Phosphorylation in Urea-Rich Eutectic Solvents

Dr. Bradley Burcar

Georgia Tech Astrobiology

1
00:00:12,499 --> 00:00:10,150
[Music]

2
00:00:16,820 --> 00:00:12,509
Thank You Moran for the nice

3
00:00:18,710 --> 00:00:16,830
introduction so before I jump into my

4
00:00:22,320 --> 00:00:18,720
solutions for the phosphorylation

5
00:00:23,650 --> 00:00:22,330
problems I thought I would just give a

6
00:00:28,370 --> 00:00:23,660
[Music]

7
00:00:30,410 --> 00:00:28,380
give a little bit of the core philosophy

8
00:00:32,930 --> 00:00:30,420
and the core view that we have in the

9
00:00:37,060 --> 00:00:32,940
center for chemical evolution so while

10
00:00:40,850 --> 00:00:37,070
the RNA world looks really good and

11
00:00:44,619 --> 00:00:40,860
probably existed at some time we don't

12
00:00:48,380 --> 00:00:44,629
believe that that was the very first

13
00:00:50,209 --> 00:00:48,390

form of life that existed on this planet

14
00:00:53,720 --> 00:00:50,219
we believe that there was something that

15
00:00:56,119 --> 00:00:53,730
preceded it and we use clues from RNA

16
00:00:59,389 --> 00:00:56,129
and DNA to figure out what could have

17
00:01:02,000 --> 00:00:59,399
preceded that and so one of the ways

18
00:01:04,670 --> 00:01:02,010
that we do that is we look at modern RNA

19
00:01:07,609 --> 00:01:04,680
in DNA and break it down into its three

20
00:01:10,310 --> 00:01:07,619
central components you have the

21
00:01:13,130 --> 00:01:10,320
recognition units the basis that it uses

22
00:01:16,310 --> 00:01:13,140
to store the information and also what

23
00:01:20,690 --> 00:01:16,320
can do the base pairing and solution you

24
00:01:23,060 --> 00:01:20,700
have the ionized linker something that

25
00:01:25,760 --> 00:01:23,070
has some sort of charge on it modern

26
00:01:28,310 --> 00:01:25,770
life uses phosphate and this helps it to

27
00:01:30,319 --> 00:01:28,320
soluble eyes in solution and also help

28
00:01:32,630 --> 00:01:30,329
the base pairing to happen by repelling

29
00:01:35,179 --> 00:01:32,640
the negative charges and then you have a

30
00:01:37,609 --> 00:01:35,189
tri functional connector a bridging

31
00:01:39,410 --> 00:01:37,619
molecule that keeps these pieces

32
00:01:41,960 --> 00:01:39,420
together and has nice chemical

33
00:01:44,990 --> 00:01:41,970
functionality to help chemistry and

34
00:01:48,499 --> 00:01:45,000
biochemistry down the line so in our

35
00:01:51,920 --> 00:01:48,509
Center we work on changing out these

36
00:01:54,230 --> 00:01:51,930
pieces from RNA and DNA and putting

37
00:01:56,120 --> 00:01:54,240
other pieces in there and seeing how

38
00:01:58,609 --> 00:01:56,130

this affects its properties and if you

39
00:02:01,209 --> 00:01:58,619
can have life without having the

40
00:02:04,520 --> 00:02:01,219
specific backbone as we see in modern

41
00:02:08,540 --> 00:02:04,530
biochemistry so we've had a good deal of

42
00:02:10,889 --> 00:02:08,550
success replacing the bases for the

43
00:02:14,190 --> 00:02:10,899
recognition units and replacing the Tri

44
00:02:16,170 --> 00:02:14,200
functional connectors but we've run into

45
00:02:18,720 --> 00:02:16,180
some problems when we tried to change

46
00:02:20,940 --> 00:02:18,730
that ionized linker into other moieties

47
00:02:24,690 --> 00:02:20,950
with our group and other groups in the

48
00:02:27,809 --> 00:02:24,700
world it just doesn't work as well so as

49
00:02:29,490 --> 00:02:27,819
opposed to trying to change it where

50
00:02:32,610 --> 00:02:29,500
some of us in the lab are trying to

51
00:02:34,860 --> 00:02:32,620
embrace it what if we had phosphate at

52
00:02:38,699 --> 00:02:34,870
the origins and it got incorporated into

53
00:02:42,750 --> 00:02:38,709
the molecules but this is fraught with

54
00:02:45,390 --> 00:02:42,760
some problems that have haunted the

55
00:02:50,490 --> 00:02:45,400
astrobiology community for the past 50

56
00:02:52,530 --> 00:02:50,500
to 60 years so one of the first issues

57
00:02:54,180 --> 00:02:52,540
is how do you even react it with early

58
00:02:58,920 --> 00:02:54,190
molecules how do you get phosphate

59
00:03:01,440 --> 00:02:58,930
incorporated in so these reactions are

60
00:03:03,899 --> 00:03:01,450
not favored in water at all it drives

61
00:03:07,490 --> 00:03:03,909
off water it's very hard to do in a

62
00:03:11,129 --> 00:03:07,500
water-based reaction and so modern

63
00:03:12,479 --> 00:03:11,139

biochemists use organic solvents and dry

64
00:03:14,819 --> 00:03:12,489
conditions to actually add the

65
00:03:17,789 --> 00:03:14,829
phosphorylation which is problematic

66
00:03:20,250 --> 00:03:17,799
because you can view the early Earth

67
00:03:22,890 --> 00:03:20,260
just covered in water and so it's nice

68
00:03:25,670 --> 00:03:22,900
to find some water or liquid based

69
00:03:28,610 --> 00:03:25,680
chemistry to make this happen and

70
00:03:31,589 --> 00:03:28,620
another major problem is the phosphate

71
00:03:34,229 --> 00:03:31,599
availability issue so a lot of the

72
00:03:36,920 --> 00:03:34,239
phosphate that we see on modern earth

73
00:03:40,289 --> 00:03:36,930
and we see from the mineral records is

74
00:03:43,280 --> 00:03:40,299
bounded insoluble species with the

75
00:03:45,149 --> 00:03:43,290
divalent cations and so this is

76
00:03:47,670 --> 00:03:45,159
traditionally been considered to be

77
00:03:50,729 --> 00:03:47,680
extremely inaccessible for prebiotic

78
00:03:53,460 --> 00:03:50,739
chemistry so how do we knock out these

79
00:03:57,240 --> 00:03:53,470
problems one at a time so how did the

80
00:04:00,409 --> 00:03:57,250
phosphorylation chemistry even happen so

81
00:04:04,559 --> 00:04:00,419
a remarkable molecule that's been

82
00:04:07,559 --> 00:04:04,569
studied for the past 40 years is urea

83
00:04:11,309 --> 00:04:07,569
this is actually the first organically

84
00:04:15,479 --> 00:04:11,319
synthesized molecule from waller in the

85
00:04:17,969 --> 00:04:15,489
early 19th century and it's a very it is

86
00:04:20,319 --> 00:04:17,979
thought to be extremely abundant on a

87
00:04:21,699 --> 00:04:20,329
prebiotic earth

88
00:04:24,279 --> 00:04:21,709

and one of the things that we all like

89
00:04:25,809 --> 00:04:24,289
to describe is you have the salt flats

90
00:04:28,869 --> 00:04:25,819
that you can see throughout the world

91
00:04:29,740 --> 00:04:28,879
now like this one depicted in Utah well

92
00:04:32,740 --> 00:04:29,750
on a prebiotic

93
00:04:34,330 --> 00:04:32,750
earth urea would have been so abundant

94
00:04:36,550 --> 00:04:34,340
that we probably would have had your

95
00:04:39,510 --> 00:04:36,560
rear flats just like we have salt flats

96
00:04:43,480 --> 00:04:39,520
so this would be very abundant and found

97
00:04:45,909 --> 00:04:43,490
everywhere and when you take a very

98
00:04:49,540 --> 00:04:45,919
simple reaction you mix in some soluble

99
00:04:52,870 --> 00:04:49,550
phosphate with urea and different sugars

100
00:04:55,059 --> 00:04:52,880
you have no water and just add heat a

101
00:04:58,899 --> 00:04:55,069
hundred degrees or more you actually

102
00:05:00,999 --> 00:04:58,909
form the phosphorylated product so this

103
00:05:03,999 --> 00:05:01,009
has been discovered about 40 years ago

104
00:05:06,040 --> 00:05:04,009
and it's a very nice reaction and a good

105
00:05:08,830 --> 00:05:06,050
way to get phosphate attached but it

106
00:05:11,740 --> 00:05:08,840
requires high temperatures that degrade

107
00:05:13,689 --> 00:05:11,750
a lot of the molecules that you want to

108
00:05:15,339 --> 00:05:13,699
phosphorylate and creates an awful lot

109
00:05:17,439 --> 00:05:15,349
of side products it's a very messy

110
00:05:22,600 --> 00:05:17,449
reaction with low yields but it does

111
00:05:24,550 --> 00:05:22,610
work so we have some chemistry that can

112
00:05:27,420 --> 00:05:24,560
work well how do you solve the problem

113
00:05:29,770 --> 00:05:27,430

of running it in the presence of water

114
00:05:32,050 --> 00:05:29,780
so as I pointed out you can see the

115
00:05:34,689 --> 00:05:32,060
reaction at the bottom just attaching

116
00:05:37,629 --> 00:05:34,699
phosphate to a simple molecule like

117
00:05:40,980 --> 00:05:37,639
glycerol it drives off water and you

118
00:05:43,749 --> 00:05:40,990
form the bus for the the phosphorylated

119
00:05:45,519 --> 00:05:43,759
organic down there it doesn't work if

120
00:05:48,580 --> 00:05:45,529
there's water because you need to remove

121
00:05:51,459 --> 00:05:48,590
the water so one of the things that

122
00:05:53,950 --> 00:05:51,469
we've started to look at based upon some

123
00:05:58,149 --> 00:05:53,960
previous work is using eutectics

124
00:06:01,089 --> 00:05:58,159
and eutectics are very unique systems

125
00:06:02,620 --> 00:06:01,099
you can take two solids like is depicted

126
00:06:05,409 --> 00:06:02,630
on the left and the right there

127
00:06:08,080 --> 00:06:05,419
mix them together and they actually form

128
00:06:09,760 --> 00:06:08,090
a liquid so it lowers the melting point

129
00:06:13,300 --> 00:06:09,770
of both of them you can take it to very

130
00:06:17,769 --> 00:06:13,310
low temperatures in a water free

131
00:06:20,200 --> 00:06:17,779
environment and so the tactics that I

132
00:06:22,990 --> 00:06:20,210
use for my experiments they do start

133
00:06:25,330 --> 00:06:23,000
with excess water but we heat them open

134
00:06:27,040 --> 00:06:25,340
to the atmosphere and drive off most of

135
00:06:28,550 --> 00:06:27,050
the excess water and create this nice

136
00:06:31,460 --> 00:06:28,560
liquid environment

137
00:06:37,159 --> 00:06:31,470
that goes down to very low temperatures

138
00:06:39,740 --> 00:06:37,169

so now we have this now we have this

139
00:06:42,470 --> 00:06:39,750
eutectic which I use urea to make

140
00:06:44,360 --> 00:06:42,480
so it's a very urea rich environment

141
00:06:47,180 --> 00:06:44,370
that's liquid and you can do a lot of

142
00:06:51,409 --> 00:06:47,190
very interesting fluid based chemistry

143
00:06:56,110 --> 00:06:51,419
at low temperatures and the tactics that

144
00:06:59,060 --> 00:06:56,120
I use are made of very valuable

145
00:07:01,730 --> 00:06:59,070
components of prebiotic ly so I have a

146
00:07:03,800 --> 00:07:01,740
ammonium formate and ammonium acetate we

147
00:07:05,540 --> 00:07:03,810
look at different chemical synthetic

148
00:07:07,340 --> 00:07:05,550
reactions that would have been likely on

149
00:07:09,860 --> 00:07:07,350
a prebiotic earth and see a lot of these

150
00:07:12,500 --> 00:07:09,870
just pop out and one of the wonderful

151
00:07:14,870 --> 00:07:12,510
things if you mix these together it

152
00:07:16,940 --> 00:07:14,880
evaporates off most of the compounds and

153
00:07:18,920 --> 00:07:16,950
you get a very specific ratio in a

154
00:07:20,510 --> 00:07:18,930
perfect ratio for doing a lot of the

155
00:07:22,219 --> 00:07:20,520
chemistry that we're looking for so you

156
00:07:24,469 --> 00:07:22,229
can start with any concentration that

157
00:07:27,650 --> 00:07:24,479
you want it all converges upon what we

158
00:07:30,980 --> 00:07:27,660
need so we've found a reaction medium

159
00:07:34,850 --> 00:07:30,990
that removes the water from it so that's

160
00:07:36,529 --> 00:07:34,860
cool we have two steps down already but

161
00:07:38,300 --> 00:07:36,539
where do we get the phosphate from and

162
00:07:40,510 --> 00:07:38,310
this has traditionally been the biggest

163
00:07:43,040 --> 00:07:40,520

problem with prebiotic phosphorylation

164
00:07:44,900 --> 00:07:43,050
so most of the minerals would be present

165
00:07:47,120 --> 00:07:44,910
in or most of the phosphate would be

166
00:07:49,909 --> 00:07:47,130
bound in a hydroxyl apatite which is

167
00:07:52,310 --> 00:07:49,919
extremely insoluble so it's bad for

168
00:07:54,830 --> 00:07:52,320
doing chemistry but it's good for modern

169
00:07:57,020 --> 00:07:54,840
life as this is what we make up when we

170
00:08:02,830 --> 00:07:57,030
used to make up bounce and if bones are

171
00:08:07,219 --> 00:08:05,390
that's a problem these don't dissolve

172
00:08:10,550 --> 00:08:07,229
you don't have any phosphate in your

173
00:08:12,620 --> 00:08:10,560
solution so we start looking at the

174
00:08:14,029 --> 00:08:12,630
modern earth as an analogue and try to

175
00:08:16,400 --> 00:08:14,039
figure out where we could have got

176
00:08:17,840 --> 00:08:16,410
phosphate from and see if it would be

177
00:08:20,300 --> 00:08:17,850
similar to what we could have on a

178
00:08:22,610 --> 00:08:20,310
prebiotic earth so what are the

179
00:08:26,510 --> 00:08:22,620
environments depict at the top is laguna

180
00:08:29,360 --> 00:08:26,520
santa maria in argentina and we see that

181
00:08:31,760 --> 00:08:29,370
this is a phosphate rich system that is

182
00:08:34,130 --> 00:08:31,770
in this aqueous pool so that seems great

183
00:08:36,740 --> 00:08:34,140
but you take a closer look and you see

184
00:08:38,779 --> 00:08:36,750
that the phosphate is coming from all of

185
00:08:39,519 --> 00:08:38,789
the biomass that's in there so it's not

186
00:08:43,209 --> 00:08:39,529
really a

187
00:08:45,579 --> 00:08:43,219
source it's just a biological source but

188
00:08:47,590 --> 00:08:45,589

then one of my collaborators remembered

189
00:08:51,939 --> 00:08:47,600
some of his work that he did in Spain

190
00:08:56,460 --> 00:08:51,949
just looking at of all things pig urine

191
00:08:59,470 --> 00:08:56,470
and waste pops so these are urea

192
00:09:04,179 --> 00:08:59,480
magnesium and calcium rich environments

193
00:09:05,860 --> 00:09:04,189
that just form giant waste pools and

194
00:09:07,960 --> 00:09:05,870
when you look at them you would expect

195
00:09:10,689 --> 00:09:07,970
to see the calcium phosphate the

196
00:09:13,079 --> 00:09:10,699
hydroxyl apatite babe depicted before

197
00:09:15,759 --> 00:09:13,089
but instead you see this mineral called

198
00:09:18,160 --> 00:09:15,769
struvite which is a much more soluble

199
00:09:20,949 --> 00:09:18,170
form and you see that to the exclusion

200
00:09:22,689 --> 00:09:20,959
of any other minerals in there so

201
00:09:24,639 --> 00:09:22,699
instead of form a hydroxyl apatite it

202
00:09:27,699 --> 00:09:24,649
gets trapped in this more soluble form

203
00:09:29,889 --> 00:09:27,709
so we started thinking can you do this

204
00:09:32,110 --> 00:09:29,899
in a laboratory so we tried to

205
00:09:35,220 --> 00:09:32,120
synthesize the struvite which would be a

206
00:09:38,079 --> 00:09:35,230
much better source for phosphorylation

207
00:09:41,439 --> 00:09:38,089
and we thought about this in terms of a

208
00:09:43,629 --> 00:09:41,449
prebiotic model so you can have a pool

209
00:09:45,879 --> 00:09:43,639
on the early earth full of calcium

210
00:09:47,829 --> 00:09:45,889
magnesium and phosphate it would

211
00:09:50,199 --> 00:09:47,839
precipitate and form this mineral at the

212
00:09:52,660 --> 00:09:50,209
bottom then you could drive off all the

213
00:09:55,420 --> 00:09:52,670

water you have some local outgassing

214
00:09:58,840 --> 00:09:55,430
from volcanoes and other hydrothermal

215
00:10:02,259 --> 00:09:58,850
vents and it could form it could provide

216
00:10:06,610 --> 00:10:02,269
magnesium and sulfate and urea and

217
00:10:10,329 --> 00:10:06,620
formate the way you come down wash it

218
00:10:14,740 --> 00:10:10,339
into the pool and then you get the

219
00:10:16,210 --> 00:10:14,750
conversion to the exact form of minerals

220
00:10:17,769 --> 00:10:16,220
that we need down there so instead of

221
00:10:21,069 --> 00:10:17,779
calcium phosphate now you have a

222
00:10:22,919 --> 00:10:21,079
struvite layer at the bottom and you've

223
00:10:26,139 --> 00:10:22,929
done it you've mobilized the phosphate

224
00:10:29,019 --> 00:10:26,149
so we tested this in the laboratory we

225
00:10:30,999 --> 00:10:29,029
simply took the hydroxyl apatite mix it

226
00:10:32,980 --> 00:10:31,009
with the eutectic heated it for seven

227
00:10:37,059 --> 00:10:32,990
days with adding an magnesium sulfate

228
00:10:39,790 --> 00:10:37,069
and lo and behold we saw the conversion

229
00:10:42,819 --> 00:10:39,800
of hydroxyl apatite disturb it-- and we

230
00:10:46,480 --> 00:10:42,829
have the XRD and the rom inspector over

231
00:10:48,610 --> 00:10:46,490
there to help to prove it but we saw

232
00:10:52,180 --> 00:10:48,620
basically quantitative conversion in

233
00:10:54,550 --> 00:10:52,190
these conditions which is fantastic so

234
00:10:56,410 --> 00:10:54,560
we wanted to put it all together and see

235
00:11:00,730 --> 00:10:56,420
if not only you could convert but if you

236
00:11:02,170 --> 00:11:00,740
could also phosphorylate so one of the

237
00:11:03,579 --> 00:11:02,180
things we looked at for prebiotic

238
00:11:05,350 --> 00:11:03,589

chemistry is you want to keep it simple

239
00:11:08,019 --> 00:11:05,360
the early Earth didn't have a lot of

240
00:11:10,329 --> 00:11:08,029
tools and neither should we so I just

241
00:11:13,300 --> 00:11:10,339
run my reactions on a simple plate by

242
00:11:16,329 --> 00:11:13,310
mixing everything together so I take

243
00:11:18,370 --> 00:11:16,339
adenosine as my model molecule mix it

244
00:11:20,769 --> 00:11:18,380
with any phosphate source mineral or

245
00:11:22,480 --> 00:11:20,779
soluble phosphate heat it from fifty

246
00:11:25,210 --> 00:11:22,490
eighty five degrees and they do get

247
00:11:27,610 --> 00:11:25,220
phosphorylated identity and one of the

248
00:11:30,130 --> 00:11:27,620
nice things I put up this chromatogram

249
00:11:32,800 --> 00:11:30,140
up here just to show how clean these

250
00:11:36,280 --> 00:11:32,810
reactions are so every peak on there

251
00:11:38,620 --> 00:11:36,290
represents a different species and so I

252
00:11:40,630 --> 00:11:38,630
get all of these phosphorylated species

253
00:11:42,400 --> 00:11:40,640
and not many other side reactions so

254
00:11:47,110 --> 00:11:42,410
this is a lot cleaner than the previous

255
00:11:49,870 --> 00:11:47,120
work and overall I've created for

256
00:11:52,389 --> 00:11:49,880
different eutectics that I show up here

257
00:11:54,880 --> 00:11:52,399
the part that outlined in red is using

258
00:11:56,430 --> 00:11:54,890
soluble phosphate and you can see I can

259
00:11:58,690 --> 00:11:56,440
get upwards of 90 percent

260
00:12:01,269 --> 00:11:58,700
phosphorylation when phosphate but when

261
00:12:04,449 --> 00:12:01,279
the phosphate is in water or in the

262
00:12:07,540 --> 00:12:04,459
eutectic soluble when I take the

263
00:12:09,940 --> 00:12:07,550

struvite or Newberry as I have depicted

264
00:12:12,370 --> 00:12:09,950
here which is moderately soluble the

265
00:12:15,370 --> 00:12:12,380
phosphorylation does go down but I can

266
00:12:17,829 --> 00:12:15,380
still get better than 20% so that is a

267
00:12:19,630 --> 00:12:17,839
pretty good mineral for phosphorylating

268
00:12:21,400 --> 00:12:19,640
you can't expect 100% yields from

269
00:12:22,840 --> 00:12:21,410
everything but one of the most

270
00:12:26,710 --> 00:12:22,850
remarkable things is with the

271
00:12:28,840 --> 00:12:26,720
hydroxyapatite down there we get a small

272
00:12:31,960 --> 00:12:28,850
amount of phosphorylation which is

273
00:12:35,050 --> 00:12:31,970
better than what is shown here with just

274
00:12:39,100 --> 00:12:35,060
the urea where you get none and even

275
00:12:41,560 --> 00:12:39,110
more interestingly when we add magnesium

276
00:12:43,900 --> 00:12:41,570
to the reaction magnesium sulfate

277
00:12:46,690 --> 00:12:43,910
a doubling or tripling of the amount of

278
00:12:49,840 --> 00:12:46,700
phosphate so we take these eutectics

279
00:12:51,250 --> 00:12:49,850
just add some magnesium sulfate and we

280
00:12:53,920 --> 00:12:51,260
get a good amount of phosphorylation

281
00:12:57,160 --> 00:12:53,930
helping to address a long-held problem

282
00:12:59,440 --> 00:12:57,170
in prebiotic chemistry so that overall

283
00:13:01,690 --> 00:12:59,450
conclusions the phosphorylation is

284
00:13:03,940 --> 00:13:01,700
robust it happens in a wide range of

285
00:13:06,730 --> 00:13:03,950
mixture it's not a very specialized

286
00:13:08,950 --> 00:13:06,740
reaction it's absorbed at moderately low

287
00:13:11,590 --> 00:13:08,960
temperatures as low as 50 degrees which

288
00:13:13,960 --> 00:13:11,600

is very easy on prebiotic earth it's

289
00:13:17,530 --> 00:13:13,970
successful with the insoluble hydroxyl

290
00:13:19,270 --> 00:13:17,540
apatite and it's significantly improved

291
00:13:21,940 --> 00:13:19,280
in the presence of magnesium sulfate

292
00:13:25,120 --> 00:13:21,950
these are very prebiotic viable

293
00:13:27,670 --> 00:13:25,130
conditions and so I'd like to

294
00:13:29,590 --> 00:13:27,680
acknowledge my collaborators from many

295
00:13:31,600 --> 00:13:29,600
different Institute's from the

296
00:13:33,820 --> 00:13:31,610
University of southern Florida and from

297
00:13:34,630 --> 00:13:33,830
the University of the University da de

298
00:13:38,710 --> 00:13:34,640
alcalá

299
00:13:52,480 --> 00:13:38,720
and my group and my funding sources and

300
00:13:58,150 --> 00:13:52,490
I'd like to take any questions now all

301
00:14:00,010 --> 00:13:58,160
right catch it's worked all right is the

302
00:14:03,070 --> 00:14:00,020
is the mechanism there more tied to the

303
00:14:06,430 --> 00:14:03,080
heating or to the drying the mechanism

304
00:14:09,010 --> 00:14:06,440
is tied to the urea in the absence of

305
00:14:10,690 --> 00:14:09,020
water and so you do need some heat there

306
00:14:12,970 --> 00:14:10,700
in order to actually activate the

307
00:14:14,440 --> 00:14:12,980
phosphate okay so it's not about driving

308
00:14:17,230 --> 00:14:14,450
though it's not about driving the water

309
00:14:19,930 --> 00:14:17,240
off it's about it's about both okay okay

310
00:14:32,920 --> 00:14:19,940
if I try to run it in water if I close

311
00:14:34,810 --> 00:14:32,930
the vials it doesn't work now yeah what

312
00:14:37,000 --> 00:14:34,820
about the pH for example we have

313
00:14:39,340 --> 00:14:37,010

hydroxyapatite which is not soluble but

314
00:14:40,360 --> 00:14:39,350
the Fiat sulfuric acid which is common

315
00:14:42,730 --> 00:14:40,370
and we'll connect settings you just

316
00:14:45,940 --> 00:14:42,740
leash all the phosphate immediately so

317
00:14:49,030 --> 00:14:45,950
it's not a problem on the more acidic

318
00:14:49,580 --> 00:14:49,040
page in 7 sure yeah and so that's one of

319
00:14:51,950 --> 00:14:49,590
the things

320
00:14:54,560 --> 00:14:51,960
can have extremely low PHS which will

321
00:14:56,720 --> 00:14:54,570
sell utilize it and that's fine but it's

322
00:14:59,060 --> 00:14:56,730
hard to preserve a lot of the chemicals

323
00:15:01,610 --> 00:14:59,070
that you need and do other reactions in

324
00:15:03,500 --> 00:15:01,620
an extremely acidic environment so you

325
00:15:05,960 --> 00:15:03,510
can view it as a multi-step problem

326
00:15:08,060 --> 00:15:05,970
where you dissolve it you possibly

327
00:15:11,000 --> 00:15:08,070
phosphorylate it and then you move it on

328
00:15:13,130 --> 00:15:11,010
to another system so that's been the

329
00:15:15,920 --> 00:15:13,140
traditional view of it before but it's

330
00:15:17,990 --> 00:15:15,930
highly contentious because of the other