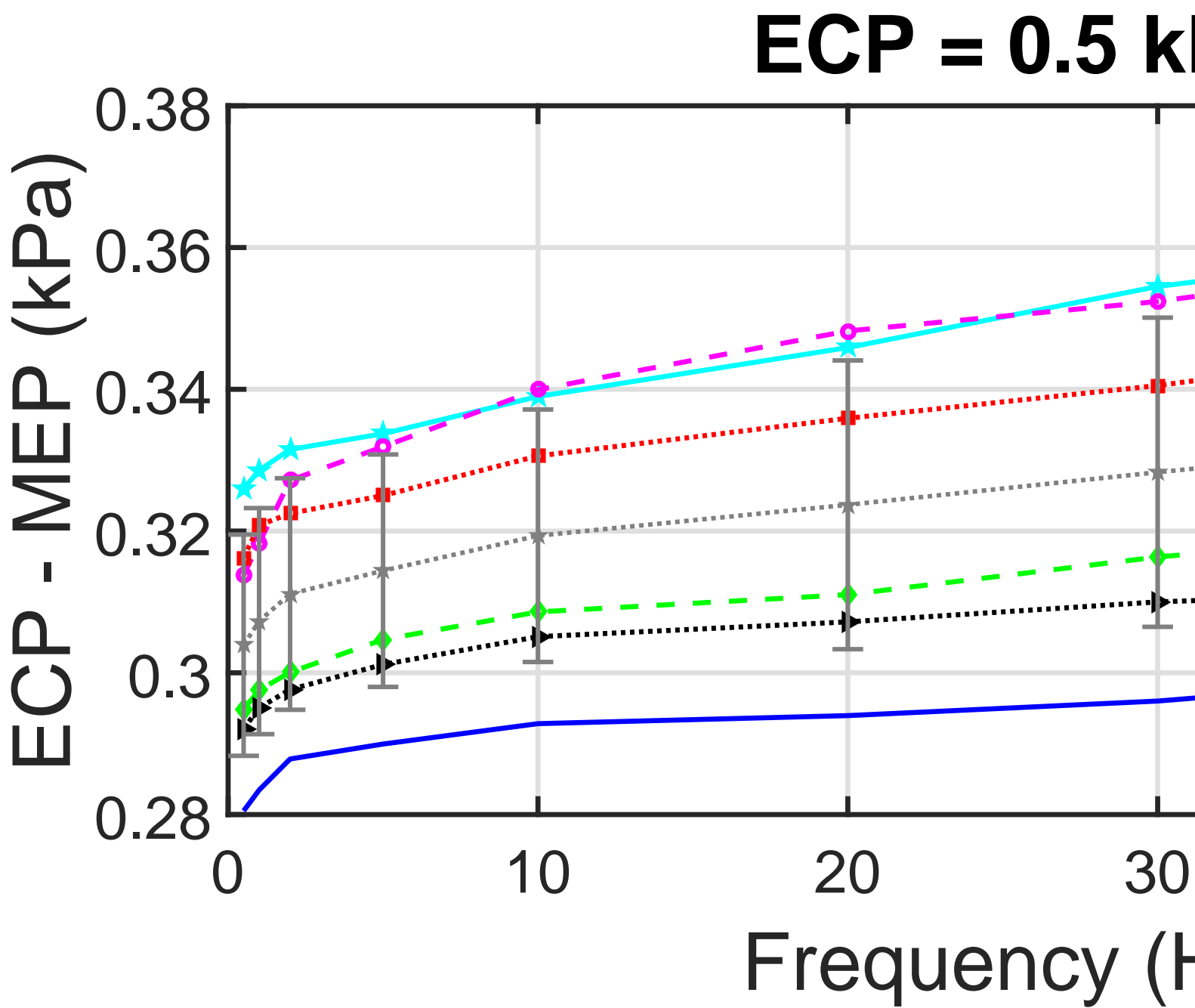
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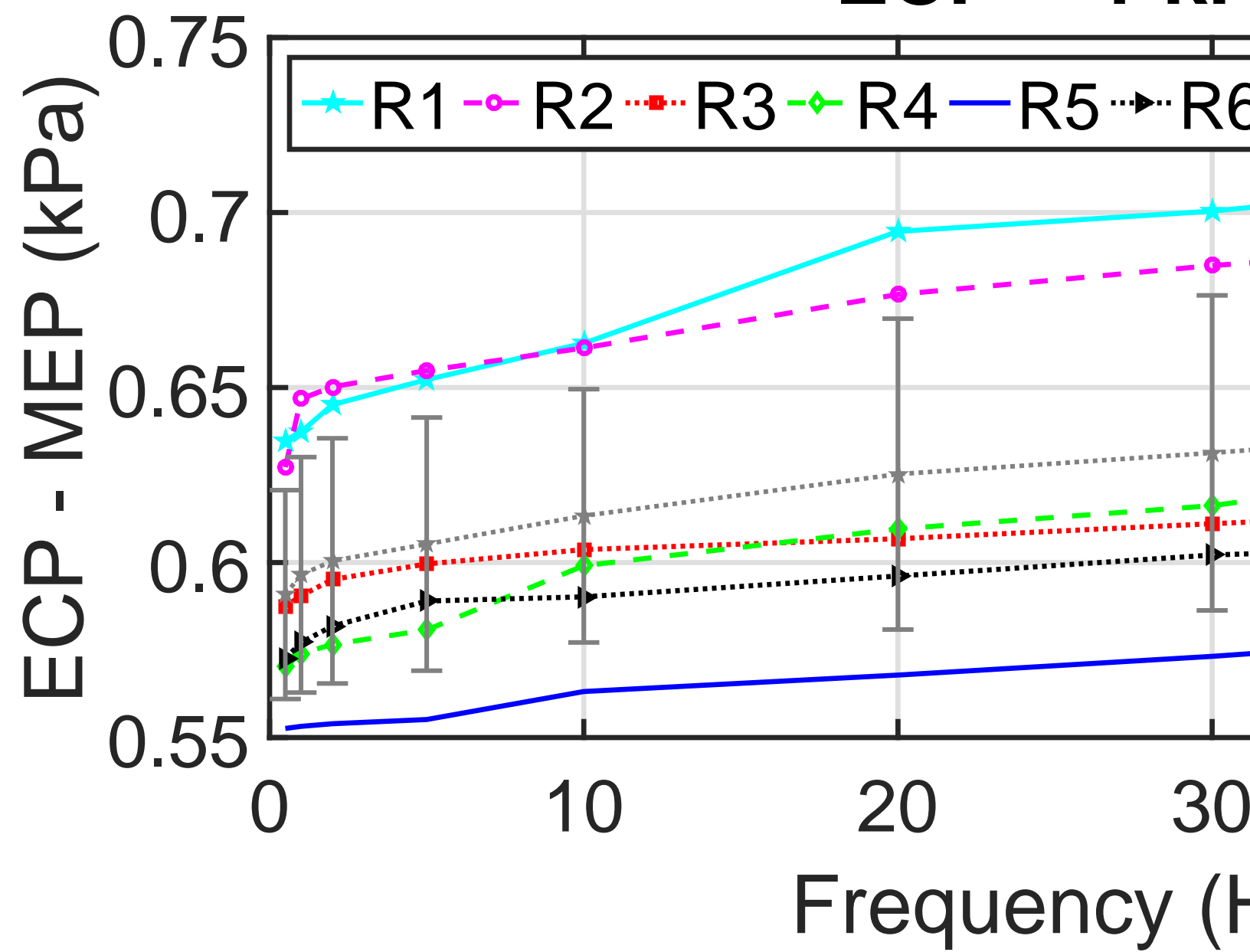
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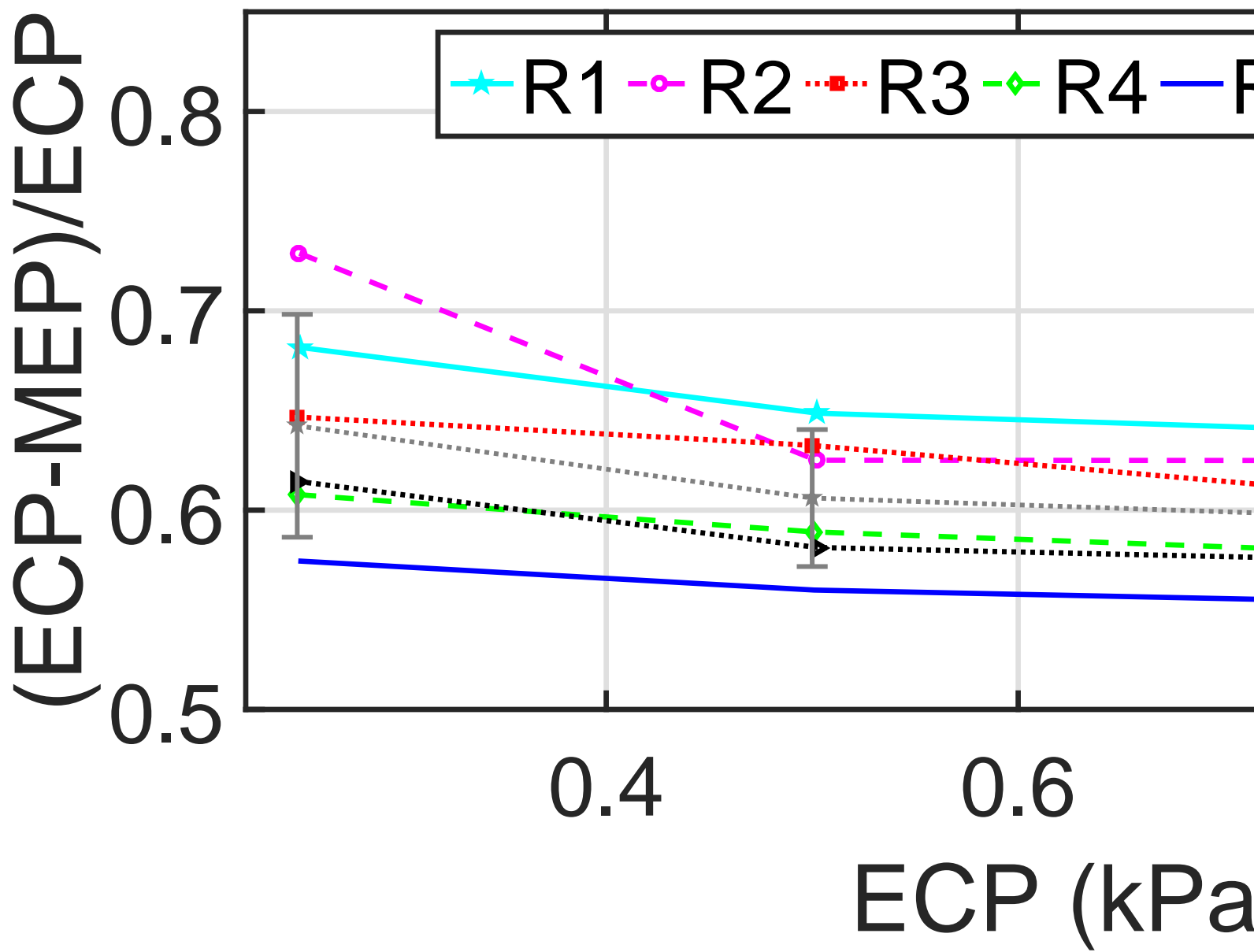




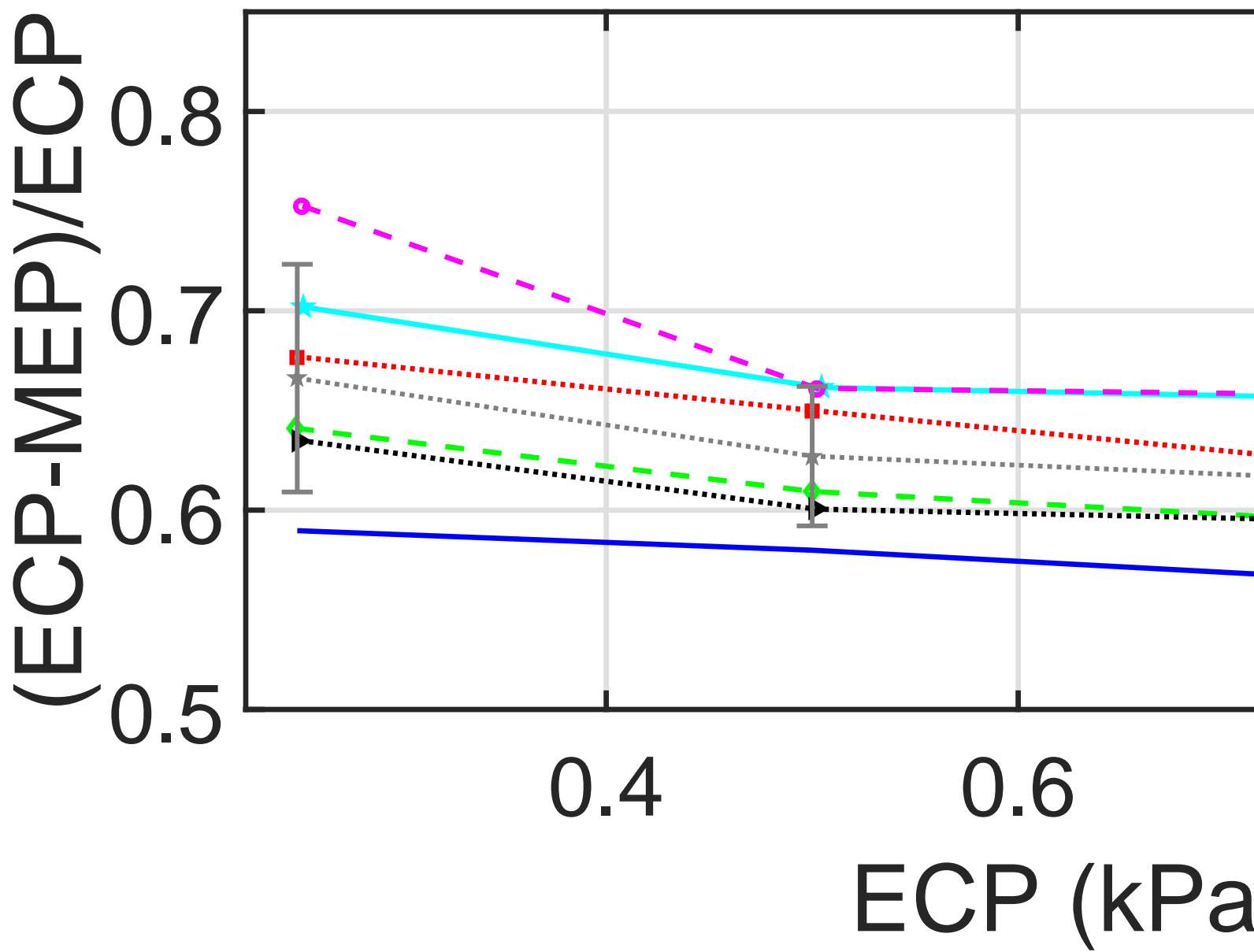


ECP = 1 kPa

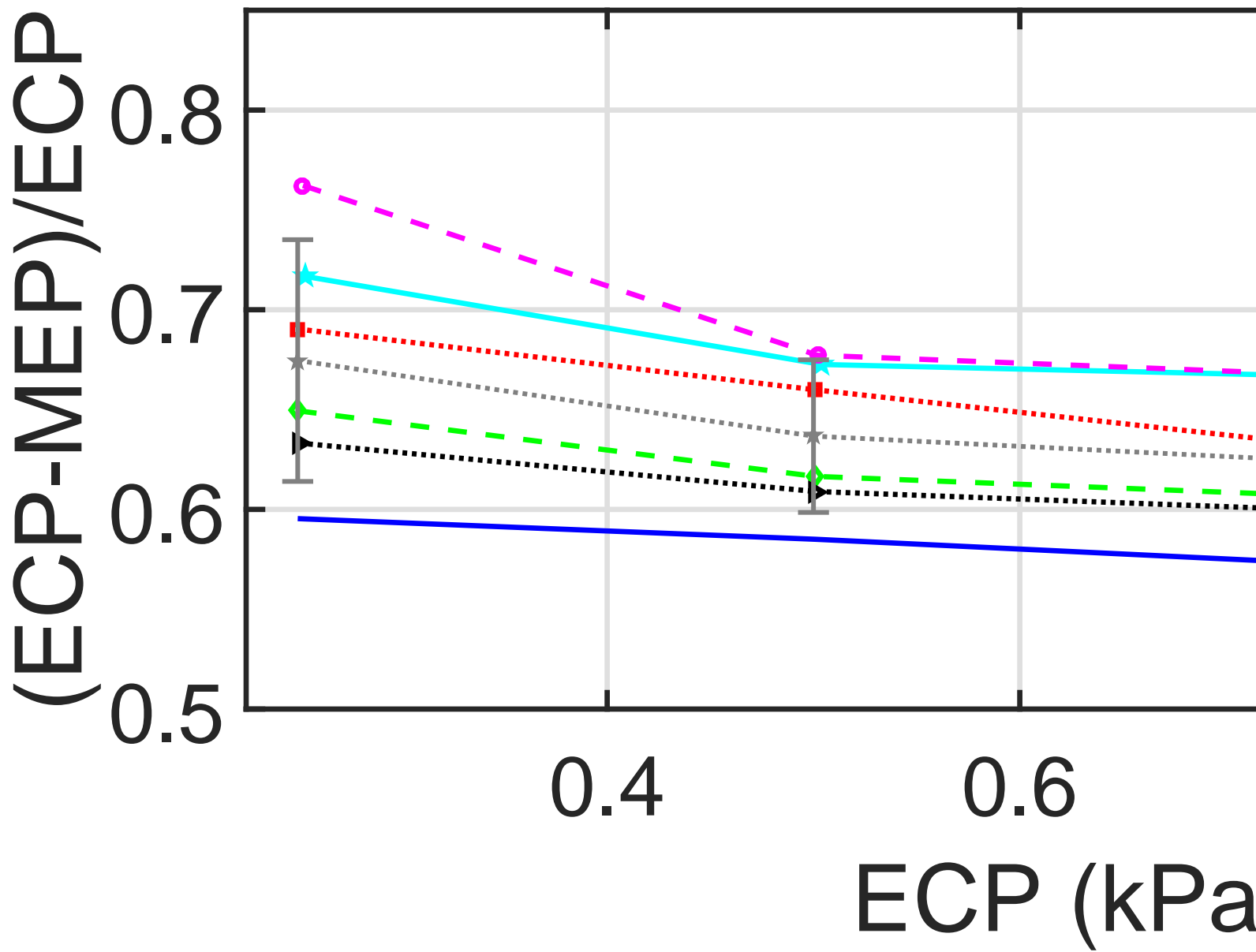
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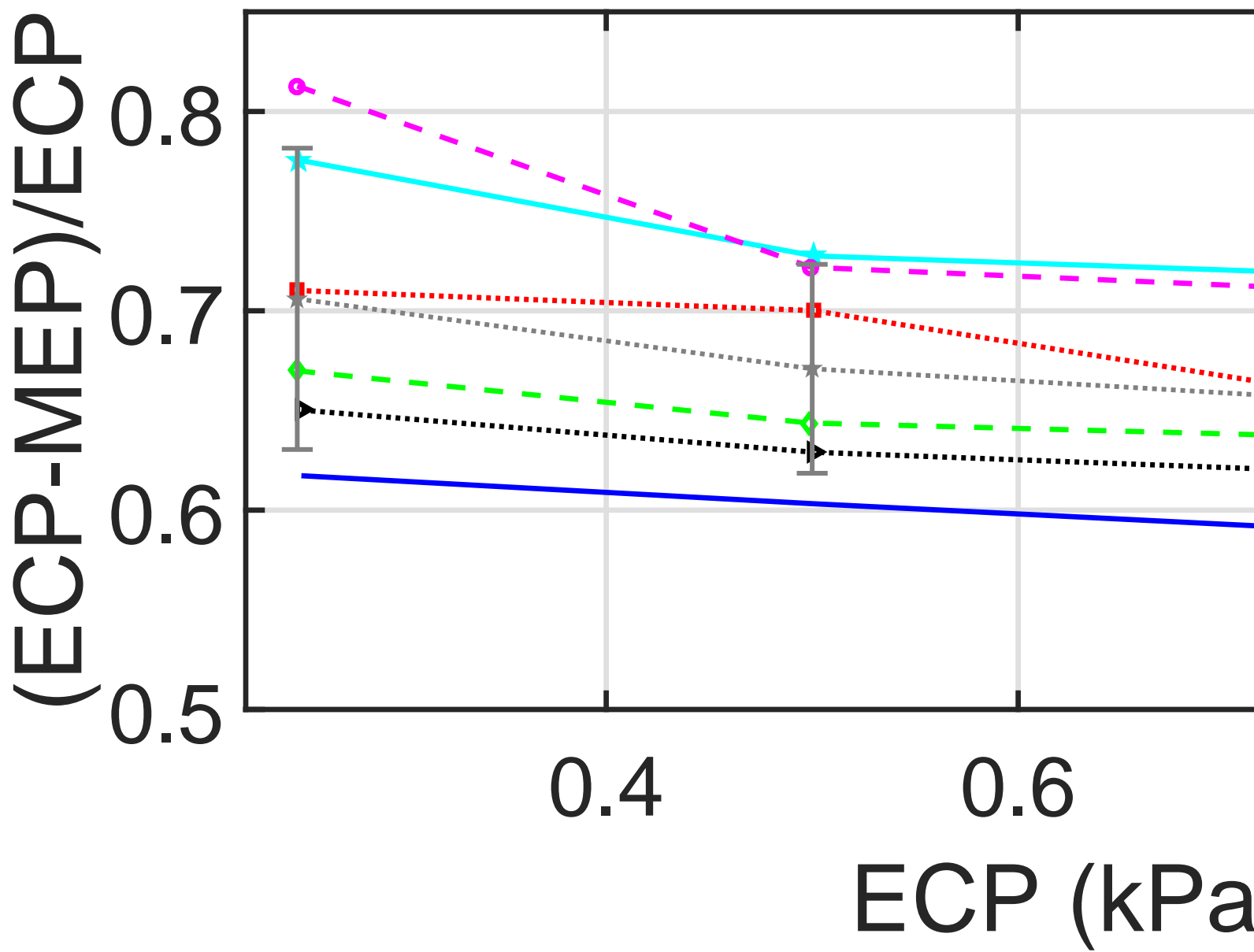
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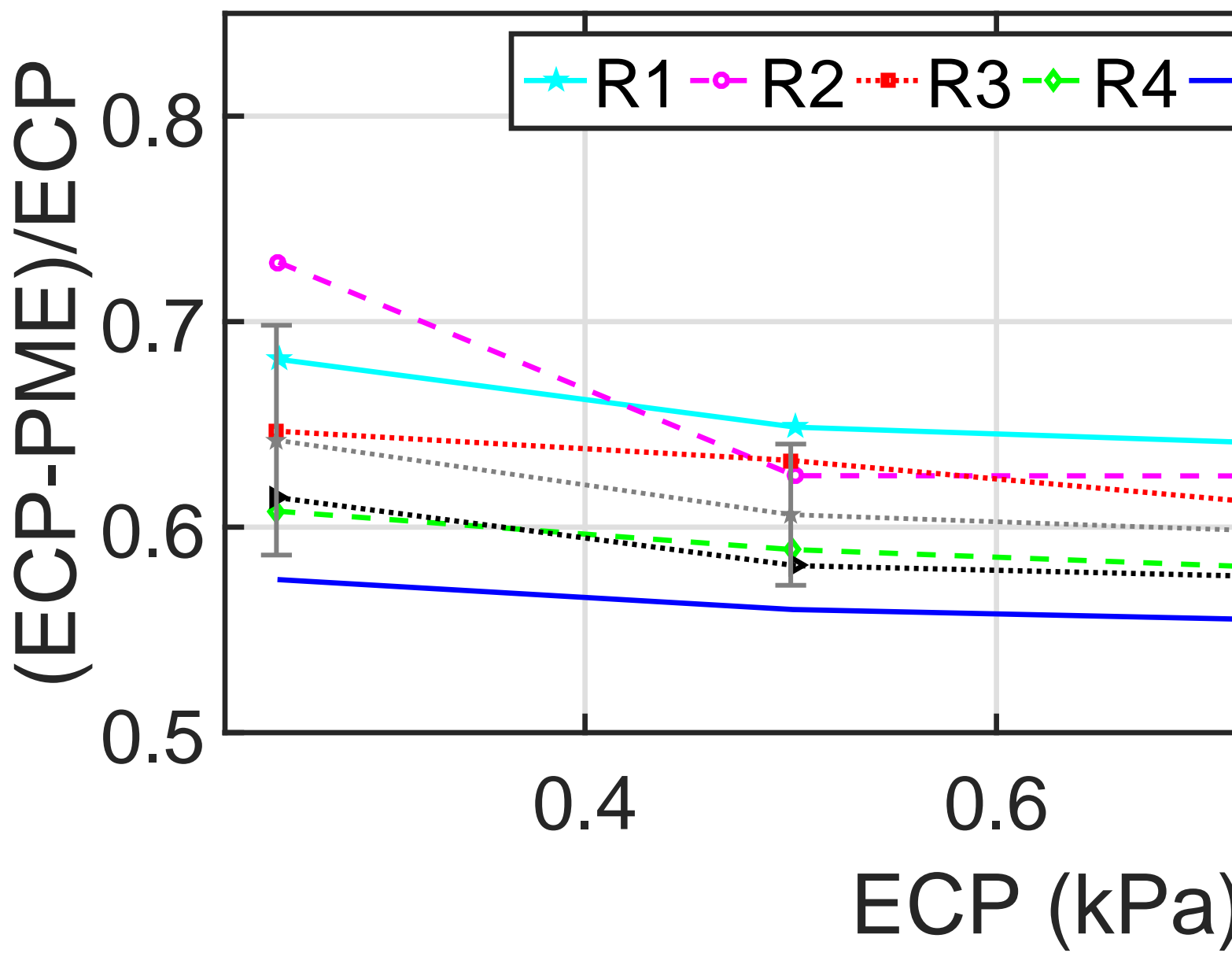
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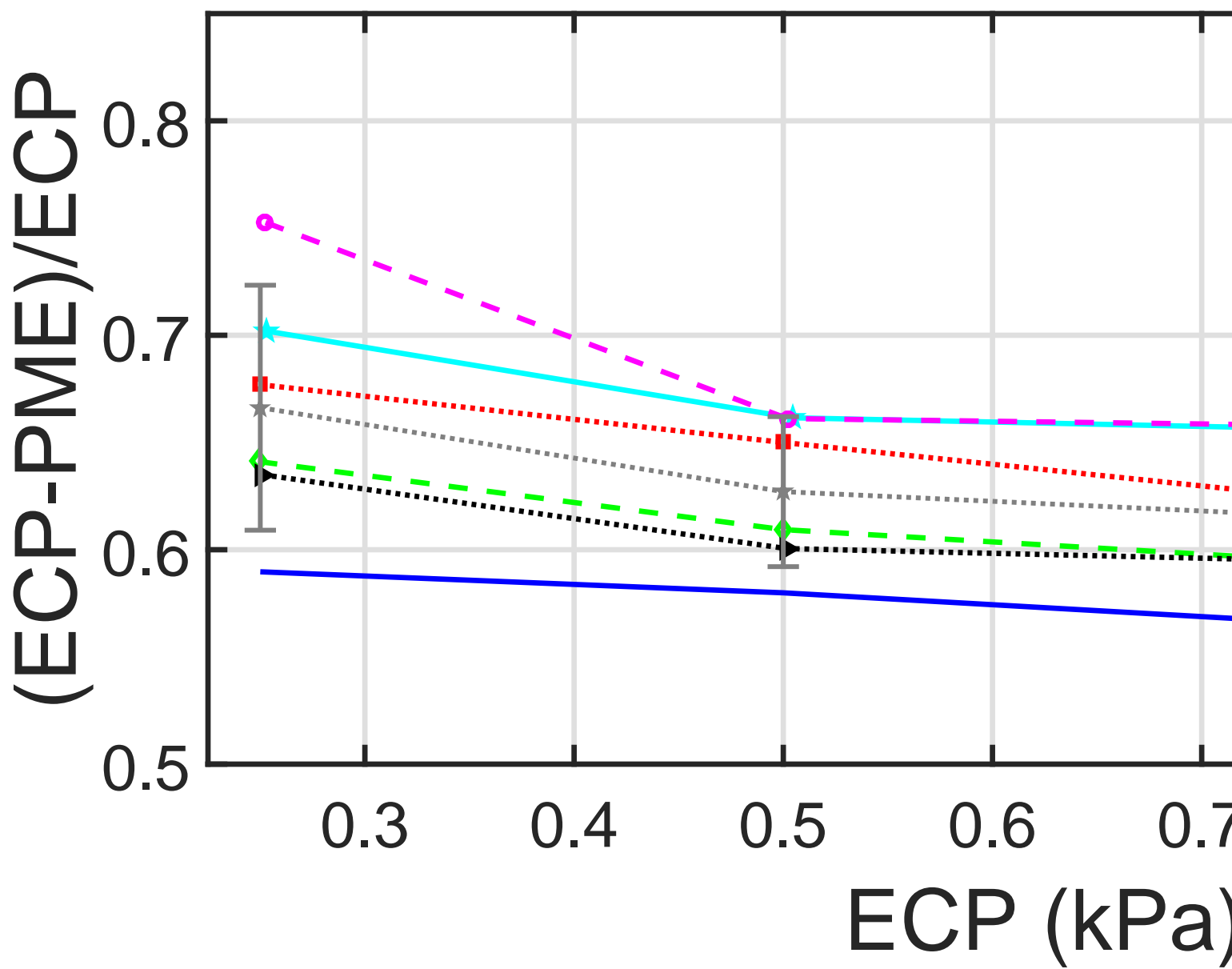
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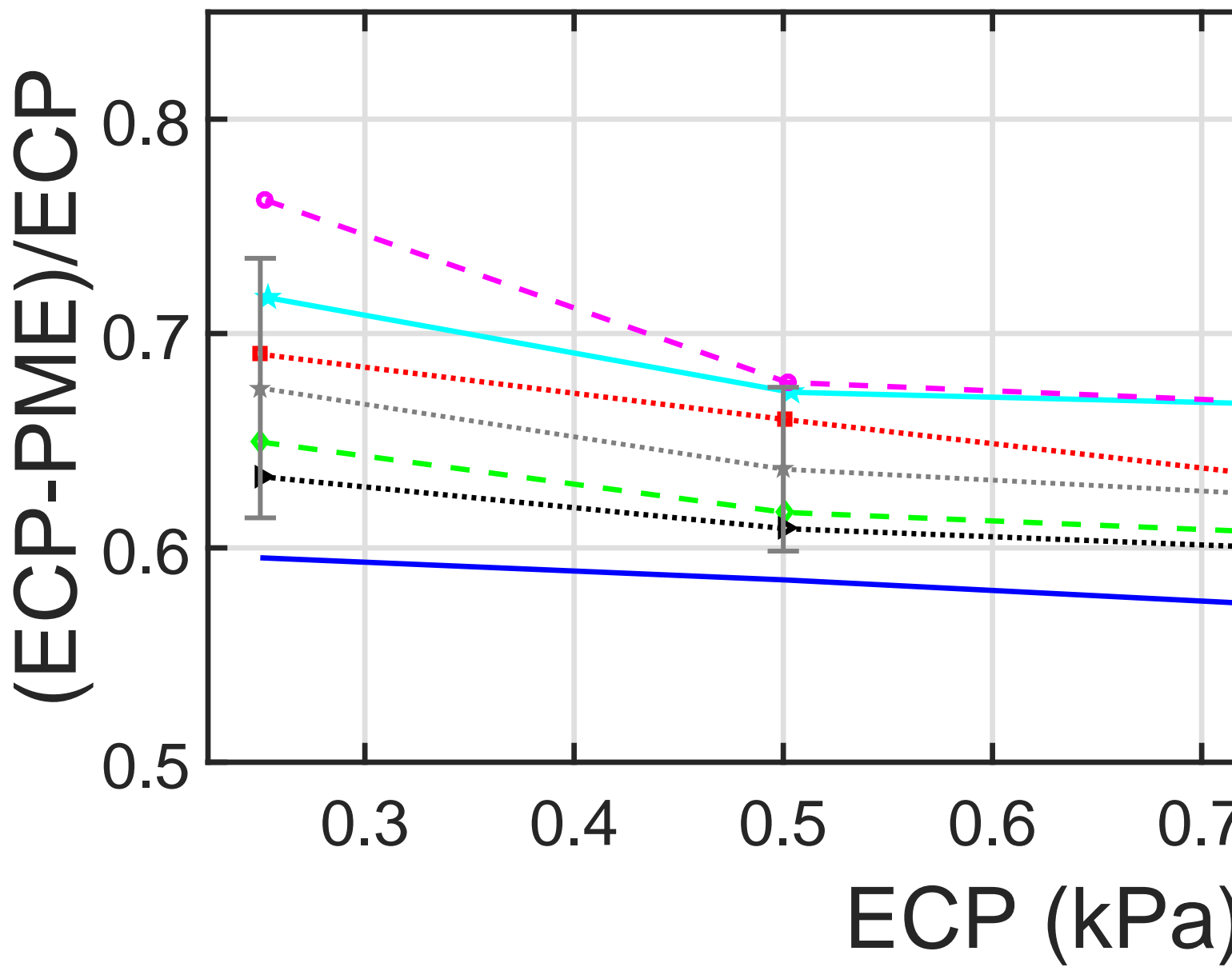
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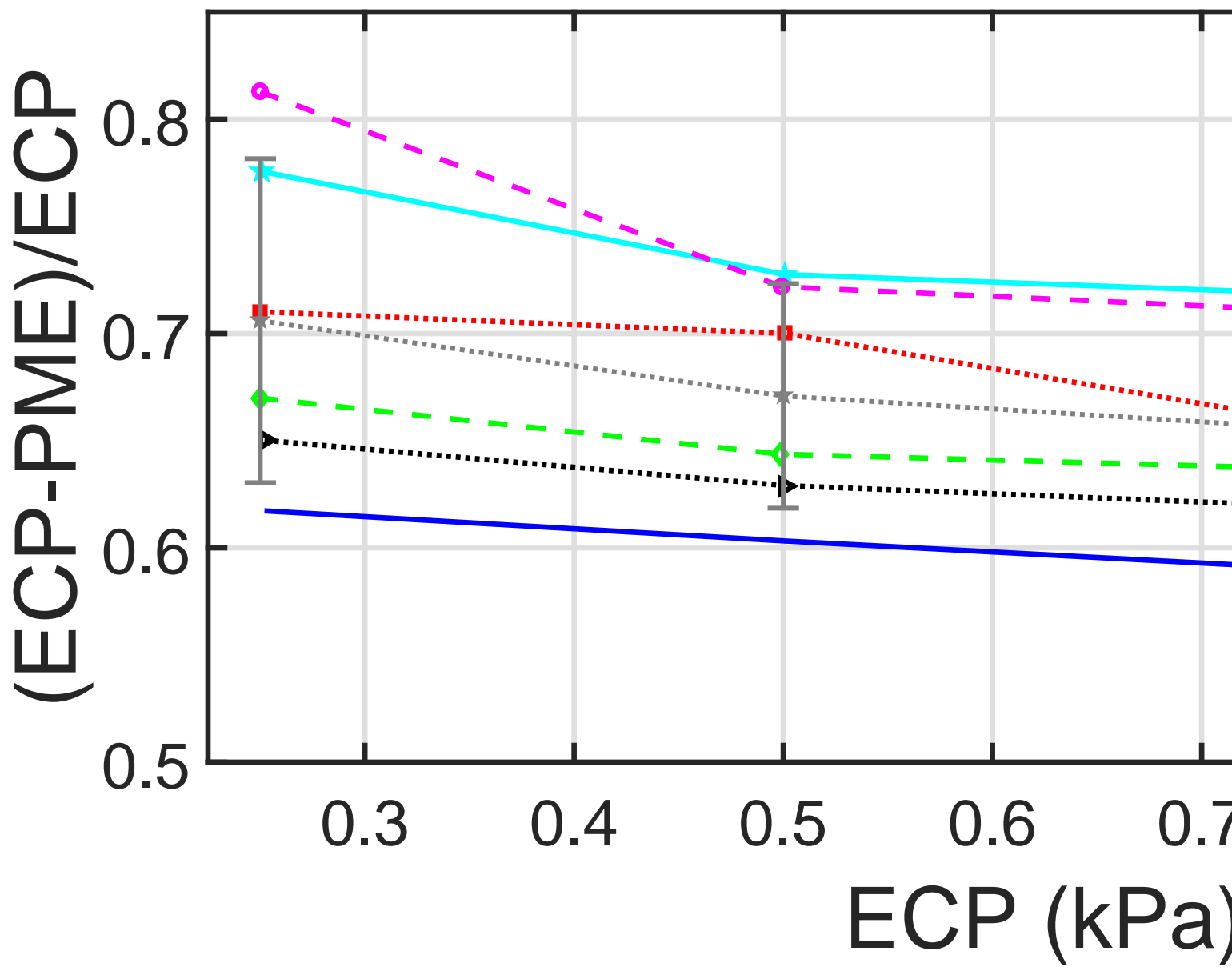
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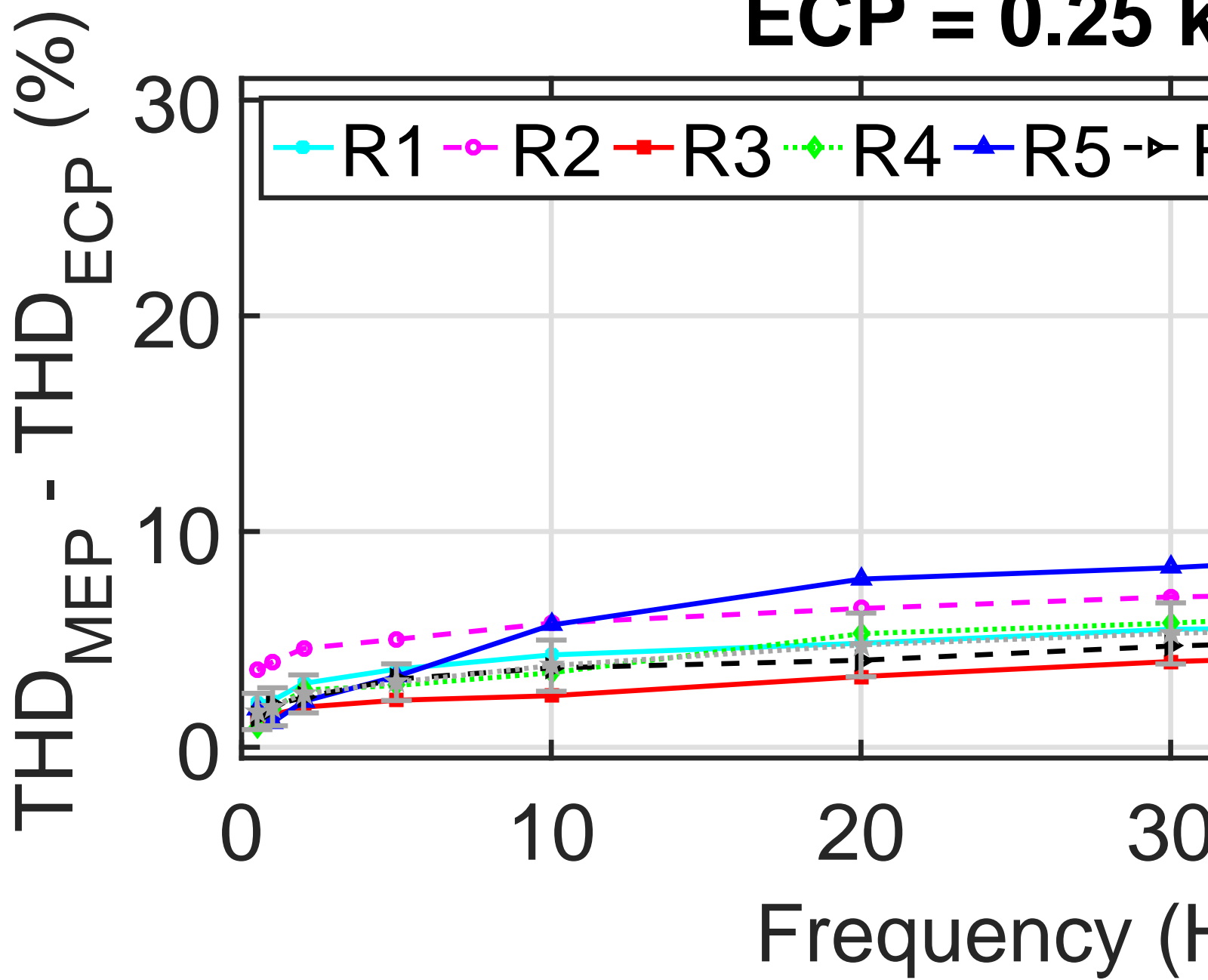


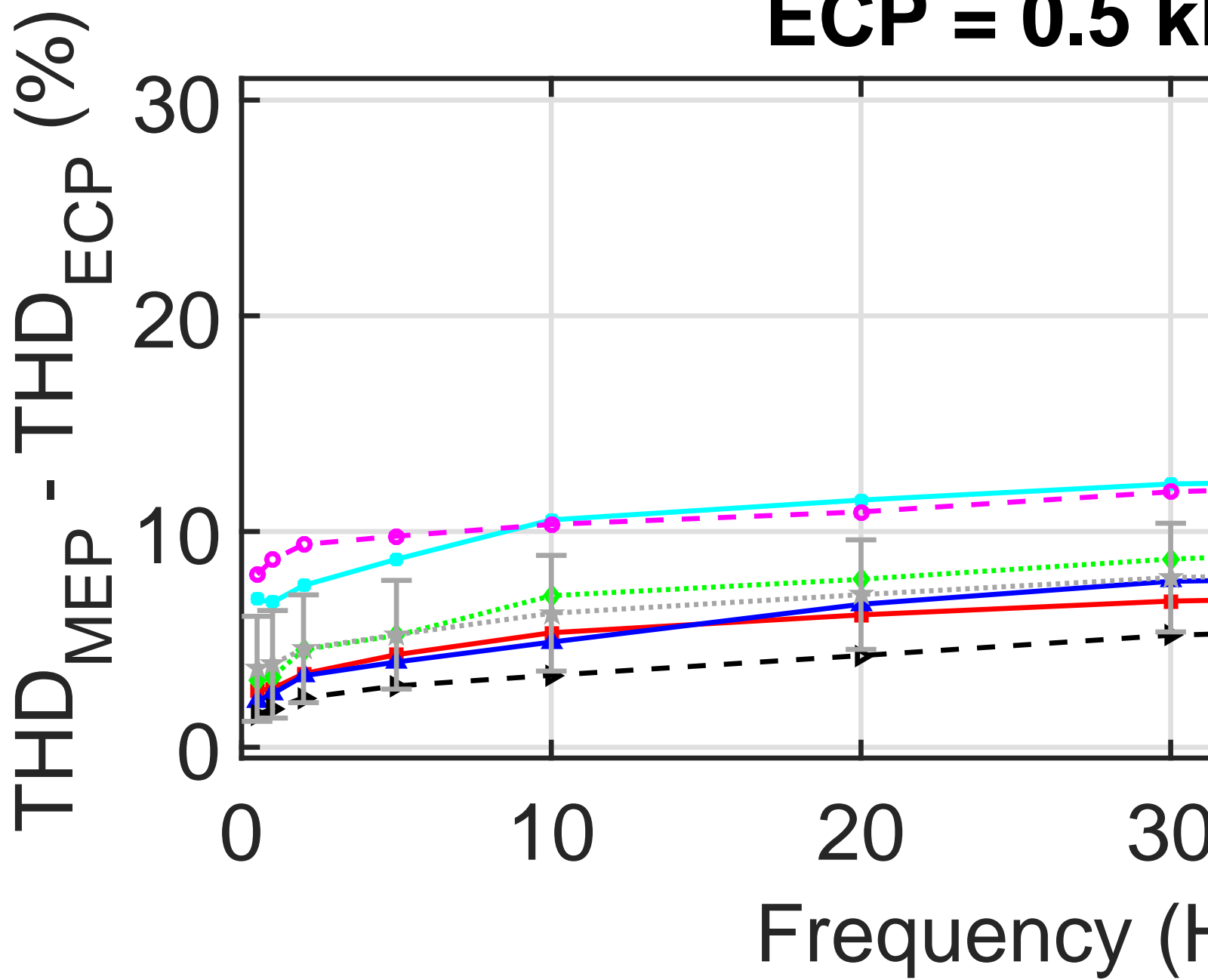
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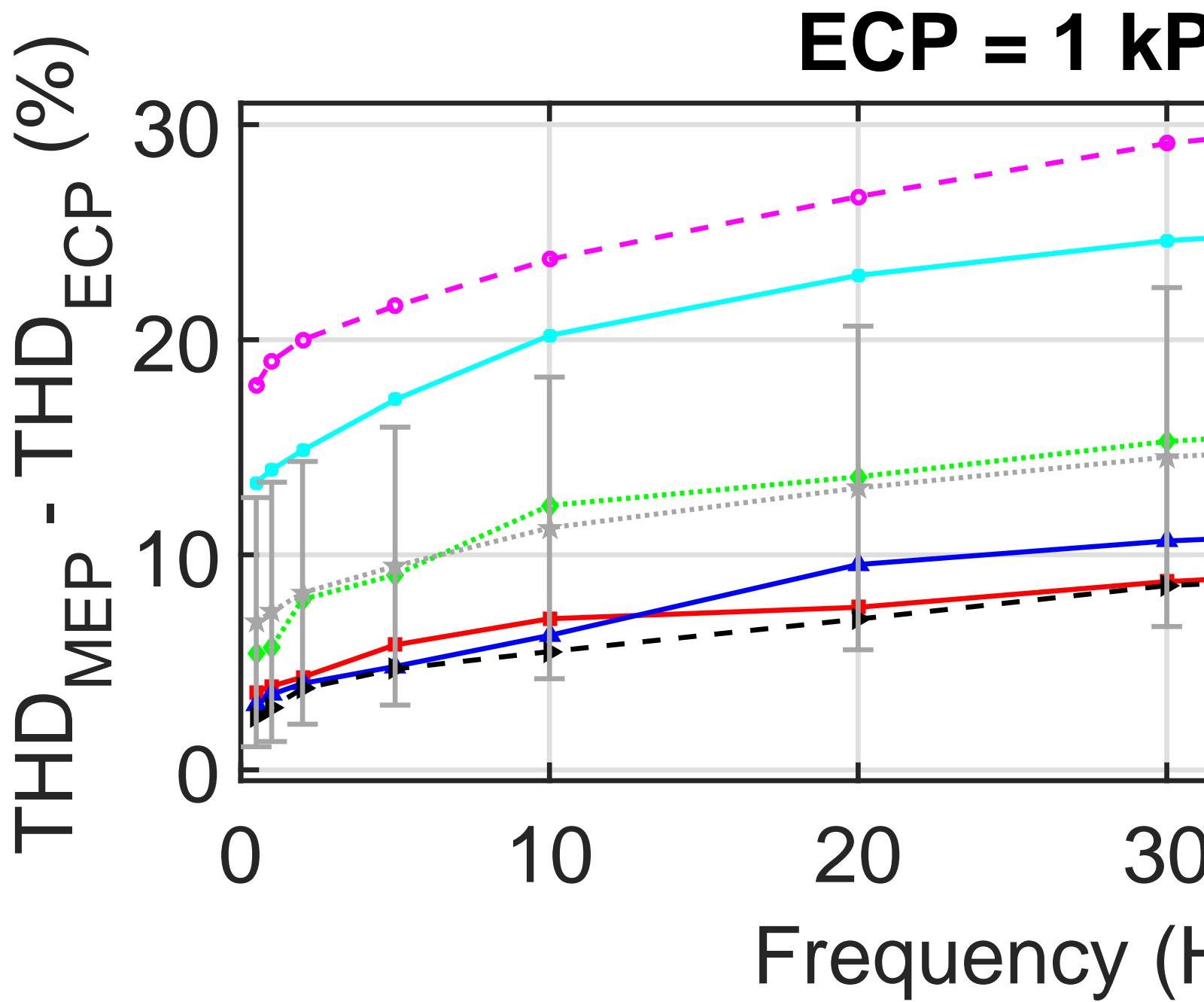


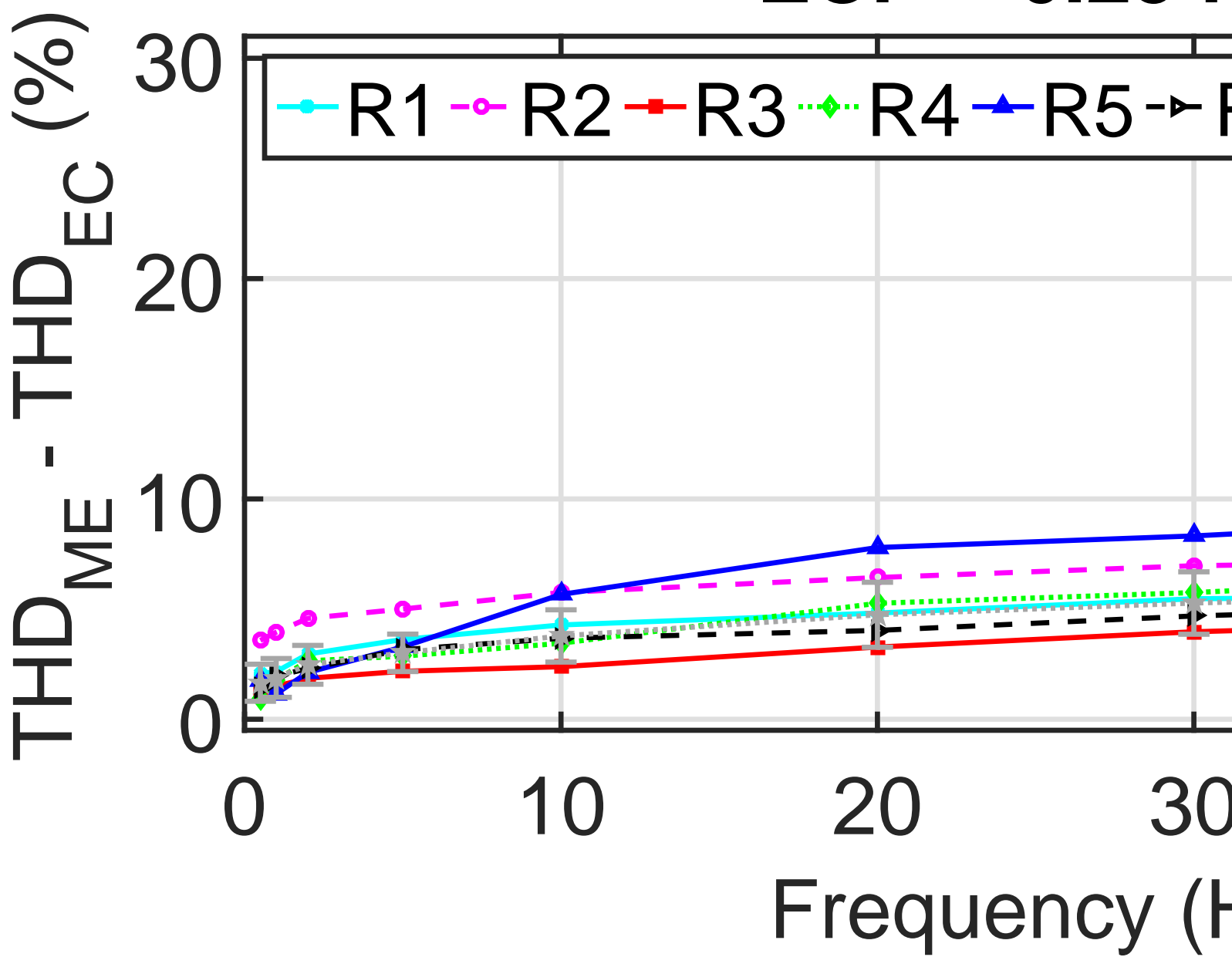
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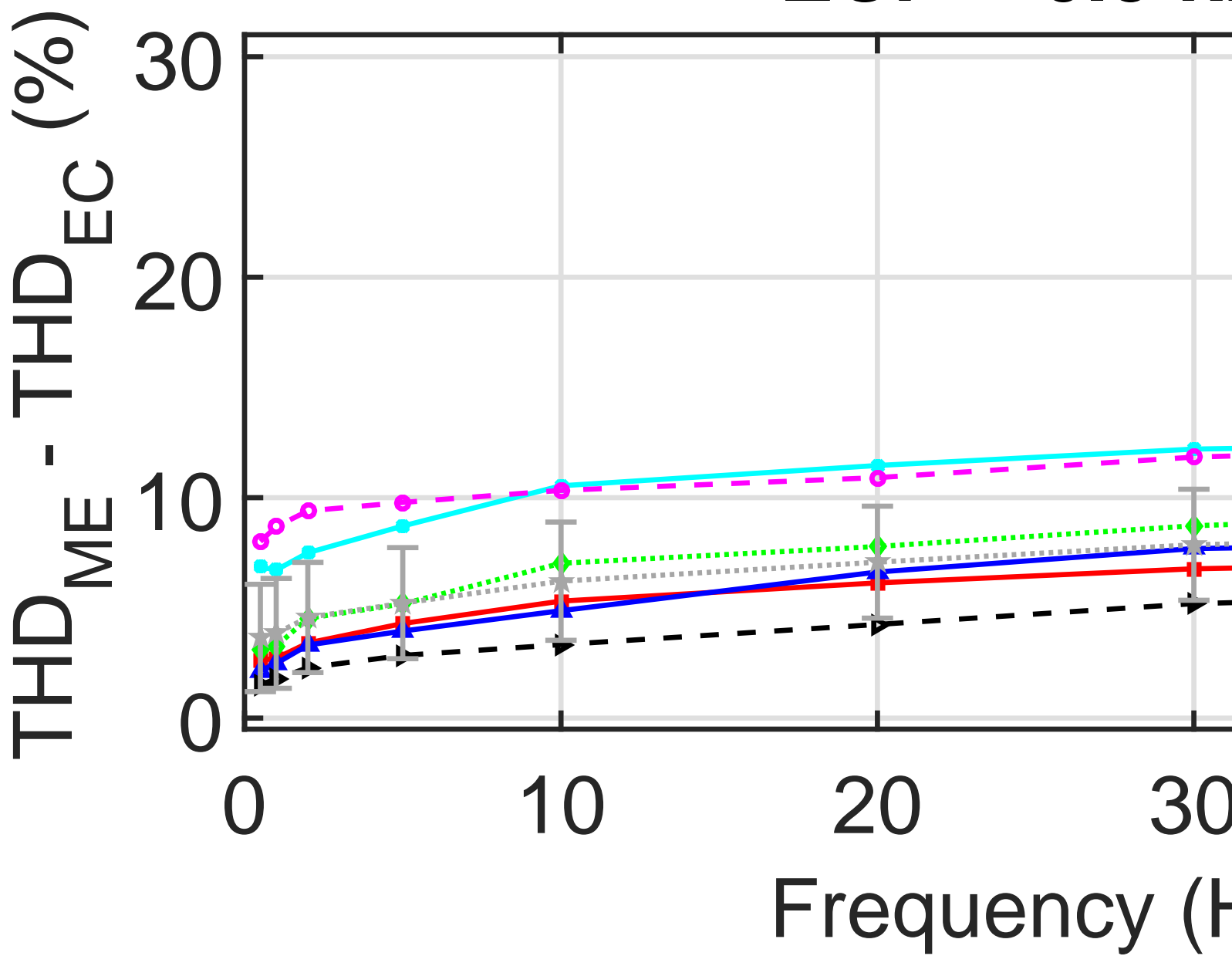


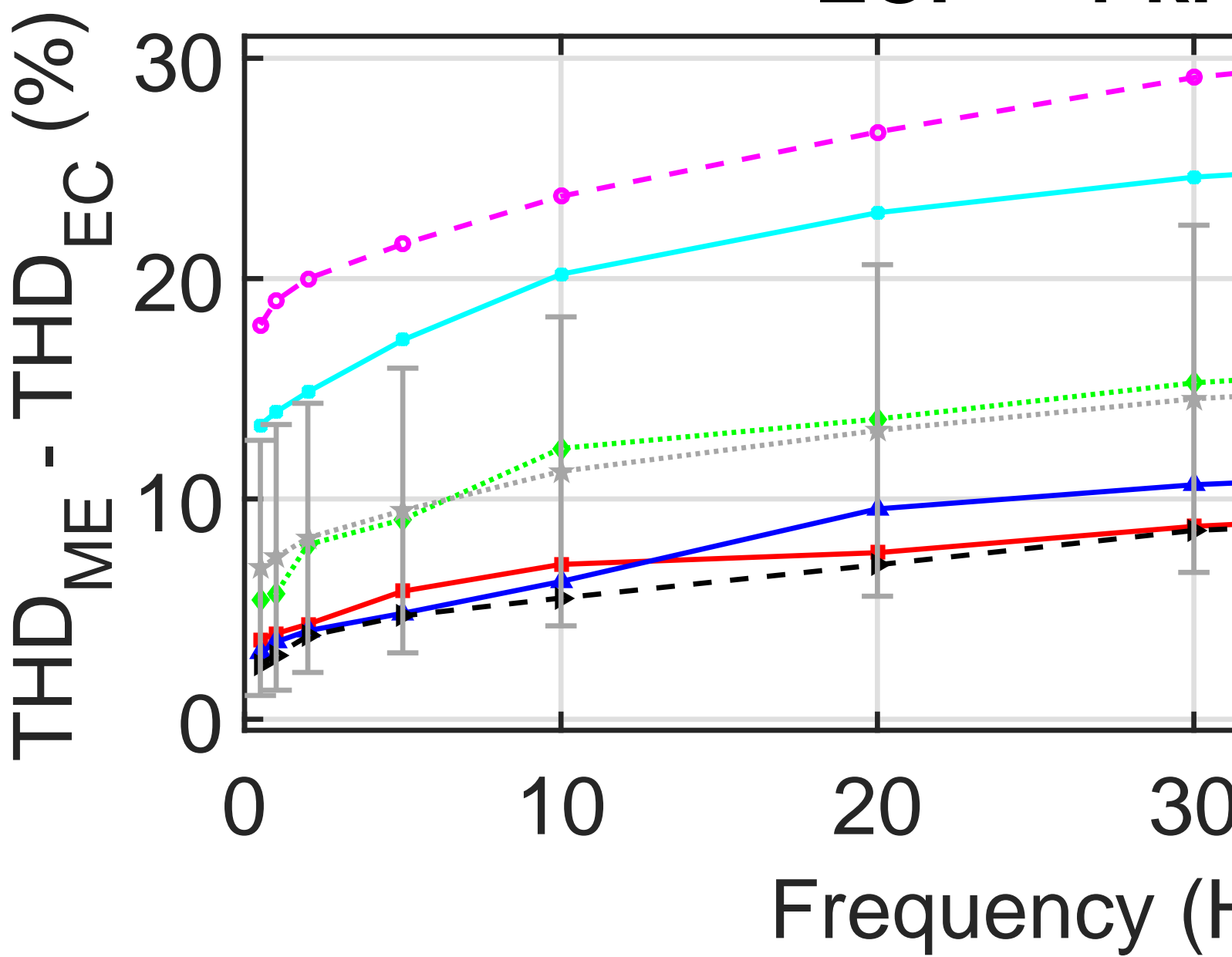


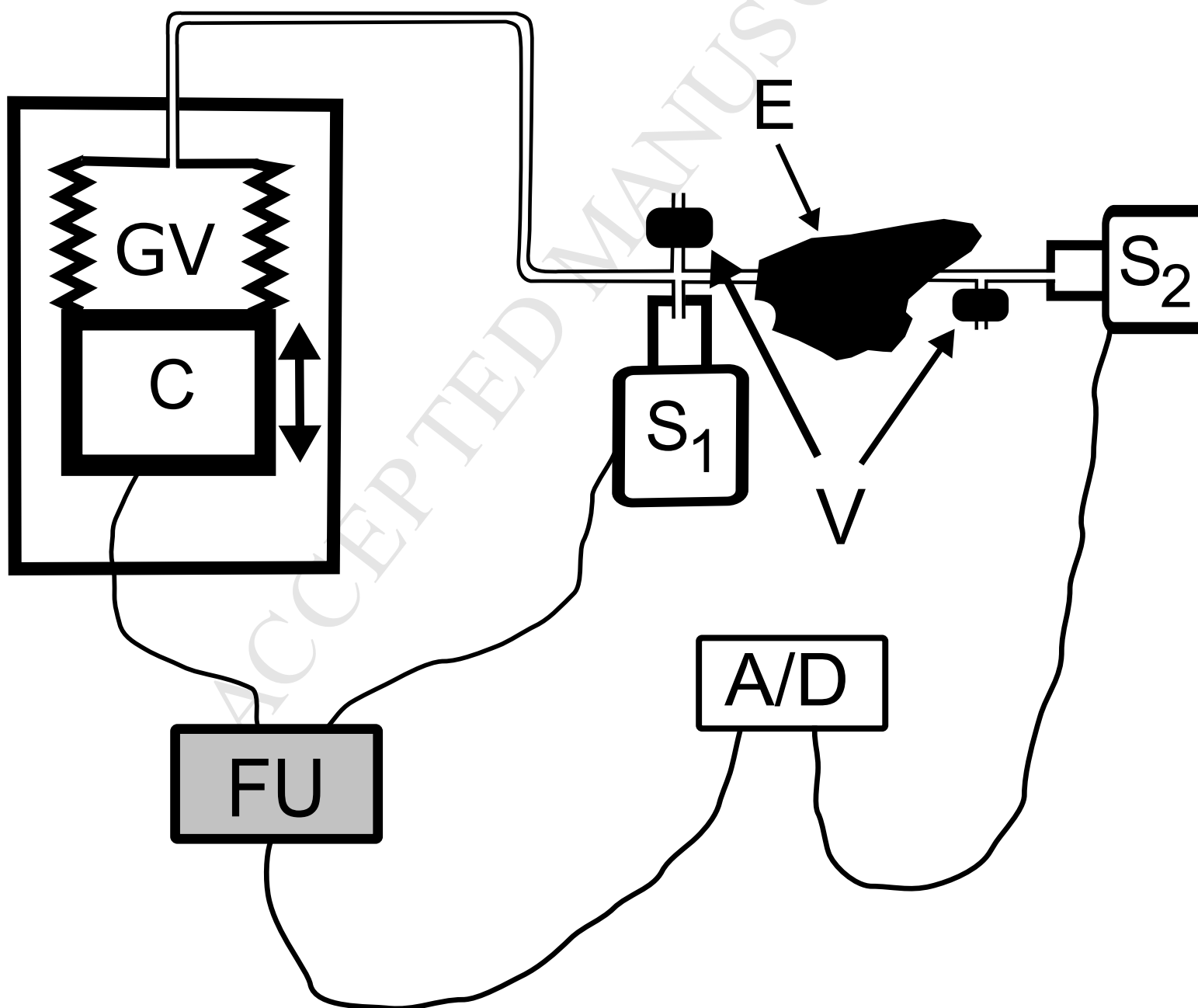




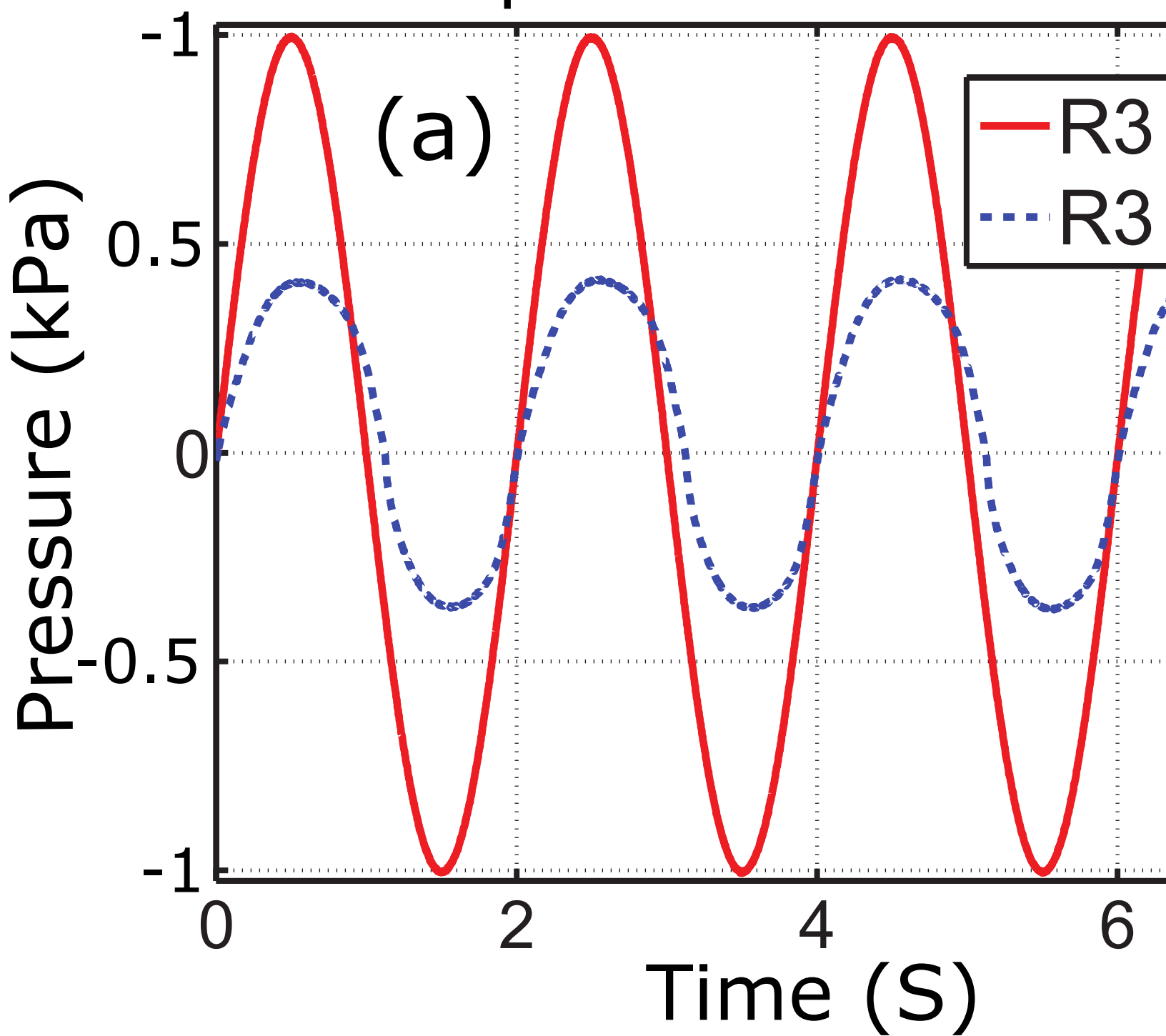
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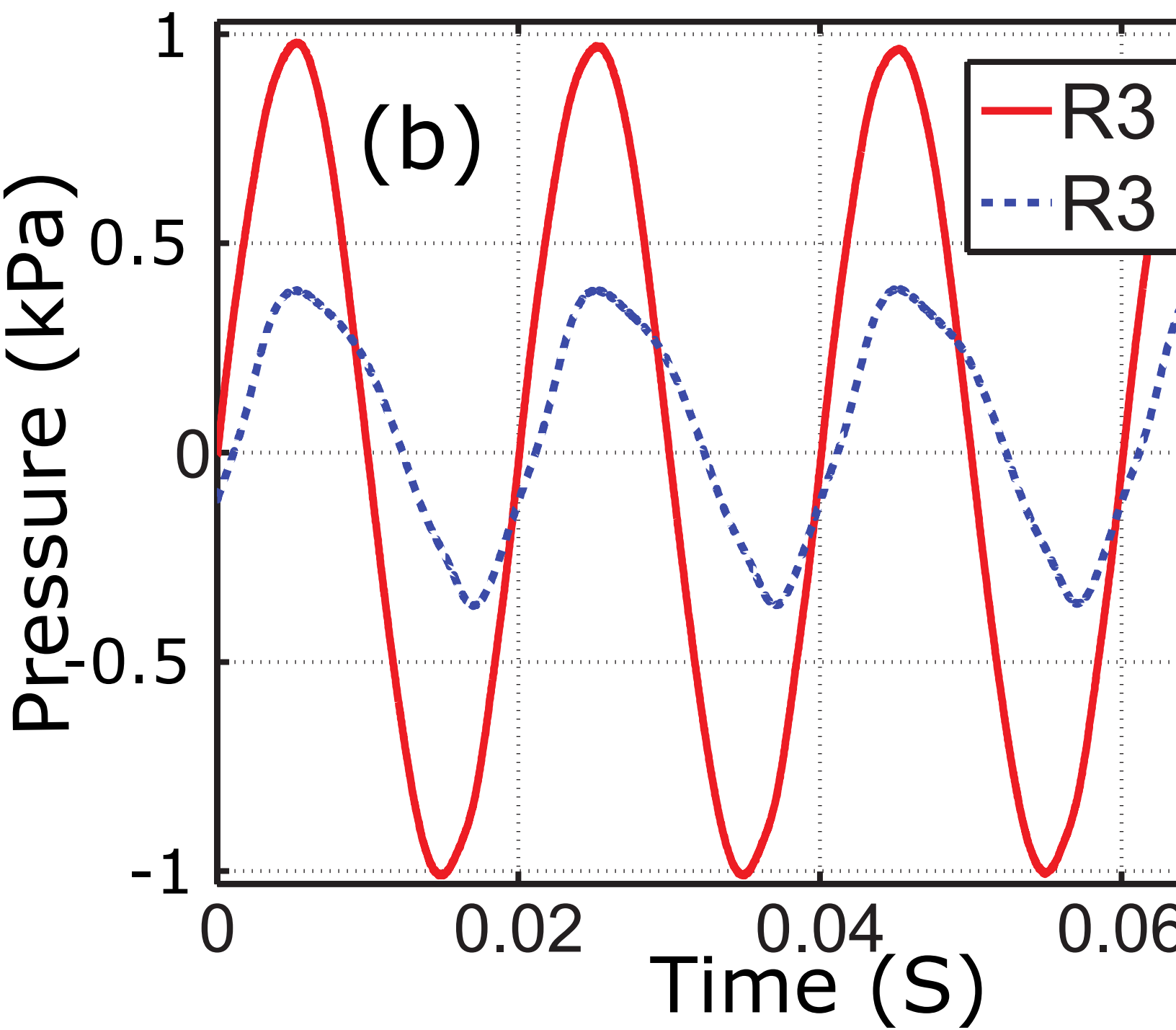
ECP = 1 kPa



Pressure amplitude of 1 kPa at



Pressure amplitude of 1 kPa and



- Middle ear pressure in rabbit is measured as a function of sinusoidal varying ear canal pressure
- Pressure amplitudes from 0.5 kPa to 2 kPa, frequencies from 0.5 Hz to 50 Hz
- Trans-tympanic pressure increases as function of frequency
- Total harmonic distortion of middle ear pressure increases as function of frequency and amplitude